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ABSTRACT

Introduction: Programmed death-1 immune checkpoint inhibitors (ICIs) have been shown to improve survival of non-small cell lung cancer (NSCLC) patients. Upon expansion of clinical administration for a variety of cancers, immure-related adverse events (ir-AEs) have been typically recognized to be associated with ICIs therefore, necessitating the monitoring and management of these patients. Among ir-AEs, immune-related interstitial lung disease (ir-ILD) is a serious complication which interrupts treatment and occasionally, is fatal. However, no prospective studies have investigated incidences of ir-ILD and associated risk factors for its development in the clinical setting.

Methods: This was a prospective cohort study consisting of NSCLC patients treated with ICIs. Baseline characteristics, including laboratory data, pulmonary function tests (PFTs), daily dyspnea defined by the modified Medical Research Council (mMRC), and anti-tumor response were assessed.

Results: Among the 138 NSCLC patients that received anti-PD-1 monotherapy, 20 patients (14.5%) developed ir-ILD within median 51.5 days [29-147: interquartile]. This was approximately three-times higher than those in clinical trials. Eleven patients (55.0%), including all of eight patients with high-grade ir-ILD (≥Grade 3), developed ir-ILD within 60 days. Impaired spirometry, decreased forced vital capacity (%FVC) and forced expiratory volume in 1.0 second (%FEV_1), and daily dyspnea measured by mMRC were identified as risk factors for ir-ILD development. Additionally, combination assessment of %FVC and %FEV_1 successfully classified patients at risk for ir-ILD development.

Conclusion: The incidences of ir-ILD were substantially higher in clinical setting. Assessment of spirometry and daily dyspnea before ICI treatment may be useful to monitor and manage NSCLC patients.

Trial Registration: This study is registered in the University Hospital Medical Information Network in Japan (http://www.umin.ac.jp/ctr/index-j.htm. UMIN000021548).

INTRODUCTION

Immune checkpoint inhibitors (ICIs), such as programmed cell death protein 1 (PD-1) inhibitors, have notable efficacy in the treatment of cancers including non-small-cell lung cancer (NSCLC) [1-6]. The PD-1 pathway is an immune escape mechanism which strongly suppresses T cell function. Blockade of PD-1 activates immune system and subsequently allows for an anti-tumor response. Consequently, PD-1 blockade can result in development of immune-related adverse events (ir-AEs) [7]. With the increased clinical usage of PD-1 inhibitors, ir-AEs are routinely reported and recognized as important adverse events in the management and monitoring of patients treated with ICIs.

Among ir-AEs, immune-related interstitial lung disease (ir-ILD) is a serious complication that typically presents with coughing, dyspnea, and hypoxia. Notably, NSCLC patients experienced significantly higher incidences of ir-ILD together with grade 3 or higher ir-ILD compared to melanoma patients [8 9]. Incidence rates of ir-ILD reported in clinical trials range between approximately 3-5% [1-4, 8-10]. However, it has recently been revealed that ir-ILD frequency in the greater population is significantly higher than the frequency reported in clinical trials. Suresh et. al. and Fukihara et. al. reported incidences of ir-ILD were 19.0%, and 15.9%, respectively [11, 12]. Despite recovery from ir-ILD, most patients required cessation/interruption of ICI treatment and changes of treatment regimens. Moreover, the frequencies of refractory disease ranged between 10-20%, with approximately 10 % mortality [11-14]. Consequently, ir-ILD was associated with poor prognosis in patients with NSCLC.

To date, several retrospective studies have reported risk factors for ir-ILD development including smoking status, line of treatment, chest radiation [15], non-adenocarcinoma [12], lower albumin [11], and fibrosis score [16]. However, these risk factors had not been validated with the broader use of ICI therapies. Therefore, this study explored risk factors for developing ir-ILD in patients with NSCLC for the first time in a prospective setting.

METHODS

Ethical approval of the study protocol

This multicenter prospective study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of each participating institution: Hamamatsu University School of Medicine, Iwata City Hospital, Shizuoka City Shizuoka Hospital, Seirei-Mikatahara Hospital, Shizuoka General Hospital, Shizuoka Saiseikai Hospital, Enshu Hospital, Shizuoka Red Cross Hospital, Fujieda City Hospital, Hamamatsu Rosai Hospital, Shizuoka City Shimizu Hospital, and Tenryu Hospital. This study was conducted in accordance with approved guidelines. Written informed consent was obtained from all subjects in accordance with institutional guidelines. The study was registered in the University Hospital Medical Information Network in Japan (UMIN000021548).

Patients

Eligible patients had documented and pathologically confirmed stage IIIB, stage IV, unresectable stage IIIA, or recurrent NSCLC after surgical resection. Most of the patients were previously received one or two lines of chemotherapy, and 2 were previously untreated. Patients were required to be > 20 years old and have an Eastern Cooperative Oncology Group performance status (ECOG) of 0, 1, or 2. Patients with treated, stable brain metastases were eligible. Additional eligibility criteria included adequate hematopoietic, hepatic, and renal function. Exclusion criteria were concomitant chest radiotherapy and prior treatment with checkpoint-targeting agents.

Study design

Patients received nivolumab at a dose of 3 mg/kg of body weight every 2 weeks or 200 mg of pembrolizumab every 3 weeks. Before anti-PD-1 inhibitor administration, pulmonary function tests (PFTs) and blood analyses were performed.

Diagnosis of immune-related interstitial lung disease

The diagnosis of ir-ILD was made based on combination of 1) clinical findings (symptoms and signs), 2) high-resolution computed tomography (HRCT) indicating occurrence of new lung parenchymal abnormalities after administration of anti-PD-1 antibody, 3) biologic findings (sputum cultures and/or bronchioalveolar lavage (BAL) fluid, and routine labs, and 4) carefully exclusion of alternative etiologies such as infection, heart failure, embolism, and tumor progression such as malignant lung infiltration, and lymphangitis carcinomatosa [12, 13, 17] . BAL was performed in two out of 20 patients with ir-ILD.

The HRCT patterns were classified according to the ATS/ERS international multidisciplinary classification of interstitial pneumonia and previous reports on drug-induced ILDs with slightly modification [15, 18, 19], which include the patterns of acute interstitial pneumonia (AIP), cryptogenic organizing pneumonia (COP), nonspecific interstitial pneumonia (NSIP), hypersensitive pneumonitis (HP), and ground-glass opacities (GGO). AIP pattern was defined as scattered or diffuse areas of GGO, consolidations and a thickening of interlobular septa with architectural distortion and traction bronchiectasis. NSIP pattern included reticulations with or without peribronchovascular infiltration and interlobular septal thickening. COP pattern was defined as discrete patchy or confluent consolidation with or without air-bronchogram, predominantly peripheral distribution or subpleural distribution. HP pattern was identified as centrilobular opacities/nodules with or without ground-glass

attenuation or consolidation. GGO pattern was displayed as discrete focal areas of ground-glass attenuation with or without smooth interlobular septal thickening.

Evaluation of responses and toxicity

Tumor responses were evaluated by referencing the CT findings initially used to define tumor development. Responses were evaluated in accordance with the Response Evaluation Criteria in Solid Tumor (version 1.1) [20]. Adverse events including ir-ILD were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0).

Measurement and pulmonary function tests

PFTs, predicted values of forced vital capacity (FVC) and forced expiratory volume in 1.0 second (FEV_1) were performed in accordance with the Japanese Respiratory Society guidelines (JRS) [21 22]. Total lung capacity (TLC), functional residual capacity (FRC), residual volume (RV), and diffusing capacity of the lung for carbon monoxide (DLCO) were measured in the limited cases.

Modified Medical Research Council questionnaire, Chronic Obstructive Pulmonary Disease Assessment Test, and Asthma Control Test

The Modified Medical Research Council (mMRC) questionnaire [23], Chronic Obstructive Pulmonary Disease (COPD) Assessment Test (CAT) [24] (Japanese version, supplied by GlaxoSmithKline, Japan), and the Asthma Control Test (ACT) [25] were used to evaluate daily instances of dyspnea. The mMRC questionnaire is a 5-point scale and ask patients to rate dyspnea from 0 (absent) to 4 (dyspnea when dressing/undressing). The CAT questionnaire consists of 8 items (cough, phlegm, chest tightness, breathlessness going up hills/stairs, activity limitations at home, confidence leaving home, sleep, and energy) assessing and quantifying the

symptoms and impacts of COPD. The Asthma Control Test (Japanese version, supplied by GlaxoSmithKline, Japan) questionnaire consists of five items used to assess the impact of asthma on everyday functioning at school or work, shortness of breath, nocturnal asthma symptoms, the use of rescue medication, and the patient's self-rating of asthma control during the previous four weeks.

PD-L1 expression

PD-L1 expression was assessed in formalin-fixed tumor samples derived from tumor-biopsy specimens at the time of NSCLC diagnosis for selected patients (n=102) using commercially available PD-1 IHC 22C3 pharmDx assay kit (Dako, North America). Samples were categorized as < 1%, 1% - 49%, or ≥ 50% based on a section that included at least 100 tumor cells eligible for evaluation [3, 4].

Statistical analysis

The primary end point was incidence of ir-ILD. Secondary end points were progression free survival (PFS), overall survival (OS) time from enrollment in the study, and safety. Based on the SWOG one-arm survival design [26], a total of 126 patients were required to achieve 90% statistical power with an α error of 0.05 assuming an expected incidences of ir-ILDP of 9.0% [27] and a threshold of 3.0% [1]. The planned cohort size was 135 patients after taking some dropouts into consideration. Discrete variables were expressed as counts and percentages, and continuous variables were expressed as median [interquartile range], unless otherwise specified. The Mann-Whitney test was used to compare continuous variables. Fisher's exact tests for independence were used to compare categorical variables. OS time was measured from the date of ICI administration until date of death or last visit. The ir-ILD-free period and PFS period were measured from the date of ICI administration until the diagnosis of ir-ILD or

date of progressive disease (PD). Cumulative survival probabilities and incidences of ir-ILD were calculated by the Kaplan-Meier method, and differences were evaluated using log-rank and Gray's test. Fine-Gray proportional hazard analyses were performed to identify predictive factors associated with ir-ILD development. Statistical analyses were performed using GraphPad Prism Version 6 (GraphPad Software, San Diego, CA, USA) and R software version 2.15.1 (The R Foundation for Statistical Computing, Austria). All analyses were two-tailed, and $p < 0.05$ was considered statistically significant.

RESULTS

Clinical characteristics

A total of 142 patients were enrolled in the study from April 2016 to May 2018. Among the patients, three did not perform PFTs and one received tracheostomy. Thus, this study analyzed 138 ICI treated patients (136 patients received nivolumab and the remaining 2 received pembrolizumab).

The clinical characteristics of the 138 NSCLC patients who received ICIs are shown in **Table 1**. The median age at enrollment was 69 years (range 40–83 years), and 112 patients (81.2%) were male. Ninety-eight (72.6%) had stage IV NSCLC, and 23 (16.7%) had stage IIIB. Approximately, 60% were diagnosed with adenocarcinoma, including 10 patients with epidermal growth factor receptor mutations. Among the 102 patients in which PD-L1 expression was assessed, in 51 (50.0%), more than 1% of tumor cells expressed PD-L1, while no PD-L1 expression was observed in 51 patients (50.0%). Thirty-eight patients exhibited partial responses (PR), 29 exhibited stable disease (SD), and 71 exhibited PD following ICI treatment.

Grades, CT findings, and outcome of immune-related interstitial lung disease.

During the observation period, 20 cases developed ir-ILD within median of 51.5 days (range=29-147 days). One had pre-existing ILD on chest CT. As to EGFR status, two patients had EGFR-mutant (Del 19) among patients with ir-ILD. One had been treated with EGFR-TKI with wash-out 14 days (**Supplemental Table 2**). Another patient, having double cancer harboring EGFR-mutant and EGFR-wild, had not received EGFR-TKI. The number of patients with ir-ILD Grades 1, 2, 3, 4, and 5 were 6, 6, 5, 0, and 3, respectively (**Figure 1**). Eleven patients (55.0%), including eight patients with ir-ILD ≥ Grade 3, developed ir-ILD within 60 days. Thirteen patients were administered corticosteroids with ICI cessation, but three patients eventually died and one did not recover (**Supplemental Table 1**). On chest CT, AIP patterns were most commonly found in severe cases (ir-ILD ≥ Grade 3), whereas 50% of patients with mild ir-ILD (<Grade2) showed COP pattern (**Supplemental Table 2**). There were no significant differences in age, sex, histology, and/or disease stage at diagnosis in NSCLC patients with or without ir-ILD (**Table 1)**. There was a trending but not significant association between PD-L1 expression and the response rate to ICI. Cells and proteins detected in peripheral blood (except basophils) did not differ in patients with or without ir-ILD **(Supplemental Table 3**). Among 20 patients developing ir-ILD, three ir-ILD patients with Grade 1 were re-challenged with ICIs: Two patients had re-challenged nivolumab after resolution from ir-ILD. Another patient had spontaneously recovered without corticosteroid after cessation of ICIs, then received atezolizumab. No recurrence was found in the three patients.

Pulmonary Function test in the presence or absence of immune-related interstitial lung disease

Next, we evaluated PFTs before the ICI administration (**Table 2**). NSLC patients who developed ir-ILD showed significantly lower %FVC (69.4% [51.6-80.5%] vs. 84.8% [69.7%-101.9%], p=0.0016) and $\%FEV_1$ (68.0% [52.3-75.2%] vs. 79.5% [63.0%-94.2%], p=0.0275) compared to patients without ir-ILD. The percentage values of FRC, TLC, and TLC (L) were also decreased in 87 of the 138 patients with ir-ILD. DLCO did not differ between the groups. According to the daily dyspnea scale, the mMRC score was higher in patients with ir-ILD, but no difference was seen in CAT and ACT scores.

Spirometry prediction of immune-related interstitial lung disease

To assess the value of spirometry as a predictor for ir-ILD, receiver-operating characteristic (ROC) analyses were performed. The area under the ROC curve (AUC) in %FVC was 0.722 (95% confidence interval (CI), 0.604-0.839). With an optimal cut-off value of 77.6%, the sensitivity was 75.0% and specificity was 60.3% (**Supplemental Table 4**). Meanwhile, the AUC in $\%FEV_1$ was 0.654, with 80.0% sensitivity and 57.8% specificity. Of note, both %FVC and $\%FEV_1$ exhibited higher negative predictive values (NPV; >90%), despite lower positive predictive values (PPV). The values of TLC, FRC, and mMRC are also shown in **Supplemental Table 3**. We then assessed the cumulative incidence of ir-ILD on either side of this cut-off value by using the Kaplan-Meier method and Gary's test. Groups with lower %FVC showed a significantly higher incidence rate of ir-ILD compared to groups with higher %FVC (23.6% vs. 4.5%, p=0.00674, **Figure 2A**). Similarly, patients with lower $\%FEV_1$ exhibited a higher incidence rate of ir-ILD (19.7% vs. 8.1%, p=0.00258, **Figure 2B**). Additionally, to estimate the risk for ir-ILD development, we further classified the patients into three groups using %FVC and $\%FEV_1$. With optimal cut-off values obtained

above, better classifications were yielded according to cumulative incidences of ir-ILD development (26.9% vs. 13.0% vs. 4.8%, p=0.00720, **Figure 2C**).

Univariate and multivariate analyses for Predicting development of immune-related interstitial lung disease

To examine the prognostic value of spirometry parameters with regards to ir-ILD prediction, we performed univariate and multivariate analyses using Fine-Gray's tests. As shown in **Table 3**, %FVC, $\%FEV_1$, mMRC, and adenocarcinoma were associated with incidences of ir-ILD, but not age or sex. The values of %FRC and %TLC were also significant. We then analyzed these associations using each optimal cut-off and a combination of %FVC, $\%FEV_1$, and mMRC. Following this assessment, %FVC and $\%FEV_1$ showed best predictive values for ir-ILD. In the multivariate analyses, %FVC significantly predicted ir-ILD.

Prognostic impact of immune-related interstitial lung disease

To evaluate the impact of ir-ILD on prognosis, PFS or ir-ILD-free period and OS were calculated. The median PFS was 51.5 days in patients who developed ir-ILD, whereas PFS in patients without ir-ILD was 65.5 days (p=0.1577, **Figure 3A**). The OS of patients who developed ir-ILD was a median of 16.2 months with a 1-year and 2-year survival rate of 55.0% and 31.3%, respectively. Meanwhile, the OS of patients who did not develop ir-ILD was median 14.0 months with a 1-year and 2-year survival rate of 57.8% and 33.1%, respectively. There were no significant differences between the groups (p=0.8078, **Figure 3B**).

Safety

The major adverse events observed during the study are listed in **Supplemental Table 5**. Among observed ir-AEs, diarrhea was most common followed by thyroiditis. Myositis, types I diabetes mellitus, and adrenal insufficiency were also observed. Among patients with ir-ILD, one patient had thyroiditis simultaneously with ir-ILD. Another, who had been recovered from ir-ILD, developed cholangitis 16 months after re-challenge of nivolumab. The incidences and onset of adverse events that disrupted ICI treatment were listed in **Supplemental Table 6**.

DISCUSSION

This is the first study to prospectively investigate the cumulative incidences and risk factors for ir-ILD in NSCLC patients treated with anti-PD-1 monotherapy. The overall incidence rate of ir-ILD was 14.5% which was approximately three times higher than the rate observed in clinical trials [2, 3, 8-10]. Notably, more than half of ir-ILD cases and all severe ir-ILD cases (≥Grade 3) developed within 60 days. The present study identified impaired spirometry and dyspnea defined by mMRC as risk factors for ir-ILD development. Importantly, classification based on %FVC and $\%FEV_1$ values enabled separation of ir-ILD incidences. Our results also suggest measurements of spirometry can offer an estimated risk for the development of ir-ILD in patients with NSCLC and may be useful for monitoring ir-ILD development.

ir-ILD is a clinically serious and potentially life-threating auto-immune toxicity. While it is considered clinically important, it is a relatively rare adverse event [8, 10]. However, the present study demonstrated that the actual occurrence of ir-ILD was considerably higher for NSCLC patients treated with PD-1 monotherapy in practice than those of reported in clinical trials [1-4, 8-10]. Similar to our result, several retrospective studies have reported that incidences of ir-ILD among patients with NSCLC ranged from 14.6% to 19.0% [11, 12, 16].

The incidences of ir-ILD (any Grades) were higher in real-world studies, including ours, than in the clinical trials [1-4, 11, 12, 14, 16]. However, the proportion of severe ir-ILD (≥Grade3) among ir-ILD in the present study was basically equivalent to that in the clinical trials (ranged 30-50%). These observations suggest that the high incidence of ir-ILD in our study may not be due to increase of mild ir-ILD (Grades 1 and 2). The differences of ir-ILD incidence between the real-world studies and clinical trials may be accounted for in part by differences in patient cohorts. Indeed, the real-world studies included more patients with low performance status and old age, both of which have been considered as high-risk factors of drug induced-ILD [28, 29]. In addition, several genetic variants to suspectable fibrotic lung disease were reported [30] and genetic factors in Japanese may be also attributable to the high incidence of ir-ILD. Japanese patients have been shown to develop drug-induced lung disease more frequently than western patients [28], though *Suresh* et al showed similar frequency of ir-ILD in western countries [12]. Interestingly, the incidence of ir-ILD in treatment with anti-PD-L1 antibody has been shown to be lower than that in treatment with anti-PD-1 antibody [5, 6]. Thus, incidences of ir-ILD might be dependent on the agents used and linked with genetic factors to some extent. Additionally, previous studies found NSCLC patients more likely to develop ir-ILD than melanoma [8] and treatment interruption due to ir-AE was reported to be associated with lower survival [31]. Indeed, ir-ILD was the most common ir-AE and frequent cause of ICI discontinuation in this study (data not shown). Collectively, these results indicated that physicians should be aware of the elevated incidence rate and clinical importance of ir-ILD during ICI therapy in the real world.

In the present study, 11 out of 20 ir-ILD cases occurred within 60 days after initiation of ICI. Interestingly, all cases of severe ir-ILD (≥Grade 3) occurred in the early phase of ICI treatment. With regards to the timing of ir-ILD development, the onset of ir-ILD occurred 2.3-2.8 months after ICI treatment initiation [12, 15, 17, 19] and the ir-ILD onset was earlier in

patients with NSCLC than that in melanoma or other malignancies [17, 19]. Although mild ir-ILD can occur more than 6 months after ICI treatment initiation, the majority of cases with severe ir-ILD developed earlier [12, 15]. Moreover, 10-20% of cases with ir-ILD were reported fatal and/or no recovery [11, 12, 15, 17]. In this study, 4 patients (20%; 3 died and 1 unchanged) deteriorated. These results suggested that cases with early onset ir-ILD included higher proportions of severe cases and a substantial number of fatal cases in the real world.

To date, several retrospective studies have reported risk factors for ir-ILD development including smoking status, line of treatment, chest radiation [15], non-adenocarcinoma [12], lower albumin [11], and fibrosis score [16]. However, these were not validated in other cohorts, and a study by Delaunay et al. did not identify any risk factors for ir-ILD [17]. Given the increased incidence rate and clinical significance of ir-ILD, identifying risk factors for ir-ILD is necessary for ICI management.

Our study prospectively evaluated lung physiology and mMRC scale. Impaired spirometry, as defined by decreased %FVC and $\%FEV_{1,}$ and increased mMRC scale, were associated with risk factors for ir-ILD. Of note, despite our findings that PPV was lower, %FVC, %FEV, and mMRC exhibited higher NPV >90% indicating possible usefulness in the practice. The mMRC score was negatively correlated with %FVC, %FEV, %TLC, and %FRC ($p<0.0001$, $p<0.0001$, $p=0.0033$, and $p=0.0034$, respectively, data not shown), suggesting mMRC alone could represent impaired spirometry. Although the AUC values of mMRC were not satisfactory, assessment of daily dyspnea allow for easier access as mMRC is a simple questionnaire and easily applicable in the clinic.

The present study identified impaired spirometry together with lower %TLC and %FRC as ir-ILD associated risk factors. The underlying association between impaired physiology and ir-ILD occurrence remains unknown. In patients with ir-ILD, an increased number of type-1 polarized activated lymphocytes as well as decreased regulatory T cells in BAL fluid

has been reported [32]. Given that decreased %TLC and %FRC were identified as risk factors for ir-ILD, total volumes of tumor, atelectasis, and presence of carcinomatous pleurisy might be implicated in ir-ILD development. These factors may interrupt drainage of activated lymphocytes from the lung, resulting in future development of ir-ILD. In addition to %FVC and $\%FEV_1$ alone, we proposed a combination assessment of %FVC and $\%FEV_1$ for predicting ir-ILD in NSCLC patients treated with ICI. Assessment of both factors combined yielded separation of NSCLC patients into three groups according to risk of ir-ILD development, though in general there is linkage between %FVC and $\%FEV_1$. These parameters showed lower PPV but higher NPV. Thus, assessment of pulmonary function test seems to have merits identifying the patients with lower risk for ir-ILD development. Although prophylactic treatment is not established, these risk-based stratification approaches may be useful for monitoring NSCLC patients treated with ICIs. Especially, patients with certain impairments of pulmonary function test would be monitored particularly in the early of ICI initiation.

Prior to this study, the effects of ir-AE on NSCLC prognosis were unknown. In this study, patients with ir-ILD exhibited shorter PFS, but no significant difference was found with regards to OS. Several studies have reported that early ir-AE was associated with better outcomes [33-36], whereas another reported that treatment interruptions due to ir-AE was associated with shorter survival [31]. Recently, two retrospective studies reported that ir-ILD was associated with decreased survival [11, 13]. Meanwhile hyper-progressive disease is common with NSCLC patients treated with ICIs than those treated with chemotherapy [37]. The median PFS in patients without ir-ILD was approximately 2 months in this study. Additionally, the frequency of best supportive care (BSC) after ICI discontinuation did not show differences between patients with or without ir-ILD. Of note, 5 out of 8 higher-grade ir-ILD (≥Grade 3) patients were treated with BSC, whereas 9 patients out of 12 lower grade ir-ILD (≤ Grade 2)

were continuously treated with chemotherapy. Thus, the impact of ir-ILD on survival seemed to depend on the severity of disease, indicating the need for a larger prospective study.

The current study had several limitations. While the combined assessment of %FVC and $\%FEV_1$ successfully classified ir-ILD risk, the predictive values were not satisfactory. Second, as previously described, the underlying associations between impaired spirometry and ir-ILD incidences remain unknown. Third, this study did not examine the baseline immune status, such as cytokine levels of serum or BAL. Fourth, the number of ir-ILD patients in this study was relatively small. Additionally, with advances in NSCLC treatment, the efficacy of ICIs and chemotherapeutics combined have resulted in limited application of monotherapy. Although this prospective study demonstrated altered incidence of ir-ILD and identified the associated risk factors in ICI monotherapy, future studies are required to address these concerns, particularly in combination therapies.

CONCLUSION

This study showed the clinical rate of ir-ILD was substantially higher than rates reported in clinical trials, the majority of ir-ILD cases developed early following ICI initiation, and decreased %FVC and $\%FEV_1$ along with daily dyspnea were identified as risk factors associated with ir-ILD development. Additionally, measuring the combination of %FVC and $\%FEV_1$ successfully classified NSCLC patients by their ir-ILD risk, suggesting the clinical usefulness of monitoring ir-ILD. Further validation of our findings and its usefulness in the context of combination therapy is necessary. Nevertheless, this prospective study is the first to demonstrate the risk factors for ir-ILD development and ir-ILD incident rates for NSCLC patients treated with ICIs in a clinical setting.

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Author contributions

YS: Conception and design, Data collection, Data analysis and interpretation, Manuscript writing, and Final approve of manuscript, MK, TU, MF, TM, KA, HK, MK, HM, SM, MT, KM, YI, TK, HY, HH, KF, NE, TF, YN: Data collection, NI: Conception and design, Data analysis. TS: Conception and design, Data analysis and interpretation Manuscript writing, Final approve of manuscript and Administrative support.

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FIGURE LEGENDS

Figure 1. Severity and onset of ir-ILD occurrences.

Severity and onset of ir-ILD occurrences.

Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; ir-ILD, immune-related interstitial lung disease.

Figure 2. Incidences of ir-ILD according to %FVC and %FEV_1.

Kaplan-Meier curves of cumulative incidences of ir-ILD according to %FVC (A), %FEV_1 (B), and combination of %FVC and %FEV_1 (C). P values were determined by Gray analyses.

Abbreviations: ir-ILD, immune-related interstitial lung disease; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1.0 second.

Figure 3. Prognostic impact of ir-ILD

Kaplan-Meier curve of ir-ILD-free and progression free period in patients with or without ir-ILD (A). Kaplan-Meier curve of overall survival in patients with or without ir-ILD (B). P values were determined by Gray analyses. Abbreviations: ir-ILD, immune-related interstitial lung disease; PFS, progression free survival; OS, overall survival.

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Table 1. Clinical characteristics of 138 NSCLC patients with or without ir-ILD

	All cases (n=138)	ir-ILD (n=20)	Non ir-ILD cases (n=118)	p-value
Age, yr	69 [65-74]	67 [61-74]	69 [65-74]	0.3433
Sex, (M/F)	112 (81.2%)/26 (18.8%)	19 (95.0%)/1 (5.0%)	93 (78.8%)/25 (21.2%)	0.1223
Observation, days	402 [151-695]	463 [112-701]	402 [165-669]	0.7463
Smoking status				
Never smoker	21 (15.2%)	2 (10.0%)	19 (16.1%)	0.7379
Former/Current smoker	117 (84.8%)	18 (90.0%)	99 (83.9%)	
Comorbidity				
COPD	56 (40.6%)	6 (30.0%)	50 (36.2%)	0.3354
Hypertension	43 (31.2%)	3 (15.0%)	40 (33.9%)	0.1191
Diabetes Mellitus	43 (11.6%)	2 (10.0%)	14 (11.9%)	1.0000
Cardiovascular disease	9 (6.5%)	0 (0%)	9 (7.6%)	0.3562
Cerebrovascular disease	9 (6.4%)	0 (0%)	9 (7.6%)	0.3562
Digestive ulcer	8 (5.8%)	2 (10.0%)	6 (5.1%)	0.3268
Hepatic disease	6 (4.3%)	1 (5.0%)	5 (3.6%)	1.0000
Bronchial asthma	4 (2.9%)	0 (0%)	4 (3.4%)	1.0000
Performance status				
0	82 (59.4%)	11 (55.0%)	71 (60.2%)	
1	50 (36.2%)	8 (40.0%)	42 (35.6%)	0.9087
2	6 (4.3%)	1 (5.0%)	5 (4.2%)	
Initial Cancer Stage				
IIIA	7 (5.1%)	1 (5.0%)	6 (4.3%)	
IIIB	23 (16.7%)	3 (15.0%)	20 (16.9%)	0.9624
IV	98 (71.0%)	14 (70.0%)	84 (71.1%)	
Recurrent	10 (7.2%)	2 (10.0%)	8 (6.8%)	
Histology				
Adenocarcinoma	81 (58.7%)	16 (80.0%)	65 (55.1%)	
SCC	52 (37.7%)	3 (15.0%)	49 (41.5%)	0.0771
Other	5 (3.6%)	1 (5.0%)	4 (3.4%)	
PD-L1 expression				
≥50%	19 (13.8%)	5 (25.0%)	14 (11.9%)	0.1079
1-49%	32 (23.2%)	2 (10.0%)	30 (25.4%)	

<1%	51 (37.0%)	10 (50.0%)	41 (34.7%)	
Not examined	36 (26.1%)	3 (15.0%)	33 (28.0%)	
EGFR mutation, yes	10 (7.2%)	2 (10.0%)	8 (6.8%)	0.6383
ALK fusion gene, yes	0 (0%)	0 (0%)	0 (0%)	-
Number of prior chemotherapy, times				
0	2 (1.4%)	1 (5.0%)	1 (0.8%)	
1	65 (47.1%)	8 (40.0%)	57 (48.3%)	0.4892
2	35 (25.4%)	5 (25.0%)	30 (25.4%)	
≥3	15 (10.9%)	6 (30.0%)	30 (25.4%)	
Chemoradiotherapy, yes	11	0	11	
Duration of ICI therapy, days	53 [28-224]	51 [14-240]	53 [30-223]	0.4732
Efficacy of ICIs				
PR	38 (27.5%)	8 (40.0%)	30 (24.1%)	
SD	29 (21.0%)	5 (25.0%)	24 (29.6%)	0.2558
PD	71 (51.4%)	7 (35.0%)	64 (46.3%)	
BSC after ICIs	52 (37.7%)	8 (40.0%)	44 (37.3%)	0.8082

COPD; chronic obstructive pulmonary disease, SCC; squamous cell carcinoma, PD-L1; programmed death ligand 1, EGFR; epidermal growth factor receptor, ALK; anaplastic lymphoma kinase, PR; partial response, SD; stable disease, PD; progressive disease, BSC; best supportive care

Table 2. Pulmonary Function tests in NSCLC patients with or without ir-ILD.

	All cases (n=138)	**ir-ILD (n=20)**	**non ir-ILD cases (n=118)**	**p-value**
FVC (L)	2.66 [2.20-3.22]	2.47 [2.09-2.89]	2.67 [2.22-3.28]	0.1083
FVC (% pred)	81.2 [68.7-100.2]	69.4 [51.6-80.5]	84.8 [69.7-101.9]	0.0016
$FEV_{1.0}$ (L)	1.88 [1.53-2.31]	1.78 [1.40-2.13]	1.93 [1.55-2.32]	0.2505
$FEV_{1.0}$ (% pred)	78.2 [61.8-92.2]	68.0 [52.3-75.2]	79.5 [63.0-94.2]	0.0275
$FEV_{1.0}$/FVC %	71.8 [64.2-79.6]	73.1 [67.5-84.0]	71.6 [63.1-79.2]	0.2480
SpO2 (%)	97 [96-98]	97 [96-98]	97 [96-98]	0.8318
mMRC	1 [0-2]	1 [1-2]	1 [0-2]	0.0461
CAT	8 [3-14]	11 [5-16]	7 [3-13]	0.2139
ACT	24 [21-25]	23 [21-25]	25 [22-25]	0.3616
	All cases (n=87)	**ir-ILD (n=15)**	**non ir-ILD cases (n=72)**	
FRC (L)	2.89 [2.40-3.58]	2.70 [2.20-3.24]	2.90 [2.50-3.60]	0.2471
FRC (% pred)	87.7 [72.2-107.7]	74.8 [61.2-89.8]	93.1 [73.7-110.0]	0.0200
RV (L)	1.86 [1.63-2.27]	1.83 [1.74-2.16]	1.87 [1.59-2.32]	0.4381
RV (% pred)	112.2 [97.6-135.3]	110.9 [99.4-135.3]	113.1 [97.4-135.3]	0.5477
TLC (L)	4.62 [3.98-5.51]	4.12 [3.18-5.14]	4.72 [4.15-5.58]	0.0461
TLC (% pred)	95.4 [77.9-108.2]	80.6 [61.3-101.7]	99.1 [84.2-110.2]	0.0051
RV/TLC (%)	41.0 [37.1-45.7]	44.0 [39.2-47.9]	40.7 [36.0-40.7]	0.0908
%DLCO (%)	69.4 [61.1-90.6]	66.5 [56.4-82.1]	70.1 [61.8-93.4]	0.1711
%DLCO/VA (%)	72.2 [54.0-86.6]	73.7 [59.9-82.4]	71.4 [53.7-87.7]	0.9242

FVC; forced vital capacity, FEV_1: forced expiratory volume in 1.0 second, FRC; functional residual capacity, RV: residual volume, TLC; total lung capacity, DLCO; diffusing capacity of the lung for carbon monoxide, VA; alveolar volume, mMRC; modified Medical Research Council, CAT; COPD assessment test

Table 3. Prediction of ir-ILD in Patients with NSCLC treated with ICIs Multivariate Fine-Gray Analyses

Predictor	HR	95% CI	p-value
Univariate analysis			
Age, yr	0.983	0.939 – 1.029	0.47
Gender, female	0.215	0.028 – 1.638	0.15
Histology, adenocarcinoma	3.054	1.031 – 9.047	0.044
Performance status,	1.249	0.593 – 2.632	0.56
PD-L1 expression, ≥1%	1.470	0.567 – 3.812	0.43
Stage, stage IV	0.940	0.362 – 2.439	0.90
Response, PR	1.292	0.855 – 1.952	0.22
FVC, %-pred	0.958	0.933 – 0.982	0.00077
FEV, %-pred	0.977	0.960 – 0.995	0.012
TLC, %	0.961	0.938 – 0.984	0.00093
FRC, %	0.967	0.943 – 0.992	0.0093
mMRC	1.477	1.021 – 2.137	0.039
Age, ≤69 yr	1.916	0.739 – 4.968	0.18
FVC, %-pred, ≤77.6%	3.851	1.420 – 10.45	0.0081
FEV, %-pred, ≤75.6%	4.857	1.650 – 14.30	0.0041
mMRC, ≥2	1.726	0.670 – 4.450	0.260
FVC, %-pred, ≤77.6% & FEV, %-pred, ≤75.6%	2.408	1.370 – 4.232	0.0023
FVC, %-pred, ≤77.6% & mMRC, ≥2	2.003	1.193 – 3.363	0.0086
FEV, %-pred, ≤75.6% & mMRC, ≥2	2.128	1.240 – 3.652	0.0061
Multivariate analysis1			
Histology, adenocarcinoma	3.000	0.993 – 9.005	0.052
FVC, %-pred	0.734	0.891 – 0.979	0.0044
FEV, %-pred	1.026	0.994 – 1.059	0.110
Multivariate analysis2			
Histology, adenocarcinoma	2.900	0.973 – 8.646	0.056
FVC, %-pred, ≤77.6%	1.838	0.446 – 7.571	0.400
FEV, %-pred, ≤75.6%	3.077	0.676 – 14.00	0.150

FVC; forced vital capacity, FEV_1: forced expiratory volume in 1.0 second, FRC; functional

residual capacity, TLC; total lung capacity, DLC, VA; alveolar volume, mMRC; modified Medical Research Council

Supplemental Table 1. Severity and outcomes of 20 NSCLC patients with ir-ILD

	ir-ILD (n=20)
CTCAE Grade	
1	6 (30%)
2	6 (30%)
3	5 (25%)
4	0 (0%)
5	3 (15%)
Treatment	
Drug Cessation only	7 (35%), [0] *
Corticosteroid	13 (65%), [8]
Outcomes	
Recovered	16 (80%), [4]
Unchanged	1 (5%), [1]
Died	3 (15%), [3]

*: Any [Grade ≥III]

13 ***Supplemental Table 2. Summary of 20 NSCLC patients with ir-ILD.***

Age/Sex	Smoking pack-years	Histology (EGFR mutants)	Initial Stage	Prior number of regimens	regimen prior to ICI	ICI	ir-ILD Grade	Time to Onset	CT pattern	Treatment	ir-ILD outcome	Treatment after ir-ILD
74M	30	Ad	IV	1	CBDCA+PEM	Nivo	1	52	COP	Cessation only	Recovered	ATZ
68M	42	Ad	IIIB	2	DOC	Nivo	1	254	COP	Cessation only	Recovered	BSC
75M	60	Ad	IV	2	DOC	Nivo	1	245	COP	Cessation only	Recovered	BSC
75M	72	Ad	IIIA	3	S-1	Nivo	1	10	GGO	Cessation only	Recovered	nPTX
55M	68	Ad	IV	1	CDDP+PEM	Nivo	1	49	HP	Cessation only	Recovered	Nivo
57M	36	Ad	IV	3	PEM	Nivo	1	826	NSIP	Corticosteroid	Recovered	Nivo
48M	15	Ad	IIIB	1	CBDCA+nPTX	Nivo	2	216	COP	Cessation only	Recovered	maintain PR
69M	0.5	SCC	IV	1	CBDCA+nPTX	Nivo	2	70	COP	Corticosteroid	Recovered	DOC
81M	30	Ad	IV	0	-	Pemb	2	119	COP	Corticosteroid	Recovered	CBDCA+nPTX
69M	40	Ad	IV	6	VNR	Nivo	2	157	GGO	Corticosteroid	Recovered	BSC
60M	20	SCC	IV	4	VNR	Nivo	2	119	HP	Corticosteroid	Recovered	nPTX
67M	0.5	Ad	IV	1	CBDCA+PEM	Nivo	2	93	NSIP	Cessation only	Recovered	DOC
67M	15	Ad	IV	2	DOC	Nivo	3	27	COP	Corticosteroid	Unchanged	BSC
65F	0	Ad (Del 19)	IV	1	CDDP+PEM	Nivo	3	28	GGO	Corticosteroid	Recovered	S1
66M	2	LCNEC	recurrent	1	CDDP+CPT-11	Nivo	3	34	HP	Corticosteroid	Recovered	CBDCA+nPTX
72M	100	Ad	IV	1	CDDP+PEM	Nivo	3	4	AIP	Corticosteroid	Recovered	BSC
54M	68	Ad	IV	2	CBDCA+nPTX	Nivo	3	12	AIP	Corticosteroid	Recovered	S-1
65M	41	Ad	IIIB	3	DOC+RAM	Nivo	5	32	AIP	Corticosteroid	Died	BSC
67M	0	Ad (Del 19)	recurrent	8	Gefitinib (washout 14 days)	Nivo	5	46	AIP	Corticosteroid	Died	BSC

81M	29	SCC	IV	2	DOC	Nivo	5	51	DAD	Corticosteroid	Died	BSC

Ad; adenocarcinoma, SCC: squarmous cell carcinoma, LCNEC; large cell neuroendocrine carcinoma, CDDP; cisplatin, CBDCA; carboplatin, nPTX; nab-paclitaxel, DOC; docetaxel, PEM: pemetrexed, S-1: tegafur/gimeracil/oteracil, VNR; vinorelbine, CPT-11; irinotecan, RAM; ramucirmab, ICI; immune checkpoint inhibitor, Nivo; nivolumab, Pemb; pembrolizumab, Atz: atezolizumab, AIP; acute interstitial pneumonia, COP; cryptogenic organizing pneumonia, NSIP; non-specific interstitial pneumonia, HP; hypersensitive pneumonia, GGO; ground glass opacities, BSC; best supportive care

Supplemental Table 3. Peripheral Blood Analyses in NSCLC patients with or without ir-ILD.

	All cases (n=138)	ir-ILD (n=20)	non ir-ILD cases (n=118)	p-value
WBC (/mm^3)	5900 [4875-7700]	7025 [5200-8243]	5900 [4875-7640]	0.1245
Neut (/mm^3)	3953 [3040-5673]	4890 [3123-6728]	3930 [2986-5528]	0.1418
Neut (%)	69.4 [63.0-77.0]	72.2 [62.9-78.0]	69.2 [63.0-76.9]	0.6738
Ly (/mm^3)	1098 [822-1586]	1368 [844-1858]	1081 [811-1514]	0.2418
Ly (%)	19.0 [12.6-25.2]	17.6 [11.3-23.8]	19.0 [13.0-26.3]	0.5050
Eo (/mm^3)	110 [58-184]	137 [75-307]	105 [56-176]	0.3498
Eo (%)	1.9 [1.0-3.2]	2.2 [1.0-4.3]	1.9 [1.0-3.1]	0.1699
Ba (/mm^3)	30 [12-51]	49 [20-88]	29 [11-48]	0.0419
Ba (%)	0.5 [0.2-0.8]	0.8 [0.3-1.0]	0.5 [0.2-0.8]	0.0637
Mo (/mm^3)	426 [326-616]	597 [370-719]	410 [320-410]	0.0630
Mo (%)	7.0 [5.7-9.0]	6.4 [6.0-8.8]	7.1 [5.5-9.0]	0.9820
IgE (IU/L)	78.0 [28.0-205.0], n=128	59.6 [15.5-1445], n=16	80.3 [31.9-206.8], n=112	0.2206
CRP (mg/dl)	0.66 [0.21-2.96]	0.74 [0.32-4.12]	0.60 [0.20-2.80]	0.5775
ESR (/mm)	53 [26-71], n=127	58 [41-82], n=18	49 [24-71], n=109	0.2731
ESR (/mm)	77 [49-98.5], n=101	79 [70-108.0], n=16	77 [47-97.5], n=85	0.4652

Supplemental Table 4. **Receiver operator curve analysis for predicting ir-ILD in patients with NSCLC administered ICIs.**

Variable :(cut-off)	AUC	95%CI	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Age: (69.0yr)	0.566	0.426 – 0.706	70.0	45.7	18.2	90.2
FVC, %-pred: (77.6%)	0.722	0.604 – 0.839	75.0	60.3	24.2	93.4
FEV, %-pred: (75.6%)	0.654	0.530 – 0.779	80.0	57.8	24.6	94.5
TLC, %: (90.7%)	0.731	0.592 – 0.869	73.3	66.7	31.4	92.3
FRC, %: (97..9%)	0.692	0.550 – 0.833	93.3	40.3	24.6	96.7
mMRC: (1)	0.639	0.509 – 0.769	83.3	38.5	18.3	93.6

FVC; forced vital capacity, FEV_1: forced expiratory volume in 1.0 second, FRC; functional residual capacity, TLC; total lung capacity mMRC, The Modified Medical Research Council; AUC, area under the curve; PPV, positive predict value; NPV, negative predict value

Supplemental Table 5. Adverse events during the ICI therapy

	All cases (n=138)	ir-ILD (n=20)	non ir-ILD cases (n=118)
Haematologic toxicity			
Leucopenia	2 (1.4%), [0]*	-	2 (1.7%), [0]
Neutropenia	2 (1.4%), [0]	-	2 (1.7%), [0]
Anemia	13 (9.4%), [0]	1 (5.0%), [0]	12 (10.2%), [0]
Thrombocytopenia	4 (2.9%), [1]	1 (5.0%), [1]	3 (2.5%), [0]
Non-haematologic toxicity			
ALT/AST increased	4 (2.9%), [2]	-	4 (3.4%), [2]
Bilirubin increased	1 (0.7%), [0]	-	1 (0.8%), [0]
Creatinine increased	5 (3.6%), [0]	-	5 (4.2%), [0]
Nausea/Appetite loss	10 (7.2%), [5]	1 (5.0%), [0]	9 (7.6%), [5]
Constipation	2 (1.4%), [0]	-	2 (1.7%), [0]
Diarrhea	11 (8.0%), [7]	1 (5.0%), [1]	10 (8.5%), [6]
Fatigue	10 (7.2%), [2]	1 (5.0%), [0]	9 (7.6%), [2]
Dysgeusia	1 (0.7%), [0]	-	1 (0.8%), [0]
Rash acneiform	6 (4.3%), [0]	2 (10.0%), [0]	4 (3.4%), [2]
Dry skin	3 (2.2%), [0]	-	3 (2.5%), [0]
Lung infection	4 (2.9%), [3]	-	4 (3.4%), [3]
Hemoptysis	2 (1.4%), [1]	-	2 (1.7%), [1]
Thromboembolic events	2 (1.4%), [0]	-	2 (1.7%), [0]
Atiroventicular block complete	1(0.7%), [1]	-	1(0.8%), [1]
Hyperglycemia	1 (0.7%), [1]	-	1 (0.8%), [1]
Immune-rerated Adverse Events			
Myositis	1 (0.7%), [1]	-	1 (0.8%), [1]
Myasthenia gravis	1 (0.7%), [1]		1 (0.8%), [1]
Type I Diabetes Mellitus, Hyperglycemia	1 (0.7%), [1]	-	1 (0.8%), [1]
Thyroiditis	6 (4.3%) [2]	1 (5.0%), [0]	5 (4.2%), [2]
Adrenal insufficiency	1 (0.7%), [0]	-	1 (0.8%), [0]
Hypophysitis	1 (0.7%), [1]		1 (0.8%), [1]
Cholangitis	1 (0.7%), [1]	1 (5.0%), [1]	-

*: Any [Grade ≥III]

Supplemental Table 6. Adverse events that disrupted the ICI therapy

ir-ILD	**Adverse Events**	**Grade**	**Onset** (days)
-	Diarrhea	2	32
-	Diarrhea	3	38
-	Diarrhea	3	98
-	Diarrhea	3	109
-	Diarrhea	3	523
-	ALT/AST increased	3	40
-	Rash acneiform	3	42
-	Creatinie increased	1	28
-	Haemoptysis	4	3
-	Atiroventicular block complete	3	327
-	Thyroiditis	3	35
-	Myasthenia gravis	3	50
-	Myositis + ALT/AST increased	3	59
-	Hypophysitis	3	133
+	Cholangitis	5	546

Figure1

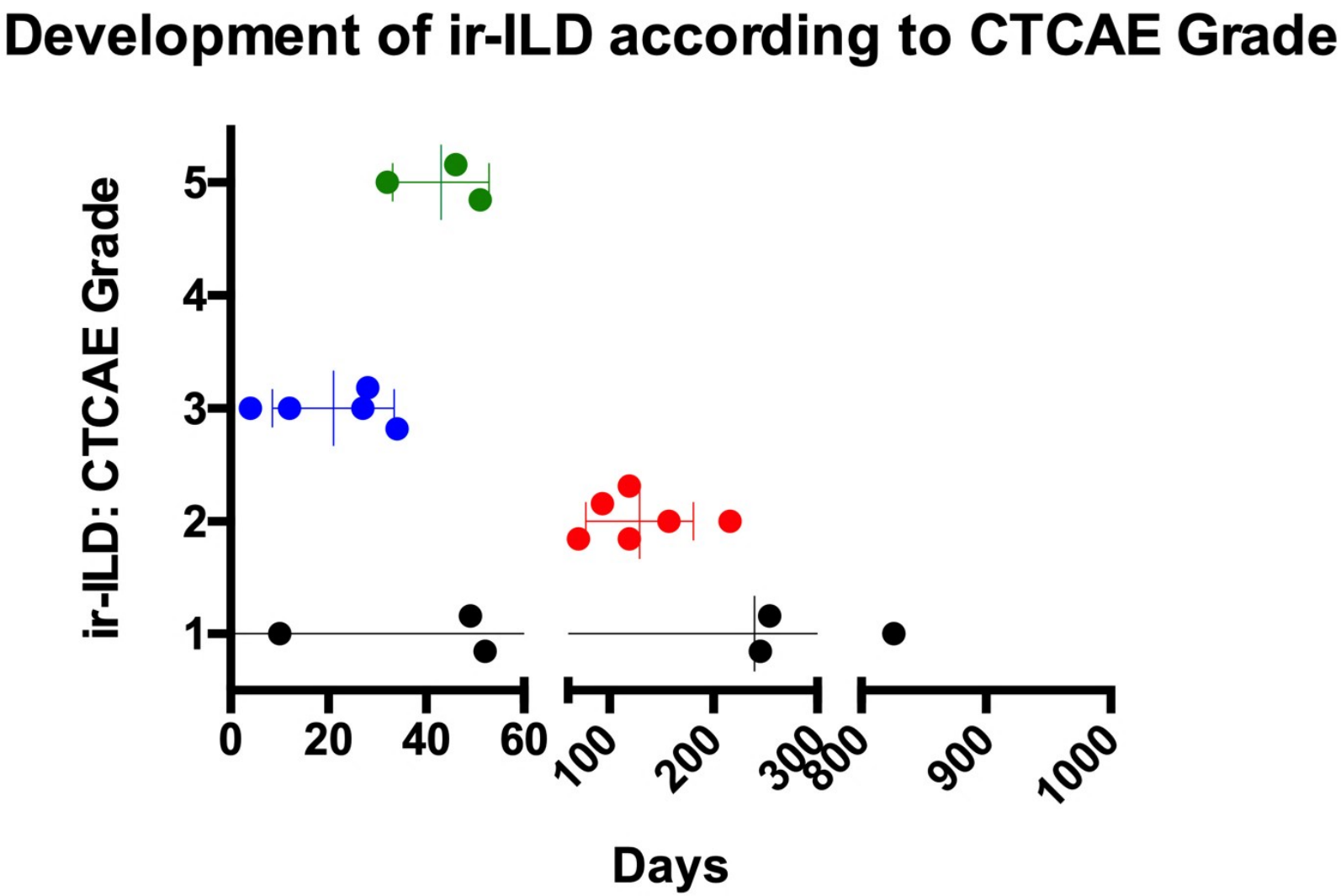
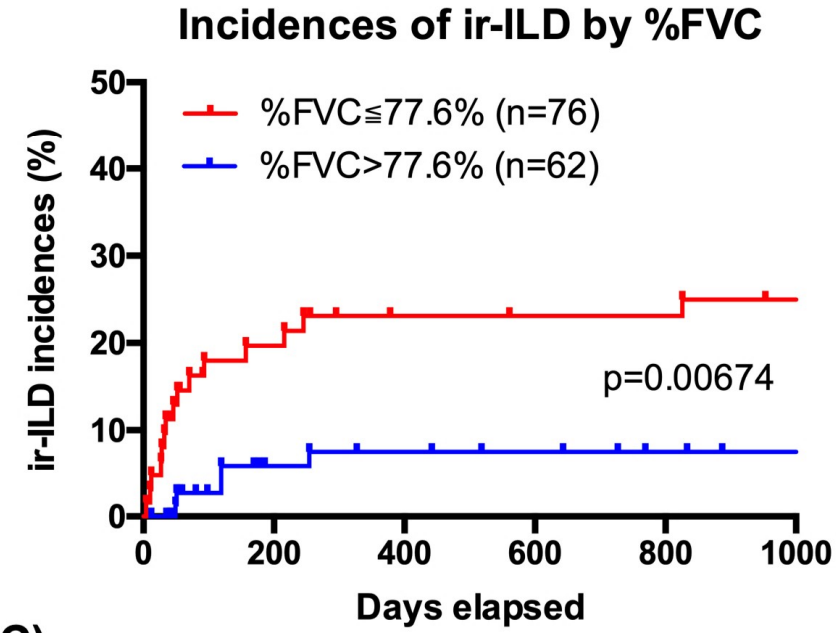
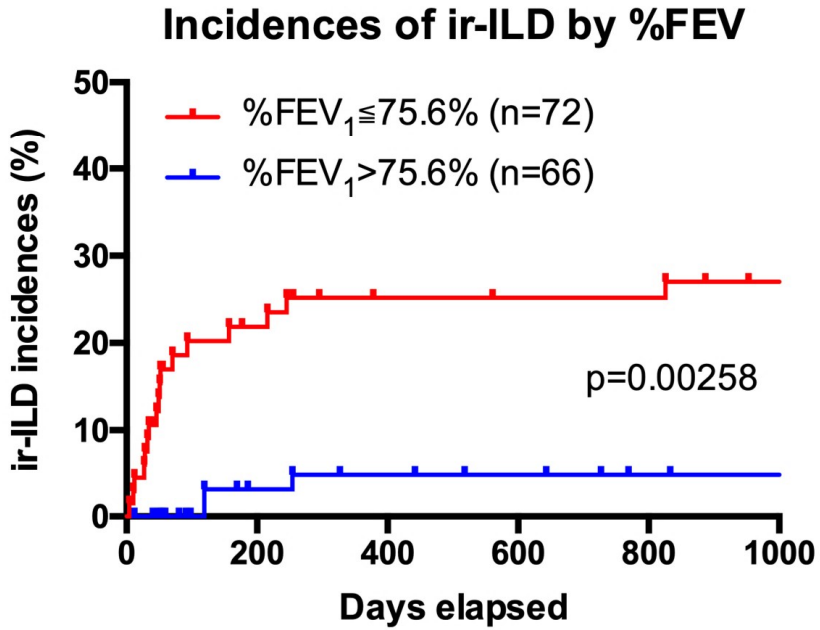

Development of ir-ILD according to CTCAE Grade
ir-ILD: CTCAE Grade
5
4
3
2
1
0
20
40
60
100
200
300
800
900
1000
Days

Figure2

A)


Incidences of ir-ILD by %FVC
%FVC≦77.6% (n=76)
%FVC>77.6% (n=62)
p=0.00674
ir-ILD incidences (%)
Days elapsed
0
10
20
30
40
50
0
200
400
600
800
1000

B)


Incidences of ir-ILD by %FEV
%FEV1≦75.6% (n=72)
%FEV1>75.6% (n=66)
p=0.00258
ir-ILD incidences (%)
Days elapsed
0
10
20
30
40
50
0
200
400
600
800
1000

C)

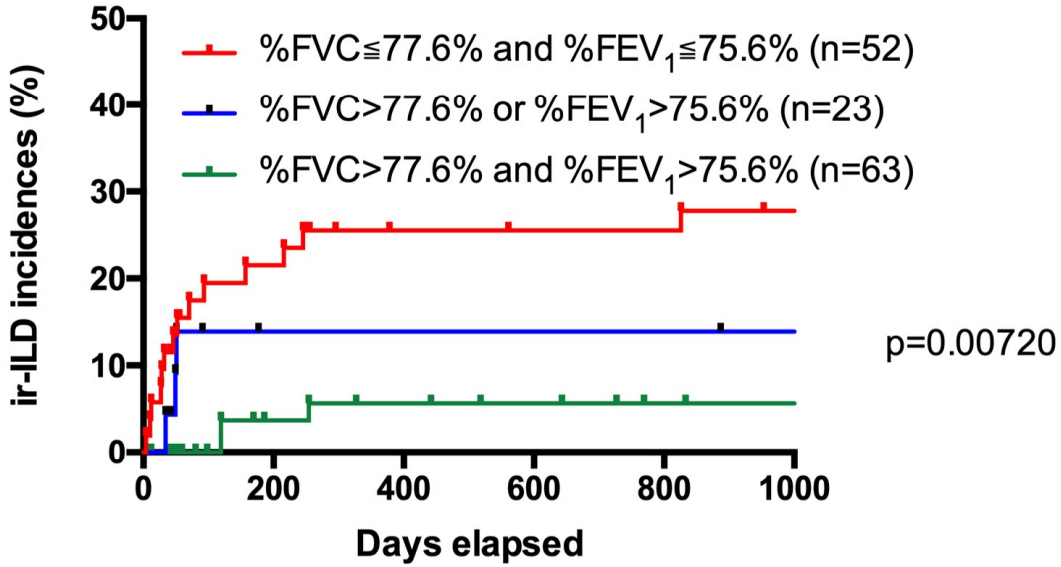
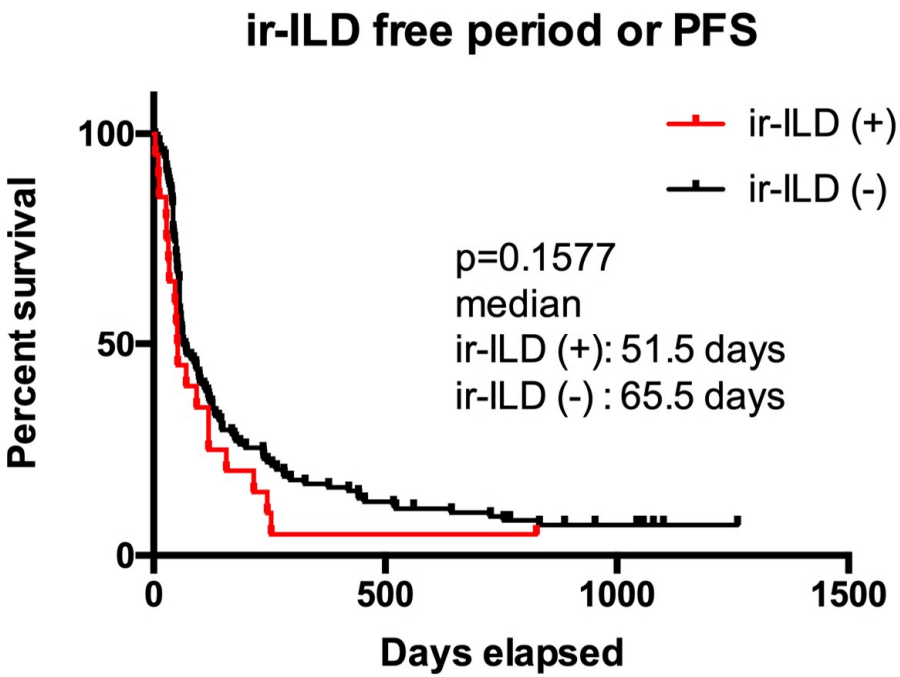
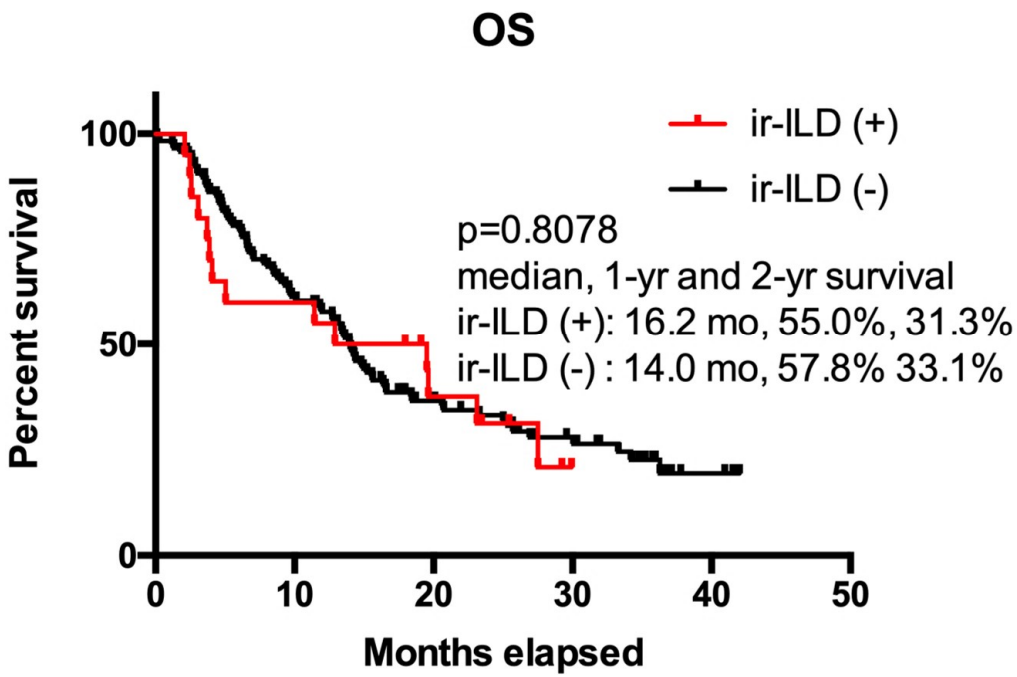

Incidences of ir-ILD by %FVC&%FEV
%FVC≦77.6% and %FEV1≦75.6% (n=52)
%FVC>77.6% or %FEV1>75.6% (n=23)
%FVC>77.6% and %FEV1>75.6% (n=63)
p=0.00720
ir-ILD incidences (%)
Days elapsed
0
10
20
30
40
50
0
200
400
600
800
1000

Figure3

A)


ir-ILD free period or PFS
ir-ILD (+)
ir-ILD (-)
p=0.1577
median
ir-ILD (+): 51.5 days
ir-ILD (-) : 65.5 days
Percent survival
100
50
0
0
500
1000
1500
Days elapsed

B)


OS
ir-ILD (+)
ir-ILD (-)
p=0.8078
median, 1-yr and 2-yr survival
ir-ILD (+): 16.2 mo, 55.0%, 31.3%
ir-ILD (-) : 14.0 mo, 57.8% 33.1%
Percent survival
100
50
0
0
10
20
30
40
50
Months elapsed