#IASLCUPDATES

Iniciativa científica de:

GECP lung cancer research

LUNG CANCER UPDATES

IASLC HIGHLIGHTS

7-10 DE SEPTIEMBRE 2019

Con la colaboración de:

Bristol-Myers Squibb

illumina

Lilly

LUNG CANCER UPDATES

IASLC HIGHLIGHTS

7-10 DE SEPTIEMBRE 2019

BARCELONA

Iniciativa científica de:

GECP lung cancer research

Patología III

Dra. Lara Pijuan

Hospital del Mar de Barcelona

Con la colaboración de:

Bristol-Myers Squibb

illumina

Lilly

P2.09-33 - Prevalence of ROS1 (SP384)-Reactive Type II Pneumocyte Staining in Lung Tissue

LUNG CANCER UPDATES
IASLC HIGHLIGHTS
7-10 DE SEPTIEMBRE 2019

ROS1 (SP384) Prevalence in Type II Pneumocytes in Lung Tissue

Catherine H. Le, Richard S.P. Huang, Tyler Foutch, Charmi Patel, Amy Hanlon Newell, Greg Pate, Ina Menzl
Roche Tissue Diagnostics, Tucson, Arizona

Background

- Availability of a positive control sample is important to ascertain that specimen preparation and immunohistochemistry (IHC) processes are performed correctly. Lack of proper control tissue may result in an invalid IHC interpretation.
- ROS1 is a integral membrane tyrosine kinase protein that functions as a growth or differentiation factor receptor. Activation of ROS1 through gene fusion functions as a targetable oncogenic-driver event.[1]
- Given the low prevalence (1-2%) of ROS1 gene fusions (which leads to overexpression of ROS1) in non-small cell lung cancer (NSCLC), the feasibility of using ROS1-fusion positive NSCLC tissue as a control sample is challenging. ROS1 wild-type (WT) protein expression has been shown to be absent in normal lung tissue; however, ROS1 IHC staining has been observed in type II pneumocytes in non-neoplastic lung tissue.[1,2,3,4] Hence, we sought to characterize the prevalence of type II pneumocytes stained with the VENTANA ROS1 (SP384) Rabbit Monoclonal Antibody in normal or benign lung tissue as well as reactive type II pneumocytes in normal portions of NSCLC samples in order to assess the feasibility of using type II pneumocytes as a control tissue for ROS1 (SP384).

Design & Methods

- One hundred seventy-seven (177) formalin-fixed, paraffin-embedded (FFPE) normal or benign lung tissue samples were randomly procured.
- 60 NSCLC tumor cases (30 ROS1 positive, 30 ROS1 negative) were evaluated for ROS1 (SP384) tumor cell staining, and ROS1 (SP384) type II pneumocyte staining within those samples was noted during evaluation.
- All tissues were sectioned at 4 µm and stained with Rabbit Monoclonal Negative Control Ig, and VENTANA ROS1 (SP384) Rabbit Monoclonal Antibody IHC; NSLC tissues were also stained with hematoxylin and eosin (H&E).
- Normal or benign lung specimens stained with the ROS1 (SP384) antibody were evaluated for presence of type II pneumocytes. If present, type II pneumocytes were evaluated for reactivity based on a 0 to 3-point stain intensity scale. Reactivity was defined as any staining greater than 0. Data was analyzed for percent distribution for a range of stain intensities.

TABLE 1. IHC was performed by the following staining protocol on the BenchMark ULTRA system.

Procedure Type	Time and Temperature Settings
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	CC1 64 minutes, 100°C
Pre Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	16 minutes, 36°C
OptiView HQ Linker	8 minutes (default)
OptiView HRP Multimer	8 minutes (default)
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Different staining intensities in type II pneumocytes of non-neoplastic lung using the ROS1 (SP384) antibody

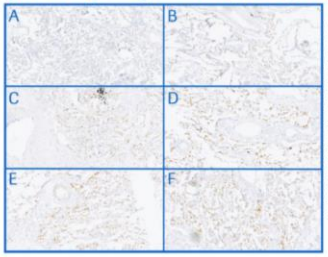


FIGURE 1. Representative images of type II pneumocytes with different staining intensities using the ROS1 (SP384) antibody in non-neoplastic lung samples. All micrographs taken at 20X. A) staining intensity = 1 B) staining intensity 1.75, C) staining intensity 2.25 D), E) and F) staining intensity 2.5.

Results

Out of the 177 normal or benign lung specimens evaluated, 165 slides stained with ROS1 (SP384) antibody were evaluable (12 cases were unacceptable due to tissue loss or were identified as neoplastic lung tissue). Type II pneumocytes were present in all 165 evaluable samples. Forty-seven type II pneumocyte samples (28.5%, 47/165) demonstrated no staining with ROS1 (SP384) antibody while 71.5% of the type II pneumocyte samples (118/165) demonstrated reactivity with ROS1 (SP384). In addition, 23.0% (38/165) of the type II pneumocyte samples demonstrated a stain intensity of at least 2. Of 60 NSCLC samples stained with the ROS1 (SP384) antibody, 31 were noted to have positive staining of reactive type II pneumocytes in the non-neoplastic cell populations.

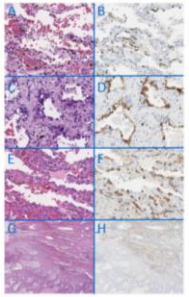
TABLE 2. Distribution of intensities of type II pneumocytes in normal or benign lung specimens stained with the ROS1 (SP384) antibody.

Observed staining intensity of type II pneumocytes	Number of non-neoplastic lung cases (out of 165 total)
0	47
(0,1]	26
[1,2]	76
[2,3]	16

Stain Intensity Score	Stain Intensity Description
0	NO staining
1+	WEAK staining
2+	MODERATE staining
3+	STRONG staining

Type II pneumocytes in NSCLC tumor samples stained with ROS1 (SP384) antibody

FIGURE 2. NSCLC samples stained with H&E (A, C, E, G) and the ROS1 (SP384) antibody (B, D, F, H) showing positive reactive type II pneumocyte staining. A-F) micrographs are taken at 20X and show reactive type II pneumocyte staining in the non-neoplastic tissue within the tumor sample. **G-H)** micrographs are taken at 4x and show tumor and non-tumor areas of the sample with non-tumor tissue containing positive type II pneumocytes.



Conclusions

- This cohort demonstrates a high prevalence of type II pneumocytes in normal or benign lung tissue that are reactive with the ROS1 (SP384) antibody, with a range of stain intensities. In addition, type II pneumocyte staining at tumor periphery is also observed in NSCLC cases. These cases must be interpreted with care, in particular not to attribute this as ROS1 expression in lepidic growth of tumor.
- In summary, type II pneumocytes can serve as internal positive control in immunostaining with the ROS1 (SP384) antibody. This finding suggests that the use of reactive type II pneumocytes in benign lung tissue as a control tissue for the ROS1 (SP384) antibody is feasible.

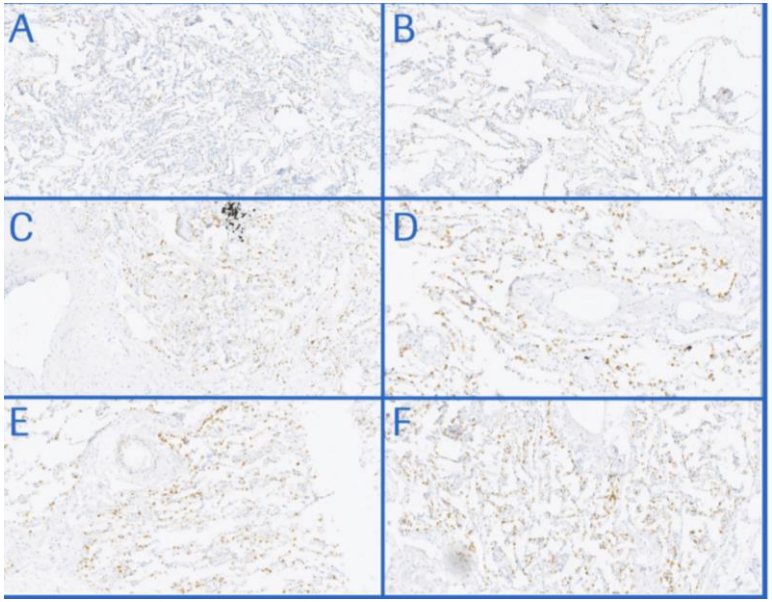
Iniciativa científica de:
GeCP lung cancer research

P2.09-33 - Prevalence of ROS1 (SP384)-Reactive Type II Pneumocyte Staining in Lung Tissue

LUNG CANCER UPDATES
IASLC HIGHLIGHTS
7-10 DE SEPTIEMBRE 2019

Observed staining intensity of type II pneumocytes	Number of non-neoplastic lung cases (out of 165 total)
0	47
(0,1]	26
(1,2]	76
(2,3]	16

Stain Intensity Score	Stain Intensity Description
0	**NO** staining
1+	**WEAK** staining
2+	**MODERATE** staining
3+	**STRONG** staining



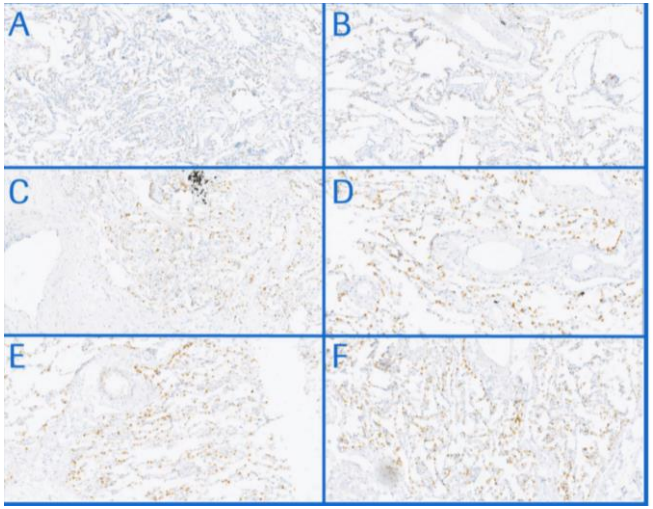
Iniciativa científica de:
GeCP
lung cancer research

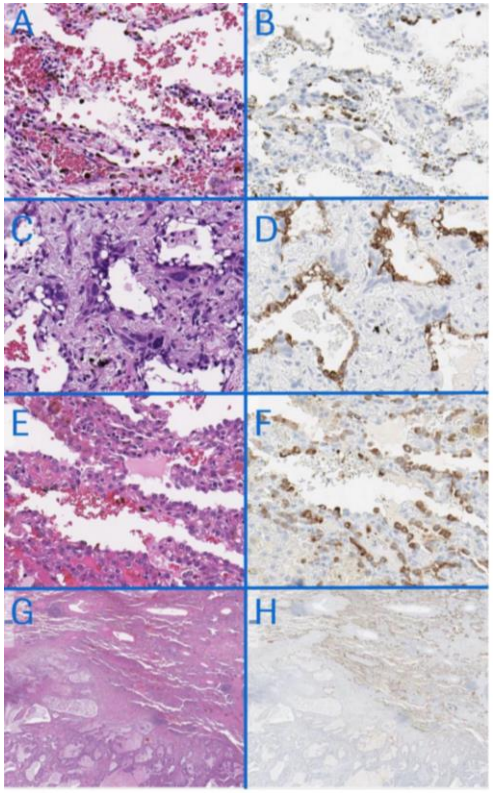
P2.09-33 - Prevalence of ROS1 (SP384)-Reactive Type II Pneumocyte Staining in Lung Tissue

LUNG CANCER UPDATES
IASLC HIGHLIGHTS
7-10 DE SEPTIEMBRE 2019

Observed staining intensity of type II pneumocytes	Number of non-neoplastic lung cases (out of 165 total)
0	47
(0,1]	26
(1,2]	76
(2,3]	16

Stain Intensity Score	Stain Intensity Description
0	**NO** staining
1+	**WEAK** staining
2+	**MODERATE** staining
3+	**STRONG** staining





Iniciativa científica de:
GeCP
lung cancer research

Bronchial washing fluid shows higher diagnostic yields tan plasma for detecting EGFR-TKI sensitizing mutation by ddPCR in lung cancer

Bronchial washing fluid shows higher diagnostic yields than plasma f detecting EGFR-TKI sensitizing mutations by ddPCR in lung cancer

Sang Hoon Lee[1], Eun Young Kim,, Arum Kim[1], Yoon Soo Chang[1]
[1]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea

Introduction

Efforts to select appropriate therapeutic agents using circulating tumor DNA (ctDNA) are widely used for the management of advanced lung adenocarcinoma, but there is still room for improvement in diagnosis rate. In this study, we compared the EGFR-tyrosine kinase sensitizing mutation (mEGFR) detection rate of bronchial washing fluid (BWF) with that of blood in lung adenocarcinoma using tissue genotype as standard reference.

Materials and Methods

Paired blood-bronchial washing specimens were recruited from the 73 patients who were pathologically confirmed to have lung cancer and tumor EGFR mutation status was clarified by tumor tissue genotyping. BWF and blood samples were obtained at the time of initial visit for pathologic examination of lung tumor and the interval for securing paired specimens was less than 24 hours. Genotyping of plasma and BWFs was performed using BioRad's digital droplet PCR (ddPCR) according to the manufacture's instruction.

Results

Table 1. Demographic characteristics of the study population.

	Total (n= 73)	Early stage (n=38)	Advanced stage (n=35)
Age (year)	65.3 ± 9.8	65.0 ± 8.1	65.7 ± 11.5
Sex (F/M)	35/38 (47.9/52.1)	20/18 (52.6/47.4)	15/20 (42.9/57.1)
Smoking status			
Never smoker	45 (61.6)	25 (65.8)	20 (57.1)
Former smoker	22 (30.1)	10 (26.3)	12 (34.3)
Current smoker	6 (8.2)	3 (7.9)	3 (8.6)
Tumor type			
Adenocarcinoma	65 (89.0)	32 (84.2)	33 (94.3)
Squamous cell carcinoma	7 (9.6)	5 (13.2)	2 (5.7)
Sarcomatoid carcinoma	1 (1.4)	1 (2.6)	-
Lung cancer stage			
I/II/III/IV	26/10/10/27	26/10/2/-	-/-/8/27
EGFR genotyping			
Wild type	35 (47.9)	18 (47.4)	17 (48.6)
E19del	19 (26.0)	12 (31.6)	7 (20.0)
L858R	19 (26.0)	8 (21.1)	11 (31.4)

Table 1 describes the characteristics of enrolled cases. The mean age of the study population was 65.3 ± 9.8 years, and 38 (52.1%) patients were male and 35 (47.9%) were female. Twenty-eight (38.4%) patients had a history of smoking and the mean smoking level of all-life was 12.6 ± 21.4 pack-year. Nearly all enrolled patients had adenocarcinoma (89.0%), and one (1.4%) had pulmonary sarcomatoid carcinoma. The average of the longest diameter of tumors was 3.5 ± 2.0 cm. Twenty-six patients were in stage I, 10 in stage II, 10 in stage III, and 27 in stage IV. Of the 73 patients, 35 (47.9%) showed wild-type EGFR and 38 showed mutations in the EGFR-tyrosine kinase domain; 19 patients had L858R substitution and 19 showed E19del, respectively. Baseline characteristics did not significantly differ between early (stages I–IIIA) and advanced (stages IIIB–IV) stages of lung cancer.

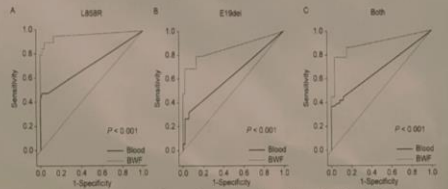


Figure 1. Comparison of receiver operator characteristic (ROC) curves according to sample (plasma or BWF) and EGFR mutational genotype. (A) L858R, (B) E19del, and (C) Both. BWF, bronchial washing fluid

When the genotype from tumor tissue was used as a reference, the AUC was 0.717 (95% CI: 0.592–0.842) when L858R was detected in a blood sample, whereas the value was 0.961 (95% CI: 0.901–1.000) for BWF (Figure 1A). Testing with BWF more accurately predicted the presence of L858R in tumor tissue than in blood, and the difference in AUC was significant ($p<0.001$, DeLong's test). Predicting the presence of E19del in tumor tissue showed similar results to the findings of the L858R test, showing that the AUC in the E19del test using blood was only 0.632 (95% CI: 0.519–0.745), while that from BWF was 0.858 (95% CI: 0.746–0.969) (Figure 1B). BWF was more useful for predicting E19del compared to blood, and the difference in the AUC was significant ($p<0.001$, DeLong's test). By combining the results of L858R and E19del, variables were simplified and the usefulness of blood and BWF were compared for predicting the tissue EGFR mutation status. The AUC for detecting tumor EGFR mutations using blood samples was 0.686 (95% CI: 0.592–0.780), whereas that using BWF was 0.895 (95% CI: 0.822–0.969), showing a significant difference between specimens ($p< 0.001$, DeLong's test) (Figure 1C).

Table 2. Sensitivity, specificity, and concordance rate of ddPCR according to EGFR mutation.

	L858R			E19del		
	Plasma	BWF	p-value	Plasma	BWF	p-value
AUC	0.717	0.961	<0.001	0.632	0.858	<0.001
Sensitivity (%)	47.37	89.47		31.58	68.42	
Specificity (%)	98.15	96.30		94.44	98.15	

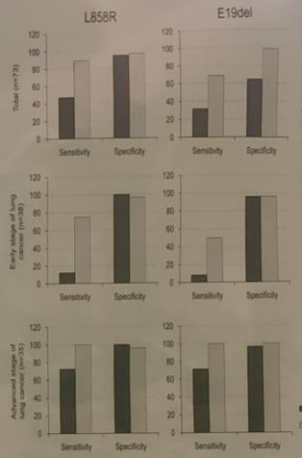


Figure 2 Comparison of sensitivity and specificity according to EGFR mutational genotype and lung cancer stage.

(A)Total patients, (B)Early stage of lung cancer (I–IIIA) (C)Advanced stage of lung cancer (IIIB–IV) BWF, bronchial washing fluid

The detection rate of mEGFR from blood samples was dependent on the disease stage. To confirm whether these findings can be applied to BWF, we investigated the mEGFR detection yields in each sample type by dividing the stage of lung cancer into early stages and advanced stages and then compared the detection rate between each type of sample in each stage group (Table 2 and Figure 2). In the early stage group (stage I–IIIA; n=38), the AUC value of blood samples for predicting tissue mEGFR was 0.504, whereas that of BWF was 0.768, showing a significant difference between sample types ($p=0.008$). In the advanced stage group (stage IIIB–IV, n=35), the AUC value obtained from BWF was higher than that from the blood; AUC from BWS was 1.000 (95% CI: 0.899–1.000), and 0.879 (95% CI: 0.724–0.964) from blood. This shows that the results from BWF predicted the tumor tissue mEGFR status more accurately than that by using plasma ($p=0.043$, DeLong's test).

Conclusions

In conclusion, compared to plasma, liquid biopsy using BWF is more effective for identifying mEGFR. This may be useful for avoiding invasive tissue biopsy and complications such as pneumothorax or bleeding following tissue biopsy. Furthermore, BWF may be an alternative method for re-biopsy to detect the presence of the T790M mutation via ddPCR.

Bronchial washing fluid shows higher diagnostic yields tan plasma for detecting EGFR-TKI sensitizing mutation by ddPCR in lung cancer

Table 2. Sensitivity, specificity, and concordance rate of ddPCR according to EGFR mutation.

	L858R			E19del		
	Plasma	BWF	p-value	Plasma	BWF	p-value
AUC	0.717	0.961	<0.001	0.632	0.858	<0.001
Sensitivity (%)	47.37	89.47		31.58	68.42	
Specificity (%)	98.15	96.30		94.44	98.15	

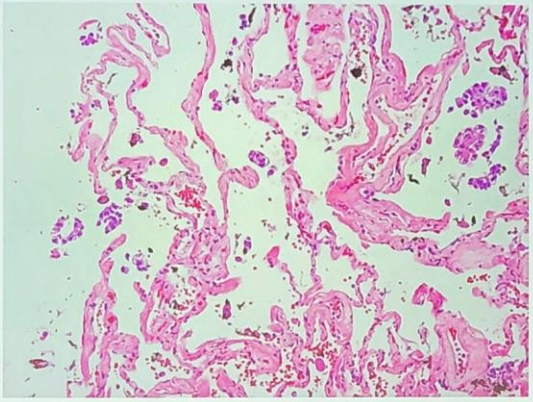
Conclusions

In conclusion, compared to plasma, liquid biopsy using BWF is more effective for identifying mEGFR. This may be useful for avoiding invasive tissue biopsy and complications such as pneumothorax or bleeding following tissue biopsy. Furthermore, BWF may be an alternative method for re-biopsy to detect the presence of the T790M mutation via ddPCR.

IASLC

2019 World Conference on Lung Cancer
September 7–10, 2019 | Barcelona, Spain

wclc2019.iaslc.com | #WCLC19
Conquering Thoracic Cancers Worldwide



Spread Through Air Spaces (STAS)

STAS is a histologic feature that carries prognostic significance, that may or may not be exacerbated by iatrogenic handling.

Not an architectural pattern as in (lepidic, acinar, etc), rather a feature of invasion (as in lymphovascular invasion)

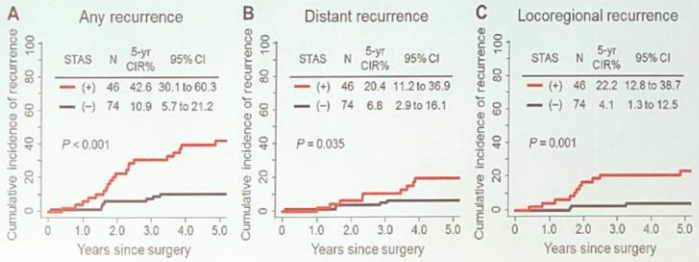
STAS should not be included in tumor size or comprehensive histologic subtyping

May also have an impact on R category status in resections

BUT

Requires standardized definition, criteria for distinction from artefact, greater understanding of the effect of iatrogenic handling

Figure 3 CIR by STAS in the limited resection group



Multivariate analysis, presence of tumor STAS remained independently associated with the risk of recurrence (hazard ratio, 3.08; *P=0.014*).
Kadota K et al; JTO 2015; 10:806-14

Correlation of Tumor Spread Through Air Spaces and Clinicopathological Characteristics in Surgically Resected Lung Adenocarcinomas

LUNG CANCER UPDATES
IASLC HIGHLIGHTS
7-10 DE SEPTIEMBRE 2019

National Taiwan University

Correlation of Tumor Spread Through Air Spaces and Clinicopathological Characteristics in Surgically Resected Lung Adenocarcinomas

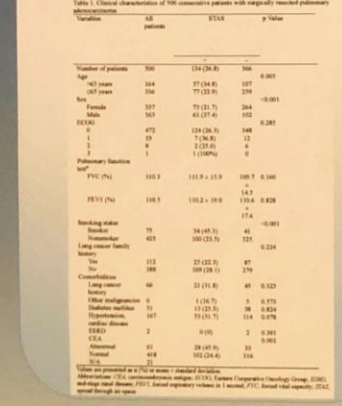
Mong-Wei Lin[1], Szu-Yen Hu[1], Min-Shu Hsieh[2], Jin-Shing Chen[1]
Department of Surgery[1] and Pathology[2], National Taiwan University Hospital, Taipei, Taiwan

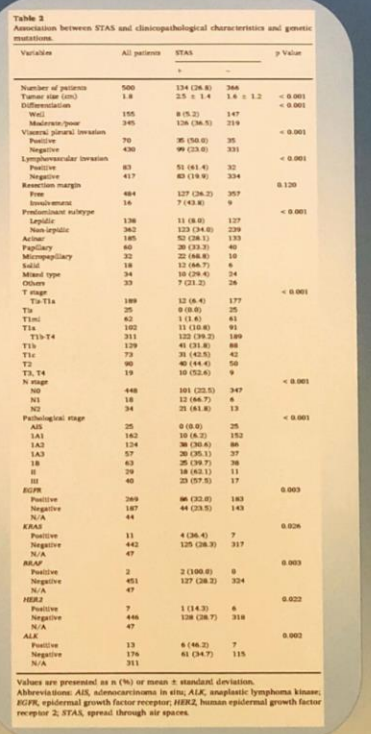
Objective

Tumor spread through air spaces (STAS) has recently been reported as a novel invasive pattern in lung adenocarcinoma, but the correlation between other clinicopathological and genetic profiles has not been well studied. The aim of this study was to investigate these correlations in patients with surgically resected lung adenocarcinoma.

Methods

Five hundred consecutive patients with lung adenocarcinoma who underwent curative lung tumor resection and with available STAS profile were reviewed retrospectively from January to December 2016. The correlations of STAS presence and clinicopathological and genetic characteristics were analyzed.





Values are presented as n (%) or mean ± standard deviation.
Abbreviations: *AIS*, adenocarcinoma in situ; *ALK*, anaplastic lymphoma kinase; *EGFR*, epidermal growth factor receptor; *HER2*, human epidermal growth factor receptor 2; *STAS*, spread through air spaces.

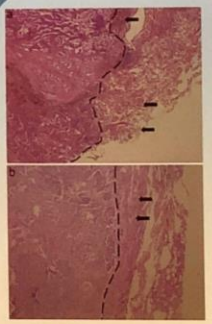
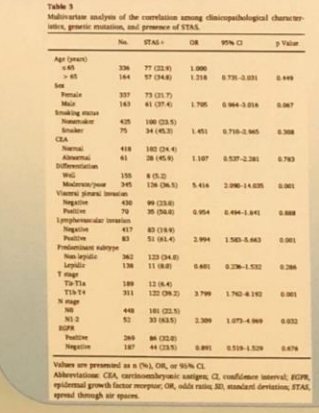


Figure: Tumor spread through air spaces was defined as tumor cells in alveolar spaces beyond the edge of the main tumor. Tumor cells were arranged in (a) mixed micropapillary clusters and single cells or (b) small solid nests (original magnification ×40).



Values are presented as n (%), OR, or 95% CI.
Abbreviations: *CEA*, carcinoembryonic antigen; *CI*, confidence interval; *EGFR*, epidermal growth factor receptor; *OR*, odds ratio; *SD*, standard deviation; *STAS*, spread through air spaces.

Results

The study included 500 consecutive patients with surgically resected lung adenocarcinoma. Most patients were female (67.4%) and nonsmokers (85%). The predominant histologic subtype was lepidic pattern in 138 (27.6%) patients. One hundred thirty-four patients (26.8%) had positive STAS. The pathological stage of these patients was adenocarcinoma in situ, IA, IB, II, and III in 25 (5%), 343 (68.6%), 63 (12.6%), 29 (5.8%), and 40 (8%), respectively. Other demographic data and clinicopathological characteristics of the total study group are given in Tables 1 and 2.

Multivariate analysis (Table 3) showed that the presence of STAS was significantly correlated to higher T ($p = 0.001$) and N ($p = 0.032$) stages, moderate/poor differentiation ($p = 0.001$), and the presence of vascular invasion ($p = 0.001$). Although positive epidermal growth factor receptor mutation and non-lepidic histologic subtypes were correlated with the presence of STAS in the univariate analysis, they were not significantly correlated with the presence of STAS in the multivariate analysis ($p = 0.676$ and 0.283, respectively).

Conclusions

Our results showed that STAS was significantly correlated with several invasive clinicopathological characteristics, including higher T and N stages, moderate/poor differentiation, and the presence of vascular invasion in surgically resected lung adenocarcinoma. The correlation may lead to poor clinical outcomes in patients with positive STAS. Based on our results and current evidence, the presence of STAS may be considered as a staging profile in future staging system.

Iniciativa científica de:
GECP lung cancer research