Intraoperative flurbiprofen alters the expression of PD-1, a molecule of immune checkpoint pathway, in patients undergoing elective thoracoscope resection of lung cancer: a randomized controlled trial

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Abstract

Background: This study aimed to determine the influence of intraoperative use of non-steroidal anti-inflammatory drugs (NSAIDs) flurbiprofen on postoperative level of programmed death 1 (PD-1) in patients undergoing thoracoscope surgery.

Methods: In this prospective double-blind trial, patients were randomized to receive intralipid (Control group, n=34, 0.1ml/kg, i.v.) or flurbiprofen axetil (Flurbiprofen group, n=34, 1mg/kg, i.v.) before the induction of anesthesia and 6 hours after the initial injection. PD-1 level on T-cell subsets, inflammation and immune markers in peripheral blood were examined before induction of anesthesia (T0), and after surgery (24 hours (T1), 72 hours (T2) and 1 week (T3)). A linear mixed model was used to examine whether the changes from baseline values (T0) between groups were different during our study.

Results: The increases in the percentages of PD-1(+)CD8(+) T-cell observed at T1 and T2 in the control group were higher than in the flurbiprofen group (T1:12.91%±1.65% versus 7.86%±5.71%, P=0.031; T2:11.54%±1.54% versus 8.75%±1.73%, P=0.004) while no difference was observed at T1 and T2 between the groups in terms of change in percentages of PD-1(+)CD4(+) T-cell. Moreover, extensive changes in the percentages of lymphocytes subsets and the concentrations of inflammatory markers was observed at T1 and T2 after surgery, and flurbiprofen seemed to attenuate the most of changes.

Conclusion: Perioperative administration of flurbiprofen attenuated postoperative PD-1 increase on CD8(+) T-cell up to 72 hours, but not after this time. The clinical relevance of changes with PD-1 to long-term outcome of surgery is still unknown.

Trial registration: Chinese Clinical Trail Registry, ChiCTR-IPR-15006482 (date of registration June 2015)

Keywords: Immune cells; Lung cancer; Flurbiprofen; Programmed death 1.

Introduction

Lung cancer is the major cause of cancer-related death in the world with approximately 80% of lung cancers occurring as non-small cell lung cancer (NSCLC) [1-3]. Some well-established immune-checkpoint molecules expressing on activated T-cell, such as programmed death 1 (PD-1, or CD279) or cytotoxic T lymphocyte-associated antigen 4 (CTLA-4, or CD152), functionally suppress T-cell-mediated immunity against tumors, and are considered a hallmark of exhausted T-cell following persistent stimulation with tumor antigens [4-8]. Increasing evidences asserts that higher levels of immune-checkpoint molecules normally could predict a worse outcome in cancer patients [9,10], furthermore immune-checkpoint blockade (e.g. anti-PD-1 therapy) elicited persistent and significant therapeutic response in multiple tumor types including lung cancer [1,11-16]. However, it is still unclear whether perioperative use of analgesic drugs can directly influence on these immune-checkpoint molecules among cancer patients receiving surgery.

Interestingly, PD-1 and its ligands were markedly inducible by cyclooxygenase (COX) enzyme activity and downstream prostaglandins (PGs) that are prominent in tumor-sustaining inflammatory mediators [17,18]. Additionally, non-steroidal anti-inflammatory drugs (NSAIDs), which exert anti-inflammatory and analgesic properties via inhibiting COX enzyme, pharmacologically act in cooperating with anti-PD-1 treatment efficiency in the preclinical cancer model [19]. In clinical settings, perioperative use of flurbiprofen, regularly prescribed as perioperative analgesic, efficiently elicited a short-term increase of innate and adaptive immune cells in postoperative peripheral blood from patients [20]. Because less is known about how NSAIDs act to adjuvant synergize with anti-PD-1 therapy in clinical settings, we aimed to determine if flurbiprofen had an effect on postoperative PD-1 level in blood circulating T-cell subset with patients undergoing elective thoracoscope resection of NSCLC.

Methods

Study Sample

This study was approved by the Biomedical Research Ethics Committee of Anhui Medical University and registered with the Chinese Clinical Trail Registry (No.ChiCTR-IPR-15006482). All the patients provided written informed consent. And this study was a prospective, double-blind, randomized and controlled clinical trial. Patients were screened at outpatient department or inpatient wards and underwent randomization between October 15, 2016 and May 10, 2017 at Anhui Provincial Hospital, China. Patients who had the following criteria were eligible for participation in the study: adults undergoing elective thoracoscope resection of lung cancer, American Society of Anesthesiologists (ASA) status of I-II, aged 40-65 years, weighing 45-80 kg for both genders. Patients were excluded if they met any of the following criteria: 1) allergy or contraindication to NSAIDs; 2) history of peptic ulceration; 3) blood coagulation disorder; 4) severe cardiac, hepatic or renal dysfunction; 5) perioperative blood transfusion; 6) bronchial asthma; 7) current or recent receipt of radiotherapy, chemotherapy, immunedepressant, glucocorticoid; 8) autoimmune disease or acute inflammation; 9) severe hypertension or diabetes mellitus; 10) pregnancy; 11) use of enoxacin, lomefloxacin, or norfloxacin; 12) duration of operation less than 120 min.

Anesthesia and Analgesia

General anesthesia was induced with midazolam (Jiang Su En Hua Medicine Co., Ltd. Batch number: 20160404; Xuzhou, Zhejiang, China) 0.05mg/kg, propofol (Batch number: MF717; AstraZeneca, London, UK) 2mg/kg, and sufentanil (Hu Bei Ren Fu Medicine Co., Ltd. Batch number: 1160903; Yichang, Hubei, China) 0.4μg/kg, and double-lumen endobronchial tube was facilitated with rocuronium (Zhe Jiang Xian Ju Medicine Co., Ltd. Batch number:160903, Tai Zhou, Zhejiang, China) 1mg/kg. The site of the tube was confirmed by fiberoptic bronchoscopy after intubation and changing patients' position before surgery. All patients received target-controlled infusion to maintain the anesthesia (i.e., propofol and remifentanil (Hu Bei Ren Fu Medicine Co., Ltd. Batch number: 6160521; Yichang, Hubei, China) with an effect concentration of 3.5-4.5 μg/ml and 2-4 ng/ml, respectively). Patients were mechanically ventilated with

suitable ventilation parameters to maintain the end-tidal carbon dioxide in a range of 35-45 mmHg. In addition, an appropriate depth of anesthesia was monitored by Narcotrend. Postoperatively, all patients received the same regimen of patient-controlled intravenous analgesia (PCIA) with sufentanil (100μg, diluted to a total volume of 100ml with 0.9% sodium chloride). PCIA was performed with a loading dose of 2ml, a background infusion of 2ml/h, a bolus of 2ml, and a lockout time of 15 minutes. In addition, tramadol as the component of rescue analgesic was required postoperatively for unbearable pain when VAS (Visual Analogue Scale) score greater than or equal to 5-point.

Intervention and Randomization

Using a computer-generated random number sequence, patients were allocated in a 1:1 ratio to receive treatment with either flurbiprofen (flurbiprofen axetil, 1mg/kg, i.v., Bei Jing Tai De Medicine Co., Ltd. Batch number: 1E016R; Beijing, China) or placebo (intralipid, 0.1ml/kg, i.v.) before the induction of anesthesia and 6 hours after the initial injection. Data collection was performed by an independent researcher who was not involved in the trial. In addition, another researcher was in charge of the preparation of study drugs. The study drugs were placed into unmarked syringes and treatment assignments concealed in sealed opaque envelopes; all of which was blinded to patients, anesthetists and other investigators involved in the study. The statistician was unaware of the assignments until all data analyses were completed.

Outcomes

Venous blood (2ml) was obtained from the non-infused peripheral vein before the induction of anesthesia (T_0), postoperative 24 hours (T_1), 72 hours (T_2), and 1 week (T_3) after surgery. The blood was preserved in EDTA anticoagulation tube at 4°C for subsequent testing within 24 hours. The primary outcome was counts of circulating PD-$1^{(+)}$$CD8^{(+)}$ T-cell or PD-$1^{(+)}$$CD4^{(+)}$ T-cell at each time point perioperatively. Secondary outcome was the percentages of lymphocyte subsets in peripheral blood mononuclear cells (PBMCs) and concentrations of inflammatory markers in peripheral blood including tumor necrosis factor-α (TNF-α); interferon-γ (IFN-γ); interleukin-6 (IL-6); C-reactive protein (CRP). Moreover, the data in the full blood count test including platelet count, total WBC (white blood cell) count, hemoglobin, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count and serum vascular endothelial growth factor (VEGF)-A were also tracked simultaneously.

Immune-checkpoint and Lymphocyte Subset Analysis

The counts of circulating PD-$1^{(+)}$$CD8^{(+)}$ T-cell or PD-$1^{(+)}$$CD4^{(+)}$ T-cell was determined as the percentages against total T lymphocytes. The percentage of $CD3^{(+)}$ total T-cell, $CD4^{(+)}$ helper T-cell, $CD8^{(+)}$ cytotoxic T-

cell, and $CD3^{(-)}CD16^{(+)}CD56^{(+)}$ NK-cell in PBMCs were measured by flow cytometry (CytoFLEX, Beckman, US). PBMCs were separated from peripheral blood by Ficoll-Hypaque density gradient centrifugation. PBMCs were resuspended in 0.2 ml phosphate buffered saline (4×106/100µl) and incubated for 30 min on ice with appropriate dilution of antibodies. The antibodies used in this study were: anti-human CD45(PercpCy5.5; 45-0459-42; Clone HI30; eBioScience; San Diego), anti-human CD3(FITC; 300306; Clone HIT3a; Biolegend; San Diego), anti-human-CD8(PE; 300908; Clone HIT8a; Biolegend; San Diego), anti-human CD4(APC; 344614; Clone SK3; Biolegend; San Diego), anti-human PD-1(Pacific Blue; 329916; Clone EH12.2H7; Biolegend; San Diego). Flow cytometric gating strategy and analysis of the PD-1 expression based on the $CD45^{(+)}CD3^{(+)}CD4^{(+)}$ and $CD45^{(+)}CD3^{(+)}CD8^{(+)}$ T cell populations (As shown in supplementary material, Figure 1).

Measurement of Inflammatory Cytokines

The concentrations of TNF-α, IFN-γ, IL-6 and VEGF-A were measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (R&D, Minneapolis, MN, USA) as per the manufacturer's protocol.

Full blood count, Serum glucose and CRP Measurement

The analysis of full blood count was performed on the EDTA peripheral blood sample using a hematology analyzer instrument (BC-6900, Mindray, China). In addition, the concentrations of serum glucose and CRP were tested from peripheral blood using the Clinical Chemistry System (ADVIA 2400, SIEMENS) and i-CHROM laser fluorescence reader, respectively.

Sample Size Calculation

The number of patients recruited for groups was based on the primary outcome by testing percentage of $PD\text{-}1^{(+)}CD8^{(+)}$ T-cell from a previous pilot study. According to these pilot data, a difference in mean $PD\text{-}1^{(+)}CD8^{(+)}$T-cell percentages between two groups was 2.08 % and a pooled SD was 2.57 %. To detect a difference of this magnitude between study groups, 32 patients were required in each group to provide 90% power at an alpha of 0.05. However, considering a loss-to-follow-up rate of about 10% in our study, we aimed to enroll a total sample size of 70 patients.

Statistical Analyses

A Shapiro-Wilk test was applied to assess the normality of continuous data. The normally distributed continuous data were recorded as mean (SD) and analyzed using the independent t-test. The skewed data were presented as the median (interquartile range), and the comparison were treated with the Mann-Whitney U test or Wilcoxon rank-sum test. The categorical variables were reported as frequency and compared using the chi-square or Fisher's exact test. Changes in percentages of PD-$1^{(+)}CD8^{(+)}$ T-cell or PD-$1^{(+)}CD4^{(+)}$ T-cell and concentrations of inflammatory markers were calculated between baseline values at T_0 and those at T_1, T_2 and T_3. Changes in both groups were analyzed with a mixed linear regression model adjusted for age and ASA status (i.e., a random effect was introduced in an effort to account for the repeated measures) in order to validate the findings of the univariable analysis. In our model, the group and time were applied as predictors, the interaction between group and time as fixed effects and patient as a random effect, and an interaction term was taken into account to test whether the change over time was different in both groups. In the univariable analyses, a Bonferroni correction was applied to post hoc analysis to compare different time changes. In addition, a sensitivity analysis using a linear mixed model with scale identity correlation matrices was applied to affirm vital differences between groups. All statistical analyses were performed on SPSS 19.0 software and all reported P values less than 0.05 were indicated to be of statistical significance.

Results

Study Population

The CONSORT diagram is shown in Figure 1. A total of 70 patients were screened for our study, with 68 patients ultimately participating. The 2 patients not included were 1 patient who refused to participate and 1 patient who did not meet inclusion criteria. 4 patients (2 in flurbiprofen group, 2 in control group) were withdrawn from the statistical analysis although they had been randomized into two groups (one patient in flubiprofen group refused phlebotomy for laboratory testing after the operation; moreover, one patient was withdrawn because of more than 1.5 liter blood loss in the flurbiprofen group; two patients in the control group due to the tumor metastasis invasion into the pleura that did not receive lung parenchyma resection operation procedure). Baseline and intraoperative characteristics of patient in both groups are shown in Table 1. No significant differences were observed between groups in terms of age, body mass index, gender, and ASA status. There were also no significant between-group differences in surgery type, duration of surgery, the use of antibiotic and propofol consumption intraoperatively. The consumption of remifentanyl in the control group was higher compared with the flurbiprofen group. Outcome values at baseline as shown in supplementary material (supplementary material, Table 1 and Table 2) were compared between groups with no significant differences observed in terms of percentage of PD-$1^{(+)}CD8^{(+)}$T-cell, PD-$1^{(+)}CD4^{(+)}$T-cell, T lymphocyte subsets, VEGF-A and other inflammatory markers concentrations.

Outcomes

As shown in Table 2, the increases in the percentages of PD-$1^{(+)}CD8^{(+)}$ T-cell observed at 24h postoperatively (T_1) and 72h postoperatively (T_2) in the control group were higher than in the flurbiprofen group (T_1, 12.91%±1.65% versus 7.86%±5.71%, P=0.031; T_2, 11.54%±1.54% versus 8.75%±1.73%, P=0.004)(Supplementary material, Figure 2, the original images regarding the PD-$1^{(+)}CD8^{(+)}$ T-cell at different time points) while no difference was observed at T_1 and T_2 between the groups in terms of change in percentages of PD-$1^{(+)}CD4^{(+)}$ T-cell (Supplementary material, Figure 3, the original images regarding the PD-$1^{(+)}CD4^{(+)}$ T-cell at different time points). However, changes in the percentages of PD-$1^{(+)}CD8^{(+)}$ T-cell from baseline (T_0), as well as PD-$1^{(+)}CD4^{(+)}$ T-cell, were similar between groups at 1 week after surgery (T_3). Moreover, as shown in Table 3, the decreases observed for all lymphocyte subsets in the control group were markedly greater at T_1 and T_2 compared to those in the flurbiprofen group except $CD8^{(+)}$ T-cell (significant change only observed at T_1). In addition, the observed significant increases in concentrations of TNF-α, IFN-γ, IL-6, CRP and VEGF-A at T_1 and T_2 was much greater in the control group than those in the flurbiprofen group. For longer postoperative follow-up (T_3), no significant differences were observed in the above-mentioned data from baseline. Except above time points, we also collect the 3 weeks (T_4) data after the surgery, however, there was no significance between groups regarding all relevant indicates (Table data not shown). We just show the expression of PD-$1^{(+)}$ on $CD8^{(+)}$ T-cell and $CD4^{(+)}$ T-cell in two groups at T_4 (Supplementary material, Figure 2 and Figure 3).

There were no significant differences between groups with respect to the proportion of patients who were unexpectedly subjected to respiratory depression after surgery, defined as a respiratory rate less than 8 breaths per minute and oxygen saturation either below 92% or a decrease of more than 5% from baseline in patients with a baseline of SPO_2 less than 90% [21] .There was also no significant between-group difference in the rate of nausea and skin pruritus. Eight patients in the control group suffered vomiting and retching, compared with one patient in the flurbiprofen group (P=0.026). Furthermore, the use of anti-emetics in the control group was higher than those in the flurbiprofen group (P=0.011). A significant reduction in 24h postoperative score for cough was observed in the flurbiprofen group but no difference was observed between groups 72h postoperatively. Moreover, ten patients in the control group required postoperative rescue analgesia (tramadol) for unbearable pain, as compared with three patients in the flurbiprofen group (P=0.030). In addition, no difference was observed between the groups in terms of wound infection and the length of hospital stay postoperatively.

Sensitivity analysis

Linear mixed models confirmed the significant differences in the percentages of PD-$1^{(+)}$ on $CD8^{(+)}$ T-cell, $CD4^{(+)}$ T-cell, NK cells and the concentrations of TNF-α, IL-6, CRP between patients who received flurbiprofen and placebo (Supplementary material, Tables 3 to 8), and the significant differences was observed at T_1 and T_2.

Discussion

PD-1, expressed in tumor-infiltrating T-cells and circulating T-cells, has been shown to predict prognosis as well as a target-therapy candidate in several malignant tumors, including NSCLC [8,12,13,16,22-26]. In peripheral blood, the latest evidence indicates a higher level of PD-1 on circulating $CD8^{(+)}$ T-cell to be correlated with a worse clinical outcome and shorter overall survival [10]. Interestingly, it is has been reported that COX inhibitors can act synergistically with immune-checkpoint blockade, implying that NSAIDs commonly used as perioperative analgesic could be a useful adjuvant for anti-PD-1/anti-CTLA-4 therapies in cancer patients [19]. To the best of our knowledge, this is the first clinical study providing evidence that NSAIDs alter postoperative level of PD-1 resulting in a smaller increase of PD-1 on $CD8^{(+)}$ T-cell in peripheral blood from lung cancer patients undergoing resection surgery.

Since the COX activity and COX-dependent inflammatory mediators such as PGs facilitate the increase of inhibitory immune-checkpoints PD-1/CTLA-4, and low levels of PD-1/CTLA-4 were confirmed in $COX\text{-}2^{MEC}$ knock-out mice bearing tumors, suggesting COX activity and downstream PGs have some potential link with PD-1 [18]. Although NSAIDs could act in cooperating with anti-PD-1 blockade in inducing eradication of tumors in preclinical study [19], our study primarily identified flurbiprofen could alter PD-1 level on the circulating $CD8^{(+)}$T-cell population from NSCLC patients until 72 hours postoperative, without any change for the percentage of circulating $PD\text{-}1^{(+)}CD8^{(+)}$ T-cell observed after this time point. Flurbiprofen had little influence on postoperative percentage of $PD\text{-}1^{(+)}CD4^{(+)}$ T-cell during the overall perioperative period. The lymphocyte percentage after surgery in the control or flurbiprofen groups was, in general, lower compared with hospital reference values. It is interesting to note that there were extensive changes in lymphocyte subsets and inflammatory markers following administration of flurbiprofen in the short-term up to postoperative 72 hours.

A possible explanation for the observed change between the groups is that prominent tumor-sustaining inflammatory factors were considered as a potent stimulator to induce PD-1/CTLA-4. This highlights that PD-1 and other inhibitory checkpoints levels involved in $CD8^{(+)}$ T-cell exhaustion were markedly enhanced with high levels of VEGF produced by a pro-angiogenic factor in the tumor's microenvironment [27]. Moreover, COX-derived PGE2 promotes tumor progression by sustaining angiogenesis through induction of VEGF that is largely required for development of a stable blood supply for tumor growth [28,29]. We speculate that inhibition of COX and PGE2 by flurbiprofen alleviates the increase of PD-1 partly due to abrogating the induction of VEGF.

There are several limitations. First, further studies are needed to focus on the exact mechanism of how NSAIDs down regulate the anti-tumor immunity and immune escape. Second, although the randomization in our study was strict, some baseline and perioperative factors were not equal between the two groups. Third, we didn't subdivide the $CD4^{(+)}$ T-cell which should be discriminated between conventional $CD4^{(+)}$ T-cell and regulatory $CD4^{(+)}$ T-cell. Fourth, our study is a single-centre investigation, a large multicenter study would be ideal to confirm our findings.

Conclusion

In conclusion, perioperative administration of flurbiprofen appears to attenuate PD-1 increase on $CD8^{(+)}$ T-cell until 72 hours postoperative, with no effect identified after this time.

Abbreviations

ASA:american society of anesthesiologists; BMI:body mass index; COX:cyclooxygenase; CRP:C-reactive protein; CTLA:cytotoxic T lymphocyte-associated antigen; ELISA:enzyme-linked immunosorbent assay; IFN-γ:interferon-γ; IL-6:interleukin-6; IQR:interquartile range; NSAIDs:non-steroidal anti-inflammatory drugs; NSCLC:non-small cell lung cancer; PBMCs:peripheral blood mononuclear cells; PCIA:patient-controlled intravenous analgesia; PD-1:programmed death 1; PGs:prostaglandins; SD:standard deviation; TNF-α:tumor necrosis factor-α; VAS:visual analogue scale; VEGF:vascular endothelial growth factor

Declarations

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

The conception and the design of the study: XQ Chai, D Wang. Patient's data collection: LX Xie, SH Shu. The statistical analysis: JC Hu, SS Hu. Interpretation of results and wrote the manuscript: JC Hu, D Wang.

Review of this manuscript: XQ Chai, C. M.

Ethics approval and consent to participate

The protocol of this study was approved by the Biomedical Research Ethics Committee of Anhui Medical University and and written informed consents have been obtained from all patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

References

[1] Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015;372(21):2018-28.

[2] Cheng TY, Cramb SM, Baade PD, Youlden DR, Nwogu C, Reid ME. The international epidemiology of lung cancer: latest trends,

disparities, and tumor characteristics. J Thorac Oncol 2016;11(10):1653-71.

[3] Reck M, Heigener DF, Mok T, Soria JC, Rabe KF. Management of non-small-cell lung cancer: recent developments. Lancet 2013;382(9893):709-19.

[4] Odorizzi PM, Pauken KE, Paley MA, Sharpe A, Wherry EJ. Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8+ T cells. J Exp Med 2015;212(7):1125-37.

[5] Lingel H, Wissing J, Arra A, et al. CTLA-4-mediated posttranslational modifications direct cytotoxic T-lymphocyte differentiation.

Cell Death Differ 2017;24(10):1739-49.

[6] Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008;26(1):677-704.

[7] Hahn AW, Gill DM, Pal SK, Agarwal N. The future of immune checkpoint cancer therapy after PD-1 and CTLA-4. Immunotherapy 2017;9(8):681-92.

[8] Boussiotis VA. Molecular and biochemical aspects of the PD-1 checkpoint pathway. N Engl J Med 2016;375(18):1767-78.

[9] Zheng H, Liu X, Zhang JH, et al. Expression of PD-1 on CD4+ T cells in peripheral blood associates with poor clinical outcome in non-small cell lung cancer. Oncotarget 2016;7(35):56233-40.

[10] Waki K, Yamada T, Yoshiyama K, et al. PD-1 expression on peripheral blood T-cell subsets correlates with prognosis in non-small cell lung cancer. Cancer Sci 2014;105(10):1229-35.

[11] Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015;373(17):123-35.

[12] Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013;369(2):122-33.

[13] Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366(26): 2443-54.

[14] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12(4):252-64.

[15] Nghiem PT, Bhatia S, Lipson EJ, et al. PD-1 Blockade with pembrolizumab in advanced merkel-cell carcinoma. N Engl J Med 2016;374(26):2542-52.

[16] Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med 2015;373(1):23-34.

[17] Chen JH, Perry CJ, Tsui YC, et al. Prostaglandin E2 and programmed cell death 1 signaling coordinately impair CTL function and survival during chronic viral infection. Nat Med 2015;21(4):327-34.

[18] Markosyan N, Chen EP, Evans RA, Ndong V, Vonderheide RH, Smyth EM. Mammary carcinoma cell derived cyclooxygenase 2 suppresses tumor immune surveillance by enhancing intratumoral immune checkpoint activity. Breast Cancer Res 2013;15(5):R75.

[19] Zelenay S, van der Veen AG, Böttcher JP, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. Cell 2015;162(6):1257-70.

[20] Wang D, Yang XL, Chai XQ, et al. A short-term increase of the postoperative naturally circulating dendritic cells subsets in flurbiprofen-treated patients with esophageal carcinoma undergoing thoracic surgery. Oncotarget 2016;7(14):18705-12.

[21] Savelloni J, Gunter H, Lee KC, et al. Risk of respiratory depression with opioids and concomitant gabapentinoids. J Pain Res 2017;10:2635-41. http://dx.doi.org/10.2147/JPR.S144963.

[22] Konishi J, Yamazaki K, Azuma M, Kinoshita I, Dosaka-Akita H, Nishimura M. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res 2004;10(15):5094-100.

[23] Thompson RH, Dong H, Lohse CM, et al. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res 2007;13(6):1757-61.

[24] Muenst S, Soysal SD, Gao F, Obermann EC, Oertli D, Gillanders WE. The presence of programmed death 1 (PD-1)-positive tumor-infiltrating lymphocytes is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat 2013;139(3):667-76.

[25] Matsuzaki J, Gnjatic S, Mhawech-Fauceglia P, et al. Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. Proc Natl Acad Sci U S A 2010;107(17):7875-80.

[26] Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 2014;515(7528):558-62.

[27] Voron T, Colussi O, Marcheteau E, et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. J Exp Med 2015;212(2):139-48.

[28] Tedore T. Regional anaesthesia and analgesia: relationship to cancer recurrence and survival. Br J Anaesth 2015;115(suppl 2):34-45.

[29] Wang D, Dubois RN. Eicosanoids and cancer. Nat Rev Cancer 2010(3);10:181-93.

Tables

Table 1. Demographic data and perioperative characteristics of patients.

Variables	Control Group (n=32)	Flurbiprofen Group(n=32)	Difference (95%CI)	P value
Age, yrs	56(5)	54(6)	2(-4 to 8)	0.455
BMI, kg/m^2	23.9(4.9)	24.3(4.1)	-0.4(-5.3 to 4.5)	0.857
Female Sex (n patients)	13	9	NA	0.292
ASA Status (n patients)			NA	0.672
I	4	2		
II	28	30		
Type of surgery (n patients)			NA	0.320
Lobectomy plus lymphadenectomy	25	28		
Segmentectomy plus lymphadenectomy	7	4		
Duration of surgery, min	134 (9)	143 (11)	-8(-19 to 2)	0.114
Total propofol, mg	838 (134)	893 (138)	-55 (-201 to 91)	0.434
Total remifentanyl, μg	1045 (106)	888 (131)	157 (29-284)	0.019
Nausea (24h postoperative) (n patients)	12	6	NA	0.095
Vomiting and dry retching(24h postoperative) (n patients)	8	1	NA	0.026
Respiratory depression (n patients)	1	0	NA	1.0
Use of anti-emetics (n patients)	13	4	NA	0.011
Skin pruritus (n patients)	1	0	NA	1.0
Length of hospital stay, day	7.5 (0.9)	7.3 (0.7)	0.3 (-0.6 to 1.1)	0.554
Tramadol rescue analgesia (n patients)	10	3	NA	0.030
VAS pain score, 0-10				
24h postoperative score at rest	3 (2-4)	2 (1-3)	NA	0.123
24h postoperative score with cough	6 (2-7)	2 (1-4)	NA	0.009
72h postoperative score at rest	2 (0-3)	1 (0-2)	NA	0.315
72h postoperative score with cough	2 (1-4)	1 (0-2)	NA	0.105

Use of antibiotic intraoperative (n patients)			NA	1.0
Cefuroxime	32	31		
Levofloxacin	0	1		
Use of antibiotic 24h postoperative,Cefuroxime (n patients)	5	2	NA	0.426
Use of antibiotic 24h postoperative,Levofloxacin (n patients)	0	0	NA	1.0
Use of antibiotic 72h postoperative, Cefuroxime (n patients)	3	2	NA	1.0
Use of antibiotic 72h postoperative, Levofloxacin (n patients)	0	0	NA	1.0
24h postoperative wound infection (n patients)	3	0	NA	0.238
72h postoperative wound infection (n patients)	1	1	NA	1.0

All continuous data are presented as mean (SD) or median (interquartile range, IQR) and compared using the independent t-test or the Mann-Whitney U test, respectively. Categorical data are presented as frequency and analyzed using the chi-square or Fisher's exact test. BMI=body mass index. ASA=American Society of Anesthesiologists. VAS= visual analogue scale. NA=not applicable.

Table 2. Changes in perioperative PD-1$^{(+)}$ on CD4$^{(+)}$ and CD8$^{(+)}$ T-cell after receiving flurbiprofen or placebo at 24 hours, 72 hours and 1 week from the baseline values before induction of anesthesia.

Variables	Control Group (n=32)	Flurbiprofen Group(n=32)	Difference (95% CI)	P value
24h postoperative data compared with baseline				
PD-1$^{(+)}$ on CD4$^{(+)}$ T-cell, %	1.76 (0.58)	1.46 (1.11)	0.30 (-0.65 to 1.25)	0.509
PD-1$^{(+)}$ on CD8$^{(+)}$ T-cell, %	12.91 (1.65)	7.86 (5.71)	5.05 (0.55 to 9.55)	0.031
72h postoperative data compared with baseline				
PD-1$^{(+)}$ on CD4$^{(+)}$ T-cell, %	1.15 (1.42)	1.79 (1.96)	-0.64 (-2.47 to 1.20)	0.469
PD-1$^{(+)}$ on CD8$^{(+)}$ T-cell, %	11.54 (1.54)	8.75 (1.73)	2.79 (1.04 to 4.55)	0.004
1 week postoperative data compared with baseline				
PD-1$^{(+)}$ on CD4$^{(+)}$ T-cell, %	0.29 (0.57)	-0.18 (0.58)	0.46 (-0.15 to 1.08)	0.130
PD-1$^{(+)}$ on CD8$^{(+)}$ T-cell, %	6.13 (1.65)	4.82 (0.78)	1.31 (-0.13 to 2.75)	0.071

Data are presented as mean (SD) or median (interquartile range, IQR) and compared using the independent t-test or the Mann-Whitney U test, respectively.

Table 3. Changes in perioperative inflammation and immune markers after receiving flurbiprofen or placebo at 24 hours, 72 hours and 1 week from the baseline values before induction of anesthesia.

Variables	Control Group (n=32)	Flurbiprofen Group(n=32)	Difference (95% CI)	P value
24h postoperative data compared with baseline				
$CD3^{(+)}$ T-cell, %	-21.25 (6.71)	-9.75 (3.37)	-11.50 (-17.20 to -5.80)	0.001
$CD4^{(+)}$ T-cell, %	-7.75 (2.44)	-4.25 (1.49)	-3.50 (-5.66 to -1.34)	0.004
$CD8^{(+)}$ T-cell, %	-3.63 (2.00)	-1.25 (1.83)	-2.38 (-4.43 to -0.32)	0.026
CD4:CD8 ratio	-0.70 (0.31)	-0.35 (0.12)	-0.35 (-0.62 to -0.08)	0.016
NK cell, %	-3.54 (1.31)	-1.49 (0.66)	-2.05 (-3.17 to -0.93)	0.001
Platelet count, 10^9/L	16.00 (-22.50 to 20.25)	2.00 (-24.50 to 24.75)	NA	0.645
Total WBC count, 10^9/L	6.80 (4.58 to 9.08)	5.90 (4.43 to 7.58)	NA	0.161
Hemoglobin, g/L	-11.38 (2.26)	-7.25 (6.27)	-4.13 (-9.18 to 0.93)	0.102
Neutrophil count, 10^9/L	5.85 (5.55 to 11.23)	10.00 (7.10 to 12.0)	NA	0.105
Lymphocyte count, 10^9/L	-0.80 (0.17)	-0.85 (0.16)	0.05 (-0.13 to 0.23)	0.554
Monocyte count, 10^9/L	0.32 (0.09)	0.31 (0.14)	0.01 (-0.12 to 0.13)	0.933
Eosinophil count, 10^9/L	0.084 (0.145)	0.021 (0.075)	0.063 (-0.065 to 0.190)	0.302
Basophil count, 10^9/L	-0.016 (0.009)	-0.015 (0.009)	-0.001 (-0.011 to 0.009)	0.790
TNF-α, pg/ml	817.13 (131.75)	552.13 (146.24)	265.0 (115.74 to 414.26)	0.002
IFN-γ, pg/ml	25.83 (3.30)	18.96 (4.87)	6.86 (2.40 to 11.33)	0.005
IL-6, pg/ml	105.25 (10.08)	68.00 (12.74)	37.25 (24.93 to 49.57)	□ 0.001
CRP, mg/ml	40.34 (11.59)	13.65 (1.92)	26.69 (16.97 to 36.41)	□ 0.001
VEGF-A, pg/ml	643 (72)	544 (64)	-99 (-172 to -26)	0.011

Serum glucose, mmol/L	3.29 (1.05)	2.26 (1.40)	1.03 (-0.30 to 2.35)	0.119
72h postoperative data compared with baseline				
$CD3^{(+)}$ T-cell, %	-20.88 (6.45)	-11.63 (4.03)	-9.25 (-15.02 to -3.48)	0.004
$CD4^{(+)}$ T-cell, %	-14.13 (5.22)	-7.88 (1.55)	-6.25 (-10.67 to – 1.83)	0.011
$CD8^{(+)}$ T-cell, %	-2.00 (1.69)	-1.38 (1.30)	-0.63 (-2.24 to 0.99)	0.421
CD4:CD8 ratio	-0.55 (-0.98 to -0.20)	-0.10 (-0.18 to -0.03)	NA	0.005
NK cell, %	-3.83 (1.23)	-2.40 (0.67)	-1.43 (-2.49 to -0.36)	0.012
Platelet count, 10^9/L	18.50 (-19.00 to 23.25)	-5.00 (-33.50 to 23.25)	NA	0.442
Total WBC count, 10^9/L	3.75 (2.45 to 4.58)	2.25 (0.83 to 3.95)	NA	0.105
Hemoglobin, g/L	-6.13 (3.04)	-6.63 (7.35)	0.50 (-5.53 to 6.53)	0.863
Neutrophil count, 10^9/L	2.05 (0.85 to 3.18)	2.65 (0.73 to 4.98)	NA	0.878
Lymphocyte count, 10^9/L	-0.69 (0.22)	-0.68 (0.26)	-0.01 (-0.27 to 0.25)	0.919
Monocyte count, 10^9/L	0.22 (0.08)	0.16 (0.13)	0.06 (-0.06 to 0.17)	0.338
Eosinophil count, 10^9/L	0.016 (0.034)	-0.006 (0.012)	0.023 (-0.006 to 0.051)	0.110
Basophil count, 10^9/L	-0.009 (0.015)	-0.005 (0.017)	-0.004 (-0.021 to 0.013)	0.642
TNF-α, pg/ml	748.00 (133.71)	422.88 (95.49)	325.13 (200.53 to 449.72)	0.000
IFN-γ, pg/ml	23.09 (3.08)	17.50 (3.46)	5.59 (2.07 to 9.10)	0.004
IL-6, pg/ml	87.25 (11.99)	59.50 (9.68)	27.75 (16.07 to 39.43)	0.001
CRP, mg/ml	44.90 (9.94)	21.46 (7.26)	23.44 (14.10 to 32.77)	0.001
VEGF-A, pg/ml	481 (75)	387 (77)	-94 (-175 to -13)	0.027

Serum glucose, mmol/L	1.88 (1.26)	0.59 (1.29)	1.29 (-0.08 to 2.65)	0.062
1 week postoperative data compared with baseline				
$CD3^{(+)}$ T-cell, %	-5.75 (10.36)	-5.13 (4.91)	-0.63 (-9.32 to 8.07)	0.880
$CD4^{(+)}$ T-cell, %	-6.50 (4.50)	-4.38 (1.19)	-2.13 (-5.93 to 1.68)	0.233
$CD8^{(+)}$ T-cell, %	-2.38 (1.51)	-2.00 (0.76)	-0.38 (-1.70 0.95)	0.543
CD4:CD8 ratio	-0.30 (-0.60 to 0.10)	-0.05 (-0.20 to 0.20)	NA	0.130
NK cell, %	-1.98 (0.80)	-1.25 (0.75)	-0.73 (-1.56 to 0.11)	0.082
Platelet count, 10^9/L	-8.00 (-42.50 to 34.00)	5.50 (-14.75 to 27.50)	NA	0.798
Total WBC count, 10^9/L	1.10 (-1.80 to 1.45)	1.55 (-1.95 to 1.80)	NA	0.645
Hemoglobin, g/L	-10.38 (3.16)	-8.88 (6.01)	-1.50 (-6.65 to 3.65)	0.542
Neutrophil count, 10^9/L	1.75 (0.50 to 3.08)	1.65 (0.33 to 2.98)	NA	0.328
Lymphocyte count, 10^9/L	-0.39 (0.29)	-0.41 (0.22)	0.03 (-0.25 to 0.30)	0.848
Monocyte count, 10^9/L	0.08 (0.10)	0.04 (0.14)	0.04 (-0.09 to 0.17)	0.524
Eosinophil count, 10^9/L	-0.013 (0.062)	0.038 (0.047)	-0.050 (-0.109 to 0.009)	0.090
Basophil count, 10^9/L	-0.006 (0.011)	-0.001 (0.020)	-0.005 (-0.022 to 0.012)	0.536
TNF-α, pg/ml	313.25 (214.96)	287.00 (146.54)	26.25 (-171.03 to 223.53)	0.780
IFN-γ, pg/ml	12.25 (3.31)	9.64 (1.56)	2.61 (-0.27 to 5.49)	0.071
IL-6, pg/ml	49.00 (15.58)	38.50 (12.71)	10.50 (-4.75 to 25.75)	0.162
CRP, mg/ml	25.44 (11.54)	20.49 (7.93)	4.95 (-5.67 to 15.57)	0.334
VEGF-A, pg/ml	67 (73)	44 (11)	-22 (-78 to 33)	0.406
Serum glucose, mmol/L	0.59 (0.31)	0.41 (1.16)	0.18 (-0.81 to	0.691

1.16)

Data are presented as mean (SD) or median (interquartile range, IQR) and compared using the independent t-test or the Mann-Whitney U test, respectively; NK=natural killer; WBC=white blood cells; TNF-α=tumor necrosis factor-α; IFN-γ=interferon-γ; IL-6=interleukin-6; CRP=C-reactive protein; VEGF=vascular endothelial growth factor; NA=not applicable.

Figures

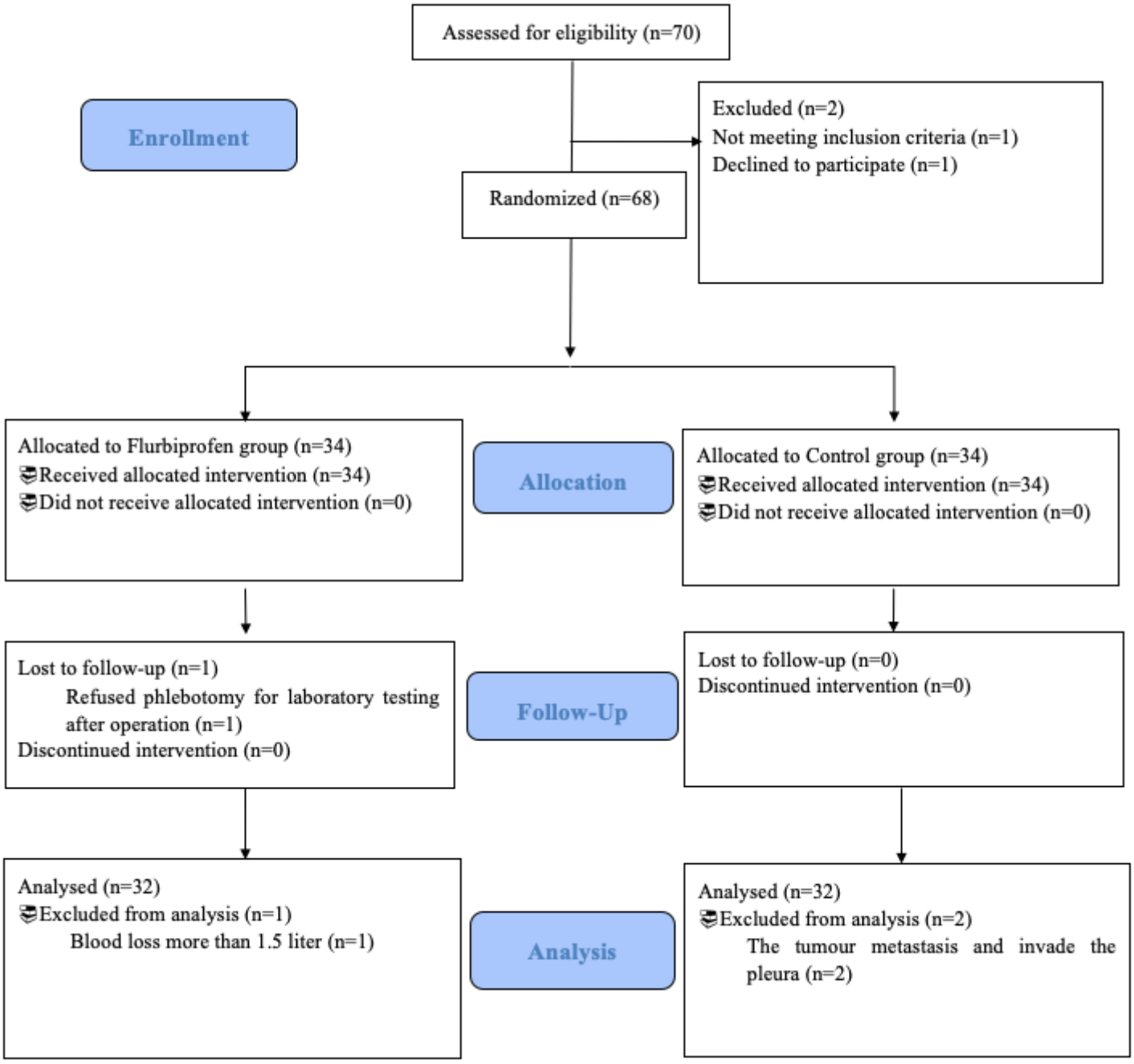

Figure 1

CONSORT diagram of trial processes.

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