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Sentinel Lymph Node in Non-Small Cell Lung Cancer: Longterm Outcomes and Immunopathological Consequences

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Abstract

Background: Lymph-node staging is pivotal in non-small-cell lung cancer (NSCLC). While systematic lymph-node dissection (SLND) is standard, it may blunt anti-tumour immunity. Sentinel lymph-node (SLN) mapping could deliver robust staging with less immune disruption. We assessed survival outcomes and lymph-node immune profiles associated with SLN mapping in NSCLC surgery.

Methods: This retrospective study of 266 patients who underwent major lung resection with SLN identification (Dec 2020–Sep 2025) assessed overall survival (OS) and disease-free survival (DFS). Translational analyses compared immune populations in SLN vs. non-SLN and node-positive (pN+) vs. node-negative (pN0) samples, using immunohistochemistry (IHC; 10 SLN and 10 pN+ patients) and flow cytometry (FCM; 13 pN0 SLN and 4 pN+ patients).

Results: SLN was detected in 80.1% of patients (213/266). In patients with NSCLC, upstaging occurred in 6.6% (11/167), with a false-negative rate of 2.6% (4/156). Median OS was 27 months for SLN pN+ and not reached for SLN pN0 (HR 9.7, 95% CI 2.61–36.22; $p<0.001$); median DFS was 12 months for SLN pN+ and not reached for SLN pN0 (HR 10.1, 95% CI 3.68–27.83; $p<0.001$). IHC and FCM demonstrated a comparable immune environment between SLNs and non-SLNs in pN0 patients for key immune compartments (CD4, CD8 T cells, memory subsets), while metastatic lymph nodes displayed disrupted architecture and altered cytotoxic Tcell markers.

Conclusions: SLN micrometastasis is a strong prognostic marker. SLN mapping may provide accurate staging while preserving nodal immunity, representing a conservative alternative to SLND in early-stage NSCLC, and warrants confirmation in larger, prospective studies.

Keywords: Lung cancer; Sentinel lymph node; Indocyanine green; Micrometastases; Anti-tumor immunity; Immunoprofilling; Immunotherapy

Introduction

Lung cancer is a major public health concern and the leading cause of cancer-related mortality worldwide [1]. Surgery remains the cornerstone of treatment across several stages of disease, often in combination with peri-operative therapies [2]. Accurate staging of lymph-node involvement is critical, as it informs prognosis and subsequent therapeutic decisions [3]. Traditionally, systematic lymph-node dissection (SLND) is undertaken at

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the time of surgical resection [4]. Although this approach improves staging accuracy, it may inadvertently compromise anti-tumour immunity by removing immunocompetent lymphoid tissue. The advent of immunotherapy has underscored the central role of the immune system in disease control and long-term survival [5-7]. Re-examining the role of lymph-node dissection in the immunotherapy era is therefore essential. The sentinel lymph node (SLN), the first node draining the tumour, is a surgical concept routinely applied in several cancers [8,9]. In breast cancer and melanoma, for example, SLN biopsy has replaced SLND in selected cases, reducing morbidity without compromising oncological outcomes [10]. By enabling enhanced detection of occult micrometastases often missed by standard pathological assessment through finer sectioning and time-consuming, dedicated immunohistochemistry (IHC) techniques, the SLN approach can yield crucial information on both metastatic status and immune contexture [11,12]. Adaptation of this strategy to thoracic surgery has been explored over many years, but has been hampered by unsatisfactory SLN detection rates, notable complications, and organisational challenges between hospital departments [13,14]. More recently, the use of indocyanine green (ICG) with near-infrared (NIR) fluorescence imaging has renewed interest in SLN mapping in thoracic surgery [15]. Several groups, including ours, have already demonstrated the safety and feasibility of this technique, with an SLN identification rate of 75% and no adverse events attributable to ICG [16-18]. We also observed strong pathological concordance between the SLN and the remainder of the lymph-node dissection [19]. When the SLN showed no neoplastic involvement, the other resected lymph nodes likewise appeared free of cancer. Finally, IHC analyses performed on the SLN in our series led to upstaging in nearly 10% of patients, demonstrating the value of the SLN for improving staging. However, the SLN technique is not currently approved for the surgical management of non-small-cell lung cancer (NSCLC). Its role in anti-tumour immunity and in predicting immunotherapy response remains under investigation. Understanding its immune composition could clarify whether SLN evaluation may serve as a reliable alternative to complete lymphadenectomy while preserving immune function. This clinical and translational study aimed, first, to assess the long-term outcomes associated with the SLN technique in NSCLC surgery and, secondly, to explore the immune environment of the SLN compared with the remainder of the lymph-node dissection in patients with NSCLC.

Materials and Methods

Clinical outcomes

This retrospective, observational, single-center study was conducted in the Thoracic Surgery Department of Nancy Regional University Hospital (France). The study protocol received ethics approval (NCT05136014), and written informed consent was obtained from each participant.

Study outcomes

The primary endpoint was five-year disease-free survival (DFS) and overall survival (OS) among patients who underwent major lung resection and SLND with the SLN technique for lung cancer in our department. DFS was defined as the interval from surgery to radiological and/or pathological confirmation of either local or distant recurrence, or to the date of last follow-up imaging. OS was defined as the interval from enrolment until death from any cause.

We also reported the SLN identification rate using NIR fluorescence imaging after ICG injection, the nodal upstaging rate, and pathological concordance between the SLN and the remaining lymph nodes removed during dissection. Nodal upstaging was defined as the presence of malignancy in one or more lymph nodes on histopathological analysis despite a negative pre-operative assessment.

Study population

Adult patients (≥ 18 years) with confirmed or suspected surgically resectable cT1a–cT2b N0 (clinical stage IA to IIA) NSCLC and no evidence of lymph-node involvement on pre-operative 18F-fluoro-deoxy-glucose positron emission tomography (18F-FDG PET) were enrolled between December 2020 and September 2025. Pre-operative thoracic computed tomography (CT), 18F-FDG PET, and brain magnetic resonance imaging (MRI) were reviewed by both a radiologist and a nuclear radiologist specialising in thoracic oncology. Pulmonary function testing and staging were performed in accordance with European Respiratory Society/European Society of Thoracic Surgeons (ERS/ESTS) guidelines [20]. Pathological staging was assigned according to the eighth edition of the TNM lung cancer classification system.

All patients received a peritumoural injection of ICG, administered either transpleurally or via electromagnetic navigational bronchoscopy (ENB), for SLN identification, following the protocol described by Phillips et al. and by our team [15,17].

Lymph-node analysis

This experimental study was undertaken in the Thoracic Surgery Department, Pathology Department, and the Cell Therapy and Tissue Bank Unit of Nancy Regional University Hospital (France). Written consent was obtained from each participant. The study was approved by our institutional review board (IRB approval NCT05136014).

Study outcomes and design

The principal objective was to describe and compare the immune populations of the SLN with those of the other

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lymph nodes removed during NSCLC surgery, to evaluate the potential immunological impact of lymph-node dissection on anti-tumour immunity and immunotherapy response in NSCLC.

We conducted a single-center study at CHRU Nancy with both retrospective and prospective components. Retrospective immunohistochemistry (IHC) was performed on archived lymph-node samples from resected NSCLC patients between January and December 2024, while prospective flow cytometry (FCM) analysis was performed on fresh lymph nodes collected during surgical resections between June and August 2025.

IHC – Study population and protocol

Two groups were defined: a "SLN" group and a "pN+" group. The "SLN" group included adult patients who underwent major lung resection with lymph-node dissection for NSCLC and who benefited from the department's SLN protocol. Exclusion criteria were: atypical resection, multiple SLNs, histology other than NSCLC, lymph nodes with major fibrohyaline involvement, and lymph-node metastasis. To form a control group, adult patients who underwent major lung resection with lymph-node dissection for NSCLC without the SLN protocol but with a single lymph-node metastasis were selected to constitute the "pN+" group. Patients with a single lymph-node metastasis were chosen because this node may correspond to the tumour-draining lymph node and can therefore be considered equivalent to the SLN. Exclusion criteria were: atypical resection, multiple metastatic nodes, histology other than NSCLC, and lymph nodes with major fibrohyaline involvement.

To compare the immune populations of the SLN or the pN+ node to the other lymph nodes, the pN0 SLN and 3 other pN0 lymph nodes from 3 different stations were chosen by patient in the "SLN" group, while the single pN+ lymph node and 3 other pN0 lymph nodes from 3 different stations were chosen by patient in the "pN+" group. Parffin-embedded lymph-node tissues were cut using a microtome to generate six microscope slides. One slide was used for HES staining (Coverstainer automated system, Agilent®, Santa Clara, United States) to confirm or rule out metastatic invasion. Five slides were dedicated to characterising the immune environment by IHC (DAKO Omnis automated system, Agilent®) with five antibodies: CD3 (T lymphocytes), CD4 (CD4 T lymphocytes), CD8 (CD8 T lymphocytes), CD20 (B lymphocytes), and CD56 (natural killer [NK] cells).

The detailed protocol is provided in the supplementary data.

FCM – Study population and protocol

As for the IHC component, we aimed to create a "SLN" group and a "pN+" group. The "SLN" group comprised patients with histologically proven or imaging-suspected NSCLC at localised stage cT1a–T2b N0 (stage IA–IIA), treated by anatomical lung resection with lymph-node dissection and the SLN protocol. The "pN+" group comprised patients with histologically proven or imaging-suspected NSCLC with a single histologically proven or suspected lymph-node involvement at staging, treated by anatomical lung resection with lymph-node dissection.

The SLN was detected as described above, and the pN+ node was identified visually during surgery. Per patient, the pN0 SLN or single pN+ node and three other pN0 lymph nodes from three different stations were sampled using an 18-gauge needle by manual aspiration; specimens were stored in cell-culture fluid (RPMI 1640, Eurobio Scientific®) at 4 °C until immunophenotyping and FCM acquisition (MACSQuant10, Miltenyi®). We established three antibody panels to identify immune-cell subsets: a "basal" panel to characterise the global immune landscape; a "T lymphocyte" panel to define T-cell subpopulations; and a "memory" panel to assess memory subsets.

The composition of each panel and the immunophenotyping protocol are detailed in the supplementary data.

Statistical analysis

Differences between continuous variables were compared using Student's t-test, while categorical variables were compared using the $\chi 2$ test. Analyses were performed using StataMP® statistical software (Stata/MP version 13.0). A p-value < 0.05 was considered statistically significant.

Clinical outcomes

Log-rank tests and hazard ratios were used to assess DFS and OS. All statistical tests and visualisations were conducted using GraphPad Prism v10.2.2 (Boston, USA).

Lymph-node analysis

To compare immune populations between lymph nodes, we used a mixed linear model to account for intra-subject correlation, thereby comparing each subject's data with their own control data. Results are presented as the mean difference estimated by the model. An equivalence analysis was conducted using the TOST (Two One-Sided Tests) method. An equivalence margin of 10% for the most abundant cells and 20% for the least abundant cells was deemed clinically relevant and prespecified. Equivalence was concluded when $p < 0.05$. These equivalence analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria).

Results

Clinical outcomes

Patient characteristics

In total, 266 patients consented to undergo SLN

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identification. The characteristics of the study population are presented in table 1.

SLN – key results

An SLN was identified in 213/266 patients, corresponding to an identification rate of 80.1%. We then focused on patients in whom an SLN was identified and analysed and for whom pathology confirmed a primary lung malignancy. Consequently, 37 patients (17.8%) with a benign lesion and 9 patients (4.2%) with a metastatic lesion were excluded from subsequent analyses.

Among the remaining 167 patients with primary lung cancer and an analysed SLN, there were 11 cases of SLN invasion by micrometastases detected by IHC, corresponding to a 6.6% nodal upstaging rate. In pN0 SLN, 4/156 (2.6%) false-negative cases were observed, i.e., metastatic invasion was found in a non-SLN.

Table 1: Demographic characteristics of the clinical part population

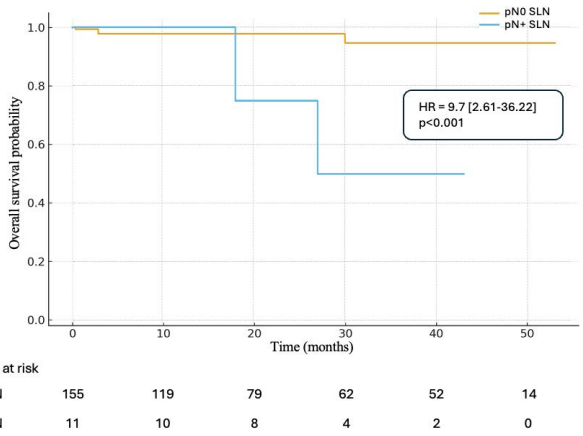
	Study population (n=266)
Sex, male/female, no (%)	141/125 (53%/47%)
Age (years), mean ± SD	67.1 ± 8.5
Smoking Status, no (%)	
Never	58 (21.8%)
Ex-Smoker	152 (57.1%)
Current	56 (21.1%)
Type of Resection, no (%)	
Lobectomy	125 (47%)
Segmentectomy	141 (53%)
Surgical Approach, no (%)	
VATS	219 (82.3%)
Robotic	34 (12.8%)
Thoracotomy	13 (4.9%)
Pulmonary Function (% Predicted)	
FEV1 (mean ± SD)	90.4 ± 20.9
DLCO (mean ± SD)	80.3 ± 20.9
Clinical Staging, no (%)	
IA1	54 (20.3%)
IA2	127 (47.7%)
IA3	50 (18.8%)
IB	29 (10.9%)
IIA	6 (2.3%)
Suspected Nodal Stage	
N0	266 (100%)
Charlson Score, mean ± SD	5.2 ± 1.8

SD, Standard Deviation ; VATS, Video-Assisted Thoracoscopic Surgery ; FEV1, Forced Expiratory Volume in one second ; DLCO, Diffusing Capacity of the Lung for Carbon Monoxide

Survival analysis

The median follow-up for this cohort was 19 months (IQR = 32).

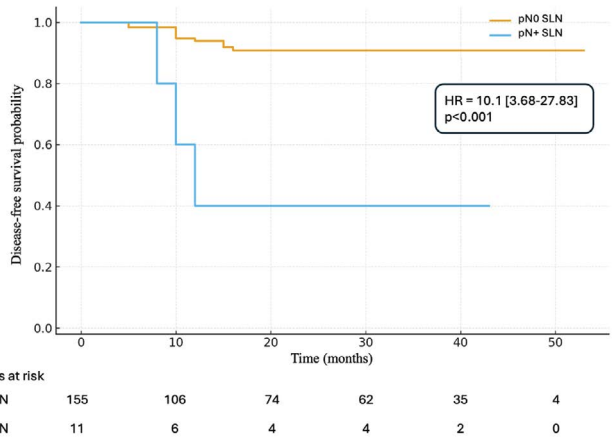
For OS, median survival was 27 months in the SLN pN+ group, whereas it was not reached in the SLN pN0 group (HR = 9.7, 95% CI 2.61–36.22, p < 0.001). OS is illustrated in figure 1.



pN0, pathologically non-invaded lymph node ; pN+, pathologically invaded lymph node ; SLN, Sentinel Lymph Node ; HR, Hazard Ratio

Figure 1: Five-year overall survival in patients with pN0 SLN and pN+ SLN.

For DFS, median survival was 12 months in the SLN pN+ group, whereas it was not reached in the SLN pN0 group (HR = 10.1, 95% CI 3.68–27.83, p < 0.001). DFS is illustrated in figure 2.



pN0, pathologically non-invaded lymph node ; pN+, pathologically invaded lymph node ; SLN, Sentinel Lymph Node ; HR, Hazard Ratio

Figure 2: Five-year disease-free survival in patients with pN0 SLN and pN+ SLN

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Lymph-node analysis

IHC

Patient characteristics

A total of 20 patients were included, divided into two groups: 10 in the "pN+" group and 10 in the "SLN" group. The two populations were statistically comparable for age, smoking status, and ASA score. However, there was a significant difference in respiratory function in favour of the SLN group, as well as in the number of segmentectomies performed.

Demographic characteristics are summarised in table 2.

Table 2: Demographic characteristics of the population studied with IHC.

	pN+ group (n=10)	SLN group (n=10)	p value
Gender, male/female	9/1	6/4	0.3
Age in year (mean ± SD)	64.3 ± 6.4	68 ± 7.2	0.24
Smoking history			
Never	0	2	0.37
Former	6	3	
Active	4	5	
ASA score (mean ± SD)	2.5 ± 0.5	2.3 ± 0.7	0.47
Respiratory function			
FEV1 (mean % ± SD)	71.3 ± 17.3	90 ± 11.1	0.01
DLCO (mean % ± SD)	67.7 ± 25.5	90.7 ± 20.3	0.06
VO2max (mean % ± SD)	15.4 ± 2.3	20.3 ± 3.1	0.02
Type of resection			
Lobectomy	10	3	0.003
Segmentectomy	0	7	
Number of lymph nodes resected (mean ± SD)	28 ± 11	12 ± 6	0.0006
Clinical stage			
I	2	10	0.005
II	3	0	
III	5	0	
IV	0	0	

SD, Standard Deviation ; ASA, American Society of Anesthesiologists ; FEV1, Forced Expiratory Volume in one second ; DLCO, Diffusing Capacity of the Lung for Carbon Monoxide ; VO2max, maximum oxygen consumption

IHC slides

In the "SLN" group, a standard distribution of B lymphocytes (CD20) was observed in the SLN and in other uninvolved lymph nodes removed during dissection, predominantly within follicles in the cortical region. These follicles were numerous and varied in size, indicating potential activation.

T lymphocytes (CD3) were mainly present in the paracortical sinuses. CD4 cells were sometimes arranged in a ring around the follicles, and a few CD8 cells were present in the germinal centers, suggesting potential B–T cell interactions. In the "pN+" group, the parenchyma of the metastatic lymph node was markedly altered by tumour infiltration. Nonetheless, various immune cells remained detectable, with some CD8 cells identified within the cancerous tissue. The other pN0 lymph nodes removed showed a cellular distribution similar to that observed in the "SLN" group.

NK-cell (CD56) staining appeared negative in both groups.

To compare lymph nodes, we performed a CD8 T-cell count. B cells were too numerous to count, and CD4 T cells were weakly labelled and confounded by CD4-positive histiocytes. CD8 T-cell counts were performed on four 0.25 mm^2 microscopic fields to obtain an average per 1 mm^2.

In the "pN+" group, the estimated difference in CD8 cells between the metastatic lymph node and healthy lymph nodes was −9.175 [−26.074; 7.724], p = 0.07, with an equivalence margin set at 10%.

In the "SLN" group, the estimated difference in CD8 cells between SLN and non-SLN values was −4.383 [−24.832; 16.066], p = 0.04, with an equivalence margin set at 10%.

FCM

Patient characteristics

Eighteen patients were initially included in the "SLN" group. However, two had intraparenchymal SLNs that could not be retrieved despite very distal dissection and were excluded; two patients initially classified as cN0 were found to have micrometastatic invasion of the SLN on final pathology (diagnosed by IHC with anti-cytokeratin AE1/AE3) without other nodal involvement and were therefore reclassified into the "pN+" group. In one patient, the SLN could not be identified (no lymph node took up ICG after exploration of the various stations) and was excluded. Thus, analyses were performed in 13 patients in the pN0 "SLN" group.

In the "pN+" group, only five patients could be included (including the two reclassified from the "SLN" group) owing to the small number of patients with a single lymph-node metastasis on pre-operative assessment during the inclusion period. One patient was excluded because FCM results were uninterpretable. The final "pN+" group therefore comprised four patients.

The two groups were statistically comparable for age, ASA score, and respiratory function. However, there were

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significant differences in the number of segmentectomies performed and in localised stages (clinical staging) in favour of the "SLN" group. Excluding the two reclassified patients, the labelling technique in the "SLN" group was performed by ENB in 46% (6/13) and by transpleural injection in 54% (7/13). Regarding histology, the "pN+" group included 4/4 (100%) adenocarcinomas (1 stage IIB and 3 stage IIIA). In the "SLN" group, there were 7/13 (53.8%) adenocarcinomas (1 stage IA1, 3 stage IA2, and 3 stage IA3), 1/13 (7.7%) squamous-cell carcinoma (stage IB), 1/13 (7.7%) neuroendocrine carcinoma (stage IB), 1/13 (7.7%) metastasis of endometrial adenocarcinoma, and 3/13 (23.1%) benign lesions (one bronchiectasis and two giant-cell epithelioid granulomas with necrosis). Patients with histology other than NSCLC were retained for immune-cell analyses, the aim being to compare populations between SLN and other nodes removed during dissection, but will, of course, be excluded from any future survival analysis. Demographic characteristics are summarised in table 3.

Table 3: Demographic characteristics of the population studied with FCM.

	pN+ group (n=4)	SLN group (n=13)	p value
Gender, male/female	1/3	5/8	0.62
Age in year (mean ± SD)	63 ± 5	67.3 ± 10.1	0.43
Smoking history			
Never	2	0	0.008
Former	2	13	
Active	0	0	
ASA score (mean ± SD)	2.25 ± 0.5	2.3 ± 0.48	0.83
Respiratory function			
FEV1 (mean % ± SD)	96.8 ± 21.4	97.9 ± 17.6	0.91
DLCO (mean % ± SD)	98 ± 32.7	93 ± 17.9	0.7
Clinical stage			
I	2	13	0.006
II	1	0	
III	1	0	
IV	0	0	
Type of resection			
Lobectomy	4	5	0.031
Segmentectomy	0	8	
Number of lymph nodes resected (mean ± SD)	19.3 ± 5.8	18.2 ± 9.4	0.84
Surgical approach			
VATS	1	5	0.64
Robotic	2	7	
Thoracotomy	1	1	

SD, Standard Deviation ; ASA, American Society of Anesthesiologists ; FEV1, Forced Expiratory Volume in one second ; DLCO, Diffusing Capacity of the Lung for Carbon Monoxide

"SLN" group

Basal panel

With a 10% equivalence margin, the SLN was statistically comparable to other lymph nodes for CD45 and CD3 cells; for CD56 cells, equivalence was demonstrated with a 20% margin.

T-lymphocyte panel

With a 10% equivalence margin, the SLN was statistically comparable to other lymph nodes for CD4 and CD8 cells; for Treg cells, equivalence was demonstrated with a 20% margin.

Memory panel

Four memory T-cell subpopulations were identified:

- T stem-cell memory (Tscm): CD45RA+ CCR7+ CD95+
- T central memory (Tcm): CD45RA− CCR7+
- T effector memory (Tem): CD45RA− CCR7−
- T effector memory re-expressing CD45RA (Temra): CD45RA+ CCR7−

With a 20% equivalence margin, the SLN was comparable to other nodes for CD3 Tscm, CD3 Tcm, CD4 Tscm, CD4 Tcm, and CD8 Tcm.

"pN+" group

Basal panel

With a 10% equivalence margin, pN+ lymph nodes were statistically comparable to pN0 lymph nodes for CD45 cells.

T-lymphocyte panel

With a 10% equivalence margin, pN+ lymph nodes were statistically comparable to pN0 lymph nodes for CD4 cells.

Memory panel

The same memory subpopulations were assessed. With a 20% equivalence margin, the pN+ lymph node was statistically comparable to pN0 lymph nodes for CD4 Tscm.

Discussion

This clinical and translational study sought to evaluate, first, five-year survival among patients undergoing major lung resection for lung cancer with an SLN protocol at Nancy University Hospital, and secondly to analyse lymph nodes at the cellular level to understand how lymph-node dissection might alter anti-tumour immunity and the capacity to respond to immunotherapy. The overarching aim was to consider whether SLN assessment could serve as an alternative to SLND in a context where the rise of immunotherapy is reshaping oncological priorities and encourages, as far as possible, the preservation of functional immune compartments.

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In our cohort, survival analyses underscore the prognostic importance of lymph-node status in patients undergoing surgery for early-stage NSCLC. The survival curves show clear separation between SLN pN0 and SLN pN+ groups for both OS and DFS. Median OS was not reached in the SLN pN0 group, compared with 27 months in the SLN pN+ group ($p < 0.001$), indicating an almost tenfold higher risk of death in the presence of nodal invasion. Similarly, median DFS was 12 months in the SLN pN+ group, while it was not reached in the SLN pN0 group ($p < 0.001$). These results confirm that micrometastatic disease in the SLN remains a major prognostic factor, even when pre-operative imaging shows no lymph-node involvement [11,21,22]. They also support the relevance of SLN assessment as a staging tool to identify a subgroup of patients whose prognosis might be improved with tailored adjuvant strategies, such as post-operative immunotherapy.

Anti-tumour immunity relies on the coordinated action of multiple immune populations [23]. Cytotoxic CD8 T lymphocytes are principal effectors, capable of recognising tumour antigens and directly destroying cancer cells, with their action reinforced by CD4 T lymphocytes that secrete IFN-γ and IL-2, stimulating both CD8 cells and macrophages. NK cells provide rapid elimination of tumour cells that evade adaptive surveillance, while dendritic cells capture and present antigens, playing a key role in initiating immune responses. B lymphocytes, through antibody production and antigen presentation, complete this defensive network. Using IHC and the FCM "basal" panel, we showed that SLNs and other dissected lymph nodes contain all these essential immune populations. In our samples, leukocytes (CD45) represented on average 96% (± 3.79) of isolated cells, underscoring the profoundly immune nature of lymph nodes. Among these, CD4 and CD8 T lymphocytes and B lymphocytes (CD19/CD20) constituted predominant subpopulations. Furthermore, in patients with benign histology, these cells were also present despite the absence of tumour cells and can therefore be mobilised if needed. These findings emphasise that lymph nodes are not merely anatomical relays for tumour dissemination, but genuine immunological sites. The functional interaction between the tumour-draining node and the tumour microenvironment, as demonstrated in prior studies [24], supports the hypothesis that SLND could deplete the cellular reservoir required for effective anti-tumour responses, potentially compromising the efficacy of adjuvant immunotherapies.

These immune cells are central to the principles of immunotherapy and condition its effectiveness. After neoadjuvant immunotherapy, patients with complete pathological responses have been shown to exhibit significantly higher levels of CD8 T lymphocytes and dendritic cells in both the tumour microenvironment and lymph nodes compared with those without complete responses [25]. Honigsberg et al. demonstrated that the tumour-draining lymph node actively orchestrates anti-tumour immune responses by producing two types of tumour-specific lymphocyte clones: local CD8 T lymphocytes with potent intranodal activity, and circulating CD8 T lymphocytes with migratory, distal surveillance capacity [7]. Immunotherapy also increases the diversity and cytotoxic activity of these two clonal pools. Removing lymph nodes during surgery may therefore excise the very site of T-cell activation and impair systemic responses. In line with this, a murine model showed that recurrence-free survival was significantly better when lung cancer surgery was performed without lymph-node dissection than with dissection [26]. Although these data derive from animals, Deng et al. reported in humans that extensive lymph-node dissection (resection of more than 16 lymph nodes) was associated with decreased anti-tumour immunity and reduced response to immunotherapy at recurrence after surgical resection [27].

Moreover, among trials of adjuvant immunotherapy, some have yielded negative results, whereas IMpower010—evaluating one year of atezolizumab (a monoclonal antibody targeting PD-L1) after surgical resection and adjuvant chemotherapy—demonstrated a benefit in recurrence-free survival [28]. One explanation may be that patients in that study did not undergo complete SLND but rather dissection of stations 7 and 4 on the right and stations 7, 5, and 6 on the left, or even simple sampling. Partial preservation of the lymphatic immune system may have allowed a more effective response to immunotherapy, which could account for these outcomes. Collectively, this raises the possibility of an immune-preserving nodal strategy i.e., a non-extended lymphadenectomy that provides high-quality staging while avoiding immune system impairment—within which the SLN technique could be particularly valuable.

We showed by IHC that the SLN harbours a CD8 T-cell population comparable to that of other nodes in the dissection, whereas the metastatic node appears depleted in CD8 cells relative to healthy lymph nodes. FCM analysis confirmed equivalence in CD8 cells within the "SLN" group and absence of equivalence in the "pN+" group, although in the latter the difference favoured the metastatic node, which contained more CD8 cells than the other nodes (positive difference of 3.014). This apparent discrepancy may reflect that the pN+ nodes studied by IHC were all invaded by metastases > 2 mm, likely creating a highly immunosuppressive environment, whereas in FCM, 50% (2/4) of pN+ nodes contained micrometastases of 0.2–2 mm, potentially triggering an anti-tumour immune response. That CD8 T lymphocytes are present in similar proportions in SLN and non-SLN nodes suggests that, when the SLN is pN0, omitting SLND could preserve an immunocompetent nodal compartment and thereby maintain the patient's immune capacity. Conversely,

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the relative paucity of CD8 cells in metastatic nodes strongly indicates locally impaired immunity, reinforcing the notion of persistent immune activity in nodes more distant from the tumour, as previously described (29). The "memory" panel enabled exploration of immune-memory subpopulations within lymph nodes. We demonstrated the presence of stem-like memory T cells (Tscm) and central memory T cells (Tcm) in SLN and in other nodes, with good concordance. Memory T cells underpin durable anti-tumour immunity through long-term persistence and rapid reactivation upon antigen re-encounter. Tscm (CD45RA+ CCR7+ CD95+) combine robust self-renewal with the capacity to differentiate into all other memory and effector T subsets (30). Their longevity and proliferative potential make them attractive targets in cancer immunotherapy, notably as reservoirs capable of sustaining long-term responses, and they are now considered strategic targets for adoptive immunotherapies (e.g., CAR-T cells, tumour-infiltrating lymphocyte transfer) [31]. Tcm (CD45RA− CCR7+) predominantly reside in lymph nodes, have high proliferative capacity, and provide an essential link between Tscm and effector memory T cells (Tem) [32]. Unlike Tem, which circulate in peripheral tissues and rapidly exert cytotoxic function, Tcm generate powerful secondary effectors after antigenic stimulation while persisting over the long term. Several studies have associated the presence of Tcm and Tscm in tumour-draining nodes with greater efficacy of immunotherapies [27]. Connolly et al. showed that stem-like CD8 T cells persist in draining lymph nodes and continuously fuel intratumoural anti-tumour responses [33], highlighting lymph nodes as active immune reservoirs.

The evolving role of the SLN in thoracic surgery mirrors experience in breast cancer, where practice has shifted from systematic axillary dissection to a more selective, conservative strategy that combines reliable staging, reduced morbidity, and immune preservation. Historically, treatment relied on systematic axillary lymph-node dissection [34], which was associated with substantial morbidity [35]. Introduction of the SLN technique profoundly changed practice: randomised trials demonstrated that SLN analysis alone provides reliable staging while significantly reducing post-operative complications [36]. Moreover, there is no difference in OS, DFS, or local control between women undergoing additional axillary dissection and those who do not when the SLN is pN0 [10]. Axillary dissection has therefore become reserved for patients with proven or extensive nodal disease, whereas in most cases SLN excision and analysis suffice to guide therapy [37]. Beyond staging, several studies have identified the SLN as a key site of immune interaction with the tumour [38], reinforcing the view that it is not simply an anatomical relay but a central immunological player. This evolution in breast cancer paves the way for similar considerations in lung cancer, where the SLN technique may allow high-quality staging alongside preservation of anti-tumour immunity.

This study has limitations: the ENB technique entails significant equipment costs and a learning curve; the sample sizes for both IHC and FCM analyses were small; patients in the "SLN" group were highly selected (stage I), reducing representativeness; and follow-up in the translational component remains insufficient to fully assess prognostic implications. It also has strengths: to our knowledge, this is the largest SLN cohort in lung cancer evaluating long-term outcomes, and the first to explore lymph-node immune populations in lung cancer patients in such an exhaustive manner. Unlike most previous studies, it relies on identification of the true SLN using a specific technique, rather than analysis of a proximal drainage node, ensuring more accurate staging and improved prognostic assessment. Integration of retrospective and prospective elements using two complementary analytical methods strengthens the validity of our findings.

Conclusion

This study supports the proposition that the SLN technique may allow limitation of the extent of lymph-node dissection while ensuring reliable and potentially more accurate staging, thereby contributing to more conservative surgery that preserves the patient's immune system. Although these findings require confirmation in larger cohorts with longer follow-up, they open the way to changes in surgical practice in thoracic oncology, integrating accurate staging with immune preservation to optimise responses to systemic treatments.

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Supplementary data

Sentinel lymph node in non-small cell lung cancer: longterm outcomes and immunopathological consequences

Florent Stasiak, Loic Reppel, Mickael Schaeffer, Guillaume Gauchotte, Arthur Streit, Joelle Siat, Lucie Schnedecker, Joseph Seitlinger, Stephane Renaud

IHC protocol

To prepare the slides, the paraffin blocks were cooled in the freezer for 10 to 15 minutes to harden the paraffin, making it easier to cut with the microtome. Once cooled, seven slides per block were prepared using the microtome, with a cut thickness of between 3 and 5 micrometres.

HES staining was performed on a Coverstainer automated system (Agilent®, Santa Clara, United States) according to the steps detailed in table 1. For IHC, the blocks were cut to 4 µm, then the slides were dried in an oven at 56°C for 45 minutes, fixed in 10% buffered formalin and then passed through the DAKO Omnis automated system (Agilent®). The antibody clones used and the protocols employed are shown in table 2.

Table 1: HES staining protocol on Coverstainer automated system

Station	Dye	Time
1	XYLENE	3 min
2	XYLENE	3 min
3	ALCOHOL 100	10 sec
4	ALCOHOL 100	2 min
5	ALCOHOL 100	2 min
6	WATER	1 min
7	HEMATOX	10 min
8	WATER	1 min
9	BLUING BUFFER	1 min
10	WATER	1 min
11	ALCOHOL 100	1 min
12	EOSIN	2 min
13	ALCOHOL 100	30 sec
14	SAFRAN	1 min
15	ALCOHOL 100	1 min
16	ALCOHOL 100	1 min
17	XYLENE	2 min

CMF protocol

Antibodies and fluorochromes

- Basal panel: CD45 VioBlue (130-110-637) for leukocytes, CD235a (Glycophorin A) FITC (130-117-688) for erythrocytes, CD3 PE (130-113-139) for T lymphocytes, CD19 PerCPVio700 (130-113-648) for B lymphocytes, CD11b PE-Vio770 (130-110-555) for monocytes, CD56 APC (130-113-310) for NK cells, CD66b APC-Vio770 (130-120-060) for granulocytes.
- 'T lymphocyte' panel: CD45RO VioBlue (130-119-620) for identification of memory/naive cells, CD4 FITC (130-114-722) for CD4 T lymphocytes, CD8 APC Vio770 (130-110-819) for CD8 T lymphocytes, CD279 PD-1 PE (130-120-388) for PD-1 labelling, CD11c PerCP-Vio700 (130-128-716) for dendritic cells, CD127 PE-Vio770 (130-113-977) and CD25 APC (130-113-846) for Treg lymphocytes.
- Memory panel: CD45 PE (130-110-632) for leukocytes, CD3 FITC (130-113-138) for T lymphocytes, CD4 PeVio770 (130-113-227) for CD4 T lymphocytes, CD8 APC Vio770 (130- 110-681) for CD8 T lymphocytes, CD95 APC (130-113-005), CD45Ra VioGreen (130-113- 369) and CCR7 VioBlue (130-117-353) for memory phenotypes.

Table 2. Clones and IHC protocols for DAKO Omnis automated systems

Antibodies	Supplier	Clone	Dilution	Protocol	
				pH	Antibody incubation time
CD20	Agilent®	L26	Ready to use	High	12 min 30
CD3	Agilent®	Polyclonal	Ready to use	Low	20 min
CD4	Agilent®	4B12	Ready to use	High	20 min
CD8	Agilent®	C8/14413	Ready to use	High	10 min
CD56	Agilent®	123C3	Ready to use	High	20 min

Immunophenotyping protocol

- Double filtration using 70µm filters (Pre-Separation Filters, 70µm, 130-095-823) and 30µm (Pre-Separation Filters, 30µm, 130-041-407) in 15mL Falcon® tubes to remove tissue fragments and aggregates.
- Centrifugation of tubes at 400G for 10 minutes.
- Removal of the supernatant, then dissociation of the pellets with 300µL of PBS.
- Transfer of 100µL per cytometry tube.
- Preparation of the antibody mix in a cone for each panel: 4µL of each antibody with 4µL of PBS. For the 'basal' and 'T lymphocyte' panels, addition of 4µL of Viobility 405/520 Fixable Dye (130-130-404) to assess cell viability. For the 'memory' panel, 5µL of 7-AAD Staining Solution (130-111-568) is added 5 minutes before passing through the cytometer as a viability marker. A quantity of 8µL of the antibody mix is added to the corresponding tubes according to the panels.

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- Incubation of the tubes for 15 minutes at room temperature and in the dark.
- Washing of the tubes with 1 mL of PBS, then centrifugation for 10 minutes at 400G.
- Removal of the supernatant and addition of 100µL of PBS to each tube.
- Run through the flow cytometer.