# Vein-first surgical technique reduces the dissemination of circulating tumor cells with potential survival benefits in patients with non-small cell lung cancer A prospective, multi-center, randomized, controlled trial combined with retrospective propensity score matching analysis based on a lung cancer registry

## Background

Recently, some studies have demonstrated that the presence of circulating tumor cells (CTCs) in the peripheral blood can be a surrogate biomarker to predict recurrence and prognosis.[1-4] CTCs are released from the primary tumor into the bloodstream and have the potential to spread to distant sites and develop into micro-metastatic deposits.[5] Numerous studies have demonstrated that surgical manipulation could promote the dissemination of tumor cells into the circulation.[6-10] A surgical technique, named “no-touch” isolation, has been reported to reduce intraoperative shedding of tumor cells into the circulation in colorectal cancer,[11-14] hepatocellular carcinoma[15,16] and pancreatic cancer.[17-19] In addition, another surgical technique that may prevent the dissemination of tumor cells into the bloodstream is to ligate the drainage veins first during surgery.[20] As reported by McCulloch and colleagues[21], tumor cells can be detected in venous blood during surgery. For lung cancer, vascular invasion within the tumor is common, which might be responsible for the high incidence of hematogenous spread of tumor cells.[22-24] Surgical manipulations of lung cancer may squeeze the tumor and further promote the spread of tumor cells

into the circulation.[9,10] The potential risk of tumor cell dissemination can theoretically be minimized if the drainage veins were ligated first (the venous-first technique). However, this technique has not yet been widely accepted as a standard of surgical oncology in the current guidelines due to the lack of sufficient evidence. As such, we conducted a prospective, multi-center, randomized, controlled trial to evaluate whether the order of vascular ligation could affect the dissemination of tumor cells into the circulation.

## Study protocol

*Purpose*

To compare the impact of different sequence of vessel ligation during surgery on the dissemination of tumor cells and survival outcomes in non-small cell lung cancer (NSCLC).

*Study setting*

This trial is a prospective, multi-center, randomized, controlled trial.

*Research sites*

West China Hospital of Sichuan University, Daping Hospital of the Third Military Medical University, and Sichuan Cancer Hospital of the University of Electronic Science and Technology of China.

*Eligibility criteria*

Inclusion criteria: patients with primary lung cancer; elder than 18 years old; tumor size more than 2cm; undergoing complete video-assisted thoracic lobectomy.

Exclusion criteria: patients undergoing segmentectomy or wedge resection; receiving a wedge resection of the lesion prior to the standard lobectomy; history of other malignant tumors; receiving neoadjuvant therapy; undergoing lobectomy with open thoracotomy.

*Randomization and masking*

Patients were randomly assigned in a 1:1 ratio according to different surgical procedures, including the vein-first procedure (ligating the pulmonary vein first, V-first) and artery-first procedure (ligating the pulmonary artery first, A-first) via computer generated randomized numbers, and both of the laboratory investigators and the patients were masked as to the allocation schedule. Sealed and numbered envelopes that contained the allocated group were prepared and opened at the beginning of surgery.

*Sample size*

Sample size was calculated based on our preliminary study. The preliminary results showed that the increasing of $FR^{+}$CTCs after surgery were observed in 40% patients in V-first group and 80% patients in A-first group. We expected that there would be at least 10% differences of the $FR^{+}$CTCs increasing rates between the two groups. And to achieve a statistical power of 80% and a two-sided type I error of 5%, 36 patients were needed in each group. With an assumption of an approximate 10% drop out rate, our aim was to enroll 80 patients.

*Procedure and intervention*

Before randomization and surgery, patients usually received physical examination,

laboratory tests, chest and upper abdominal computed tomography (CT) scan, brain magnetic resonance imaging (MRI), single-photon emission tomography computed tomography (SPECT) bone scan, or positron emission tomography CT (PET-CT) as an alternative to try to find out distant metastasis. Generally, surgical resection is not considered for patients with distant metastasis.

Thoracoscopic lobectomy was performed with the standard technique, with the only difference being the sequence of vessel ligation. For V-first technique, the pulmonary veins in the hilum of pulmonary lobes were dissected and transected firstly, followed by the bronchus and PA branches, and leaving the fissure finally, which was described as the single-direction thoracoscopic lobectomy.[25] For A-first technique, all arteries should be completely ligated before venous interruption. Taking the right upper lobectomy as an example, there were often multiple arteries that required ligation first. The apicoanterior trunk of the right upper artery was fully exposed from the anterior aspect of hilum, a stapler was used to divide this branch. Then the upper lobe of lung was pulled forward, and the upper lobar bronchus was dissected through the posterior approach. After the upper lobar bronchus was transected, the posterior ascending branch of the right upper artery was exposed and ligated. The remaining right upper lobe veins were transected finally. All operations were carried out by certified thoracic surgeons who had at least three-year experience of thoracoscopic lobectomy. Three milliliters of peripheral blood was sampled from radial artery in all the enrolled patients before and after surgery, using an EDTA anti-coagulant vacuum tube (BD Diagnostics, Sparks, MD). Preoperative blood samples were collected from patients before making

the incision. Postoperative blood samples were harvested immediately after the chest closed. Samples were then stored at 4℃ and processed within 24 hours.

*CTCs detection*

CTCs are analyzed and quantified using the Folate Receptor-positive Circulating Tumor Cells ($FR^+$-CTCs) Detection Kit (Geno Biotech Co. Ltd., Hong Kong, China), which has been approved by the China Food and Drug Administration (CFDA). The enrichment of CTCs is initially achieved by lysing erythrocytes, followed by immuno-magnetic depletion of leukocytes from the whole blood. Then, $FR^+$ CTCs in each sample are quantified by ligand-targeted polymerase chain reaction (LT-PCR) as published before.[26] The primer sequences are listed as follows: detection probe (an oligonucleotide that is conjugated to the tumor-specific ligand folic acid), 5'–CTCAA CTGGT GTCGT GGAGT CGGCA ATTCA GTTGA GGGTT CTAA–3'; forward primer, 5'–TATGA TTATG AGGCA TGA–3'; reverse primer, 5'–GGTGT CGTGG AGTCG–3'; TaqMan probe, 5'–FAM–CAGTT GAGGG TTC–MGB–3'. The LT-PCR reaction is performed on an ABI StepOne instrument as follows: denaturation at 95℃ for 2 minutes, annealing at 40℃ for 30 seconds, extension at 72℃ for 30 seconds, and then cooling at 8℃ for 5 minutes; 40 cycles of denaturation at 95℃ for 10 seconds, annealing at 35℃ for 30 seconds, and extension at 72℃ for 10 seconds. A self-referenced CTC unit (denoted "FU") derived from standard curve is used to indicate the abundance of $FR^+$-CTCs in 3 mL peripheral blood. A serial of standards containing oligonucleotides ($10^{-14}$ to $10^{-9}$ M, corresponding to 2 to $2\times10^5$ CTC units/3 mL blood) are used for $FR^+$-CTC quantification.

*Outcome assessment*

The primary endpoints are the differences in the level of $FR^+$CTCs detected before and after surgery in the two groups. Secondary endpoints are disease-free survival and overall survival.

*Follow-up strategy*

All patients visit the surgical outpatient office one month after surgery for follow-up assessment. Patients are then evaluated every three to six months during the first two years, every six-month intervals in the next three years and annually thereafter. During routine follow-up, physical examination, chest and abdominal CT scans, and brain CT or MRI scan are performed. SPECT bone scan is performed once a year, and PET-CT is also conducted if necessary. Telephone follow-up is conducted if the outpatient follow-up is not available.

*Statistical analysis*

Continuous data are presented in means and compared using Student's t-test in case of normal distribution, and described in median and compared using Mann-Whitney *U*-test if non-normal distribution. Categorical data are presented in counts and percentages and compared with $\chi$-square test or the Fisher's exact test according to the cell size examined among groups. Logistic regression is performed to examine the risk factors associated with the change of $FR^+$ CTC levels during surgery. Survival curves are estimated by the Kaplan-Meier method and compared by the log-rank test. Factors potentially affecting the survival are assessed by univariable and multivariable Cox regression analysis.

*Clinical trial registry*

This trial was registered at the *ClinicalTrials.gov* (NCT03436329).

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**Conflict of interest statement**

None declared.

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