Supplement 1

For: Ya-Ting Du, Ya-Wei Li, Bin-Jiang Zhao, et al. Long-term survival after combined epidural-general anesthesia or general anesthesia alone: Follow-up of a randomized trial.

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan, final statistical analysis plan, summary of changes.

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Original study protocol

Impacts of epidural anesthesia and analgesia on long-term survival in elderly patients after major surgery: 3-year follow-up of a multicenter, randomized controlled trail

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Date of last revision: June 13, 2014

Version of protocol: V2.0

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1. Background

Cancer is one of the leading causes of surgery in elderly patients. In our previous study of the "Effects of two different anesthesia-analgesia methods on incidence of postoperative delirium in elderly patients: a multicenter randomized controlled trial (PUCRP 201101)", about 92% of enrolled patients underwent surgery for cancer. Surgery is an important part of cancer treatment; in fact, it is the main treatment for resectable solid organ cancers. Cancer recurrence/metastasis is the main reason that affects the long-term outcome after cancer surgery. According to previous studies, cancer recurrence develops in about 30-40% of patients after radical surgery for stage II-III colorectal cancer; cancer recurrence mainly occurred within 2 years.[1] And metastasis is responsible for about 90% of all cancer deaths.[2]

The development of recurrence/metastasis after cancer surgery depends largely on the balance between the anti-metastatic immune function of the body and the ability of the residual cancer cells to implant, proliferate and attract new blood vessels.[3] It is true that the primary cancer is resected during surgery; however, surgery or surgery-related response may promote cancer recurrence/metastasis, possibly through the following ways. Firstly, even with the most advanced surgical techniques, cancer cells enter the lymphatic system and blood circulation during surgery in a significant proportion of patients.[4,5] Secondly, cancer resection reduces tumor-related anti-angiogenic factors [6] but increases pro-angiogenic factors such as vascular endothelial growth factor (VEGF);[7] these may promote the growth of local and distant cancer.[8] Thirdly, surgery may induce inhibition of cell-mediated immunity including the function of cytotoxic T cells and natural killer cells which are the primary anti-tumor cells,[9,10] possibly by surgery-related stress response,[11-14] blood loss and transfusion,[15,16] hypothermia,[17,18] and even perioperative anxiety.[9,19-24] The peak of immunosuppression was thought to occur on the third day after surgery.[25]

Anesthesia and perioperative management may also affect long-term outcomes after cancer surgery.[26] Many evidences come from experimental studies. For example, isoflurane and halothane inhibit the toxicity of interferon-enhanced NK cells; sevoflurane suppresses cytokine release from NK cells and NK cell-related cells.[27,28] Hypoxia inducible factors (HIFs) plays an important role in tumor formation and metastasis, high levels HIFs are closely associated with poor prognosis.[29-34] Studies found that inhalation of anesthetic drugs (including isoflurane, sevoflurane, desflurane, nitric oxide and xenon) can upregulate the expression of HIFs,[35-44] but the effects on cancer patients remain unclear. In addition, inhaled anesthetics can modulate gene expression in breast and brain tumor cells.[45] Among intravenous anesthetics, ketamine and thiopental are found to suppress NK cell activity and promote tumor metastasis.[46] On the other hand, propofol and barbiturates may block the translation of HIFs mRNA, and then down-regulate HIFs.[47,48] Experimental studies also showed that propofol inhibits the invasion of cancer cells;[49] but clinical significance is not clear. Opioids are used to relieve perioperative pain, but may also inhibits cellular and humoral immune function. In animal studies, clinically relevant dose opioids can inhibit NK cell activity, and promote tumor growth and angiogenesis;[50,51] but conflicting evidence also exists.[52,53] On the other hand, local anesthetic drugs have effect of membrane stabilization and can inhibit invasion and proliferation of cancer cells in experimental studies.[54,55]

Intense and persistent stress response during the perioperative period may promote metastasis by suppressing immune function.[9] Regional anesthesia such as neuraxial anesthesia can block the noxious afferent stimuli to the central nervous system; and thus, blunt the activation of the sympathetic nervous system, relieve the neuroendocrine response, and reduce the requirement for opioids. In clinical studies, use of thoracic epidural anesthesia is associated with lower concentrations of stress hormones, less release of endogenous opioids, and milder immunosuppression after surgery.[56,57] In another study, women who underwent breast cancer surgery with paravertebral block and propofol sedation had lower serum concentration of vascular endothelial growth factor (VEGF) than those with general anesthesia.[58] Theoretically, use of epidural anesthesia/analgesia during cancer surgery may help to reduce postoperative recurrence and metastasis.

Whether regional anesthesia can provide beneficial effects on tumor recurrence and metastasis remain unclear. Current evidence mainly comes from retrospective studies. Biki et al.[59] analyzed data of patients after radical prostatectomy; after adjusting for confounding factors, use of epidural anesthesia was associated with a reduced risk of recurrence by about 57%. Exadaktylos et al.[60] also reported that women who received paravertebral block during breast cancer surgery had lower recurrence and metastasis rate at 36 months. In some studies, the advantages of epidural anesthesia appear only in some subgroups. For example, in a study of patients undergoing colorectal cancer surgery, epidural anesthesia was associated with a lower cancer

recurrence only in patients older than 64 years of age.[61] In another study, epidural anesthesia was associated with a reduced long-term mortality in patients after rectal cancer surgery but not colon cancer surgery.[62] However, there are also studies that reported no beneficial effects. In a post-hoc analysis of randomized trial, Myles et al.[63] reported that use of epidural anesthesia during major abdominal cancer surgery did not improve cancer-free survival. Recently, Chen et al.[64] conducted a meta-analysis and found that use of epidural anesthesia/analgesia might be associated with improved overall survival after surgery for operable cancer; however, no advantage was found regarding recurrence and metastasis. Therefore, prospective randomized controlled trials are required to further clarify the effect of epidural anesthesia on long-term outcomes after cancer surgery.

We hypothesize that use of epidural anesthesia/analgesia during cancer surgery may reduce recurrence/metastasis and improve long-term survival, possibly by blunting stress response and relieving immunosuppression.

2. Objective

To investigate whether "combined epidural-general anesthesia and postoperative epidural analgesia" compared with "general anesthesia and postoperative intravenous analgesia" can improve long-term survival in elderly patients after major thoracic and abdominal surgery for cancer.

3. Study design

3.1 Type of the study

It is a long-term follow-up of patients enrolled in the underlying trial "Effects of two anesthesia-analgesic methods on incidence of postoperative delirium in elderly patients undergoing major thoracic and abdominal surgery: a multicenter randomized controlled trial (PUCRP 201101)". The outcome assessors are not aware of the group assignment and results of the previous trial during the follow-up period.

3.2 Participating centers

This multicenter trial is conducted in seven tertiary care hospitals affiliated with Peking University in Beijing, China. The seven participating centers include Peking University First Hospital, Peking University People's Hospital, Peking University Third Hospital, Beijing Hospital, Beijing Shijitan Hospital, Beijing Cancer Hospital, and China-Japan Friendship Hospital.

4. Study population

4.1 Participants

Potential participants are patients who are enrolled in the underlying trial "Effects of two anesthesia-analgesic methods on incidence of postoperative delirium in elderly patients undergoing major thoracic and abdominal surgery: a multicenter randomized controlled trial (PUCRP 201101)", completed the trial and agreed to receive postoperative follow-up via telephone. The underlying trial was initiated from November 21, 2011, and planned to enroll 1800 patients. Up to now, there are more than 900 patients recruited; about 170 patients has been recruited for more than 2 years.

4.2 Inclusion and exclusion criteria of the underlying trial

The inclusion and exclusion criteria of the underlying trial include the following.

4.2.1 Inclusion criteria:

4.2.1.1 Elderly patients (aged 60-90 years).

4.2.1.2 Scheduled to undergo non-cardiac thoracic or abdominal surgery with an expected duration of 2 hours or longer.

4.2.1.3 Agreed to receive postoperative patient-controlled analgesia.

4.2.2 Exclusion criteria:

4.2.2.1 Previous history of schizophrenia, epilepsy or Parkinson disease, or unable to complete preoperative assessment due to severe dementia, language barrier or end-stage disease.

4.2.2.2 History of myocardial infarction or stroke within 3 months before surgery.

4.2.2.3 Any contraindication for epidural anesthesia and analgesia, including abnormal vertebral anatomy, previous spinal trauma or surgery, severe chronic back pain, coagulation disorder (prothrombin time or activated partial prothrombin time longer than 1.5 times of the upper normal limit, or platelet count of less than 80×10^9/L), local infection near the site of puncture, and severe sepsis.

4.2.2.4 Severe heart dysfunction (New York Heart Association functional classification 3 or above), hepatic insufficiency (Child-Pugh grade C), or renal insufficiency (serum creatinine of 442 μmol/L or above, with or without serum potassium of 6.5 mmol/L or above, or requirement of renal replacement therapy).

4.2.2.5 Any other conditions that were considered unsuitable for the study participation.

4.3 Criteria of drop-out of the underlying trial

4.3.1 Study intervention was not administered (due to failed epidural puncture, failed epidural catheterization, epidural catheter obstruction, blood appear in epidural catheter, inadequate epidural analgesia, etc.).

4.3.2 Intervention was interrupted by the investigators/anesthesiologists (due to adverse events).

4.3.3 Use of a prohibited drugs.

The causes of protocol deviation should be recorded and corrected when possible. The cases would be followed up according to the study protocol and included in the intention-to-treat analysis.

4.3.4 Withdraw consent after intervention started.

The situation should be recorded. The primary therapeutic effects recorded in the last time will be regarded as the final results. The cases will be included in the intention-to-treat analysis.

4.4 Criteria of elimination of the underlying trial

Included patients will be excluded if they meet any of the following criteria:

4.4.1 Withdraw consents before intervention.

4.4.2 Surgery cancelled.

4.4.3 No assessment result of the primary outcome.

The causes of elimination should be explained. The case will be excluded from the intention-to-treat analysis. The case report forms will be preserved for reference.

5. Long-term follow-ups

5.1 Requirements for investigators

Investigators who perform the long-term follow-ups are not involved in patient management during the underlying and have no knowledge of study group assignment. They will be specially trained for long-term follow-ups and authorized by the principle investigator.

5.2 Preparation before follow-ups

History of surgery will be re-checked in the electronic medical record system; the results of pathological examination of surgical specimens (including the Tumor-Node-Metastasis [TNM] stage of cancer) and the diagnoses at hospital discharge will be collected.

5.3 Conduct of long-term followed-ups

5.3.1 Long-term follow-ups will be performed from the second year after surgery via telephone interview (after ethic approval), for a period of up to 3 years after surgery.

5.3.2 Data collection during long-term follow-ups includes the following:

5.3.2.1 Results of postoperative re-examinations. For patients after cancer surgery, cancer recurrence is defined as reappearance of the same cancer in the original place or in the organs/lymph nodes near the place it first started; cancer metastasis is defined as reappearance of the same cancer in another part of the body, with some distance from where it started. For patients with confirmed cancer recurrence and/or metastasis, the date of earliest diagnoses and subsequent therapies will be documented.

5.3.2.2 Postoperative therapies for the primary surgical disease. For patients after cancer surgery, anti-cancer therapies may include radiotherapy, chemotherapy, interventional therapy, reoperation, and others therapies.

5.3.2.3 Re-hospitalization after surgery. Reasons leading to re-hospitalization will be documented, including recurrence/metastasis of the original cancer, new cancer, new serious non-cancer disease, or other conditions. The diagnostic evidences and treatments will be documented.

5.3.2.4 For 3-year survivors, cognitive function will be assessed with the modified Telephone Interview for Cognitive Status (TICS-m; a 12-item questionnaire that assesses global cognitive function by verbal communication via telephone. The score ranges from 0 to 50, with higher score indicating better function);[65] quality of life will be assessed with the World Health Organization Quality of Life-brief version (WHOQOL-BREF; a 24-item questionnaire that provides assessments of the quality of life in physical, psychological, and social relationship, and environmental domains. For each domain, the score ranges from 0 to 100, with higher score indicating better function).[66]

5.3.2.5 For patients who die during the follow-up period after surgery, the exact date of death will be recorded (consistent with death certificate). The causes of death will be documented. Cancer-specific death is defined as death fully attributable to a specific cancer, i.e., the cancer for which the surgery is performed during the underlying trial.[67] Cancer-specific death usually occur after cancer recurrence and/or metastasis, with other causes including stroke, myocardial infarction or accident excluded.

5.4 Management of patients lost to follow-up

5.4.1 Patients or their family members will be contacted for at least 5 attempts on 5 different days before they are marked as lost to follow-up.

5.4.2 For patients who are lost to contact, the following systems will be checked in order to minimize the rate of loss-to-follow-up, i.e., the laboratory test system, imaging examination system, medical insurance system, and outpatient registration system of the hospital.

5.4.3 For patients who are lost-to-follow-up, the time of last hospital visits after surgery recorded in the in- or outpatient medical record system will be adopted as the censoring time.

6. Efficacy outcomes

6.1 Primary outcome

Overall survival, which is defined as the time from surgery to the date of all-cause death.

6.2 Secondary outcomes

6.2.1 Overall survival in cancer patients. Overall survival is defined as the time from surgery to the date of all-cause death.

6.2.2 Time to cancer recurrence and/or metastasis after surgery in all and cancer patients.

6.2.3 Time to re-hospitalization after surgery in all and cancer patients.

6.2.4 All-cause mortality rate at 1, 2 and 3 years after surgery in all and cancer patients.

6.2.5 Cognitive function in 3-year survivors, which will be assessed with the TICS-m at the end of the 3rd year after surgery.

6.2.6 Quality of life in 3-year survivors, which will be assessed with the WHOQOL-BREF at the end of the 3rd year after surgery.

7. Withdrawal from long-term follow-ups

7.1 Participants will be withdrawn from long-term follow-ups in the following conditions:

7.1.1 Requested by participants or legal representatives of participants.

7.1.2 Considered to be unsuitable for continued follow-ups by the investigators.

7.2 For patients who are withdrawn from the long-term follow-ups, detailed reasons leading to withdrawal will be recorded. Follow-up data of the last visit will be recorded.

8. Safety consideration

This is a long-term follow-up of patients enrolled in our underlying study “Effects of two different anesthesia-analgesia methods on incidence of postoperative delirium in elderly patients undergoing major thoracic and abdominal surgery: a multicenter randomized controlled trial (PUCRP 201101)”. There are no additional interventions; and, therefore, there are no additional risks for the patients.

9. Unmasking of group assignment

9.1 After the follow-ups of all cases have been completed, the data of case report forms have been checked as correct and the data entry have been finished, the database will be checked by the data manager.

9.2 After the database is locked, unmasking will be conducted. The database will be sent to the statisticians for statistical analysis.

10. Data management

10.1 The investigators should record data timely, completely and correctly according to the original examinations and follow-up assessments.

10.2 The completed case report forms, after signed by the supervisors, will be sent to a clinical data custodian.

10.3 After the data in the case report forms have been input and checked, the case report forms will be stored in sequence order.

10.4 Data management will be inspected by Peking University Clinical Research Institute.

11. Statistical analysis

11.1 General principles

11.1.1 Numeric variables will be presented as mean ± standard deviation or median (interquartile range). Categorical variables will be presented as number (%).

11.1.2 Two-tailed tests will be used in all statistical analysis unless otherwise indicated, and $P<0.05$ will be considered statistically significant.

11.1.3 The primary and secondary outcomes will be analyzed in an intention-to-treat population, i.e., all patients are analyzed in the group to which they are randomized and received at least part of study intervention. If epidural anesthesia and/or analgesia fail, these patients will be converted to general anesthesia and intravenous analgesia but will be analyzed in the group to which they are randomized. Also, we will do per-protocol analysis for the primary endpoint, in this case, drop-out patients will be excluded.

11.2 Patient enrolment, drop-out/elimination and lost-to-follow-up

The status of patient recruitment, drop-out/elimination and lost-to-follow-up will be summarized and listed. Comparison of the overall elimination/drop-out and lost-to-follow-up rate between the two groups will be performed with chi square test.

*11.3 **Demographics** and baseline characteristics*

11.3.1 Demographics and baseline data will be presented.

11.3.2 For between-group differences, numeric variables will be compared with independent-sample t test or Wilcoxon test. Categorical variables will be compared with chi square test, continuity-corrected chi-square test or Fisher's exact test. Ordinal variables will be compared with Wilcoxon test or Cochran-Mantel-Haenszel test.

11.4 Efficacy analysis

11.4.1 Evaluation of primary endpoint

The overall survival after surgery will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; a Cox proportional hazards model will be used to adjust for potential confounding factors. The estimated difference between two groups will be expressed as hazard ratio and 95% CI. Patients who are lost to follow-up will be censored at the time of last contact.

11.4.2 Evaluation of secondary endpoints

11.4.2.1 Time-to-event variables (time to cancer recurrence/metastasis and time to rehospitalization after surgery) will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; Cox proportional hazards models will be used to adjust for potential confounding factors. The estimates hazard ratios and 95% CIs will be provided. Patients who are lost to follow-up will be censored at the time of last contact.

11.4.2.2 All-cause mortality rate at 1, 2 and 3 years will be obtained from survival analysis tables and the difference between groups will be calculated with tests based on a cloglog transformation of the Kaplan–Meier estimator.[68]

11.4.2.3 The scores of cognitive function and quality of life in 3-year survivors after surgery will be analyzed using the independent sample t test or the Mann-Whitney U test. The difference between two means/medians and 95% CI will be provided. The difference between two medians (and 95% CI) will be calculated with the Hodges-Lehmann estimator. Missing data will not be replaced.

11.4.2.4 For the subgroup of cancer patients, time-to-event variables (overall survival, time to cancer recurrence/metastasis, and time to rehospitalization after surgery) will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; Cox proportional hazards models will be used to adjust for potential confounding factors. The estimates hazard ratios and 95% CIs will be provided. Patients who are lost to follow-up will be censored at the time of last contact.

12. Quality control and quality assurance

12.1 Investigators who perform long-term follow-ups are not involved in patient management during the underlying trial and have no knowledge of study group assignment. They are trained to do the long-term follow-ups according to the study protocol and authorized by the principle investigator.

12.2 The conduct of the study will be monitored by the Peking University Clinical Research Institute.

12.3 Statistical analysis will be performed by the Department of Biostatistics of Peking University First Hospital.

13. Ethics requirements

13.1 Ethics Committee

The study protocol must be approved by the local Clinical Research Ethics Committees before the study can be started. The investigators must strictly follow the Helsinki Declaration and China's relevant clinical trial management regulations. The principal investigator is responsible to report the status and the progress of the study to the Ethics Committee.

13.2 Oral consent of study participation

As the follow-up is pure observational without any interventions, we apply to waive the written informed consent for this subsequent telephone contact. However, for each patient, investigators are responsible to explain the purpose of this study in an oral manner. The investigators must let every patient know that he/she has the right to refuse follow-up at any time. Every patient must give an oral consent before follow-up can be performed. The explanation by the investigators and oral consents of the participants will be recorded and kept as a part of the clinical trial documents. For patients with mild dementia or incompetence, oral consents must be obtained from legal representatives.

13.3 Privacy and confidentiality

13.3.1 During the study period, the collected data from participants are labelled with special recruitment numbers and acronyms of names.

13.3.2 All personal information of the participants will be kept confidential. The filing cabinets storing the study documents will be locked. Apart from the study investigators, only authorized inspectors from the Scientific Research Department or members from the Ethics Committee of Peking University First Hospital are allowed to access the information.

13.3.3 Results of the study will be published as scientific articles. But all personal data (including name and age, etc.) are strictly confidential.

14. Study termination

Long-term follow-up will be performed for a period of up to 3 years (from the recruitment of the last patient). Decision to terminate follow-up will be made by the principal investigator.

15. Preservation of documents

Investigators will carefully preserve all documents and data of the clinical trial according to the requirements of Good Clinic Practice for a period of 5 years.

16. Declaration of interests

This trial is funded by the National Key R&D Program of China (2018YFC2001800) and the Peking University Clinical Research Program (PUCRP201101). The investigators declare no conflict of interests.

17. References

1. Fu C: Indication and opportunity of recurrent colonic carcinoma reoperation. *Zhong Guo Shi Yong Wai Ke Za Zhi* 2004;24:400-2
2. Fokas E, Engenhart-Cabillic R, Daniilidis K, Rose F, An HX: Metastasis: the seed and soil theory gains identity. Cancer Metastasis Rev 2007; 26: 705-15
3. Shakhar G, Ben-Eliyahu S: Potential prophylactic measures against postoperative immunosuppression: could they reduce recurrence rates in oncological patients? Ann Surg Oncol 2003; 10: 972-92
4. Denis MG, Lipart C, Leborgne J, LeHur PA, Galmiche JP, Denis M, Ruud E, Truchaud A, Lustenberger P: Detection of disseminated tumor cells in peripheral blood of colorectal cancer patients. Int J Cancer 1997; 74: 540-4
5. Yamaguchi K, Takagi Y, Aoki S, Futamura M, Saji S: Significant detection of circulating cancer cells in the blood by reverse transcriptase-polymerase chain reaction during colorectal cancer resection. Ann Surg 2000; 232: 58-65
6. Holmgren L, O'Reilly MS, Folkman J: Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nat Med 1995; 1: 149-53
7. Hofer SO, Molema G, Hermens RA, Wanebo HJ, Reichner JS, Hoekstra HJ: The effect of surgical wounding on tumour development. Eur J Surg Oncol 1999; 25: 231-43
8. Eggermont AM, Steller EP, Marquet RL, Jeekel J, Sugarbaker PH: Local regional promotion of tumor growth after abdominal surgery is dominant over immunotherapy with interleukin-2 and lymphokine activated killer cells. Cancer Detect Prev 1988; 12: 421-9
9. 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 1999; 80: 880-8
10. Brittenden J, Heys SD, Ross J, Eremin O: Natural killer cells and cancer. Cancer 1996; 77: 1226-43
11. Nelson CJ, Lysle DT: Severity, time, and beta-adrenergic receptor involvement in surgery-induced immune alterations. J Surg Res 1998; 80: 115-22
12. Lennard TW, Shenton BK, Borzotta A, Donnelly PK, White M, Gerrie LM, Proud G, Taylor RM: The influence of surgical operations on components of the human immune system. Br J Surg 1985; 72: 771-6
13. Carter JJ, Whelan RL: The immunologic consequences of laparoscopy in oncology. Surg Oncol Clin N Am 2001; 10: 655-77
14. Targarona EM, Balague C, Knook MM, Trias M: Laparoscopic surgery and surgical infection. Br J Surg 2000; 87: 536-44
15. Xu YX, Ayala A, Chaudry IH: Prolonged immunodepression after trauma and hemorrhagic shock. J Trauma 1998; 44: 335-41
16. Klein HG: Immunomodulatory aspects of transfusion: a once and future risk? Anesthesiology 1999; 91: 861-5
17. Salman H, Bergman M, Bessler H, Alexandrova S, Beilin B, Djaldetti M: Hypothermia affects the phagocytic activity of rat peritoneal macrophages. Acta Physiol Scand 2000; 168: 431-6

18. 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 1996; 334: 1209-15
19. Cohen S, Herbert TB: Health psychology: psychological factors and physical disease from the perspective of human psychoneuroimmunology. Annu Rev Psychol 1996; 47: 113-42
20. Moynihan JA, Ader R: Psychoneuroimmunology: animal models of disease. Psychosom Med 1996; 58: 546-58
21. Ishihara Y, Matsunaga K, Iijima H, Fujii T, Oguchi Y, Kagawa J: Time-dependent effects of stressor application on metastasis of tumor cells in the lung and its regulation by an immunomodulator in mice. Psychoneuroendocrinology 1999; 24: 713-26
22. Freire-Garabal M, Nunez MJ, Balboa JL, Fernandez-Rial JC, Vallejo LG, Gonzalez-Bahillo J, Rey-Mendez M: Effects of alprazolam on cellular immune response to surgical stress in mice. Cancer Lett 1993; 73: 155-60
23. Andersen BL, Farrar WB, Golden-Kreutz D, Kutz LA, MacCallum R, Courtney ME, Glaser R: Stress and immune responses after surgical treatment for regional breast cancer. J Natl Cancer Inst 1998; 90: 30-6
24. Larson MR, Duberstein PR, Talbot NL, Caldwell C, Moynihan JA: A presurgical psychosocial intervention for breast cancer patients. psychological distress and the immune response. J Psychosom Res 2000; 48: 187-94
25. Coffey JC, Wang JH, Smith MJ, Bouchier-Hayes D, Cotter TG, Redmond HP: Excisional surgery for cancer cure: therapy at a cost. Lancet Oncol 2003; 4: 760-8
26. Sessler DI: Long-term consequences of anesthetic management. Anesthesiology 2009; 111: 1-4
27. Mitsuhata H, Shimizu R, Yokoyama MM: Suppressive effects of volatile anesthetics on cytokine release in human peripheral blood mononuclear cells. Int J Immunopharmacol 1995; 17: 529-34
28. Markovic SN, Knight PR, Murasko DM: Inhibition of interferon stimulation of natural killer cell activity in mice anesthetized with halothane or isoflurane. Anesthesiology 1993; 78: 700-6
29. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL: The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 2000; 157: 411-21
30. Generali D, Berruti A, Brizzi MP, Campo L, Bonardi S, Wigfield S, Bersiga A, Allevi G, Milani M, Aguggini S, Gandolfi V, Dogliotti L, Bottini A, Harris AL, Fox SB: Hypoxia-inducible factor-1alpha expression predicts a poor response to primary chemoendocrine therapy and disease-free survival in primary human breast cancer. Clin Cancer Res 2006; 12: 4562-8
31. Rohwer N, Lobitz S, Daskalow K, Jons T, Vieth M, Schlag PM, Kemmner W, Wiedenmann B, Cramer T, Hocker M: HIF-1alpha determines the metastatic potential of gastric cancer cells. Br J Cancer 2009; 100: 772-81
32. Dai CX, Gao Q, Qiu SJ, Ju MJ, Cai MY, Xu YF, Zhou J, Zhang BH, Fan J: Hypoxia-inducible factor-1 alpha, in association with inflammation, angiogenesis and MYC, is a critical prognostic factor in patients with HCC after surgery. BMC Cancer 2009; 9: 418
33. Baba Y, Nosho K, Shima K, Irahara N, Chan AT, Meyerhardt JA, Chung DC, Giovannucci EL, Fuchs CS, Ogino S: HIF1A overexpression is associated with poor prognosis in a cohort of 731 colorectal cancers. Am J Pathol 2010; 176: 2292-2301
34. Osada R, Horiuchi A, Kikuchi N, Yoshida J, Hayashi A, Ota M, Katsuyama Y, Melillo G, Konishi I: Expression of hypoxia-inducible factor 1alpha, hypoxia-inducible factor 2alpha, and von Hippel-Lindau protein in epithelial ovarian neoplasms and allelic loss of von Hippel-Lindau gene: nuclear expression of hypoxia-inducible factor 1alpha is an independent prognostic factor in ovarian carcinoma. Hum Pathol 2007; 38: 1310-20
35. Ma D, Lim T, Xu J, Tang H, Wan Y, Zhao H, Hossain M, Maxwell PH, Maze M: Xenon preconditioning protects against renal ischemic-reperfusion injury via HIF-1alpha activation. J Am Soc Nephrol 2009; 20: 713-20
36. De Hert SG, Turani F, Mathur S, Stowe DF: Cardioprotection with volatile anesthetics: mechanisms and clinical implications. Anesth Analg 2005; 100: 1584-93
37. Beck-Schimmer B, Breitenstein S, Urech S, De Conno E, Wittlinger M, Puhan M, Jochum W, Spahn DR, Graf R, Clavien PA: A randomized controlled trial on pharmacological preconditioning in liver surgery using a volatile anesthetic. Ann Surg 2008; 248: 909-18
38. Kawaguchi M, Furuya H, Patel PM: Neuroprotective effects of anesthetic agents. J Anesth 2005; 19: 150-6
39. Wang C, Weihrauch D, Schwabe DA, Bienengraeber M, Warltier DC, Kersten JR, Pratt PF, Jr., Pagel PS: Extracellular signal-regulated kinases trigger isoflurane preconditioning concomitant with upregulation of hypoxia-inducible factor-1alpha and vascular endothelial growth factor expression in rats. Anesth Analg 2006; 103: 281-8
40. Porta C, Figlin RA: Phosphatidylinositol-3-kinase/Akt signaling pathway and kidney cancer, and the

therapeutic potential of phosphatidylinositol-3-kinase/Akt inhibitors. J Urol 2009; 182: 2569-77
41. Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, Michiels C, Kietzmann T, Gorlach A: Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site. Arterioscler Thromb Vasc Biol 2007; 27: 755-61
42. Maranchie JK, Zhan Y: Nox4 is critical for hypoxia-inducible factor 2-alpha transcriptional activity in von Hippel-Lindau-deficient renal cell carcinoma. Cancer Res 2005; 65: 9190-3
43. Mullenheim J, Ebel D, Frassdorf J, Preckel B, Thamer V, Schlack W: Isoflurane preconditions myocardium against infarction via release of free radicals. Anesthesiology 2002; 96: 934-40
44. Hanouz JL, Zhu L, Lemoine S, Durand C, Lepage O, Massetti M, Khayat A, Plaud B, Gerard JL: Reactive oxygen species mediate sevoflurane- and desflurane-induced preconditioning in isolated human right atria in vitro. Anesth Analg 2007; 105: 1534-9
45. Huitink JM, Heimerikxs M, Nieuwland M, Loer SA, Brugman W, Velds A, Sie D, Kerkhoven RM: Volatile anesthetics modulate gene expression in breast and brain tumor cells. Anesth Analg 2010; 111: 1411-5
46. Melamed R, Bar-Yosef S, Shakhar G, Shakhar K, Ben-Eliyahu S: Suppression of natural killer cell activity and promotion of tumor metastasis by ketamine, thiopental, and halothane, but not by propofol: mediating mechanisms and prophylactic measures. Anesth Analg 2003; 97: 1331-9
47. Tanaka T, Takabuchi S, Nishi K, Oda S, Wakamatsu T, Daijo H, Fukuda K, Hirota K: The intravenous anesthetic propofol inhibits lipopolysaccharide-induced hypoxia-inducible factor 1 activation and suppresses the glucose metabolism in macrophages. J Anesth 2010; 24: 54-60
48. Wakamatsu T, Tanaka T, Oda S, Nishi K, Harada H, Daijo H, Takabuchi S, Kai S, Fukuda K, Hirota K: The intravenous anesthetics barbiturates inhibit hypoxia-inducible factor 1 activation. Eur J Pharmacol 2009; 617: 17-22
49. Mammoto T, Mukai M, Mammoto A, Yamanaka Y, Hayashi Y, Mashimo T, Kishi Y, Nakamura H: Intravenous anesthetic, propofol inhibits invasion of cancer cells. Cancer Lett 2002; 184: 165-70
50. Gupta K, Kshirsagar S, Chang L, Schwartz R, Law PY, Yee D, Hebbel RP: Morphine stimulates angiogenesis by activating proangiogenic and survival-promoting signaling and promotes breast tumor growth. Cancer Res 2002; 62: 4491-8
51. Beilin B, Martin FC, Shavit Y, Gale RP, Liebeskind JC: Suppression of natural killer cell activity by high-dose narcotic anesthesia in rats. Brain Behav Immun 1989; 3: 129-37
52. Gaspani L, Bianchi M, Limiroli E, Panerai AE, Sacerdote P: The analgesic drug tramadol prevents the effect of surgery on natural killer cell activity and metastatic colonization in rats. J Neuroimmunol 2002; 129: 18-24
53. Sasamura T, Nakamura S, Iida Y, Fujii H, Murata J, Saiki I, Nojima H, Kuraishi Y: Morphine analgesia suppresses tumor growth and metastasis in a mouse model of cancer pain produced by orthotopic tumor inoculation. Eur J Pharmacol 2002; 441: 185-91
54. Mammoto T, Higashiyama S, Mukai M, Mammoto A, Ayaki M, Mashimo T, Hayashi Y, Kishi Y, Nakamura H, Akedo H: Infiltration anesthetic lidocaine inhibits cancer cell invasion by modulating ectodomain shedding of heparin-binding epidermal growth factor-like growth factor (HB-EGF). J Cell Physiol 2002; 192: 351-8
55. Martinsson T: Ropivacaine inhibits serum-induced proliferation of colon adenocarcinoma cells in vitro. J Pharmacol Exp Ther 1999; 288: 660-4
56. Ahlers O, Nachtigall I, Lenze J, Goldmann A, Schulte E, Hohne C, Fritz G, Keh D: Intraoperative thoracic epidural anaesthesia attenuates stress-induced immunosuppression in patients undergoing major abdominal surgery. Br J Anaesth 2008; 101: 781-7
57. Chae BK, Lee HW, Sun K, Choi YH, Kim HM: The effect of combined epidural and light general anesthesia on stress hormones in open heart surgery patients. Surg Today 1998; 28: 727-31
58. Looney M, Doran P, Buggy DJ: Effect of anesthetic technique on serum vascular endothelial growth factor C and transforming growth factor beta in women undergoing anesthesia and surgery for breast cancer. Anesthesiology 2010; 113: 1118-25
59. 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 2008; 109: 180-7
60. Exadaktylos AK, Buggy DJ, Moriarty DC, Mascha E, Sessler DI: Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? Anesthesiology 2006; 105: 660-4
61. Gottschalk A, Ford JG, Regelin CC, You J, Mascha EJ, Sessler DI, Durieux ME, Nemergut EC: Association between epidural analgesia and cancer recurrence after colorectal cancer surgery. Anesthesiology 2010; 113: 27-34
62. 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. Br J Anaesth 2011; 107: 164-70
63. Myles PS, Peyton P, Silbert B, Hunt J, Rigg JR, Sessler DI: Perioperative epidural analgesia for major

abdominal surgery for cancer and recurrence-free survival: randomised trial. Bmj 2011; 342: d1491
64. Chen WK, Miao CH: The effect of anesthetic technique on survival in human cancers: a meta-analysis of retrospective and prospective studies. PLoS One 2013; 8: e56540
65. Meng C, Zhang XQ, Bi ZY, Wei GZ, Zhou JS, Wang H, Sun HL: Telephone interview for cognitive status-modified used in screening cognitive impairment. Zhong Hua Shen Jing Ke Za Zhi 2005; 38: 30–33
66. Hao YT, Fang JQ, Power M, Wu SM, Zhu SM: The Equivalence of WHOQOLBREF among 13 culture versions. Zhong Guo Xin Li Wei Sheng Za Zhi 2006; 20: 71-75
67. Sarfati D, Blakely T, Pearce N: Measuring cancer survival in populations: relative survival vs cancer-specific survival. Int J Epidemiol 2010; 39: 598-610
68. Klein JP, Logan B, Harhoff M, Andersen PK: Analyzing survival curves at a fixed point in time. Stat Med 2007; 26:4505–19

Final study protocol

Impacts of combined epidural-general anesthesia versus general anesthesia alone on long-term survival in elderly after major thoracic and abdominal surgery: 5-year follow-up of a randomized controlled trail

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Date of last revision: May 1, 2017

Version of protocol: V4.0

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1. Background

Cancer is one of the leading causes of surgery in elderly patients. In our previous study of the "Effects of two different anesthesia-analgesia methods on incidence of postoperative delirium in elderly patients: a multicenter randomized controlled trial (PUCRP 201101)", about 92% of enrolled patients underwent surgery for cancer. Surgery is an important part of cancer treatment; in fact, it is the main treatment for resectable solid organ cancers. Cancer recurrence/metastasis is the main reason that affects the long-term outcome after cancer surgery. According to statistics in China, the estimated 5-year survival after cancer diagnosis was 37% in 2015.[1] And metastasis is responsible for about 90% of all cancer deaths.[2]

The development of recurrence/metastasis after cancer surgery depends largely on the balance between the anti-metastatic immune function of the body and the ability of the residual cancer cells to implant, proliferate and attract new blood vessels.[3] It is true that the primary cancer is resected during surgery; however, surgery or surgery-related response may promote cancer recurrence/metastasis, possibly through the following ways. Firstly, even with the most advanced surgical techniques, cancer cells enter the lymphatic system and blood circulation during surgery in a significant proportion of patients.[4,5] Secondly, cancer resection reduces tumor-related anti-angiogenic factors[6] but increases pro-angiogenic factors such as vascular endothelial growth factor (VEGF);[7] these may promote the growth of local and distant cancer.[8] Thirdly, surgery may induce inhibition of cell-mediated immunity including the function of cytotoxic T cells and natural killer cells which are the primary anti-tumor cells,[9,10] possibly by surgery-related stress response,[11-14] blood loss and transfusion,[15,16] hypothermia,[17,18] and even perioperative anxiety.[9,19-24] The peak of immunosuppression was thought to occur on the third day after surgery.[25]

Anesthesia and perioperative management may also affect long-term outcomes after cancer surgery.[26] Many evidences come from experimental studies. For example, isoflurane and halothane inhibit the toxicity of interferon-enhanced NK cells; sevoflurane suppresses cytokine release from NK cells and NK cell-related cells.[27,28] Hypoxia inducible factors (HIFs) plays an important role in tumor formation and metastasis, high levels HIFs are closely associated with poor prognosis.[29-34] Studies found that inhalation of anesthetic drugs (including isoflurane, sevoflurane, desflurane, nitric oxide and xenon) can upregulate the expression of HIFs,[35-44] but the effects on cancer patients remain unclear. In addition, inhaled anesthetics can modulate gene expression in breast and brain tumor cells.[45] Among intravenous anesthetics, ketamine and thiopental are found to suppress NK cell activity and promote tumor metastasis.[46] On the other hand, propofol and barbiturates may block the translation of HIFs mRNA, and then down-regulate HIFs.[47,48] Experimental studies also showed that propofol inhibits the invasion of cancer cells;[49] but clinical significance is not clear. Opioids are used to relieve perioperative pain, but may also inhibits cellular and humoral immune function. In animal studies, clinically relevant dose opioids can inhibit NK cell activity, and promote tumor growth and angiogenesis;[50,51] but conflicting evidence also exists.[52,53] On the other hand, local anesthetic drugs have effect of membrane stabilization and can inhibit invasion and proliferation of cancer cells in experimental studies.[54,55]

Intense and persistent stress response during the perioperative period may promote metastasis by suppressing immune function.[9] Regional anesthesia such as neuraxial anesthesia can block the noxious afferent stimuli to the central nervous system; and thus, blunt the activation of the sympathetic nervous system, relieve the neuroendocrine response, and reduce the requirement for opioids. In clinical studies, use of thoracic epidural anesthesia is associated with lower concentrations of stress hormones, less release of endogenous opioids, and milder immunosuppression after surgery.[56,57] In another study, women who underwent breast cancer surgery with paravertebral block and propofol sedation had lower serum concentration of vascular endothelial growth factor (VEGF) than those with general anesthesia.[58] Theoretically, use of epidural anesthesia/analgesia during cancer surgery may help to reduce postoperative recurrence and metastasis.

Whether regional anesthesia can provide beneficial effects on tumor recurrence and metastasis remain unclear. Current evidence mainly comes from retrospective studies. Biki et al.[59] analyzed data of patients after radical prostatectomy; after adjusting for confounding factors, use of epidural anesthesia was associated with a reduced risk of recurrence by about 57%. Exadaktylos et al.[60] also reported that women who received paravertebral block during breast cancer surgery had lower recurrence and metastasis rate at 36 months. In some studies, the advantages of epidural anesthesia appear only in some subgroups. For example, in a study of patients undergoing colorectal cancer surgery, epidural anesthesia was associated with a lower cancer recurrence only in patients older than 64 years of age.[61] In another study, epidural anesthesia was associated

with a reduced long-term mortality in patients after rectal cancer surgery but not colon cancer surgery.[62] However, there are also studies that reported no beneficial effects. In a post-hoc analysis of randomized trial, Myles et al.[63] reported that use of epidural anesthesia during major abdominal cancer surgery did not improve cancer-free survival. Recently, Chen et al.[64] conducted a meta-analysis and found that use of epidural anesthesia/analgesia might be associated with improved overall survival after surgery for operable cancer; however, no advantage was found regarding recurrence and metastasis. Therefore, prospective randomized controlled trials are required to further clarify the effect of epidural anesthesia on long-term outcomes after cancer surgery.

We hypothesize that use of epidural anesthesia/analgesia during cancer surgery may reduce recurrence/metastasis and improve long-term survival, possibly by blunting stress response and relieving immunosuppression.

2. Objective

To investigate whether "combined epidural-general anesthesia and postoperative epidural analgesia" compared with "general anesthesia and postoperative intravenous analgesia" can improve long-term survival in elderly patients after major thoracic and abdominal surgery for cancer.

3. Study design

3.1 Type of the study

It is a long-term follow-up of patients enrolled in the underlying trial "Effects of two anesthesia-analgesic methods on incidence of postoperative delirium in elderly patients undergoing major thoracic and abdominal surgery: a multicenter randomized controlled trial (PUCRP 201101)". The outcome assessors are not aware of the group assignment and results of the previous trial during the follow-up period.

3.2 Participating centers

This multicenter trial is conducted in five tertiary care hospitals affiliated with Peking University in Beijing, China. The five participating centers include Peking University First Hospital, Peking University People's Hospital, Peking University Third Hospital, Beijing Hospital, and Beijing Shijitan Hospital.

4. Study population

4.1 Participants

Potential participants are patients who were enrolled in the underlying trial "Effects of two anesthesia-analgesic methods on incidence of postoperative delirium in elderly patients undergoing major thoracic and abdominal surgery: a multicenter randomized controlled trial (PUCRP 201101)", completed the trial and agreed to receive postoperative follow-up via telephone. The underlying trial was conducted from November 2011 to May 2015. A total of 1,802 patients were recruited, of whom 901 received combined epidural-general anesthesia plus postoperative epidural analgesia and 901 received general anesthesia plus postoperative intravenous analgesia. Of these, a total of 1,720 patients completed the underlying trial and were included in this long-term follow-up study, including 857 in the combined epidural-general anesthesia group and 863 in the general anesthesia group.

4.2 Inclusion and exclusion criteria of the underlying trial

The inclusion and exclusion criteria for the underlying trial include the following.

4.2.1 Inclusion criteria:

4.2.1.1 Elderly patients (aged 60-90 years).

4.2.1.2 Scheduled to undergo non-cardiac thoracic or abdominal surgery with an expected duration of 2 hours or longer. For those who undergo thoracoscopic or laparoscopic surgery, the expected length of incision must be 5 centimeters or more.

4.2.1.3 Agreed to receive postoperative patient-controlled analgesia.

4.2.2 Exclusion criteria:

4.2.2.1 Previous history of schizophrenia, epilepsy or Parkinson disease, or unable to complete preoperative assessment due to severe dementia, language barrier or end-stage disease.

4.2.2.2 History of myocardial infarction or stroke within 3 months before surgery.

4.2.2.3 Any contraindication for epidural anesthesia and analgesia, including abnormal vertebral anatomy, previous spinal trauma or surgery, severe chronic back pain, coagulation disorder (prothrombin time or activated partial prothrombin time longer than 1.5 times of the upper normal limit, or platelet count of less than 80×10^9/L), local infection near the site of puncture, and severe sepsis.

4.2.2.4 Severe heart dysfunction (New York Heart Association functional classification 3 or above), hepatic insufficiency (Child-Pugh grade C), or renal insufficiency (serum creatinine of 442 μmol/L or above, with or without serum potassium of 6.5 mmol/L or above, or requirement of renal replacement therapy).

4.2.2.5 Any other conditions that were considered unsuitable for the study participation.

4.3 Criteria of drop-out of the underlying trial

4.3.1 Study intervention was not administered (due to failed epidural puncture, failed epidural catheterization, epidural catheter obstruction, blood appear in epidural catheter, inadequate epidural analgesia, etc.).

4.3.2 Intervention was interrupted by the investigators/anesthesiologists (due to adverse events).

4.3.3 Use of a prohibited drugs.

The causes of protocol deviation should be recorded and corrected when possible. The cases would be followed up according to the study protocol and included in the intention-to-treat analysis.

4.3.4 Withdraw consent after intervention started.

The situation should be recorded. The primary therapeutic effects recorded in the last time will be regarded as the final results. The cases will be included in the intention-to-treat analysis.

4.4 Criteria of elimination of the underlying trial

Included patients will be excluded if they meet any of the following criteria:

4.4.1 Withdraw consents before intervention.

4.4.2 Surgery cancelled.

4.4.3 No assessment result of the primary outcome.

The causes of elimination should be explained. The case will be excluded from the intention-to-treat analysis. The case report forms will be preserved for reference.

5. Long-term follow-ups

5.1 Requirements for investigators

Investigators who perform the long-term follow-ups are not involved in patient management during the underlying trial and have no knowledge of study group assignment. They will be specially trained for long-term follow-ups and authorized by the principle investigator.

5.2 Preparation before follow-ups

History of surgery will be re-checked in the electronic medical record system; the results of pathological examination of surgical specimens (including the Tumor-Node-Metastasis [TNM] stage of cancer) and the diagnoses at hospital discharge will be collected.

5.3 Conduct of long-term followed-ups

5.3.1 Long-term follow-ups will be performed once a year after surgery via telephone interview (after ethic approval), for a period of up to 5 years (from the recruitment of the last patient).

5.3.2 Data collection during long-term follow-ups includes the following:

5.3.2.1 Results of postoperative re-examinations. For patients after cancer surgery, cancer recurrence is defined as reappearance of the same cancer in the original place or in the organs/lymph nodes near the place it first started; cancer metastasis is defined as reappearance of the same cancer in another part of the body, with some distance from where it started. The development of cancer recurrence and/or metastasis will be diagnosed by surgeons (and/or radiologists when necessary) according to the results of re-examination. For patients with confirmed cancer recurrence and/or metastasis, the date of earliest diagnoses and subsequent therapies will be documented.

5.3.2.2 Postoperative therapies for the primary surgical disease. For patients after cancer surgery, postoperative therapies may include radiotherapy, chemotherapy, interventional therapy, reoperation, and others therapies.

5.3.2.3 Re-hospitalization after surgery. Reasons leading to re-hospitalization will be documented, including recurrence/metastasis of the original cancer, new cancer, new serious non-cancer disease, or other conditions. The diagnostic evidences and treatments will be documented.

5.3.2.3.1 New cancers are defined as those that are confirmed by pathological examinations but different from the primary ones. For patients with new cancers, the date of earliest diagnoses and subsequent therapies will be documented.

5.3.2.3.2 New serious non-cancer diseases are defined as those that required hospital readmission and/or another surgical therapy. For patients with new serious non-cancer diseases, the date of earliest diagnosis and subsequent therapies will be documented.

5.3.2.4 For 3-year survivors, cognitive function will be assessed with the modified Telephone Interview for Cognitive Status (TICS-m; a 12-item questionnaire that assesses global cognitive function by verbal communication via telephone. The score ranges from 0 to 50, with higher score indicating better function);[65] quality of life will be assessed with the World Health Organization Quality of Life-brief version (WHOQOL-BREF; a 24-item questionnaire that provides assessments of the quality of life in physical, psychological, and social relationship, and environmental domains. For each domain, the score ranges from 0 to 100, with higher score indicating better function).[66]

5.3.2.5 For patients who die during the follow-up period after surgery, the exact date of death will be recorded (consistent with death certificate). The causes of death will be documented. Cancer-specific death is defined as death fully attributable to a specific cancer, i.e., the cancer for which the surgery is performed during the underlying trial.[67] Cancer-specific death usually occur after cancer recurrence and/or metastasis, with other causes including stroke, myocardial infarction or accident excluded.

5.4 Management of patients lost to follow-up

5.4.1 Patients or their family members will be contacted for at least 5 attempts on 5 different days before they are marked as lost to follow-up.

5.4.2 For patients who are lost to contact, the following systems will be checked in order to minimize the rate of

loss-to-follow-up, i.e., the laboratory test system, imaging examination system, medical insurance system, and outpatient registration system of the hospital.

5.4.3 For patients who are lost-to-follow-up, the time of last hospital visits after surgery recorded in the in- or outpatient medical record system will be adopted as the censoring time.

6. Efficacy outcomes

6.1 Primary outcome

Overall survival, which is defined as the time from surgery to the date of all-cause death.

6.2 Secondary outcomes

6.2.1 Cancer-specific survival, which is defined as the time from surgery to the date of cancer-specific death, i.e., death fully attributable to a specific cancer for which surgery is performed during the underlying trial. Patients who die of a cause other than the specific cancer are “censored” at the time of death.

6.2.2 Recurrence-free survival, which is defined as the time from surgery to the date of cancer recurrence/metastasis or all-cause death, whichever occurs first.

6.2.3 Event-free survival, which is defined as the time from surgery to the date of cancer recurrence/metastasis, new cancer, new serious non-cancer disease or all-cause death, whichever occurs first.

6.3 Other outcomes

6.3.1 Overall survival of cancer patients, which is defined as the time from surgery to the date of all-cause death.

6.3.2 Cancer-specific survival of cancer patients, which is defined as the time from surgery to the date of cancer-specific death, i.e., death fully attributable to a specific cancer for which surgery is performed during the underlying trial. Patients who die of a cause other than the specific cancer are “censored” at the time of death.

6.3.3 Recurrence-free survival of cancer patients, which is defined as the time from surgery to the date of cancer recurrence/metastasis or all-cause death, whichever occurs first.

6.3.4 Event-free survival of cancer patients, which is defined as the time from surgery to the date of cancer recurrence/metastasis, new cancer, new serious non-cancer disease or all-cause death, whichever occurs first.

6.3.5 Cognitive function in 3-year survivors, which will be assessed with the TICS-m at the end of the 3rd year after surgery.

6.3.6 Quality of life in 3-year survivors, which will be assessed with the WHOQOL-BREF at the end of the 3rd year after surgery.

7. Withdrawal from long-term follow-ups

7.1 Participants will be withdrawn from long-term follow-ups in the following conditions:

7.1.1 Requested by participants or legal representatives of participants.

7.1.2 Considered to be unsuitable for continued follow-ups by the investigators.

7.2 For patients who are withdrawn from the long-term follow-ups, detailed reasons leading to withdrawal will be recorded. Follow-up data of the last visit will be recorded.

8. Safety consideration

This is a long-term follow-up of patients enrolled in our previous study "Effects of two different anesthesia-analgesia methods on incidence of postoperative delirium in elderly patients undergoing major thoracic and abdominal surgery: a multicenter randomized controlled trial (PUCRP 201101)". There are no additional interventions; and, therefore, there are no additional risks for the patients.

9. Unmasking of group assignment

9.1 After the follow-ups of all cases have been completed, the data of case report forms have been checked as correct and the data entry have been finished, the database will be checked by the data manager.

9.2 After the database is locked, unmasking will be conducted. The database will be sent to the statisticians for statistical analysis.

10. Data management

10.1 The investigators should record data timely, completely and correctly according to the original examinations and follow-up assessments.

10.2 The completed case report forms, after signed by the supervisors, will be sent to a clinical data custodian.

10.3 After the data in the case report forms have been input and checked, the case report forms will be stored in sequence order.

10.4 Data management will be inspected by Peking University Clinical Research Institute.

11. Statistical analysis

11.1 General principles

11.1.1 Numeric variables will be presented as mean ± standard deviation or median (minimum, maximum; or interquartile range). Categorical variables will be presented as number of cases (%).

11.1.2 For each hypothesis, two-tailed tests will be used in all statistical analysis, and $P<0.05$ will be considered statistically significant. For the treatment-by-covariate interaction in predefined subgroup analyses, a $P<0.10$ will be defined as statistically significant.

11.1.3 The primary and secondary outcomes will be analyzed in a modified intention-to-treat population, i.e., all patients are analyzed in the group to which they are randomized and received at least part of study intervention. If epidural anesthesia and/or analgesia fail, these patients will be converted to general anesthesia and intravenous analgesia but will be analyzed in the group to which they are randomized. For patients with repeated recruitment for different surgeries, only the first was considered for this analysis. Also, we will do per-protocol analysis for the primary endpoint, in this case, drop-out patients will be excluded.

11.2 Patient enrolment, drop-out/elimination and lost-to-follow-up

The status of patient recruitment, drop-out/elimination and lost-to-follow-up will be summarized and listed. Comparison of the overall elimination/drop-out and lost-to-follow-up rate between the two groups will be performed with chi square test.

*11.3 **Demographics** and baseline characteristics*

11.3.1 Demographics and baseline data will be presented.

11.3.2 Between-group differences will be compared using the absolute standardized differences (ASDs), which are defined as the absolute difference in means, mean ranks or proportions divided by the pooled standard deviation and calculated with the formula published by Austin.[68] Baseline variables with an absolute standardized difference of $\geq 1.96\times\sqrt{(n1+n2)/(n1\times n2)}$ will be considered imbalanced between the two groups and adjusted for in all analyses if considered necessary.

*11.4 **Intra- and postoperative variables***

Numeric variables will be analyzed using the independent-samples t test or Mann-Whitney U test; categorical variables will be analyzed using the chi square test, continuity correction chi square test or Fisher exact test. Missing data will not be replaced.

11.5 Efficacy analysis

11.5.1 Evaluation of primary endpoint

11.5.1.1 The overall survival after surgery will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; a Cox proportional hazards model will be used to adjust for predetermined factors including age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. The estimated difference between two groups will be expressed as hazard ratio and 95% CI. Patients who are lost to follow-up will be censored at the time of last contact.

11.5.1.2 The differences of the primary outcome in predefined subgroups will be tested using the Cox proportional hazard models adjusted for preselected factors of age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. Treatment-by-covariate interactions will be assessed separately for each predefined factor, adjusting for the same variables. The hazard ratio (and 95% CI) for each subgroup and the p values of treat-by-covariate interactions will be displayed in a forest plot.

11.5.2 Evaluation of secondary endpoints

11.5.2.1 The time-to-event variables (cancer-specific survival, recurrence-free survival, and event-free survival after surgery) will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; Cox proportional hazards models will be used to adjust for predetermined factors including age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. The estimates hazard ratios and 95% CIs will be provided. For cancer-specific survival, patients who die of a cause other than the specific cancer will be censored at the time of death. Patients who are lost to follow-up will be censored at the time of last contact.

11.5.2.2 The scores of cognitive function and quality of life in 3-year survivors after surgery will be analyzed using the independent sample t test or the Mann-Whitney U test. The difference between two means/medians and 95% CI will be provided. The difference between two medians (and 95% CI) will be calculated with the Hodges-Lehmann estimator. Missing data will not be replaced.

11.5.3 Analysis for the subgroup of cancer patients

11.5.3.1 For demographics and baseline data, between-group differences will be compared using the absolute standardized differences (ASDs), which are defined as the absolute difference in means, mean ranks or proportions divided by the pooled standard deviation and calculated with the formula published by Austin.[68] Baseline variables with an absolute standardized difference $\geq 1.96\times\sqrt{(n1+n2)/(n1\times n2)}$ will be considered imbalanced between the two groups and adjusted for in all analyses if considered necessary.

11.5.3.2 For other intra- and postoperative variables, numeric variables will be analyzed using the independent-samples t test or Mann-Whitney U test; categorical variables will be analyzed using the chi square test, continuity correction chi square test or Fisher exact test. Missing data will not be replaced.

11.5.3.3 The time-to-event variables (overall survival, cancer-specific survival, recurrence-free survival, and event-free survival after surgery) will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; Cox proportional hazards models will be used to adjust for predetermined factors including age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. The estimates hazard ratios and 95% CIs will be provided. For cancer-specific survival, patients who die of a cause other than the specific cancer will be censored at the time of death. Patients who are lost to follow-up will be censored at the time of last contact.

11.5.3.4 The differences of overall survival in predefined subgroups will be tested using the Cox proportional hazard models adjusted for preselected factors of age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. Treatment-by-covariate interactions will be assessed separately for each predefined factor, adjusting for the same variables. The hazard ratio (and 95% CI) for each subgroup and the p values of treat-by-covariate interactions will be displayed in a forest plot.

12. Quality control and quality assurance

12.1 Investigators who perform long-term follow-ups are not involved in patient management during the underlying trial and have no knowledge of study group assignment. They are trained to do the long-term follow-ups according to the study protocol and authorized by the principle investigator.

12.2 The conduct of the study will be monitored by the Peking University Clinical Research Institute.

12.3 Statistical analysis will be performed by the Department of Biostatistics of Peking University First Hospital.

13. Ethics requirements

13.1 Ethics Committee

The study protocol must be approved by the local Clinical Research Ethics Committees before the study can be started. The investigators must strictly follow the Helsinki Declaration and China's relevant clinical trial management regulations. The principal investigator is responsible to report the status and the progress of the study to the Ethics Committee.

13.2 Oral consent of study participation

As the follow-up is pure observational without any interventions, we apply to waive the written informed consent for this subsequent telephone contact. However, for each patient, investigators are responsible to explain the purpose of this study in an oral manner. The investigators must let every patient know that he/she has the right to refuse follow-up at any time. Every patient must give an oral consent before follow-up can be performed. The explanation by the investigators and oral consents of the participants will be recorded and kept as a part of the clinical trial documents. For patients with mild dementia or incompetence, oral consents must be obtained from legal representatives.

13.3 Privacy and confidentiality

13.3.1 During the study period, the collected data from participants are labelled with special recruitment numbers and acronyms of names.

13.3.2 All personal information of the participants will be kept confidential. The filing cabinets storing the study documents will be locked. Apart from the study investigators, only authorized inspectors from the Scientific Research Department or members from the Ethics Committee of Peking University First Hospital are allowed to access the information.

13.3.3 Results of the study will be published as scientific articles. But all personal data (including name and age, etc.) are strictly confidential.

14. Study termination

Long-term follow-up will be performed for a period of up to 5 years (from the recruitment of the last patient). Decision to terminate follow-up will be made by the principal investigator.

15. Preservation of documents

Investigators will carefully preserve all documents and data of the clinical trial according to the requirements of Good Clinic Practice for a period of 5 years.

16. Declaration of interests

This trial is funded by the National Key R&D Program of China (2018YFC2001800) and the Peking University Clinical Research Program (PUCRP201101). The investigators declare no conflict of interests.

17. References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J: Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-32
2. Fokas E, Engenhart-Cabillic R, Daniilidis K, Rose F, An HX: Metastasis: the seed and soil theory gains identity. Cancer Metastasis Rev 2007; 26: 705-15
3. Shakhar G, Ben-Eliyahu S: Potential prophylactic measures against postoperative immunosuppression: could they reduce recurrence rates in oncological patients? Ann Surg Oncol 2003; 10: 972-92
4. Denis MG, Lipart C, Leborgne J, LeHur PA, Galmiche JP, Denis M, Ruud E, Truchaud A, Lustenberger P: Detection of disseminated tumor cells in peripheral blood of colorectal cancer patients. Int J Cancer 1997; 74: 540-4
5. Yamaguchi K, Takagi Y, Aoki S, Futamura M, Saji S: Significant detection of circulating cancer cells in the blood by reverse transcriptase-polymerase chain reaction during colorectal cancer resection. Ann Surg 2000; 232: 58-65
6. Holmgren L, O'Reilly MS, Folkman J: Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nat Med 1995; 1: 149-53
7. Hofer SO, Molema G, Hermens RA, Wanebo HJ, Reichner JS, Hoekstra HJ: The effect of surgical wounding on tumour development. Eur J Surg Oncol 1999; 25: 231-43
8. Eggermont AM, Steller EP, Marquet RL, Jeekel J, Sugarbaker PH: Local regional promotion of tumor growth after abdominal surgery is dominant over immunotherapy with interleukin-2 and lymphokine activated killer cells. Cancer Detect Prev 1988; 12: 421-9
9. 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 1999; 80: 880-8
10. Brittenden J, Heys SD, Ross J, Eremin O: Natural killer cells and cancer. Cancer 1996; 77: 1226-43
11. Nelson CJ, Lysle DT: Severity, time, and beta-adrenergic receptor involvement in surgery-induced immune alterations. J Surg Res 1998; 80: 115-22

12. Lennard TW, Shenton BK, Borzotta A, Donnelly PK, White M, Gerrie LM, Proud G, Taylor RM: The influence of surgical operations on components of the human immune system. Br J Surg 1985; 72: 771-6
13. Carter JJ, Whelan RL: The immunologic consequences of laparoscopy in oncology. Surg Oncol Clin N Am 2001; 10: 655-77
14. Targarona EM, Balague C, Knook MM, Trias M: Laparoscopic surgery and surgical infection. Br J Surg 2000; 87: 536-44
15. Xu YX, Ayala A, Chaudry IH: Prolonged immunodepression after trauma and hemorrhagic shock. J Trauma 1998; 44: 335-41
16. Klein HG: Immunomodulatory aspects of transfusion: a once and future risk? Anesthesiology 1999; 91: 861-5
17. Salman H, Bergman M, Bessler H, Alexandrova S, Beilin B, Djaldetti M: Hypothermia affects the phagocytic activity of rat peritoneal macrophages. Acta Physiol Scand 2000; 168: 431-6
18. 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 1996; 334: 1209-15
19. Cohen S, Herbert TB: Health psychology: psychological factors and physical disease from the perspective of human psychoneuroimmunology. Annu Rev Psychol 1996; 47: 113-42
20. Moynihan JA, Ader R: Psychoneuroimmunology: animal models of disease. Psychosom Med 1996; 58: 546-58
21. Ishihara Y, Matsunaga K, Iijima H, Fujii T, Oguchi Y, Kagawa J: Time-dependent effects of stressor application on metastasis of tumor cells in the lung and its regulation by an immunomodulator in mice. Psychoneuroendocrinology 1999; 24: 713-26
22. Freire-Garabal M, Nunez MJ, Balboa JL, Fernandez-Rial JC, Vallejo LG, Gonzalez-Bahillo J, Rey-Mendez M: Effects of alprazolam on cellular immune response to surgical stress in mice. Cancer Lett 1993; 73: 155-60
23. Andersen BL, Farrar WB, Golden-Kreutz D, Kutz LA, MacCallum R, Courtney ME, Glaser R: Stress and immune responses after surgical treatment for regional breast cancer. J Natl Cancer Inst 1998; 90: 30-6
24. Larson MR, Duberstein PR, Talbot NL, Caldwell C, Moynihan JA: A presurgical psychosocial intervention for breast cancer patients. psychological distress and the immune response. J Psychosom Res 2000; 48: 187-94
25. Coffey JC, Wang JH, Smith MJ, Bouchier-Hayes D, Cotter TG, Redmond HP: Excisional surgery for cancer cure: therapy at a cost. Lancet Oncol 2003; 4: 760-8
26. Sessler DI: Long-term consequences of anesthetic management. Anesthesiology 2009; 111: 1-4
27. Mitsuhata H, Shimizu R, Yokoyama MM: Suppressive effects of volatile anesthetics on cytokine release in human peripheral blood mononuclear cells. Int J Immunopharmacol 1995; 17: 529-34
28. Markovic SN, Knight PR, Murasko DM: Inhibition of interferon stimulation of natural killer cell activity in mice anesthetized with halothane or isoflurane. Anesthesiology 1993; 78: 700-6
29. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL: The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. Am J Pathol 2000; 157: 411-21
30. Generali D, Berruti A, Brizzi MP, Campo L, Bonardi S, Wigfield S, Bersiga A, Allevi G, Milani M, Aguggini S, Gandolfi V, Dogliotti L, Bottini A, Harris AL, Fox SB: Hypoxia-inducible factor-1alpha expression predicts a poor response to primary chemoendocrine therapy and disease-free survival in primary human breast cancer. Clin Cancer Res 2006; 12: 4562-8
31. Rohwer N, Lobitz S, Daskalow K, Jons T, Vieth M, Schlag PM, Kemmner W, Wiedenmann B, Cramer T, Hocker M: HIF-1alpha determines the metastatic potential of gastric cancer cells. Br J Cancer 2009; 100: 772-81
32. Dai CX, Gao Q, Qiu SJ, Ju MJ, Cai MY, Xu YF, Zhou J, Zhang BH, Fan J: Hypoxia-inducible factor-1 alpha, in association with inflammation, angiogenesis and MYC, is a critical prognostic factor in patients with HCC after surgery. BMC Cancer 2009; 9: 418
33. Baba Y, Nosho K, Shima K, Irahara N, Chan AT, Meyerhardt JA, Chung DC, Giovannucci EL, Fuchs CS, Ogino S: HIF1A overexpression is associated with poor prognosis in a cohort of 731 colorectal cancers. Am J Pathol 2010; 176: 2292-2301
34. Osada R, Horiuchi A, Kikuchi N, Yoshida J, Hayashi A, Ota M, Katsuyama Y, Melillo G, Konishi I: Expression of hypoxia-inducible factor 1alpha, hypoxia-inducible factor 2alpha, and von Hippel-Lindau protein in epithelial ovarian neoplasms and allelic loss of von Hippel-Lindau gene: nuclear expression of hypoxia-inducible factor 1alpha is an independent prognostic factor in ovarian carcinoma. Hum Pathol 2007; 38: 1310-20
35. Ma D, Lim T, Xu J, Tang H, Wan Y, Zhao H, Hossain M, Maxwell PH, Maze M: Xenon preconditioning protects against renal ischemic-reperfusion injury via HIF-1alpha activation. J Am Soc Nephrol 2009; 20:

713-20
36. De Hert SG, Turani F, Mathur S, Stowe DF: Cardioprotection with volatile anesthetics: mechanisms and clinical implications. Anesth Analg 2005; 100: 1584-93
37. Beck-Schimmer B, Breitenstein S, Urech S, De Conno E, Wittlinger M, Puhan M, Jochum W, Spahn DR, Graf R, Clavien PA: A randomized controlled trial on pharmacological preconditioning in liver surgery using a volatile anesthetic. Ann Surg 2008; 248: 909-18
38. Kawaguchi M, Furuya H, Patel PM: Neuroprotective effects of anesthetic agents. J Anesth 2005; 19: 150-6
39. Wang C, Weihrauch D, Schwabe DA, Bienengraeber M, Warltier DC, Kersten JR, Pratt PF, Jr., Pagel PS: Extracellular signal-regulated kinases trigger isoflurane preconditioning concomitant with upregulation of hypoxia-inducible factor-1alpha and vascular endothelial growth factor expression in rats. Anesth Analg 2006; 103: 281-8
40. Porta C, Figlin RA: Phosphatidylinositol-3-kinase/Akt signaling pathway and kidney cancer, and the therapeutic potential of phosphatidylinositol-3-kinase/Akt inhibitors. J Urol 2009; 182: 2569-77
41. Bonello S, Zahringer C, BelAiba RS, Djordjevic T, Hess J, Michiels C, Kietzmann T, Gorlach A: Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site. Arterioscler Thromb Vasc Biol 2007; 27: 755-61
42. Maranchie JK, Zhan Y: Nox4 is critical for hypoxia-inducible factor 2-alpha transcriptional activity in von Hippel-Lindau-deficient renal cell carcinoma. Cancer Res 2005; 65: 9190-3
43. Mullenheim J, Ebel D, Frassdorf J, Preckel B, Thamer V, Schlack W: Isoflurane preconditions myocardium against infarction via release of free radicals. Anesthesiology 2002; 96: 934-40
44. Hanouz JL, Zhu L, Lemoine S, Durand C, Lepage O, Massetti M, Khayat A, Plaud B, Gerard JL: Reactive oxygen species mediate sevoflurane- and desflurane-induced preconditioning in isolated human right atria in vitro. Anesth Analg 2007; 105: 1534-9
45. Huitink JM, Heimerikxs M, Nieuwland M, Loer SA, Brugman W, Velds A, Sie D, Kerkhoven RM: Volatile anesthetics modulate gene expression in breast and brain tumor cells. Anesth Analg 2010; 111: 1411-5
46. Melamed R, Bar-Yosef S, Shakhar G, Shakhar K, Ben-Eliyahu S: Suppression of natural killer cell activity and promotion of tumor metastasis by ketamine, thiopental, and halothane, but not by propofol: mediating mechanisms and prophylactic measures. Anesth Analg 2003; 97: 1331-9
47. Tanaka T, Takabuchi S, Nishi K, Oda S, Wakamatsu T, Daijo H, Fukuda K, Hirota K: The intravenous anesthetic propofol inhibits lipopolysaccharide-induced hypoxia-inducible factor 1 activation and suppresses the glucose metabolism in macrophages. J Anesth 2010; 24: 54-60
48. Wakamatsu T, Tanaka T, Oda S, Nishi K, Harada H, Daijo H, Takabuchi S, Kai S, Fukuda K, Hirota K: The intravenous anesthetics barbiturates inhibit hypoxia-inducible factor 1 activation. Eur J Pharmacol 2009; 617: 17-22
49. Mammoto T, Mukai M, Mammoto A, Yamanaka Y, Hayashi Y, Mashimo T, Kishi Y, Nakamura H: Intravenous anesthetic, propofol inhibits invasion of cancer cells. Cancer Lett 2002; 184: 165-70
50. Gupta K, Kshirsagar S, Chang L, Schwartz R, Law PY, Yee D, Hebbel RP: Morphine stimulates angiogenesis by activating proangiogenic and survival-promoting signaling and promotes breast tumor growth. Cancer Res 2002; 62: 4491-8
51. Beilin B, Martin FC, Shavit Y, Gale RP, Liebeskind JC: Suppression of natural killer cell activity by high-dose narcotic anesthesia in rats. Brain Behav Immun 1989; 3: 129-37
52. Gaspani L, Bianchi M, Limiroli E, Panerai AE, Sacerdote P: The analgesic drug tramadol prevents the effect of surgery on natural killer cell activity and metastatic colonization in rats. J Neuroimmunol 2002; 129: 18-24
53. Sasamura T, Nakamura S, Iida Y, Fujii H, Murata J, Saiki I, Nojima H, Kuraishi Y: Morphine analgesia suppresses tumor growth and metastasis in a mouse model of cancer pain produced by orthotopic tumor inoculation. Eur J Pharmacol 2002; 441: 185-91
54. Mammoto T, Higashiyama S, Mukai M, Mammoto A, Ayaki M, Mashimo T, Hayashi Y, Kishi Y, Nakamura H, Akedo H: Infiltration anesthetic lidocaine inhibits cancer cell invasion by modulating ectodomain shedding of heparin-binding epidermal growth factor-like growth factor (HB-EGF). J Cell Physiol 2002; 192: 351-8
55. Martinsson T: Ropivacaine inhibits serum-induced proliferation of colon adenocarcinoma cells in vitro. J Pharmacol Exp Ther 1999; 288: 660-4
56. Ahlers O, Nachtigall I, Lenze J, Goldmann A, Schulte E, Hohne C, Fritz G, Keh D: Intraoperative thoracic epidural anaesthesia attenuates stress-induced immunosuppression in patients undergoing major abdominal surgery. Br J Anaesth 2008; 101: 781-7
57. Chae BK, Lee HW, Sun K, Choi YH, Kim HM: The effect of combined epidural and light general anesthesia on stress hormones in open heart surgery patients. Surg Today 1998; 28: 727-31
58. Looney M, Doran P, Buggy DJ: Effect of anesthetic technique on serum vascular endothelial growth factor C and transforming growth factor beta in women undergoing anesthesia and surgery for breast cancer.

Anesthesiology 2010; 113: 1118-25
59. 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 2008; 109: 180-7
60. Exadaktylos AK, Buggy DJ, Moriarty DC, Mascha E, Sessler DI: Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? Anesthesiology 2006; 105: 660-4
61. Gottschalk A, Ford JG, Regelin CC, You J, Mascha EJ, Sessler DI, Durieux ME, Nemergut EC: Association between epidural analgesia and cancer recurrence after colorectal cancer surgery. Anesthesiology 2010; 113: 27-34
62. 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. Br J Anaesth 2011; 107: 164-70
63. Myles PS, Peyton P, Silbert B, Hunt J, Rigg JR, Sessler DI: Perioperative epidural analgesia for major abdominal surgery for cancer and recurrence-free survival: randomised trial. Bmj 2011; 342: d1491
64. Chen WK, Miao CH: The effect of anesthetic technique on survival in human cancers: a meta-analysis of retrospective and prospective studies. PLoS One 2013; 8: e56540
65. Meng C, Zhang XQ, Bi ZY, Wei GZ, Zhou JS, Wang H, Sun HL: Telephone interview for cognitive status-modified used in screening cognitive impairment. Zhong Hua Shen Jing Ke Za Zhi 2005; 38: 30–33
66. Hao YT, Fang JQ, Power M, Wu SM, Zhu SM: The Equivalence of WHOQOLBREF among 13 culture versions. Zhong Guo Xin Li Wei Sheng Za Zhi 2006; 20: 71-75
67. Sarfati D, Blakely T, Pearce N: Measuring cancer survival in populations: relative survival vs cancer-specific survival. Int J Epidemiol 2010; 39: 598-610
68. Austin PC: Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med 2009; 28: 3083-107

Summary of changes from the original study protocol approved by the ethics committee

	Version	Date of version	Drafter/reviser	Contents of revision
1	2.0	Jun. 13, 2014	FC, D-XW.	(This was the first version approved by the ethics committee before initiating follow-up).
2	2.1	Oct. 20, 2014	Y-WL, D-XW.	1) Closed two study centers (clause 3.2). 2) Added "For those who undergo thoracoscopic or laparoscopic surgery, the expected length of incision must be five centimeters or more" (clause 4.2.1.2).
2	3.0	Jun. 29, 2016	M-HL, D-XW.	1) Clarified that "Long-term follow-ups will be performed once a year after surgery via telephone interview (after ethic approval)" (clause 5.3.1). 2) Clarified that "The development of cancer recurrence and/or metastasis will be diagnosed by surgeons (and/or radiologists when necessary) according to the results of re-examination" (clause 5.3.2.1). 3) Added "New cancers are defined as those that are confirmed by pathological examinations but different from the primary ones. For patients with new cancers, the date of earliest diagnoses and subsequent therapies will be documented" (clause 5.3.2.3.1). 4) Added "New serious non-cancer diseases are defined as those that required hospital readmission and/or another surgical therapy. For patients with new serious non-cancer diseases, the date of earliest diagnosis and subsequent therapies will be documented" (clause 5.3.2.3.2). 5) Added "Other outcomes" for the subgroup of cancer patients (clauses 6.3.1 to 6.3.4). 6) Revised the section of statistical analysis (clause 11). 7) Revised informed consent. 8) Revised case report form.
3	4.0	May 1, 2017	W-JZ, D-XW.	1) Revised the duration of long-term follow-up: "Long-term follow-ups will be performed once a year after surgery via telephone interview (after ethic approval), for a period of up to 5 years (from the recruitment of the last patient)" (clause 5.3.1). 2) Revised informed consent. 3) Revised case report form.

Original statistical analysis plan

This is a copy from the original study protocol.

1. Efficacy outcomes

1.1 Primary outcome

Overall survival, which is defined as the time from surgery to the date of all-cause death.

1.2 Secondary outcomes

1.2.1 Overall survival in cancer patients. Overall survival is defined as the time from surgery to the date of all-cause death.

1.2.2 Time to cancer recurrence and/or metastasis after surgery in all and cancer patients.

1.2.3 Time to re-hospitalization after surgery in all and cancer patients.

1.2.4 All-cause mortality rate at 1, 2 and 3 years after surgery in all and cancer patients.

1.2.5 Cognitive function in 3-year survivors, which will be assessed with the TICS-m at the end of the 3rd year after surgery.

1.2.6 Quality of life in 3-year survivors, which will be assessed with the WHOQOL-BREF at the end of the 3rd year after surgery.

2. Statistical analysis

2.1 General principles

2.1.1 Numeric variables will be presented as mean ± standard deviation or median (interquartile range). Categorical variables will be presented as number (%).

2.1.2 Two-tailed tests will be used in all statistical analysis unless otherwise indicated, and $P<0.05$ will be considered statistically significant.

2.1.3 The primary and secondary outcomes will be analyzed in an intention-to-treat population, i.e., all patients are analyzed in the group to which they are randomized and received at least part of study intervention. If epidural anesthesia and/or analgesia fail, these patients will be converted to general anesthesia and intravenous analgesia but will be analyzed in the group to which they are randomized. Also, we will do per-protocol analysis for the primary endpoint, in this case, drop-out patients will be excluded.

2.2 Patient enrolment, drop-out/elimination and lost-to-follow-up

The status of patient recruitment, drop-out/elimination and lost-to-follow-up will be summarized and listed. Comparison of the overall elimination/drop-out and lost-to-follow-up rate between the two groups will be performed with chi square test.

2.3 Demographics and baseline characteristics

2.3.1 Demographics and baseline data will be presented.

2.3.2 For between-group differences, numeric variables will be compared with independent-sample t test or Wilcoxon test. Categorical variables will be compared with chi square test, continuity-corrected chi-square test or Fisher's exact test. Ordinal variables will be compared with Wilcoxon test or Cochran-Mantel-Haenszel test.

2.4 Efficacy analysis

2.4.1 Evaluation of primary endpoint

The overall survival after surgery will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; a Cox proportional hazards model will be used to adjust for potential confounding factors. The estimated difference between two groups will be expressed as hazard ratio and 95% CI. Patients who are lost to follow-up will be censored at the time of last contact.

2.4.2 Evaluation of secondary endpoints

2.4.2.1 Time-to-event variables (time to cancer recurrence/metastasis and time to rehospitalization after surgery) will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; Cox proportional hazards models will be used to adjust for potential confounding factors. The estimates hazard ratios and 95% CIs will be provided. Patients who are lost to follow-up will be censored at the time of last contact.

2.4.2.2 All-cause mortality rate at 1, 2 and 3 years will be obtained from survival analysis tables and the difference between groups will be calculated with tests based on a cloglog transformation of the Kaplan–Meier estimator.[1]

2.4.2.3 The scores of cognitive function and quality of life in 3-year survivors after surgery will be analyzed using the independent sample t test or the Mann-Whitney U test. The difference between two means/medians and 95% CI will be provided. The difference between two medians (and 95% CI) will be calculated with the Hodges-Lehmann estimator. Missing data will not be replaced.

2.4.2.4 For the subgroup of cancer patients, time-to-event variables (overall survival, time to cancer recurrence/metastasis, and time to rehospitalization after surgery) will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; Cox proportional hazards models will be used to adjust for potential confounding factors. The estimates hazard ratios and 95% CIs will be provided. Patients who are lost to follow-up will be censored at the time of last contact.

3. Reference

1. Klein JP, Logan B, Harhoff M, Andersen PK: Analyzing survival curves at a fixed point in time. Stat Med 2007; 26:4505–19

Final statistical analysis plan

1. Overview of analyses

This document contains the statistical analysis plan for the trial. The aim is to clarify analyses and to avoid misleading inference from post-hoc analyses. Therefore, the statistical analysis plan has been completed prior to the availability of any outcome data.

2. Background of the trial

The principal research question is whether "combined epidural-general anesthesia and postoperative epidural analgesia" compared with "general anesthesia and postoperative intravenous analgesia" can improve long-term survival in elderly patients after major noncardiac thoracic and abdominal surgery for cancer.

The study is a long-term follow-up of a multicenter randomized controlled trial.

3. Efficacy outcomes

3.1 Primary outcome

Overall survival, which is defined as the time from surgery to the date of all-cause death.

3.2 Secondary outcomes

3.2.1 Cancer-specific survival, which is defined as the time from surgery to the date of cancer-specific death, i.e., death fully attributable to a specific cancer for which surgery is performed during the underlying trial. Patients who die of a cause other than the specific cancer are "censored" at the time of death.

3.2.2 Recurrence-free survival, which is defined as the time from surgery to the date of cancer recurrence/metastasis or all-cause death, whichever occurs first.

3.2.3 Event-free survival, which is defined as the time from surgery to the date of cancer recurrence/metastasis, new cancer, new serious non-cancer disease or all-cause death, whichever occurs first.

3.3 Other outcomes

3.3.1 Overall survival of cancer patients, which is defined as the time from surgery to the date of all-cause death.

3.3.2 Cancer-specific survival of cancer patients, which is defined as the time from surgery to the date of cancer-specific death, i.e., death fully attributable to a specific cancer for which surgery is performed during the underlying trial. Patients who die of a cause other than the specific cancer are "censored" at the time of death.

3.3.3 Recurrence-free survival of cancer patients, which is defined as the time from surgery to the date of cancer recurrence/metastasis or all-cause death, whichever occurs first.

3.3.4 Event-free survival of cancer patients, which is defined as the time from surgery to the date of cancer recurrence/metastasis, new cancer, new serious non-cancer disease or all-cause death, whichever occurs first.

3.3.5 Cognitive function in 3-year survivors, which will be assessed with the TICS-m at the end of the 3rd year after surgery.

3.3.6 Quality of life in 3-year survivors, which will be assessed with the WHOQOL-BREF at the end of the 3rd year after surgery.

4. Statistical analysis

4.1 General principles

4.1.1 Numeric variables will be presented as mean ± standard deviation or median (minimum, maximum; or interquartile range). Categorical variables will be presented as number of cases (%).

4.1.2 For each hypothesis, two-tailed tests will be used in all statistical analysis, and $P<0.05$ will be considered statistically significant. For the treatment-by-covariate interaction in predefined subgroup analyses, a $P<0.10$ will be defined as statistically significant.

4.1.3 The primary and secondary outcomes will be analyzed in a modified intention-to-treat population, i.e., all patients are analyzed in the group to which they are randomized and received at least part of study intervention. If epidural anesthesia and/or analgesia fail, these patients will be converted to general anesthesia and intravenous analgesia but will be analyzed in the group to which they are randomized. For patients with repeated recruitment for different surgeries, only the first was considered for this analysis. Also, we will do per-protocol analysis for the primary endpoint, in this case, drop-out patients will be excluded.

4.2 Patient enrolment, drop-out/elimination and lost-to-follow-up

The status of patient recruitment, drop-out/elimination and lost-to-follow-up will be summarized and listed. Comparison of the overall elimination/drop-out and lost-to-follow-up rate between the two groups will be performed with chi square test.

4.3 Demographics and baseline characteristics

4.3.1 Demographics and baseline data will be presented.

4.3.2 Between-group differences will be compared using the absolute standardized differences (ASDs), which are defined as the absolute difference in means, mean ranks or proportions divided by the pooled standard deviation and calculated with the formula published by Austin.[1] Baseline variables with an absolute standardized difference of $\geq 1.96\times\sqrt{(n1+n2)/(n1\times n2)}$ will be considered imbalanced between the two groups and adjusted for in all analyses if considered necessary.

4.4 Intra- and postoperative variables

Numeric variables will be analyzed using the independent-samples t test or Mann-Whitney U test; categorical variables will be analyzed using the chi square test, continuity correction chi square test or Fisher exact test. Missing data will not be replaced.

4.5 Efficacy analysis

4.5.1 Evaluation of primary endpoint

4.5.1.1 The overall survival after surgery will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; a Cox proportional hazards model will be used to adjust for predetermined factors including age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. The estimated difference between two groups will be expressed as hazard ratio and 95% CI. Patients who are lost to follow-up will be censored at the time of last contact.

4.5.1.2 The differences of the primary outcome in predefined subgroups will be tested using the Cox proportional hazard models adjusted for preselected factors of age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. Treatment-by-covariate interactions will be assessed separately for each predefined factor, adjusting for the same variables. The hazard ratio (and 95% CI) for each subgroup and the p values of treat-by-covariate interactions will be displayed in a forest plot.

4.5.2 Evaluation of secondary endpoints

4.5.2.1 The time-to-event variables (cancer-specific survival, recurrence-free survival, and event-free survival after surgery) will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; Cox proportional hazards models will be used to adjust for predetermined factors including age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. The estimates hazard ratios and 95% CIs will be provided. For cancer-specific survival, patients who die of a cause other than the specific cancer will be censored at the time of death. Patients who are lost to follow-up will be censored at the time of last contact.

4.5.2.2 The scores of cognitive function and quality of life in 3-year survivors after surgery will be analyzed using the independent sample t test or the Mann-Whitney U test. The difference between two means/medians and 95% CI will be provided. The difference between two medians (and 95% CI) will be calculated with the Hodges-Lehmann estimator. Missing data will not be replaced.

4.5.3 Analysis for the subgroup of cancer patients

4.5.3.1 For demographics and baseline data, between-group differences will be compared using the absolute standardized differences (ASDs), which are defined as the absolute difference in means, mean ranks or proportions divided by the pooled standard deviation and calculated with the formula published by Austin.[1] Baseline variables with an absolute standardized difference $\geq 1.96\times\sqrt{(n1+n2)/(n1\times n2)}$ will be considered imbalanced between the two groups and adjusted for in all analyses if considered necessary.

4.5.3.2 For other intra- and postoperative variables, numeric variables will be analyzed using the independent-samples t test or Mann-Whitney U test; categorical variables will be analyzed using the chi square test, continuity correction chi square test or Fisher exact test. Missing data will not be replaced.

4.5.3.3 The time-to-event variables (overall survival, cancer-specific survival, recurrence-free survival, and event-free survival after surgery) will be analyzed using a Kaplan-Meier estimator with differences between groups assessed by log-rank test; Cox proportional hazards models will be used to adjust for predetermined factors including age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. The estimates hazard ratios and 95% CIs will be provided. For cancer-specific survival, patients who die of a cause other than the specific cancer will be censored at the time of death. Patients who are lost to follow-up will be censored at the time of last contact.

4.5.3.4 The differences of overall survival in predefined subgroups will be tested using the Cox proportional hazard models adjusted for preselected factors of age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. Treatment-by-covariate interactions will be assessed separately for each predefined factor, adjusting for the same variables. The hazard ratio (and 95% CI) for each subgroup and the p values of treat-by-covariate interactions will be displayed in a forest plot.

5. Reference

1. Austin PC: Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med 2009; 28: 3083-107

Summary of changes from the original statistical analysis plan

In "General principles" section, we added the significant level for treat-by-covariate interaction: "For the treatment-by-covariate interaction in predefined subgroup analyses, a $P<0.10$ will be defined as statistically significant" (clause 4.1.2). We changed from "an intention-to-treat population" to "a modified intention-to-treat population", and added "For patients with repeated recruitment for different surgeries, only the first was considered for this analysis" (clause 4.1.3).

In "Demographics and baseline characteristics" section, we changed the method to compare between-group differences: "Between-group differences will be compared using the absolute standardized differences (ASDs), which are defined as the absolute difference in means, mean ranks or proportions divided by the pooled standard deviation and calculated with the formula published by Austin.[1] Baseline variables with an absolute standardized difference $\geq 1.96\times\sqrt{(n1+n2)/(n1\times n2)}$ will be considered imbalanced between the two groups and adjusted for in all analyses if considered necessary (clause 4.3.2)."

We added a "Intra- and postoperative variables" section to describe methods to compare between-group differences regarding intra- and postoperative variables: "Numeric variables will be analyzed using the independent-samples t test or Mann-Whitney U test; categorical variables will be analyzed using the chi square test, continuity correction chi square test or Fisher exact test. Missing data will not be replaced" (clause 4.4).

In the "Evaluation of primary endpoint" section, we clarified factors that will be included in the Cox proportional hazard model: "…; a Cox proportional hazards model will be used to adjust for predetermined factors including age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy (clause 4.5.1.1)". We added methods to assess differences of the primary outcome in predefined subgroups and treat-by-covariate interactions: "The differences of the primary outcome in predefined subgroups will be tested using the Cox proportional hazard models adjusted for preselected factors of age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. Treatment-by-covariate interactions will be assessed separately for each predefined factor, adjusting for the same variables. The hazard ratio (and 95% CI) for each subgroup and the p values of treat-by-covariate interactions will be displayed in a forest plot (clause 4.5.1.2)."

In the "Evaluation of secondary endpoints" section, we changed time-to-event variables from "time to cancer recurrence/metastasis and time to rehospitalization after surgery" to "cancer-specific survival, recurrence-free survival, and event-free survival after surgery" because the latter variables are collected during the study period and are more commonly used to reflect outcomes of cancer patients; we clarified factors that will be included in the Cox proportional hazard model: "…; Cox proportional hazards models will be used to adjust for predetermined factors including age, sex, body mass index, Charlson Comorbidity Index, type of surgery, location of surgery, type of cancer, TNM stage, study center, and postoperative anti-cancer therapy. We added that "For cancer-specific survival, patients who die of a cause other than the specific cancer will be censored at the time of death" (clause 4.5.2.1). To be noted, event-free survival and cancer-specific mortality are added after data collection, but before analysis.

In the "Evaluation of secondary endpoints" section, we deleted "All-cause mortality rate at 1, 2 and 3 years (original clause 2.4.2.2)" because this information is included in the primary endpoint "overall survival".

We added a section of "Analysis for the subgroup of cancer patients (clauses 4.5.3.1 to 4.5.3.4)", which was previously a clause in the "Evaluation of secondary endpoints" section of the original statistical analysis plan (original clause 2.4.2.4). We added this section in order to describe the methods of analyses for the subgroup of cancer patients in more detail.

Reference

1. Austin PC: Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med 2009; 28: 3083-107