# Effect of Equipotent Doses of Propofol *versus* Sevoflurane Anesthesia on Regulatory T Cells after Breast Cancer Surgery

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## ABSTRACT

**Background:** Clusters of differentiation 39 and 73, enzymes expressed on the surface of regulatory T cells, promote cancer recurrence and metastasis by suppressing immune cells. The authors hypothesized that propofol is less immunosuppressive than volatile anesthetics. The objective of this randomized trial was to compare the changes in cluster of differentiation 39 and 73 expression on regulatory T cells between propofol- and sevoflurane-based anesthesia during breast cancer surgery.

**Methods:** A total of 201 patients having breast cancer surgery were randomly assigned and analyzed (n = 99 for propofol, n = 102 for sevoflurane). Blood samples were obtained immediately before anesthesia induction and 1 and 24 h postoperatively. The frequency of cluster of differentiation 39 and 73 expression on circulating regulatory T cells (primary outcome) and the frequency of circulating type 1 and type 17 helper T cells, natural killer cells, and cytotoxic T cells were investigated. Serum cytokines and the neutrophil-to-lymphocyte ratio were also evaluated.

**Results:** Changes in cluster of differentiation 39 and 73 expression on regulatory T cells over time did not differ with propofol and sevoflurane groups (difference [95% confidence interval]: 0.01 [−2.04 to 2.06], $P = 0.995$ for cluster of differentiation 39; −0.93 [−3.12 to 1.26], $P = 0.403$ for cluster of differentiation 73). There were no intergroup differences in type 1, type 17 helper T cells, natural killer cells, cytotoxic T cells, cytokines, or the neutrophil-to-lymphocyte ratio.

**Conclusions:** Changes in immune cells were similar with propofol and sevoflurane during breast cancer surgery. The effect of anesthetics on the perioperative immune activity may be minimal during cancer surgery. **(Anesthesiology 2018; 129:921-31)**



WORLDWIDE, breast cancer is the most common cancer, and the second most common cause of death, in females.[1] Surgical removal of breast cancer is the first-line treatment, and other therapies, such as chemotherapy and radiation therapy, continue to play important roles.[2] However, the surgical procedure itself is associated with immune suppression and cancer metastasis.[3,4] Anesthesia can trigger immunosuppression and accelerate the progression of cancer metastasis,[5,6] although it is important to achieve optimal conditions for surgical procedures. Previous studies suggest that anesthetics can affect cancer progression,[7–9] although no guidelines state which anesthetic is best for patients having cancer surgery. However, several studies suggest that volatile anesthetics promote breast cancer recurrence and metastasis compared with propofol-based anesthesia.[10–12]

Various factors are involved in cancer immunity. Ectonucleoside triphosphate diphosphohydrolase-1, also known as cluster of differentiation 39, and ecto 5′-nucleotidase, also known as cluster of differentiation 73, are associated with cancer progression. Cluster of differentiation 39 and 73 expression plays important roles in the suppression of cancer immunity and promotes cancer recurrence and metastasis.[13] High expression of clusters of differentiation 39 and 73 on regulatory T cells suppress types 1 and 17 helper T cells and impair the tumor cell-killing effect of natural killer cells and cytotoxic T cells.[14] Volatile anesthetics may promote cancer progression and metastasis more than propofol, since volatile anesthetics induce immunosuppression in the cancer environment.[7,15,16] We therefore tested the hypothesis that propofol is less immunosuppressive and reduces the frequency of cluster of differentiation 39

### Editor's Perspective

#### What We Already Know about This Topic

- Cluster of differentiation enzymes on regulatory T cells are immunosuppressive and promote cancer recurrence
- The investigators tested the hypothesis that cluster of differentiation 39 and 73 expression is increased less with propofol than sevoflurane

#### What This Article Tells Us That Is New

- A total of 201 women having breast cancer surgery were randomly assigned to propofol or sevoflurane anesthesia
- Cluster of differentiation 39 and 73 expression did not differ nor did any other evaluated immune functions
- These results do not support the putative protective effect of propofol on cancer recurrence

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and 73 expression on circulating regulatory T cells than an equipotent amount of volatile anesthetic during cancer surgery.

## Materials and Methods

### Study Population

This study was approved by the Institutional Review Board of Konkuk University Medical Center, Seoul, Korea (approval number KUH1160086) and registered at clinicaltrials.gov (NCT02567929). There was a discrepancy between the actual study and initial trial registration in terms of the primary outcome (explained further in "Discussion"). The study was conducted at a single tertiary medical center (Konkuk University Medical Center), and written, informed consent was obtained from all patients. The study was conducted according to the original protocol, which remained unchanged from first enrollment in March 2016 with a prospective randomized design (the full protocol can be obtained on request).

Korean women having elective breast cancer surgery were enrolled in this randomized trial. Patients were excluded based on the following criteria: (1) age less than 20 yr, (2) re-do surgery, (3) history of previous cancer, (4) other concurrent surgery excluding breast reconstruction surgery, and (5) history of drug abuse.

Before anesthesia induction, patients were randomly allocated to a propofol-based (propofol) or sevoflurane-based (sevoflurane) anesthesia group by opening a sequentially numbered envelope containing the randomization assignment (third-party allocation). The allocation sequence was generated by the clinical research coordination center in our hospital that was not otherwise involved in the trial, with random-permuted block randomization. Anesthesia team and care providers including surgical team and nursing team were blinded to the study goals and were asked to follow the protocol. A research assistant, responsible for eligibility and enrollment for the patients, obtained informed consents. A data collector, responsible for data gathering including blood sampling, was blinded to allocation. The research assistant and data collector did not participate in patient care. The research team members were blinded to the study throughout the perioperative period and until the completion of statistical analysis.

### Anesthesia

Patients were not premedicated. Anesthesia was induced after establishing routine noninvasive monitoring with the Bispectral Index. If required, invasive arterial blood pressure monitoring was performed. Lidocaine, 0.5 mg/kg, was given intravenously to all patients.

For the propofol group, an initial target concentration of 4.0 μg/ml propofol (effect-site, modified Marsh model with $k_{e0}$ = 1.21 $min^{-1}$)[17] was administered intravenously by using a target-controlled infusion device (Orchestra Base Primea; Fresenius Vial, France). For the sevoflurane group, 5 mg/kg thiopental sodium was administered intravenously to induce anesthesia.

After loss of consciousness, adequate mask ventilation was confirmed, and 0.6 mg/kg rocuronium was administered intravenously for muscle relaxation under the guidance of peripheral neuromuscular transmission monitoring. A fixed target concentration of 5.0 ng/ml remifentanil (plasma site, Minto model)[18,19] was administered intravenously and maintained until the end of surgery. Tracheal intubation was performed at a train-of-four count of zero. After anesthesia induction, 0.3 mg ramosetron was administered intravenously to prevent postoperative nausea and vomiting. Additional rocuronium was administered guided by neuromuscular transmission monitoring. Anesthesia was maintained with propofol by using target-controlled infusion for the propofol group, and with sevoflurane inhalation for the sevoflurane group, by titrating the Bispectral Index between 40 and 60 to provide comparable anesthesia in each group. During the anesthesia, the mean systemic blood pressure was maintained to within 20% of baseline or above 60 mmHg.

At the end of the surgery, the administration of propofol or sevoflurane with remifentanil for each group was stopped, and 0.5 mg/kg ketorolac was given intravenously for postoperative analgesia. Residual neuromuscular paralysis was antagonized with 0.03 mg/kg neostigmine and 0.008 mg/kg glycopyrrolate with neuromuscular transmission monitoring. Intravenous patient-controlled analgesia (PCA) was applied on demand for patients having radical mastectomies. The total PCA volume was 200 ml, consisting of 2,000 μg (40 ml) fentanyl, 0.6 mg (4 ml) ramosetron, and 156 ml normal saline. The PCA device (Gemstar Pump; Hospira, USA) was programed to deliver 0.03 ml · $kg^{-1}$ · $h^{-1}$ as the basal infusion rate and 0.05 ml/kg on demand with 15 min as the lock-out time. After tracheal extubation, patients were transferred to the postanesthesia care unit. Postoperative medical treatment and decision making were performed by the responsible surgeon according to the standard institutional regimens.

### Blood Samples

We examined the frequency of cluster of differentiation 39 and 73 expression on circulating regulatory T cells, as well as the frequency of circulating immune cells, such as types 1 and 17 helper T cells. In addition, the frequency and apoptosis of circulating natural killer cells and cluster of differentiation $8^+$ T cells were explored. Proinflammatory cytokines, such as interleukin 6 and interleukin 12, antiinflammatory cytokines, such as interleukin 10, and transforming growth factor β were also monitored. Venous blood samples were collected immediately before anesthesia induction, on arrival to the postanesthesia care unit, and at 24 h postoperatively. Samples were collected in ethylenediaminetetraacetic acid tubes.

### Flow Cytometric Analysis

Flow cytometric analysis was performed to evaluate immune cells. Peripheral blood mononuclear cells were isolated from

blood samples by using density-gradient centrifugation over a Ficoll-Hypaque gradient (GE Healthcare, USA). The peripheral blood mononuclear cells were washed with phosphate-buffered saline (137 mM NaCl, 2.7 M KCl, 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$; pH 7.4) and resuspended in Roswell Park Memorial Institute medium 1640 with 1% penicillin and 10% fetal bovine serum.

**Assay for Frequency of Cluster of Differentiation 39 and 73 Expression on Circulating Regulatory T Cells.** To investigate the frequency of cluster of differentiation 39 and 73 expression on regulatory T cells in peripheral blood mononuclear cells, fluorescein isothiocyanate–conjugated antihuman cluster of differentiation 39 (catalog number 561444; BD Biosciences, USA), phycoerythrin-cyanine 7–conjugated antihuman cluster of differentiation 73 (catalog number 561258; BD Biosciences), peridinin chlorophyll–conjugated antihuman cluster of differentiation 4 (catalog number 347324; BD Biosciences), and allophycocyanin-conjugated antihuman cluster of differentiation 25 (catalog number 555434; BD Biosciences) were used. After 30 min, the cells were washed with a fluorescence-activated cell sorting buffer (0.1% bovine serum albumin–phosphate-buffered saline) and fixed for 20 min with Fix/Perm buffer (catalog number 421401; BioLegend, USA). After fixation, the cells were permeabilized with FACS Perm 2 (catalog number 421403; BioLegend) according to the manufacturer's instructions and stained with phycoerythrin-conjugated antihuman forkhead box P3 (catalog number 320208; BioLegend) antibodies for 30 min.

**Assay for Frequency of Circulating Types 1 and 17 Helper T Cells.** To determine the frequency of cluster of differentiation $4^+$ interferon-$\gamma^+$ on type 1 helper T cells and cluster of differentiation $4^+$ interleukin $17A^+$ on type 17 helper T cells in peripheral blood mononuclear cells, the peripheral blood mononuclear cells were washed with a fluorescence-activated cell sorting buffer (0.1% bovine serum albumin–phosphate-buffered saline). After washing, cells were stained with peridinin chlorophyll–conjugated antihuman cluster of differentiation 4 (catalog number 347324; BD Biosciences) at room temperature for 30 min. After washing with a fluorescence-activated cell sorting buffer (0.1% bovine serum albumin–phosphate-buffered saline), cells were stimulated with 50 ng/ml phorbol myristate acetate (Sigma Aldrich, USA) and 1 μg/ml ionomycin (Sigma Aldrich) in the presence of Golgi stop (catalog number 554724; BD Biosciences) for 4 h at 37°C. Stimulated cells were washed with a fluorescence-activated cell sorting buffer and fixed for 10 min with 4% paraformaldehyde (catalog number 554655; BD Biosciences). After fixation, cells were permeabilized with FACS Perm 2 (catalog number 340973; BD Biosciences) according to the manufacturer's instructions and stained with fluorescein isothiocyanate–conjugated antihuman interferon-γ (catalog number 554700; BD Biosciences) and phycoerythrin-conjugated antihuman interleukin 17A (catalog number 12-7179-42; eBioscience, USA) antibody for 30 min.

**Assay for Frequency of Circulating Natural Killer and Cluster of Differentiation $8^+$ T Cells.** To isolate natural killer cells in peripheral blood mononuclear cells, cells were stained with phycoerythrin-cyanine 7–conjugated antihuman cluster of differentiation 16 (catalog number 25-0168-42; eBioscience) and allophycocyanin-conjugated anti-human cluster of differentiation 56 (catalog number 557711; BD Biosciences) for 30 min. To isolate cluster of differentiation $8^+$ cells in peripheral blood mononuclear cells, cells were stained with phycoerythrin-conjugated antihuman cluster of differentiation 8 (catalog number 555367; BD Biosciences). After 30 min, cluster of differentiation $56^+$ cluster of differentiation $16^+$ cells (natural killer cell) or cluster of differentiation $8^+$ cells were purified from peripheral blood mononuclear cells by using FACS Aria (BD Biosciences) according to the manufacturer's protocol.

**Assay for the Apoptosis Rate of Circulating Natural Killer and Cluster of Differentiation $8^+$ T Cells.** To determine the apoptosis rate of natural killer cells and cluster of differentiation $8^+$ T cells in peripheral blood mononuclear cells, phycoerythrin-conjugated antihuman cluster of differentiation 8 (catalog number 555367; BD Biosciences), phycoerythrin-cyanine 7–conjugated antihuman cluster of differentiation 16 (catalog number 25-0168-42; eBioscience), and allophycocyanin-conjugated antihuman cluster of differentiation 56 (catalog number 557711; BD Biosciences) were used. After 30 min, cells were washed with a cell-staining buffer (catalog number 420201; BioLegend). Next, 5y3 cells were mixed with 300 μl Annexin-V binding buffer (catalog number 422201; BioLegend) and fluorescein isothiocyanate–conjugated Annexin-V (catalog number 640906; BioLegend) antibody at room temperature for 15 min. All data were collected on a FACS Aria or FACSCalibur (BD Biosciences) and analyzed with FlowJo software (Tree Star, USA).

### *Enzyme-linked Immunosorbent Assay*

Blood samples were centrifuged at 3,000*g* for 5 min, and the serum was stored at −20°C for future measurements. Concentration of interleukin 6, 10, and 12 and transforming growth factor β were measured by means of enzyme-linked immunosorbent assay (triplicate measurements) with specific monoclonal antibodies against these cytokines by using commercial enzyme-linked immunosorbent assay kits (Quantikine Colorimetric Sandwich ELISA kits; R&D Systems, USA). Cytokine concentrations of the samples and the quality controls were determined by reading from the standard curve by using fluorescence microplate reader (SpectraMax Gemini EM; Molecular Devices, USA). The sensitivity thresholds of assay were 0.7, 3.9, 5.0, and 15.4 pg/ml for interleukin 6, 10, and 12 and transforming growth factor β, respectively.

### *Determination of the Differential Count of Leukocytes*

The data of differential count of leukocytes were collected immediately before anesthesia induction and 24 h postoperatively. The absolute neutrophil count was divided by the

absolute lymphocyte count to calculate the neutrophil-to-lymphocyte ratio, a robust prognostic marker for breast cancer.[20]

### Clinical Measurements

Postoperative pain was assessed by using a visual analog scale ranging from 0 mm (no pain) to 100 mm (worst pain imaginable). Intravenous 0.5 mg/kg ketorolac was administered on demand as an additional rescue analgesic treatment. All doses of opioids that were administered during the perioperative periods were recorded.

### Statistical Analysis

The primary outcome measure was the frequency of cluster of differentiation 39 and 73 expression on circulating regulatory T cells over time, and the secondary outcome measure was the frequency of circulating natural killer cell over time in the propofol and sevoflurane groups. The first 10 enrolled patients per group were examined in an interim analysis to estimate sample size by using G*power (version 3.1.9.2; Universität Kiel, Germany). No *P* value adjustments were made for this interim analysis. First, we calculated the standardized effect size, the magnitude of the difference between groups, for deduction of sample size. We calculated the standardized effect size of 0.222 for change in cluster of differentiation 39 expression, 0.234 for change in cluster of differentiation 73 expression, and 0.293 for change in natural killer cell. This yielded a sample size of 99 in each group for cluster of differentiation 39 expression, 89 in each group for cluster of differentiation 73 expression, and 57 in each group for natural killer cell. The sample size achieved 80% power to detect differences in the mean frequency value of cluster of differentiation 39 and 73 on regulatory T cells between the two groups by using two-way ANOVA for repeated measurements at the 5% significance level (α). Finally, we assigned 99 patients to each group and enrolled 218 patients in the study to allow for a 10% drop-out rate.

The intergroup differences in the frequency of cluster of differentiation 39 and 73 on circulating regulatory T cells and the frequency of circulating types 1 and 17 helper T cells, natural killer cells, and cytotoxic T cells over time were analyzed by using two-way ANOVA for repeated measurements. The independent two-tailed Student's *t* test was used to compare means for continuous, normally distributed data between the propofol and sevoflurane groups. When the data were not normally distributed (*e.g.*, demographic data, perioperative clinical characteristics, cytokine concentration, and neutrophil-to-lymphocyte ratio), the Mann–Whitney U test was used. To compare the changes in cytokine over time in both groups, the Friedman test with Bonferroni correction was used. The chi-square test was used to compare the means for categorical variables (*e.g.*, operation type and cancer stage) between the propofol and sevoflurane groups. Normally distributed continuous data are presented as the mean ± SD, and data that are not normally distributed are presented as the median (25 to 75%). For categorical variables, the number of patients (n) and proportion (%) were calculated. All calculations were performed by using SPSS (version 20.0; SPSS Inc., USA). A value of $P < 0.05$ was taken to indicate statistical significance.

## Results

A total of 218 patients were enrolled in this randomized trial from March 31 to December 2, 2016. Seventeen patients were excluded: eight had re-do cases, six had a history of previous cancer, and three underwent other concurrent surgery. Ultimately, 201 patients were included in the final analysis: 99 given propofol and 102 given sevoflurane. The participant flow diagram is shown in figure 1.

Table 1 shows patient characteristics. The propofol and sevoflurane groups did not differ in age, height, or weight. No differences were observed between the two groups in operation type, cancer stage, duration of anesthesia or surgery, or analgesics, including intraoperative remifentanil and postoperative ketorolac and fentanyl for PCA. The visual analog scale pain score when patients were at rest and moving also did not differ between the two groups. The dose of thiopental sodium was significantly higher in the sevoflurane group because it was administered only in the sevoflurane group.

Figure 2 shows the frequency of cluster of differentiation 39 and 73 expression on circulating regulatory T cells. The mean frequency of cluster of differentiation 39 expression in the propofol *versus* sevoflurane group (17.1 [interquartile range, 11.6 to 22.2%] *vs.* 17.6 [11.3 to 21.2%], 16.7 ± 7.6% *vs.* 16.5 ± 7.9%, and 16.9 [11.6 to 21.9%] *vs.* 17.6 [11.8 to 21.6%] at immediately before anesthesia induction, on arrival to the postanesthesia care unit, and 24 h postoperatively, respectively; fig. 2) on circulating regulatory T cells did not change significantly over time ($P = 0.680$) and did not differ between the two groups (difference [95% CI], 0.01 [−2.04 to 2.06]; $P = 0.995$). The mean frequency of cluster of differentiation 73 expression of propofol *versus* sevoflurane group (19.4 [13.9 to 23.7%] *vs.* 19.0 [15.6 to 23.9%], 18.5 [12.1 to 22.8%] *vs.* 19.2 [15.6 to 23.9%], 19.2 [13.9 to 22.1%] *vs.* 19.6 [14.1 to 23.5%] at immediately before anesthesia induction, on arrival to the postanesthesia care unit, and 24 h postoperatively, respectively; fig. 2) on circulating regulatory T cells did not change significantly over time ($P = 0.658$) and did not differ between the two groups (difference [95% CI], −0.93 [−3.12 to 1.26]; $P = 0.403$).

Figure 3 shows the frequency of circulating helper T cells. The frequency of circulating type 1 helper T cells did not change over time and did not differ between the two groups ($P = 0.126$; fig. 3). The frequency of circulating type 17 helper T cells decreased over time, but the reduction was not significant and did not differ between the two groups ($P = 0.740$; fig. 3).

Figure 4 shows the frequency of circulating natural killer cells and cytotoxic T cells, as well as the apoptosis rate for each cell type. The frequency of circulating natural killer cells

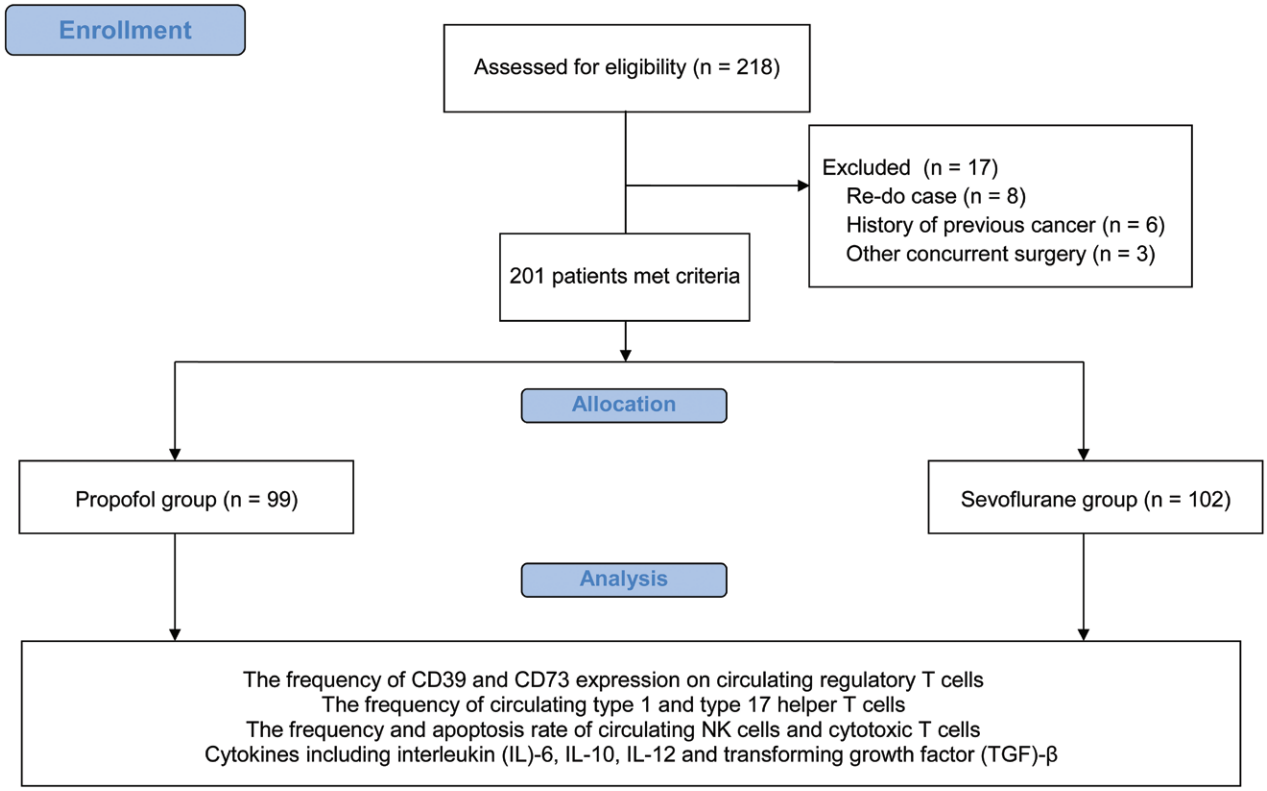


**Fig. 1.** Consolidated Standards of Reporting Trials flow diagram. CD = cluster of differentiation; NK = natural killer.

and cytotoxic T cells did not change over time, and there were no significant intergroup differences ($P = 0.738$ and $P = 0.305$, respectively; fig. 4A). The apoptosis rate of circulating natural killer cells and cytotoxic T cells also did not change over time, and there were no significant intergroup differences ($P = 0.957$ and $P = 0.397$, respectively; fig. 4B).

Table 2 presents additional data on the inflammatory cytokines in peripheral blood mononuclear cells related to circulating helper T cells and natural killer cells. The levels of interleukin 10, interleukin 12, and transforming growth factor β did not change over time and did not show significant differences between the two groups. The interleukin 6 level increased, and the change over time was significant in the both groups, but it did not differ between the two groups. Table 2 also shows the neutrophil-to-lymphocyte ratio, which is a good prognostic marker in patients with breast cancer. The median value of neutrophil-to-lymphocyte ratios of the propofol and sevoflurane groups (1.55 [interquartile range, 1.24 to 1.75] *vs.* 1.76 [1.21 to 2.48] preoperatively; 1.62 [1.29 to 2.43] *vs.* 1.68 [1.30 to 2.21] postoperatively) stayed within the normal range[21] and did not show significant intergroup differences ($P = 0.202$ and $P = 0.883$, respectively).

## Discussion

We showed that propofol- and sevoflurane-based anesthesia had similar effects on the frequency of cluster of differentiation 39 and 73 expression on circulating regulatory T cells. They also had similar effects on the frequency of circulating types 1 and 17 helper T cells, natural killer cells, and cytotoxic T cells during breast cancer surgery.

Cluster of differentiation 39 and 73 expression promotes cancer progression by inducing adenosine formation and is also associated with immunosuppression in cancer patients.[22,23] In addition, by suppressing helper T cells, cluster of differentiation 39 and 73 expression on regulatory T cells interferes with the activation of cytotoxic T cells and consequently promotes cancer progression, metastasis, and recurrence.[22] The inflammatory response is an important factor in cancer progression, and propofol is known to have an antiinflammatory action compared with sevoflurane.[24] Several studies suggest that propofol inhibits cancer progression and metastasis more than sevoflurane, because propofol does not induce immunosuppression in a cancerous environment.[7,25–27] We therefore postulated that propofol might attenuate the inhibitory actions of cluster of differentiation 39 and 73 expression on regulatory T cells. However, propofol and sevoflurane showed a similar effect on cluster of differentiation 39 and 73 expression on circulating regulatory T cells in the present study. In addition, the frequency of circulating types 1 and 17 helper T cells and cytotoxic T cells did not differ between the propofol and sevoflurane groups. Therefore, propofol and sevoflurane have similar effects on cancer immunity based on the frequency of cluster

**Table 1.** Patient Demographic Data and Perioperative Clinical Characteristics

| | Propofol (n = 99) | Sevoflurane (n = 102) | *P* Value |
|---|---|---|---|
| Age (yr) | 52 (45–58) | 52 (45–56) | 0.361 |
| Height (cm) | 157 (154–161) | 158 (155–161) | 0.212 |
| Weight (kg) | 57 (52–63) | 57 (53–62) | 0.956 |
| Operation type (%) | | | 0.748 |
| Breast-conserving surgery | 75 (73.9) | 75 (76.1) | |
| Modified radical mastectomy | 24 (25.1) | 27 (25.9) | |
| Cancer stage (%) | | | 0.772 |
| 0 | 15 (15.1) | 14 (13.7) | |
| I | 45 (45.5) | 51 (50.0) | |
| II | 30 (30.3) | 29 (28.4) | |
| III | 7 (7.1) | 8 (7.9) | |
| IV | 2 (2.0) | 0 (0) | |
| Anesthetic | | | |
| Remifentanil (μg) | 1,319 (1,118–1,665) | 1,403 (1,100–1,808) | 0.512 |
| Propofol (mg) | 823 (658–1,035) | 0 (0–0) | NS |
| Thiopental sodium (mg) | 0 (0–0) | 275 (250–300) | NS |
| Min-$C_e$ of propofol (μg/ml) | 2.5 (2.0– 3.0) | 0 (0–0) | NS |
| Max-$C_e$ of propofol (μg/ml) | 3.5 (3.0–4.0) | 0 (0–0) | NS |
| $Min_{et}$ sevoflurane (Vol%) | 0 (0–0) | 1.0 (1.0–1.5) | NS |
| $Max_{et}$ sevoflurane (Vol%) | 0 (0–0) | 2.0 (2.0–2.0) | NS |
| Duration of anesthesia (min) | 147 (119–175) | 135 (115–170) | 0.251 |
| Duration of operation (min) | 110 (85–133) | 103 (80–135) | 0.461 |
| Analgesic | | | |
| Ketorolac (mg) | 0 (0–30) | 0 (0–30) | 0.456 |
| Fentanyl (μg) | 50 (0–100) | 50 (0–100) | 0.934 |
| VAS-R | | | |
| Preop | 34 (24–44) | 30 (20–50) | 0.164 |
| Post 1 h | 30 (20–30) | 30 (20–40) | 0.126 |
| Post 24 h | 10 (5–20) | 10 (10–30) | 0.185 |
| VAS-M | | | |
| Preop | 37 (27–57) | 40 (30–51) | 0.335 |
| Post 1 h | 30 (20–40) | 30 (25–48) | 0.174 |
| Post 24 h | 20 (10–30) | 20 (10–40) | 0.137 |

Data are expressed as median (25–75%) or mean (SD).

Max-$C_e$ of propofol = maximal effect-site target concentration of propofol; $Max_{et}$ sevoflurane = maximal end-expiratory concentration of sevoflurane; Min-$C_e$ of propofol = minimal effect-site target concentration of propofol; $Min_{et}$ sevoflurane = minimal end-expiratory concentration of sevoflurane; NS = nonspecific; Preop = immediately before anesthesia induction; Post 1 h = at 1 h postoperatively; Post 24 h = at 24 h postoperatively; VAS-M = visual analog scale score on moving; VAS-R = visual analog scale pain score at rest.

of differentiation 39 and 73 expression on circulating regulatory T cells.

Natural killer cells play an important role in suppressing cancer progression.[28] In a previous report, high expression of clusters of differentiation 39 and 73 on regulatory T cells inhibited natural killer cell activation.[29] During this sequence, interleukin 12 activates natural killer cells and promotes the release of tumor necrosis factor α and interleukin 10, which kills tumor cells. However, the frequency of circulating natural killer cells and related cytokines, such as tumor necrosis factor α, interleukin 10, and interleukin 12, did not differ between the propofol and sevoflurane groups in our study. In addition to the frequency of circulating natural killer cells, we also explored the frequency of apoptosis of these cells, and it was also similar in both groups. These results suggest that these two anesthetics have similar effects on cluster of differentiation 39 and 73 expression on circulating regulatory T cells and produce changes in natural killer cells.

Various factors can affect the perioperative immune status, including surgical stress, the release of various endogenous opioids, hypothermia, blood transfusion, and the patient's baseline immune status.[8,26,30,31] Cluster of differentiation 39 and 73 expression on circulating regulatory T and related immune cells may be affected by these factors and by anesthetics. Some *in vitro* studies have supported the superiority of propofol over volatile anesthetics for slowing cancer progression and reducing recurrence and metastasis.[27,32–34] However, Tylman *et al.*[5] reported similar effects of propofol and volatile anesthetics on perioperative immune status during colorectal surgery, and Lindholm *et al.*[35] observed that volatile anesthetics did not increase the risk of cancer recurrence in a large clinical cohort study. The lack of a definitive conclusion regarding the choice of

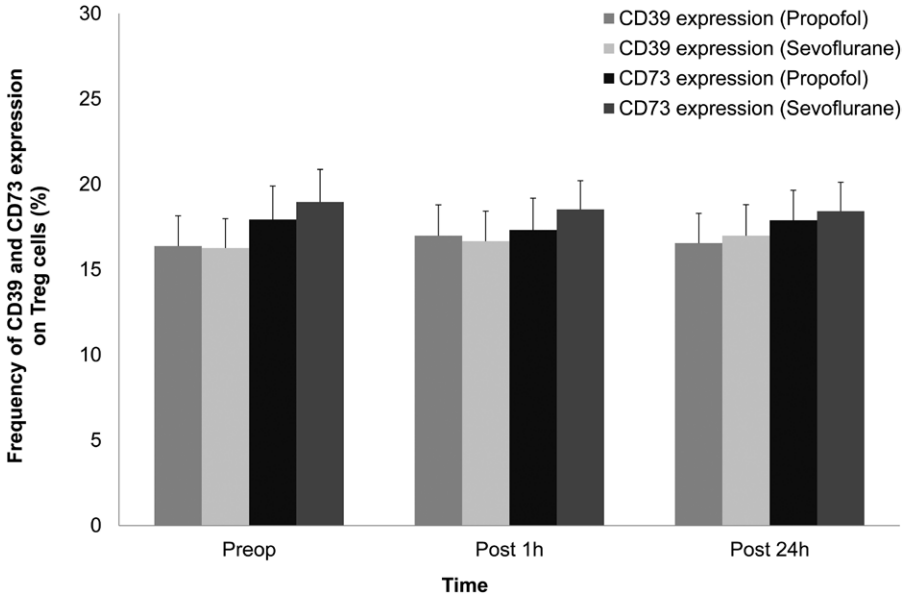


**Fig. 2.** Frequency of cluster of differentiations (CD) 39 and CD73 expression on circulating regulatory T (Treg) cells. Changes in frequency of CD39 and CD73 expression on circulating Treg cells were not significantly different in the two groups ($P = 0.995$ and $P = 0.403$, respectively). Data are expressed as mean values with *error bars* of 95% CI. Post 1h = at 1 h postoperatively; Post 24h = at 24 h postoperatively; Preop = immediately before anesthesia induction.

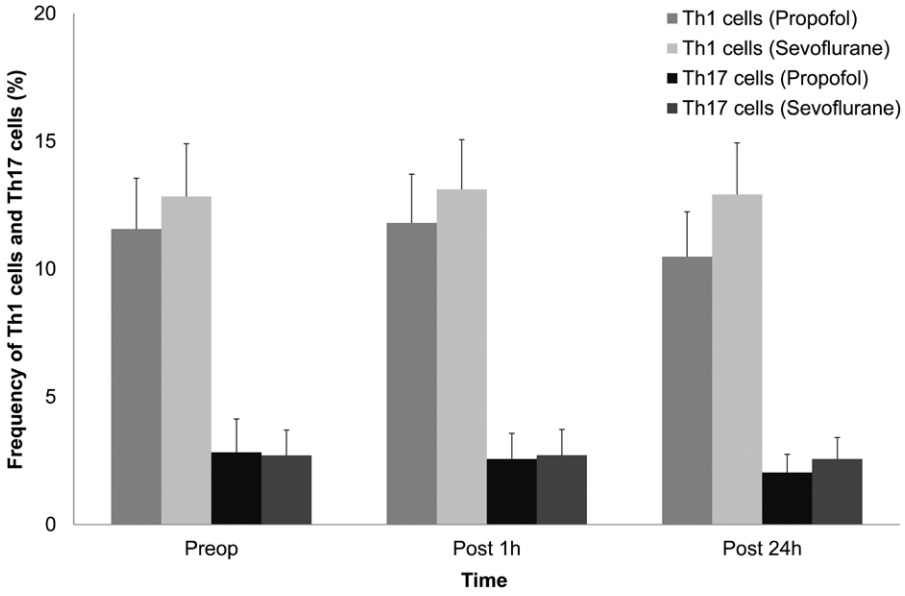


**Fig. 3.** Changes in the frequency of circulating type 1 helper T (Th1) cells and type 17 helper T (Th17) cells. Changes in the frequency of circulating Th1 and Th17 cells were not significantly different in the two groups ($P = 0.126$ and $P = 0.740$, respectively). Data are expressed as mean values with *error bars* of 95% CI. Post 1h = at 1 h postoperatively; Post 24h = at 24 h postoperatively; Preop = immediately before anesthesia induction.

propofol or sevoflurane for cancer surgery may be associated with the influence of the above factors on cancer immunity during cancer surgery. In addition, we could not exclude the effect of opioids on cancer immunity. Opioids are often given intraoperatively regardless of whether intravenous or volatile anesthetics are used. Previous studies suggest intraoperative use of opioids may promote cancer prognosis and metastasis.[36,37] In addition, previous studies have shown the superiority of propofol compared with volatile anesthetics for reducing immunosuppression in cancer patients; however, these studies were not performed under conditions of equipotent use of opioids between the propofol and volatile anesthetic groups.[11,12] We administered opioids to the same target-controlled estimated plasma concentration in both groups and were therefore able to evaluate the specific effects of propofol and sevoflurane on cells that expressed

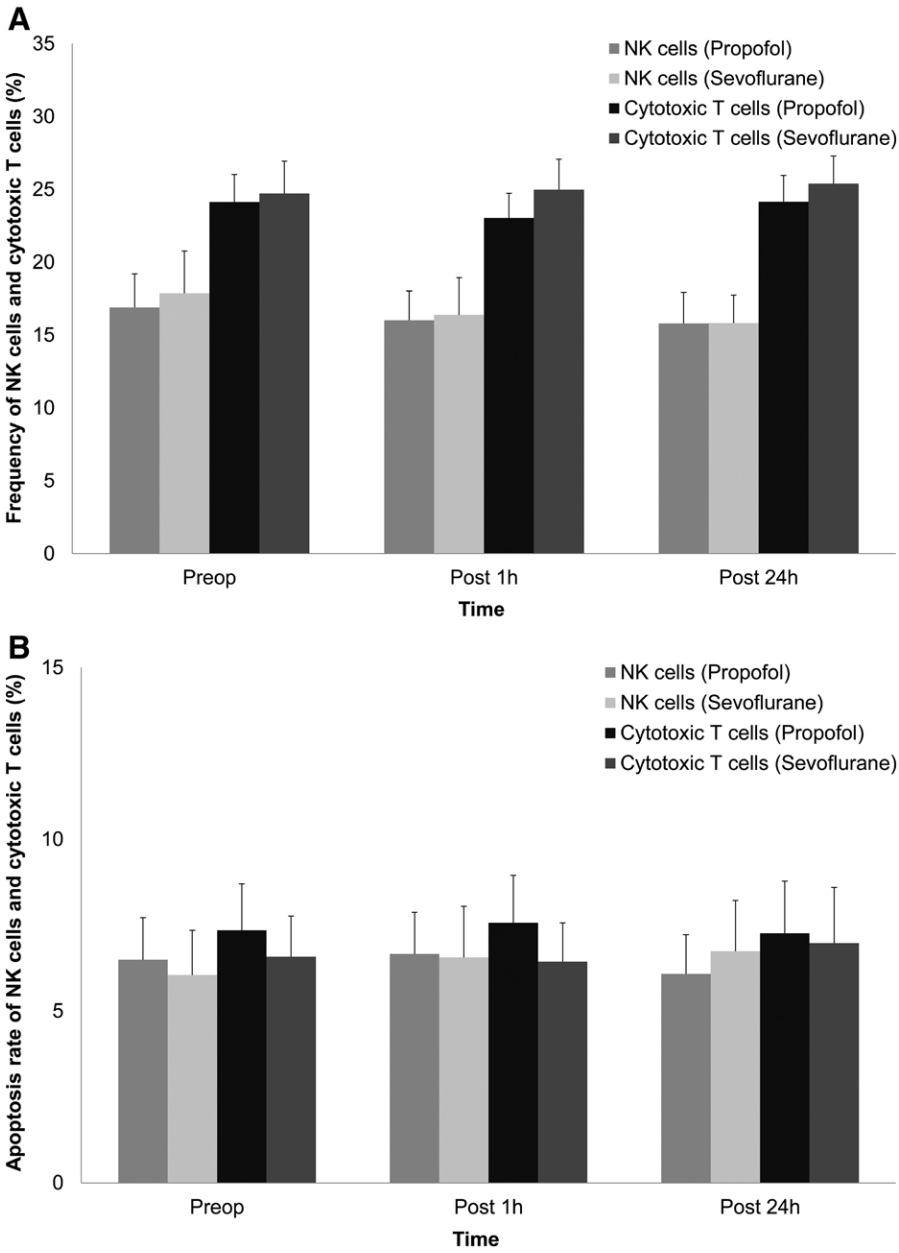


**Fig. 4.** The frequency and apoptosis rate of circulating natural killer (NK) cells and cytotoxic T cells. (*A*) The frequency of circulating NK and cytotoxic T cells was not significantly different in the two groups ($P = 0.738$ and $P = 0.305$, respectively). (*B*) The apoptosis rate of circulating NK and T cells was also not significantly different in the two groups ($P = 0.957$ and $P = 0.397$, respectively). Data are expressed as mean values with *error bars* of 95% CI. Post 1h = at 1 h postoperatively; Post 24h = at 24 h postoperatively; Preop = immediately before anesthesia induction.

clusters of differentiation 39 and 73 and on natural killer cells.

There were several limitations to this clinical trial. First, the opioids (remifentanil and fentanyl) and nonsteroidal antiinflammatory drug (ketorolac) used in both groups could mask the pure effect of propofol or sevoflurane on the immunity of patients with cancer, although they were used similarly. Opioids have immunosuppressive effects,[38] and nonsteroidal antiinflammatory drugs improve the survival of patients with cancer.[39] However, the immunosuppressive effect of opioids in clinical practice is still controversial because of the balance of pain and opioids related to immunosuppression.[40,41] We therefore suggest that further studies should investigate the pure effect of anesthetics by minimizing the effects of opioids. Second, the use of immune markers such as cluster of differentiation 39 and 73 expression might be limited because of their lack of clinical relevance. Although various animal studies suggest that cluster of differentiation 39 and 73 expression contribute to a poor prognosis in patients with cancer,[14] the effects of cluster of

**Table 2.** Perioperative Cytokine Concentrations and Neutrophil-to-lymphocyte Ratio in Blood during Breast Cancer Surgery

| | Propofol (n = 99) | Sevoflurane (n = 102) | Difference (95% CI) | *P* Value |
|---|---|---|---|---|
| IL-6 (ng/ml) | | | | |
| Preop | 0.27 (0.23–0.30) | 0.28 (0.23–0.32) | −0.02 (−0.04 to 0.01) | 0.174 |
| Post 1 h | 0.30 (0.23–0.36)* | 0.28 (0.23–0.31) | 0.02 (−0.01 to 0.04) | 0.130 |
| Post 24 h | 0.33 (0.23–0.40)*† | 0.34 (0.29–0.37) *† | −0.10 (−0.04 to 0.02) | 0.604 |
| IL-10 (ng/ml) | | | | |
| Preop | 0.62 (0.51–0.75) | 0.60 (0.53–0.67) | 0.03 (−0.01 to 0.07) | 0.141 |
| Post 1 h | 0.62 (0.53–0.76) | 0.60 (0.53–0.66) | 0.01 (−0.04 to 0.05) | 0.154 |
| Post 24 h | 0.61 (0.50–0.73) | 0.61 (0.53–0.67) | 0.01 (−0.04 to 0.05) | 0.770 |
| IL-12 (ng/ml) | | | | |
| Preop | 0.27 (0.24–0.30) | 0.27 (0.23–0.32) | 0.00 (−0.02 to 0.02) | 0.946 |
| Post 1 h | 0.26 (0.24–0.30) | 0.26 (0.23–0.30) | 0.00 (−0.01 to 0.02) | 0.786 |
| Post 24 h | 0.27 (0.24–0.31) | 0.27 (0.24–0.31) | 0.00 (−0.02 to 0.01) | 0.982 |
| TGF-β (ng/ml) | | | | |
| Preop | 2.11 (2.06–2.16) | 2.13 (2.08–2.18) | −0.02 (−0.04 to 0.01) | 0.165 |
| Post 1 h | 2.11 ± 0.10 | 2.11 ± 0.07 | −0.01 (−0.03 to 0.02) | 0.525 |
| Post 24 h | 2.09 (2.04–2.15) | 2.13 (2.06–2.16) | −0.02 (−0.05 to 0.00) | 0.095 |
| Neutrophil (%) | | | | |
| Preop | 55 (49–61) | 58 (48–65) | −1.85 (−4.80 to 1.10) | 0.229 |
| Post 24 h | 55 (50–64) | 56 (51–62) | 0.30 (−2.60 to 3.20) | 0.854 |
| Lymphocyte (%) | | | | |
| Preop | 35 ± 9 | 33 ± 9 | 1.91 (−0.66 to 4.47) | 0.144 |
| Post 24 h | 34 (26–39) | 33 (27–40) | −0.40 (−3.20 to 2.20) | 0.781 |
| NLR | | | | |
| Preop | 1.55 (1.24–2.05) | 1.76 (1.21–2.48) | −0.14 (−0.35 to 0.73) | 0.202 |
| Post 24 h | 1.62 (1.29–2.43) | 1.68 (1.30–2.21) | 0.01 (−0.19 to 0.23) | 0.883 |

Data are expressed as median (interquartile range) or mean ± SD with difference (95% CI).

*$P < 0.017$ compared with Preop with Bonferroni correction. †$P < 0.017$ compared to Post 1 h with Bonferroni correction.

IL = interleukin; NLR = neutrophil-to-lymphocyte ratio; Preop = immediately before anesthesia induction; Post 1 h = at 1 h postoperatively; Post 24 h = at 24 h postoperatively; TGF-β = transforming growth factor β.

differentiation 39 and 73 expression on cancer progression are not yet proven in clinical fields. Therefore, we examined the neutrophil-to-lymphocyte ratio, which is a known prognostic marker,[20] and found that it did not differ between the two groups. Therefore, we could indirectly assume that the effects of anesthetics on cancer prognosis might be minimal. Nevertheless, further studies with long-term follow-up should investigate the prognostic value of cluster of differentiation 39 and 73 expression according to the anesthetic used, because there have been increasing attempts to regulate the cluster of differentiation 39 and 73 expression as potent targets of cancer immunotherapy.[14,29,42] Third, we measured only the frequency and apoptosis rate of natural killer cells and cytotoxic T cells in peripheral blood mononuclear cells without investigating their function or activity. However, we assumed that the immune cell activity would not differ, because the frequency of apoptosis of natural killer cells and cytotoxic T cells was similar between the two groups. Fourth, we investigated frequency of immune changes up to 24 h postoperatively. Therefore, we could not assess the overall perioperative immune status, because this might be affected for 1 to 4 weeks postoperatively.[43,44] However, we explored the effect of anesthetics that were administered during the intraoperative period and believe that we captured the most important time period in this study. Finally, there was a discrepancy between the primary outcome of the actual study and the primary outcome listed in the initial trial registration. At the time of initial trial registration, natural killer cell was selected as the primary outcome. However, actual data collection was delayed because we found cluster of differentiation 39 and 73 expression on regulatory T cells regulates natural killer cell activity.[29] We considered that cluster of differentiation 39 and 73 expression on regulatory T cells would be more meaningful than natural killer cell and ultimately selected cluster of differentiation 39 and 73 expression as the primary outcome. Actual data collection was initiated 7 months after initial trial registration, and we recalculated the sample size in this interim analysis. However, no *P* value adjustments were made for this interim analysis, and the trial registration was not updated accordingly. The registration was revised after completion of the study to reflect the accurate primary outcome and sample size information. However, the protocol was not changed during enrollment or analysis and corresponds to the current listing at clinicaltrials.gov. Adequately powered, large, randomized clinical trials are required to evaluate the effect of anesthetics on diverse immune cells during cancer surgery.

In conclusion, cluster of differentiation 39 and 73 expression on circulating regulatory T cells, as well as the frequency

of circulating types 1 and 17 helper T cells, natural killer cells, and cytotoxic T cells, were not affected by propofol or sevoflurane anesthesia during breast cancer surgery. The effect of anesthetics on cancer recurrence may be minimal.

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## Competing Interests

The authors declare no competing interests.

## Reproducible Science

Full protocol available at: yshkim75@daum.net. Raw data available at: yshkim75@daum.net.

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