Ex Vivo Interferon Gamma Production by Peripheral Immune Cells Predicts Survival in Lung Adenocarcinoma

Sung Soo Ahn,[1] Minkyung Kwon,[3] Mindong Sung,[1] Seung Min Jung,[1] Sang-Won Lee,[1] Yong-Beom Park,[1] Sang Taek Kim,[4] Jason Jungsik Song[1,2]

Abstract

Immunotherapies targeting the immune checkpoint receptor have shown promising results in non–small-cell lung cancer. Nevertheless, there are limitations in current biomarkers for evaluating the function of immune cells. Interferon gamma (IFN-γ) is a proinflammatory cytokine that contributes to cancer recognition and elimination. This study demonstrated ex vivo IFN-γ production might be a biomarker for predicting patient prognosis in lung adenocarcinoma.

Background: Lung cancer is one of the most lethal malignancies, with a 5-year survival rate < 20% in patients with stage IV lung cancer. Impaired host immunity is associated with lung cancer pathogenesis, and interferon gamma (IFN-γ) plays an important role in antitumor immune surveillance. We evaluated the clinical significance of ex vivo production of IFN-γ in patients with lung adenocarcinoma. **Patients and Methods:** We reviewed the medical records of 109 treatment-naive patients with lung adenocarcinoma who had undergone IFN-γ releasing assay. Differences in the IFN-γ level in nil and mitogen tubes were defined as ex vivo IFN-γ production. Correlation analysis was performed to evaluate the correlation between ex vivo IFN-γ production, cancer staging, and Eastern Cooperative Oncology Group performance status. The optimal cutoff values of low and high ex vivo IFN-γ production were estimated using receiver operator characteristic curve analysis. Cox proportional hazard analyses were used to evaluate the prognostic factors of 1-year overall patient survival. **Results:** Ex vivo IFN-γ production correlated with N stage, M stage, cancer staging, and Eastern Cooperative Oncology Group performance status. Low ex vivo IFN-γ production (ex vivo IFN-γ production ≤ 7.79 IU/mL) was independently associated with 1-year overall survival (odds ratio = 3.289; 95% confidence interval, 1.573-6.872; $P = .002$). Additionally, low ex vivo IFN-γ production was an independent predictor of 1-year overall survival in patients with stage IV cancer (odds ratio = 3.156; 95% confidence interval, 1.473-6.760; $P = .003$). **Conclusion:** Ex vivo IFN-γ production before treatment might be a useful biomarker for predicting prognosis in patients with lung adenocarcinoma.

Clinical Lung Cancer, Vol. 20, No. 3, e299-308
Keywords: Biomarker, IFN-γ, IFN-γ releasing assay, Lung cancer, Prognosis

Introduction

Lung cancer is one of the leading causes of death worldwide, despite the expanding knowledge of cancer biology and targeted treatment.[1,2] The 5-year survival of patients with stage IV lung cancer remains < 20%.[3,4] To overcome this, there has been growing interest in cancer immunotherapy.[5] Immunotherapies targeting the immune checkpoint receptor programmed cell death 1 or programmed cell death ligand 1, and cytotoxic T-lymphocyte–associated protein 4 have shown promising results in non–small-cell lung cancer.[6-9]

Interferon gamma (IFN-γ) is a proinflammatory cytokine mainly produced by T cells and natural killer (NK) cells that contributes to cancer recognition and elimination.[10] Mice with impaired IFN-γ

[1]Division of Rheumatology, Department of Internal Medicine
[2]Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul, South Korea
[3]Department of Pulmonary and Critical Care Medicine, Mayo Clinic, Jacksonville, FL
[4]Department of General Internal Medicine, Section of Rheumatology and Clinical Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX

Submitted: Aug 28, 2018; Revised: Jan 11, 2019; Accepted: Jan 17, 2019; Epub: Jan 26, 2019

Address for correspondence: Jason Jungsik Song, MD, PhD, Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, South Korea
Fax: +82-2-393-6884; e-mail contact: JSKSONG@yuhs.ac

1525-7304/$ - see frontmatter
https://doi.org/10.1016/j.cllc.2019.01.002

Table 1 Baseline Characteristics of 109 Enrolled Patients

Variable	Value
Clinical Variables	
Age (years)	60.0 (52.5-70.3)
Male sex	62 (56.9)
Current smoker	31 (28.4)
Smoking (pack-years)	0.0 (0.0-25.0)
T Stage	
I	17 (15.6)
II	22 (20.2)
III	24 (22.0)
IV	46 (42.2)
N Stage	
0	25 (22.9)
I	11 (10.1)
II	19 (17.4)
III	54 (49.5)
M Stage	
0	24 (22.0)
I	85 (78.0)
Cancer Stage	
I	11 (10.1)
II	2 (1.8)
III	11 (10.1)
IV	85 (78.0)
ECOG PS	
0-I	81 (74.3)
II-IV	28 (25.7)
Gene Mutation	
EGFR mutation[a]	39 (35.8)
KRAS mutation[b]	4 (3.7)
ALK translocation[c]	8 (7.3)
Comorbidities	
Hypertension	34 (31.2)
Diabetes mellitus	14 (12.8)
Coronary/cerebrovascular disease	6 (5.5)
Chronic obstructive pulmonary disease	3 (2.8)
Arrhythmia	3 (2.8)
Treatment After Diagnosis	
Chemotherapy	76 (69.7)
Radiotherapy	49 (45.0)
Follow-up duration (months)	13.7 (2.4-31.7)
IGRA Results	
Negative	57 (52.3)
Positive	47 (43.1)
Indeterminate	5 (4.6)
IFN-γ Level (IU/mL)	
Nil (IU/mL)	0.07 (0.05-0.13)
Tuberculosis antigen (IU/mL)	0.31 (0.12-2.31)
Mitogen (IU/mL)	13.57 (10.73-17.74)
Ex vivo IFN-γ production (IU/mL)[d]	13.32 (10.67-17.67)

Values are expressed as median (interquartile range) or n (%).
Abbreviations: ALK = anaplastic lymphoma kinase; ECOG PS = Eastern Cooperative Oncology Group performance status; EGFR = epidermal growth factor receptor; IFN = interferon; IGRA = interferon-γ releasing assay; KRAS = Kirsten rat sarcoma viral oncogene.
[a]Total number of patients tested was 88.
[b]Total number of patients tested was 53.
[c]Total number of patients tested was 67.
[d]Ex vivo IFN-γ production was estimated by calculating difference in IFN-γ production between mitogen tube and nil tube (mitogen minus nil).

function were not able to eliminate cancer.[10,11] However, in previous studies that evaluated serum IFN-γ levels in lung cancer, inconsistent results were shown. Martin et al[12] reported decreased serum IFN-γ level in cancer patients was associated with poor prognosis despite similar serum level of IFN-γ between lung cancer patients and healthy control group members. Li et al[13] have reported lower IFN-γ level in lung cancer patients but lower IFN-γ level was not predictive of patient prognosis.

IFN-γ releasing assay (IGRA) is a test originally designed to detect latent *Mycobacterium tuberculosis* (TB) infection. IGRA is composed of 3 tubes (nil, mitogen, and TB antigen), and its results are interpreted according to the absolute IFN-γ production and the difference of IFN-γ production between the tubes.[14] The IFN-γ level in the nil tube measures baseline IFN-γ production, whereas the IFN-γ level in the mitogen tube indicates IFN-γ production after nonspecific immune-cell stimulation with phytohemagglutinin (PHA). Because IFN-γ plays an important role in host immune function against cancer, we sought to examine whether the IFN-γ production in peripheral blood immune cells stimulated by mitogen is associated with patient survival in lung adenocarcinoma.

Patients and Methods

Patient Selection

We retrospectively reviewed the medical records of patients who were newly diagnosed of lung adenocarcinoma and who had undergone IGRA at the time of lung cancer diagnosis in Severance Hospital between August 2009 and May 2017. We searched our electronic medical record data by using the keywords of "lung cancer" and "IGRA." First, 301 patients were screened, and we excluded patients according to the following criteria: patients without evidence of primary lung cancer; patients with TB or non-*tuberculosis Mycobacterium* infection, active concurrent infection, and tested IGRA for tumor necrosis factor α inhibitor usage; patients with confirmed pathologic findings other than lung adenocarcinoma; and patients tested for IGRA after cancer treatment initiation (Supplemental Figure 1 in the online version). Patients were routinely screened for TB infection by sputum culture or bronchoscopy at cancer diagnosis, and patients who had positive acid-fast bacilli test on sputum culture or evidence of TB on pathology were excluded. Finally, 109 patients with lung adenocarcinoma were included. This study was approved by the institutional review board of Severance Hospital (approval 4-2018-0623) and was conducted in accordance with the principles set forth in the Declaration of Helsinki. The requirement for informed consent was waived because of the retrospective study design.

Table 2 Correlation Between IFN-γ Level in Individual Tubes With Cancer Burdens and ECOG PS

Characteristic	Nil (IU/mL)	Tuberculosis Antigen (IU/mL)	Mitogen (IU/mL)	Ex Vivo IFN-γ Production (IU/mL)[a]
T stage	0.159 (.099)	−0.111 (.251)	−0.118 (.221)	−0.123 (.202)
N stage	−0.010 (.915)	−0.093 (.335)	−0.273 (.004)	−0.273 (.004)
M stage	−0.022 (.819)	0.061 (.526)	−0.210 (.028)	−0.210 (.029)
Lung cancer stage[b]	0.040 (.682)	0.075 (.437)	−0.264 (.006)	−0.265 (.005)
ECOG PS	−0.039 (.689)	−0.121 (.209)	−0.318 (<.001)	−0.316 (<.001)

Values are presented as correlation coefficient (*r*) and *P* (in parentheses).
Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status; IFN = interferon.
[a]Ex vivo IFN-γ production was estimated by calculating difference in IFN-γ production between mitogen tube and nil tube (mitogen minus nil).
[b]Lung cancer stage was determined according to the 7th edition of the International Union Against Cancer's tumor, node, metastasis classification staging system.

Acquisition of Clinical Data

We collected clinical patient data by reviewing the hospital's electronic charting system. Demographic data included age, sex, current smoking status, and amount of cigarette exposure (pack-years). The Eastern Cooperative Oncology Group performance status (ECOG PS) was collected using previously reported criteria.[15] Lung cancer staging and the T stage, N stage, and M stage were determined according to the 7th edition of the International Union Against Cancer's tumor, node, metastasis classification staging system.[16] The presence of epidermal growth factor receptor (*EGFR*) and Kirsten rat sarcoma viral oncogene (*KRAS*) mutation as well as anaplastic lymphoma kinase (*ALK*) translocation status were also collected. The presence of comorbidities was identified using the 10th revision of the International Classification of Diseases.[17]

IGRA Test Method and Interpretation

Testing and interpretation of IGRA results with whole blood samples was performed using the QuantiFERON-TB Gold In-Tube test (Cellestis) according to the manufacturer's instructions. In brief, 1 mL of blood was drawn directly into each of the blood collection tubes: the nil tube (negative control: whole blood without antigens or mitogen), mitogen tube (positive control: whole blood with PHA), and TB antigen tube (whole blood with peptides of ESAT-6, CFP-10, and TB7.7 proteins simulating TB-specific antigens). Tubes were incubated overnight at 37°C. The level of IFN-γ (IU/mL) was determined by enzyme-linked immunosorbent assays using an automated microplate processor (Evolis Twin Plus; Bio-Rad Laboratories), and the results were reported as previously described.[18] Ex vivo IFN-γ production was defined as the difference in IFN-γ production between the mitogen tube and the nil tube (mitogen minus nil).[19]

Statistical Analysis

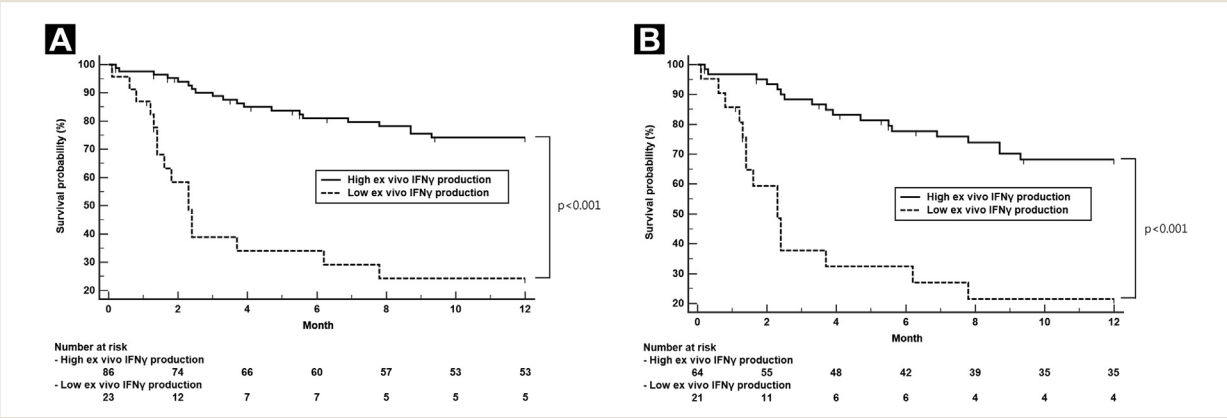
Data analysis was conducted using MedCalc 18.6.0 statistical software (MedCalc Software). Data are presented as medians with interquartile ranges, and categorical variables are expressed as frequencies and percentages. Continuous variables were compared using the Mann-Whitney *U* test or Kruskal-Wallis test, and categorical data were compared by the chi-square test or the Fisher exact test, as appropriate. To compare categorical data among multiple groups, the chi-square test for trend was used. Correlations between the IFN-γ level in the separate tubes and the T stage, N stage, M stage, lung cancer staging, and ECOG PS were analyzed using the Pearson correlation analysis. Univariate and multivariate Cox proportional hazard analyses with a forward stepwise method were used to compare variables for predicting 1-year overall patient survival. In multivariate analysis, only variables that were statistically significant in univariate analysis were included. Kaplan-Meier analysis and the log-rank test were used to compare 1-year overall patient survival according to ex vivo IFN-γ production. The optimal cutoff values

Table 3 Cox Proportional Hazard Analysis of Variables Related to 1-Year Survival (N = 109)

	Univariate Analysis			Multivariate Analysis		
Characteristic	OR	95% CI	*P*	OR	95% CI	*P*
Age	1.049	1.022-1.076	< .001	1.049	1.016-1.082	.003
Male sex	1.678	0.839-3.355	.144			
Current smoker	1.462	0.740-2.886	.274			
Smoking (pack-years)	1.023	1.004-1.042	.017	1.034	1.012-1.056	.002
Initial cancer stage	2.619	1.093-6.278	.031	2.790	1.099-7.086	.031
ECOG PS	2.339	1.606-3.407	< .001			
Chemotherapy after diagnosis	0.357	0.183-0.697	.003	0.303	0.134-0.685	.004
Radiotherapy after diagnosis	0.938	0.487-1.804	.847			
Presence of any comorbidities	1.011	0.521-1.962	.974			
Ex vivo IFN-γ production (IU/mL)	0.914	0.869-0.960	< .001			
Low ex vivo IFN-γ production[a]	5.372	2.756-10.472	< .001	3.289	1.573-6.872	.002

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; IFN = interferon; OR = odds ratio.
[a]Low ex vivo IFN-γ production was defined as ex vivo IFN-γ production ≤ 7.79 IU/mL.

Figure 1 Comparison of Overall 1-Year Survival in Patients With Low Versus High Ex Vivo IFN-γ Production. Overall 1-Year Survival Rate Is Significantly Lower in Patients With Low Ex Vivo IFN-γ Production Than in Those With High Ex Vivo IFN-γ Production Among (A) Total Patients (n = 109) and (B) Those With Stage IV Lung Adenocarcinoma (n = 85). Low Ex Vivo IFN-γ Production Was Defined as Ex Vivo IFN-γ Production ≤ 7.79 IU/mL; High, > 7.79 IU/mL



Abbreviation: IFN = interferon.

of low and high ex vivo IFN-γ production in predicting survival were set at ≤ 7.79 IU/mL and > 7.79 IU/mL, which were estimated using receiver operator characteristic curve analysis. In all statistical analyses, a 2-tailed *P* value of < .05 was considered statistically significant.

Results

Baseline Characteristics of Patients

Baseline characteristics of patients enrolled onto this study are shown in Table 1. A total of 109 patients with newly diagnosed lung adenocarcinoma were included. Median age was 60 years, and 56.9% were men. Thirty-one patients (28.4%) were current smokers. The majority of the patients had stage IV disease (78.0%), followed by 11 with stage I disease (10.1%), 2 with stage II (1.8%), and 11 with stage III (10.1%). Eighty-one patients (74.3%) had ECOG PS 0 or 1 during IGRA testing. Among the comorbidities included, hypertension (31.2%) and diabetes mellitus (12.8%) were the most prevalent. After the lung cancer diagnosis, 76 patients (69.7%) had undergone chemotherapy and 49 (45.0%) had undergone radiotherapy. The median duration of follow-up was 13.7 month.

Baseline IGRA Results

Fifty-seven patients (52.3%) had positive IGRA, 47 (43.1%) had negative IGRA, and 5 (4.6%) had indeterminate results. Median IFN-γ levels in the nil, TB antigen, and mitogen tube were 0.07, 0.31, and 13.57 IU/mL, respectively. The median ex vivo IFN-γ production was 13.32 IU/mL (Table 1).

Association of IFN-γ Level in Individual Tubes With Cancer Burdens and ECOG PS

A statistically significant correlation was detected between the IFN-γ level from the mitogen tube and ex vivo IFN-γ production and N stage, M stage, cancer staging, and ECOG PS (Table 2). The strongest correlation was observed between ECOG PS and IFN-γ level in the mitogen tube ($r = -0.318$, $P < .001$) and ex vivo IFN-γ production ($r = -0.316$, $P < .001$). However, there was no statistically significant association between the IFN-γ level in nil and TB antigen tubes and T stage, N stage, M stage, cancer staging, and ECOG PS.

Factors Related to 1-Year Overall Survival and Comparison of 1-Year Overall Survival Rate According to Ex Vivo IFN-γ Production

In univariate analysis, age, smoking (pack-years), cancer stage, ECOG PS, treatment with chemotherapy, and ex vivo IFN-γ production were significant variables associated with 1-year overall survival (OS). In multivariate analysis, age, smoking (pack-years), cancer stage, treatment with chemotherapy, and low ex vivo IFN-γ production (odds ratio = 3.289; 95% confidence interval, 1.573-6.872; $P = .002$) were associated with 1-year OS (Table 3). A comparison of patients with low ex vivo IFN-γ production (n = 23) and high ex vivo IFN-γ production (n = 86) by the log-rank test showed that the 1-year OS rate was significantly lower in those with low ex vivo IFN-γ production ($P < .001$) (Figure 1A). In subgroup patients of stage IV disease, 1-year OS rate was significantly lower in patients with low ex vivo IFN-γ production than in patients with high ex vivo IFN-γ production ($P < .001$) (Figure 1B).

Characteristics of Stage IV Lung Adenocarcinoma Patients With Low Versus High Ex Vivo IFN-γ Production

We compared characteristics of low ex vivo IFN-γ production patients with high ex vivo IFN-γ production patients among those with stage IV disease (Table 4). Of 85 patients with stage IV disease, 64 (75.3%) had high and 21 (24.7%) low ex vivo IFN-γ production. Although differences were not noted in age, sex, and smoking status, the proportion of patients with an ECOG PS of 2 to 4 was higher in patients with low versus high ex vivo IFN-γ production ($P = .003$). Patients with high ex vivo IFN-γ production had a higher frequency of *EGFR* mutation than those with low

Table 4 Baseline Characteristics of Patients With Stage IV Lung Adenocarcinoma

Characteristic	Low Ex Vivo IFN-γ Production (N = 21)	High Ex Vivo IFN-γ Production (N = 64)	*P*
Clinical Variables			
Age (years)	67.0 (55.5-72.0)	59.5 (50.5-70.5)	.203
Male sex	14 (66.7)	34 (53.1)	.280
Current smoker	6 (28.6)	15 (23.4)	.638
Smoking (pack-years)	0.0 (0.0-30.0)	0.0 (0.0-20.0)	.243
ECOG PS			.003
0-I	9 (42.9)	50 (78.1)	
II-IV	12 (57.1)	14 (21.9)	
Gene Mutation			
EGFR mutation[a]	4 (19.0)	29 (45.3)	.040
KRAS mutation[b]	1 (4.8)	3 (4.7)	.999
ALK translocation[c]	2 (9.5)	5 (7.8)	.999
Treatment			
Chemotherapy after diagnosis	14 (66.7)	50 (78.1)	.294
Radiotherapy after diagnosis	11 (52.4)	30 (46.9)	.663
Comorbidities			
Chronic obstructive pulmonary disease	1 (4.8)	2 (3.1)	.999
Coronary/ cerebrovascular disease	2 (9.5)	3 (4.7)	.593
Arrhythmia	0 (0.0)	3 (4.7)	.571
Hypertension	7 (33.3)	20 (31.3)	.860
Diabetes mellitus	5 (23.8)	6 (9.4)	.089
IGRA Results			
Negative	11 (52.4)	35 (54.7)	.855
Positive	6 (28.6)	29 (45.3)	.179
Indeterminate	4 (19.0)	0 (0.0)	.003
IFN-γ Level (IU/mL)			
Nil (IU/mL)	0.07 (0.03-0.16)	0.07 (0.05-0.15)	.491
Tuberculosis antigen (IU/mL)	0.22 (0.08-0.98)	0.30 (0.12-2.87)	.278
Mitogen (IU/mL)	3.26 (0.70-6.64)	14.76 (12.41-18.36)	< .001

Data are expressed as median (interquartile range) or n (%). Low ex vivo IFN-γ production was defined as ex vivo IFN-γ production ≤ 7.79 IU/mL; high, > 7.79 IU/mL.
Abbreviations: ALK = anaplastic lymphoma kinase; ECOG PS = Eastern Cooperative Oncology Group performance status; EGFR = epidermal growth factor receptor; EGFR = epidermal growth factor receptor; IFN = interferon; IGRA = interferon-γ releasing assay; KRAS = Kirsten rat sarcoma viral oncogene.
[a]Total number of patients tested was 71.
[b]Total number of patients tested was 42.
[c]Total number of patients tested was 54.

production ($P = .040$). There were no differences in the presence of comorbidities and treatment options after lung cancer diagnosis between these groups. Indeterminate IGRA results were only observed in patients with low ex vivo IFN-γ production. Median IFN-γ levels in the mitogen tube were 3.26 in patients with low and 14.76 in those with high ex vivo IFN-γ production ($P < .001$).

Factors Associated With 1-Year OS in Patients With Stage IV Lung Adenocarcinoma

In univariate analysis, age, smoking (pack-years), ECOG PS, treatment with chemotherapy, and ex vivo IFN-γ production were statistically significantly associated with 1-year OS. In multivariate analysis, age, smoking (pack-years), ECOG PS, treatment with chemotherapy, and low ex vivo IFN-γ production (odds ratio = 3.156; 95% confidence interval, 1.473-6.760; $P = .003$) were associated with 1-year OS (Table 5).

Comparison of 1-Year OS According to EGFR *Mutation and Ex Vivo IFN-γ Production*

We compared the survival rate among 4 subgroups on the basis of *EGFR* mutation positivity and ex vivo IFN-γ production. Regardless of EGFR positivity, low ex vivo IFN-γ production patients had lower 1-year survival (Figure 2). Interestingly, patients with both high ex vivo IFN-γ production and positive *EGFR* mutation had the highest 1-year OS rate. There were no significant differences in age, sex, and cancer staging among the 4 subgroups (Supplemental Table 1 in the online version). No significant difference was noted in the use of first-line EGFR tyrosine kinase inhibitors between *EGFR* mutation–positive patients with high and low ex vivo IFN-γ production (31.4% vs. 50.0%; $P = .589$).

Discussion

Although it is evident that the advent of immunotherapy is encouraging in the field of immuno-oncology, the relationship between host immunity and cancer still remains largely unclear. In this study, decreased ex vivo IFN-γ production by peripheral immune cells was associated with patient prognosis along with N and M stage. Importantly, the 1-year OS rate was significantly lower in patients with low ex vivo IFN-γ production (≤ 7.79 IU/mL) than in those with high ex vivo IFN-γ production (> 7.79 IU/mL). There was no significant correlation between the baseline IFN-γ production by unstimulated immune cells and patient prognosis. Despite great efforts made in the discovery of biomarkers for predicting patient prognosis in lung cancer to date, there are substantial limitations in the current biomarkers for evaluating the function of immune cells.[20] Our findings imply that evaluation of ex vivo IFN-γ production could be a useful prognostic marker in patients with lung adenocarcinoma.

IFN-γ production by immune cells could be decreased because of cancer progression. Interleukin 10 derived by cancer has been shown to decrease interleukin 2 and IFN-γ production.[21] Furthermore, in patients with gastric cancer, IFN-γ production by NK cells decreased in relation to tumor burdens.[22] During cancer progression, development of T-cell exhaustion and high level of T-regulatory cells are also reported as immune dysregulation.[23,24]

Table 5 Cox Proportional Hazard Analysis of Variables Related to 1-Year Survival in Patients With Stage IV Lung Adenocarcinoma (N = 85)

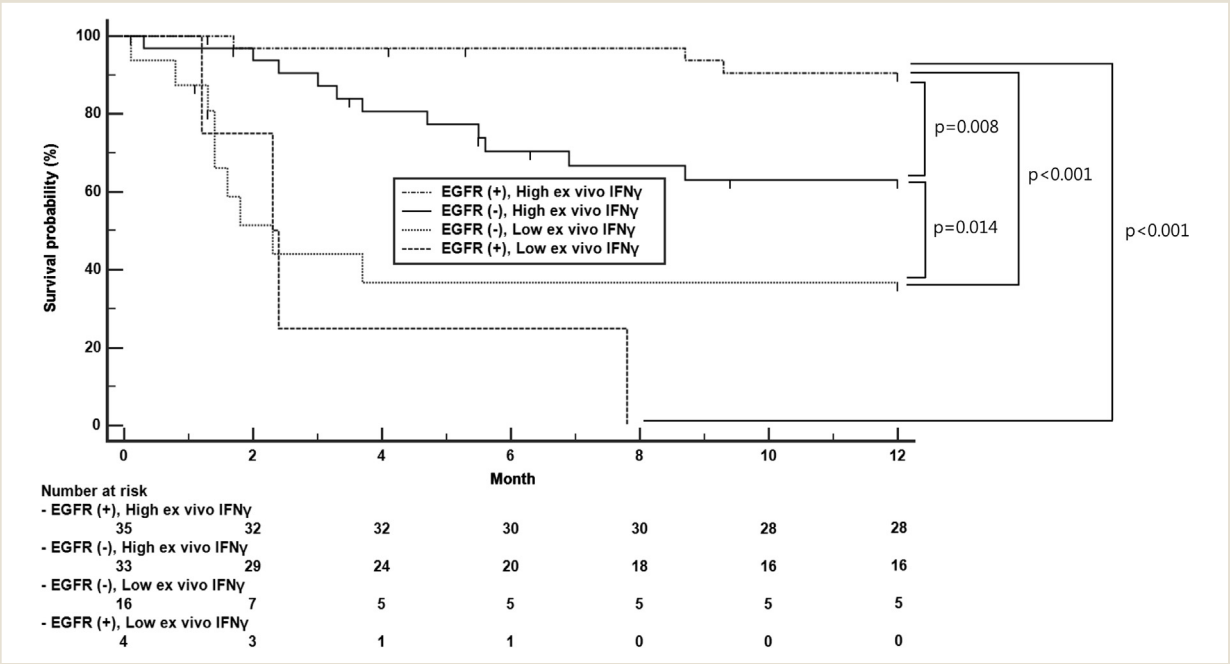
Characteristic	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	*P*	OR	95% CI	*P*
Age	1.045	1.018-1.073	< .001	1.050	1.017-1.085	.003
Male sex	1.562	0.768-3.177	.218			
Current smoker	1.375	0.654-2.890	.400			
Smoking (pack-years)	1.028	1.005-1.051	.015	1.026	1.002-1.051	.034
ECOG PS	2.339	1.547-3.535	< .001	1.755	1.017-3.029	.043
Chemotherapy after diagnosis	0.142	0.068-0.297	< .001	0.320	0.132-0.776	.012
Radiotherapy after diagnosis	0.695	0.350-1.381	.299			
Presence of any comorbidities	1.405	0.708-2.789	.331			
Ex vivo IFN-γ production (IU/mL)	0.916	0.869-0.965	< .001			
Low ex vivo IFN-γ production[a]	4.695	2.339-9.423	< .001	3.156	1.473-6.760	.003

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; IFN = interferon; OR = odds ratio.
[a]Low ex vivo IFN-γ production was defined as ex vivo IFN-γ production ≤ 7.79 IU/mL.

The mechanism between immune dysregulation and decreased ex vivo IFN-γ production is unknown. In our study, ex vivo IFN-γ production was not associated with T stage, but it was significantly associated with the N stage and M stage. It is possible that diminished IFN-γ production by immune cells could hamper the host immunity to inhibit cancer cells migrating into adjacent lymph nodes or distant organs. Identifying the causal relationship between decreased ex vivo IFN-γ production and patient prognosis is beyond our study's scope. Detailed molecular and cellular analysis of IFN-γ–producing cells in lung adenocarcinoma patients would provide us with potential mechanisms of our observations.

Given that the IGRA test is used for TB screening, our study population might be skewed toward concurrent TB infection, which could provide systemic immune activation. Therefore, we excluded patients with active TB infection and only included patients with latent or previous TB infection. Interestingly, the proportion of patients with positive IGRA result tended to be higher in the high ex vivo IFN-γ production group (Table 4). However, the difference

Figure 2 Comparison of Overall 1-Year Survival According to *EGFR* Mutation and Ex Vivo IFN-γ Production. Overall 1-Year Survival Rate is Significantly Higher for Lung Cancer Patients With *EGFR* Mutation–Positive and High Ex Vivo IFN-γ Production Than for Those in Other 3 Groups. Low Ex Vivo IFN-γ Production Was Defined as Ex Vivo IFN-γ Production ≤ 7.79 IU/mL; High, > 7.79 IU/mL



Abbreviations: EGFR = epidermal growth factor receptor; IFN = interferon.

was not statistically significant and could be biased because indeterminate results were only found in those with low ex vivo IFN-γ production.

When we compared the gene mutation in patients with stage IV cancer, *EGFR* mutation was less frequently observed in patients with low ex vivo IFN-γ production (Table 4). Further analysis demonstrated that the 1-year OS rate was highest in patients with high ex vivo IFN-γ production and positive *EGFR* mutation (Figure 2). This could be due to treatment benefit by EGFR inhibitors or favorable prognostic effect by *EGFR* mutation in patients with high ex vivo IFN-γ production. As a result of the small number of patients in our study, prospective studies with larger populations are necessary to evaluate the role of *EGFR* mutation in patients with high ex vivo IFN-γ production.

Only a few studies have evaluated this subject so far. Huang et al[25] reported that a PHA-stimulated IFN-γ level was associated with progression-free survival and OS, and it was an independent predictor of patient prognosis in non−small-cell lung cancer. Although there are similarities between our study and that of Huang et al, there are several differences. First, we evaluated the clinical significance of ex vivo IFN-γ production rather than IFN-γ production in the mitogen tube. Because PHA-stimulated IFN-γ production can be falsely increased as a result of increased baseline IFN-γ production, it might be more accurate to remove the amount of baseline IFN-γ from the amount of IFN-γ after PHA stimulation to assess ex vivo IFN-γ production. Second, we directly evaluated the correlation between cancer burdens (T stage, N stage, M stage, and cancer staging) and ex vivo IFN-γ production. Third, because immunoresponse can be different depending on pathologic subtypes and/or stages of lung cancer, we included a homogeneous population of patients with lung adenocarcinoma, which is the most common subtype, and subgroup analysis was performed in stage IV cancer.

The main strength of this study was that we enrolled a homogeneous population of patients with lung adenocarcinoma who had undergone IGRA at diagnosis. Our study is not without limitations. First, because of its retrospective nature, the study is prone to bias and cannot define causality. Second, because IGRA is not routinely performed in patients with lung cancer, selection bias is present. Third, within our data, only a limited number of patients with early stage lung adenocarcinoma were available. Fourth, even though ex vivo IFN-γ production was independently associated with patient prognosis in Cox proportional hazard analysis, ex vivo IFN-γ production was significantly associated with ECOG PS in our patients, and a possibility of reverse causality cannot be completely excluded.

In conclusion, we demonstrated that decreased ex vivo IFN-γ production was independently associated with the 1-year OS rate in lung adenocarcinoma, even when other prognostic factors were considered. These findings suggest that ex vivo IFN-γ production before treatment might be a useful marker for predicting prognosis in patients with lung adenocarcinoma.

Clinical Practice Points

- Immunotherapies targeting the immune checkpoint receptor have shown promising results in non−small-cell lung cancer, but there are substantial limitations in the current biomarkers for evaluating the function of immune cells.
- IFN-γ is a proinflammatory cytokine mainly produced by T cells and NK cells that contributes to cancer recognition and elimination.
- In this study, decreased ex vivo IFN-γ production by peripheral immune cells was associated with patient prognosis, along with the N stage and M stage.
- Ex vivo IFN-γ production before treatment might be a useful marker for predicting prognosis in patients with lung adenocarcinoma.

Acknowledgment

Supported in part by a new faculty research seed money grant from Yonsei University College of Medicine for 2018 (2018-32-0028). The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the report.

Disclosure

The authors have stated that they have no conflict of interest.

Supplemental Data

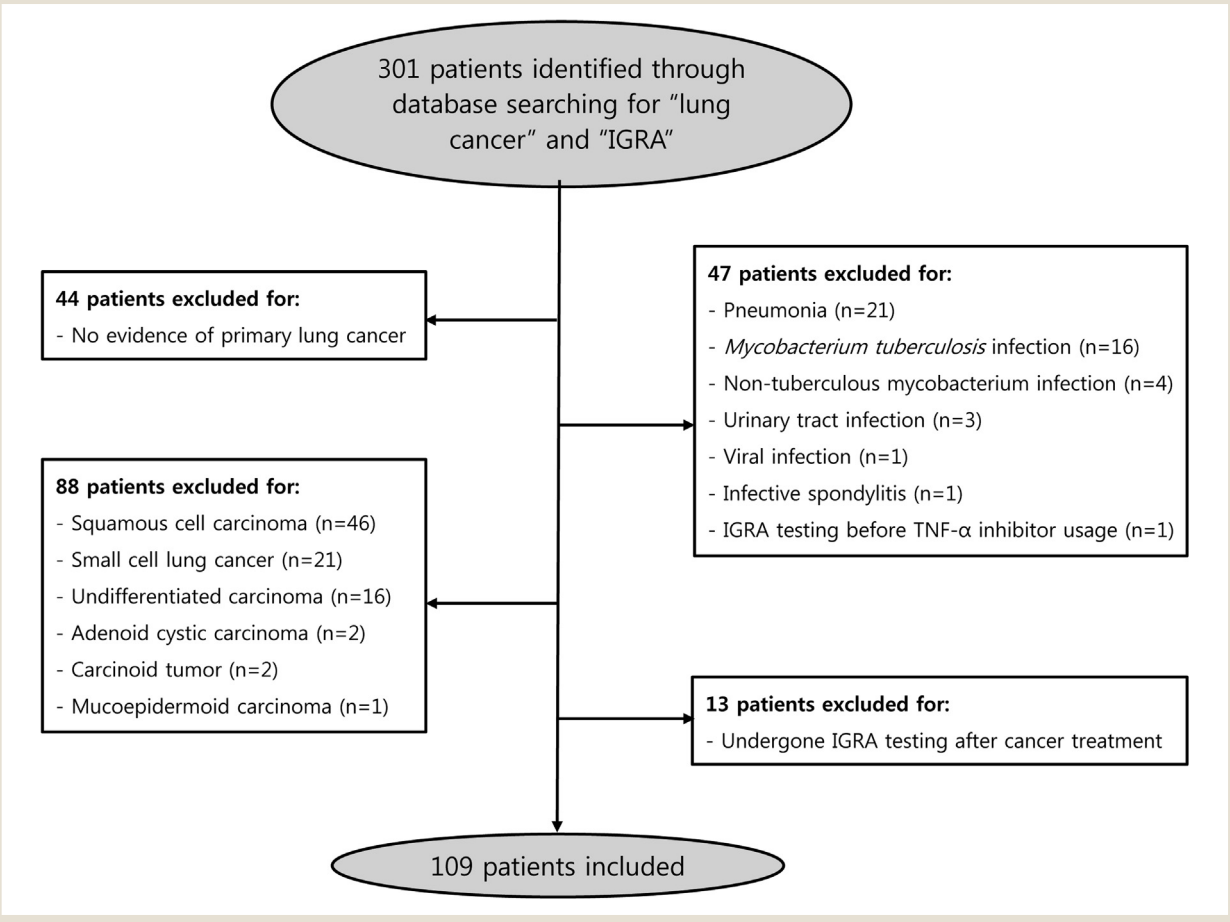
A supplemental figure and table accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2019.01.002.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; 65:87-108.
2. Torre LA, Siegel RL, Jemal A. Lung cancer statistics. *Adv Exp Med Biol* 2016; 893: 1-19.
3. Cetin K, Ettinger DS, Hei YJ, O'Malley CD. Survival by histologic subtype in stage IV nonsmall cell lung cancer based on data from the Surveillance, Epidemiology and End Results Program. *Clin Epidemiol* 2011; 3:139-48.
4. Massarelli E, Papadimitrakopoulou V, Welsh J, Tang C, Tsao AS. Immunotherapy in lung cancer. *Transl Lung Cancer Res* 2014; 3:53-63.
5. Subramaniam DS, Liu SV, Giaccone G. Novel approaches in cancer immunotherapy. *Discov Med* 2016; 21:267-74.
6. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non−small-cell lung cancer. *N Engl J Med* 2015; 372:2018-28.
7. Rizvi NA, Mazieres J, Planchard D, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non−small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. *Lancet Oncol* 2015; 16:257-65.
8. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non−small-cell lung cancer. *N Engl J Med* 2018; 378:2078-92.
9. Seetharamu N, Budman DR, Sullivan KM. Immune checkpoint inhibitors in lung cancer: past, present and future. *Future Oncol* 2016; 12:1151-63.
10. Ikeda H, Old LJ, Schreiber RD. The roles of IFN gamma in protection against tumor development and cancer immunoediting. *Cytokine Growth Factor Rev* 2002; 13:95-109.
11. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. *Nat Rev Immunol* 2006; 6:836-48.
12. Martin F, Santolaria F, Batista N, et al. Cytokine levels (IL-6 and IFN-gamma), acute phase response and nutritional status as prognostic factors in lung cancer. *Cytokine* 1999; 11:80-6.
13. Li J, Wang Z, Mao K, Guo X. Clinical significance of serum T helper 1/T helper 2 cytokine shift in patients with non−small cell lung cancer. *Oncol Lett* 2014; 8: 1682-6.
14. Lalvani A, Pareek M. Interferon gamma release assays: principles and practice. *Enferm Infecc Microbiol Clin* 2010; 28:245-52.
15. Buccheri G, Ferrigno D, Tamburini M. Karnofsky and ECOG performance status scoring in lung cancer: a prospective, longitudinal study of 536 patients from a single institution. *Eur J Cancer* 1996; 32A:1135-41.
16. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the *TNM Classification of Malignant Tumours*. *J Thorac Oncol* 2007; 2:706-14.
17. Steindel SJ. *International Classification of Diseases,* 10th edition, clinical modification and procedure coding system: descriptive overview of the next generation HIPAA code sets. *J Am Med Inform Assoc* 2010; 17:274-82.

18. Pai M, Denkinger CM, Kik SV, et al. Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. *Clin Microbiol Rev* 2014; 27:3-20.
19. Ahn SS, Park ES, Shim JS, et al. Decreased ex vivo production of interferon-gamma is associated with severity and poor prognosis in patients with lupus. *Arthritis Res Ther* 2017; 19:193.
20. Thakur MK, Gadgeel SM. Predictive and prognostic biomarkers in non−small cell lung cancer. *Semin Respir Crit Care Med* 2016; 37:760-70.
21. Neuner A, Schindel M, Wildenberg U, Muley T, Lahm H, Fischer JR. Prognostic significance of cytokine modulation in non−small cell lung cancer. *Int J Cancer* 2002; 101:287-92.
22. Lee J, Park KH, Ryu JH, et al. Natural killer cell activity for IFN-gamma production as a supportive diagnostic marker for gastric cancer. *Oncotarget* 2017; 8:70431-40.
23. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* 2015; 15:486-99.
24. Kotsakis A, Koinis F, Katsarou A, et al. Prognostic value of circulating regulatory T cell subsets in untreated non−small cell lung cancer patients. *Sci Rep* 2016; 6: 39247.
25. Huang HC, Su WJ, Chiang CL, et al. The predictive value of the interferon-gamma release assay for chemotherapy responses in patients with advanced non−small-cell lung cancer. *Lung Cancer* 2018; 115:64-70.

Supplemental Figure 1 **Flowchart for Patient Inclusion**



Abbreviation: IGRA = interferon-γ releasing assay.

Supplemental Table 1 Clinical Characteristics of Patients According to *EGFR* Mutation and Ex Vivo IFN-γ Production

Characteristic	*EGFR* Mutation		No *EGFR* Mutation		*P*
	Low Ex Vivo IFN-γ Production (N = 4)	High Ex Vivo IFN-γ Production (N = 35)	Low Ex Vivo IFN-γ Production (N = 16)	High Ex Vivo IFN-γ Production (N = 33)	
Age (years)	69.5 (7.5)	58.0 (16.0)	62.0 (17.5)	61.0 (23.0)	.137
Male sex	1 (25.0)	17 (48.6)	11 (68.8)	21 (63.6)	.088
Patients treated with first-line EGFR tyrosine kinase inhibitors	2 (50.0)	11 (31.4)	0 (.0)	0 (0.0)	< .001
Cancer Stage					.170
I-III	0 (0.0)	6 (17.1)	2 (12.5)	9 (27.3)	
IV	4 (100.0)	29 (82.9)	14 (87.5)	24 (72.7)	
ECOG PS					.003
0-I	2 (50.0)	31 (88.6)	7 (43.8)	27 (81.8)	
II-IV	2 (50.0)	4 (11.4)	9 (56.3)	6 (18.2)	

Data are expressed as n (%). Low ex vivo IFN-γ production was defined as ex vivo IFN-γ production ≤ 7.79 IU/mL; high, > 7.79 IU/mL.
Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status; EGFR = epidermal growth factor receptor; IFN = interferon.