



## ORIGINAL ARTICLE

# Characterization of tumour mutation burden in patients with non-small cell lung cancer and interstitial lung disease

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## ABSTRACT

**Background and objective:** The efficacy expectation of immune checkpoint inhibitors against NSCLC in patients with ILD seems to be high because these populations are supposed to have high TMB. However, information about the characterization of TMB in patients with NSCLC and ILD is limited. Therefore, this study aimed to evaluate TMB in samples of NSCLC with ILD and clarify factors that influence TMB values.

**Methods:** The medical records of patients with NSCLC who underwent thoracic surgery at our institution between January 2014 and January 2017 were retrospectively reviewed. Whole-exome sequencing with an Ion Proton system and gene expression profiling of fresh surgical specimens were performed.

**Results:** Among 367 patients with NSCLC, 62 (16.9%) were diagnosed with ILD. All samples were collected from primary tumours with a median TMB of approximately 2.1 (range: 0.1–64.4) mutation/Mb. Among 81 squamous cell carcinomas, we compared 27 tumours with concomitant ILD and 54 tumours without ILD. Univariate analyses revealed that tumours with concomitant ILD showed lower TMB values than those without ILD. Multivariate analysis revealed that concomitant ILD was significantly associated with low TMB values. Conversely, no difference was noted in the TMB value of adenocarcinoma between patients with and without ILD.

**Conclusion:** Squamous cell carcinoma and adenocarcinoma with ILD do not have high TMB values. Therefore, considering the risk of severe pneumonitis, immune

## SUMMARY AT A GLANCE

Patients with NSCLC and ILD did not have high TMB and could develop severe pneumonitis if immune checkpoint inhibitors are used. Therefore, immune checkpoint inhibitors should not be used based on expectations of high TMB in patients with NSCLC and ILD.

**checkpoint inhibitors should not be used routinely against patients with NSCLC and ILD based on the expectation of high TMB values.**

**Key words:** interstitial lung diseases, lung cancer, non-small cell lung cancer, tumour mutation burden.

## INTRODUCTION

Interstitial lung disease (ILD) occurs at a frequency of approximately 10% among patients with lung cancer undergoing surgery in Japan.<sup>1</sup> Patients with advanced non-small cell lung cancer (NSCLC) and ILD are commonly excluded from clinical trials because of the acute exacerbation of ILD caused by chemotherapy; therefore, data on the efficacy and feasibility of chemotherapy are limited. Recently, patients with advanced NSCLC and ILD were reported to be possibly sensitive to immune checkpoint inhibitors.<sup>2–4</sup> The authors hypothesized that patients with advanced NSCLC with ILD potentially have higher tumour mutation burden (TMB) related to the efficacy of immune checkpoint inhibitors than those without ILD because ILD is associated with microsatellite instability, smoking history and male sex, which are in turn associated with higher mutation burden.<sup>5–7</sup> However, reports about TMB characterization in patients with NSCLC and ILD are limited. Moreover, in Japan, the TMB value

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cannot be measured in the clinical settings because this value is yet to be established as a biomarker for immune checkpoint inhibitors, and it can only be obtained through research on limited population. Therefore, this study evaluated TMB in patients with NSCLC and ILD, and evaluated the factors that influenced TMB values.

## METHODS

Between January 2014 and April 2017, 454 patients with NSCLC who underwent surgery at the Shizuoka Cancer Center and provided informed consent were enrolled in this study. The study protocol was approved by the institutional review board of the Shizuoka Cancer Center (IRB No. 25-33).

Whole-exome sequencing was performed on an Ion Proton system (Thermo Fisher Scientific, Waltham, MA, USA) using an Ion Torrent AmpliSeq RDY Exome Kit (Thermo Fisher Scientific). Corresponding peripheral blood samples were used to identify tumour-specific mutations. Ion Reporter AmpliSeq Exome Tumor-Normal workflow template (ver. 4.4) (Thermo Fisher Scientific) was used for the detection of tumour-specific mutations. TMB (mutations/Mb) was calculated by dividing the total number of detected tumour-specific mutations, including base substitutions, and indels by the total examined read length with  $\geq 20\times$  sequencing coverage. Samples with an estimated tumour purity of  $<20\%$  were calculated using PurBayes programme<sup>8</sup> and/or samples in which TMB is outlier in low range (TMB  $\leq 0.1$ ) were excluded because of the risk of false negatives; finally, 367 (81%) samples were analysed. The 20/20+ programme<sup>9</sup> was used to identify significantly mutated genes, and genes with 'driver q-value' of  $<0.1$  were defined statistically significant. Medical records were retrospectively reviewed, and patients were divided according to the presence/absence of ILD and histology subtype of the tumour. Patients with TMB of  $\geq 10$  mutations/Mb were defined as 'high TMB'.<sup>10</sup> In this study, ILD was defined as chronic, progressive fibrosing interstitial pneumonia of unknown cause occurring primarily in older adults or associated with connective tissue disease and occupation. ILD diagnosis is based on high-resolution computed tomography (HRCT) features as defined by the International Consensus Statement of the American Thoracic Surgery and the European Respiratory Society.<sup>11,12</sup> All chest CT scans taken just prior to surgery were evaluated before surgery by one radiologist (M.E.), approximately 10 thoracic surgeons and one pulmonologist (H.K.) who were blinded to patient outcomes. Moreover, pathological findings associated with ILD were judged by the pathology report. The tumour, node and metastasis (TNM) stages were evaluated based on the TNM classification of lung cancer (7th edition).

Univariate analyses of the correlation between TMB values and categorical variables were performed using the Wilcoxon rank-sum test, and multivariate analyses of correlation between TMB values and all categorical variables were performed using the least squares approximation. Categories assessed with regard to TMB value were sex, age ( $<75/\geq 75$  years), pack-years ( $<20/\geq 20$ ), pathological stage (stage I/II/III/IV) and existence

of ILD diagnosed by HRCT or pathological findings in resected specimens (yes/no).

All *P*-values were two-sided, with values  $<0.05$  considered statistically significant. Statistical analyses were performed using JMP 11.2.0 software (SAS Institute, Cary, NC, USA).

## RESULTS

### Patient characteristics

Overall, 62 (16.9%) of the 367 patients with NSCLC were diagnosed with ILD by HRCT or pathological findings, and 35 and 27 patients were identified with adenocarcinoma and ILD and with squamous cell carcinoma and ILD, respectively. Of the 35 adenocarcinomas with ILD, 19 were diagnosed by both HRCT and pathological findings. The remaining 16 adenocarcinomas with ILD were diagnosed only by HRCT or pathological findings. Conversely, all squamous cell carcinoma with ILD could be diagnosed by both HRCT and pathological findings.

In our study, 35 patients with usual interstitial pneumonia (UIP) pattern and 24 patients with non-UIP pattern, that is, 13 non-specific interstitial pneumonia pattern, 8 airway centred fibrosis pattern, 1 desquamative interstitial pneumonia pattern, 1 pleuroparenchymal fibroelastosis pattern and 1 unclassifiable ILD, were included.

In the subset analysis of patients with squamous cell carcinoma, there was no significant difference in smoking status between patients with and without ILD ( $P = 1.0000$ ).

### TMB characterization

Overall, 367 samples were collected from primary tumours with a median TMB of 2.1 (range: 0.1–64.4) mutation/Mb (Table 1). The median TMB values were approximately 1.7 (range: 0.1–64.4) and 5.9 (range: 0.2–25.9) mutation/Mb in adenocarcinoma and squamous cell carcinoma, respectively.

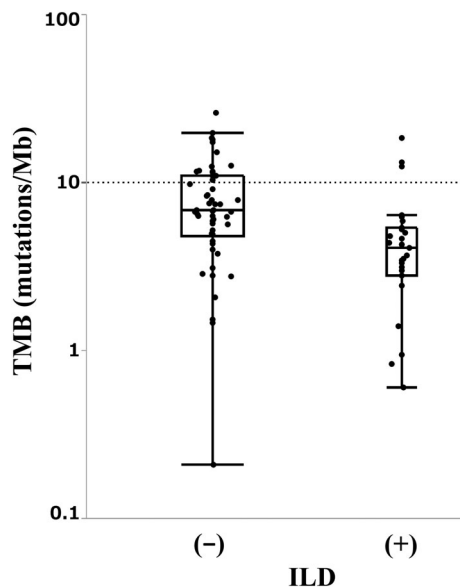
Of the 81 squamous cell carcinomas, we analysed the correlation between TMB values and categorical variables, which included comparison between 27 tumours with concomitant ILD and 54 tumours without ILD. In univariate analyses, tumours with concomitant ILD showed lower TMB values than those without ILD (Fig. 1). The median TMB values were 4.1 and 6.8 in squamous cell carcinomas with ILD and without ILD, respectively. Multivariate analysis revealed that concomitant ILD was significantly associated with low TMB ( $P = 0.0122$ ; Table 2). Moreover, the proportion of high TMB (TMB  $\geq 10$  mutations/Mb) in patients with ILD was 11.1%, whereas that without ILD was 29.6% ( $P = 0.0945$ ).

We analysed the correlation between TMB values and categorical variables in patients with adenocarcinoma. In univariate analyses, tumours in male patients and heavy smokers showed higher TMB values than those in female patients and never or light smokers. The 35 of the 286 adenocarcinoma tumours with ILD were compared with 251 tumours without ILD. No significant difference in the TMB value of adenocarcinoma between patients with ILD and those without ILD was noted (Fig. 2A). Multivariate analysis showed that pack-years  $\geq 20$  was significantly associated with high TMB ( $P < 0.0001$ ; Table 3A). Then, we evaluated 160 adenocarcinomas not

**Table 1** Baseline characteristics of patients

| Characteristics                    |             |
|------------------------------------|-------------|
| No. of patients                    | 367         |
| Age at the time of surgery (years) |             |
| Median                             | 70          |
| Range                              | 39–87       |
| Sex                                |             |
| Male                               | 223 (60.8%) |
| Female                             | 144 (39.2%) |
| Pack-years                         |             |
| ≥20                                | 209 (56.9%) |
| <20                                | 158 (43.1%) |
| Existence of ILD                   |             |
| Yes                                | 62 (16.9%)  |
| No                                 | 305 (83.1%) |
| Pathological stage                 |             |
| I                                  | 245 (66.8%) |
| II                                 | 72 (19.6%)  |
| III                                | 46 (12.5%)  |
| IV                                 | 4 (1.1%)    |
| Pathological subtype               |             |
| Squamous                           | 81 (22.1%)  |
| Adenocarcinoma                     | 286 (77.9%) |
| TMB (mutations/Mb)                 |             |
| Median                             | 2.1         |
| Range                              | 0.1–64.4    |

ILD, interstitial lung disease; TMB, tumour mutation burden.



**Figure 1** Analysis of the association between TMB and ILD in patients with squamous cell carcinoma (with ILD,  $n = 27$ ; without ILD,  $n = 54$ ).  $P = 0.0005$ . ILD, interstitial lung disease; TMB, tumour mutation burden.

harbouring driver mutation, which included 28 tumours with concomitant ILD and 132 tumours without ILD. In univariate analyses, tumours in male patients, heavy smoker and advance stage showed higher TMB. However, no significant difference in the TMB value of adenocarcinoma between patients with ILD and those

**Table 2** Univariate and multivariate analyses of TMB value in patients with SqLC ( $n = 81$ )

| Variable                              | Univariate analysis | Multivariate analysis |
|---------------------------------------|---------------------|-----------------------|
|                                       | $P$ -value          | $P$ -value            |
| Sex (female vs male)                  | 0.5719              | 0.5839                |
| Age ( $\leq 75$ vs $> 75$ years)      | 0.4171              | 0.7300                |
| Pack-years ( $< 20$ or $\geq 20$ )    | 0.7937              | 0.2794                |
| Pathological staging (I vs II vs III) | 0.8893              | 0.9663                |
| Existence of ILD (yes vs no)          | 0.0005              | 0.0122                |

ILD, interstitial lung disease; SqLC, squamous cell lung cancer; TMB, tumour mutation burden.

without ILD was found (Fig. 2B). Multivariate analysis showed that pack-years  $\geq 20$  was also significantly associated with high TMB ( $P = 0.0024$ ; Table 3B). Furthermore, the proportion of high TMB (TMB  $\geq 10$  mutations/Mb) in patients with ILD was 17.9%, whereas that without ILD was 18.9% ( $P = 1.0000$ ).

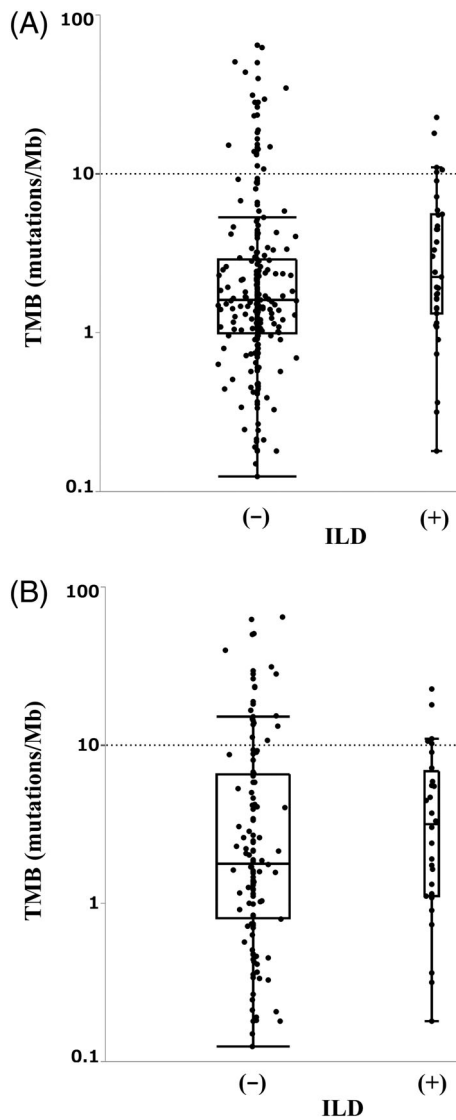
Moreover, we compared the TMB value between tumours located outside the radiological findings of ILD and those within. However, no significant difference was observed in both tumours ( $P = 0.8377$ ).

Furthermore, no significant difference was observed in the TMB value of NSCLC between patients with UIP pattern and those with non-UIP pattern ( $P = 0.4778$ ).

Nine and 10 genes were detected as significantly mutated genes in squamous cell carcinomas with and without ILD, respectively. Among them, *TP53* and *RBI* were commonly detected. Significant differences in frequencies in each gene were not observed between squamous cell carcinomas with and without ILD (Table S1 in Supplementary Information). In adenocarcinoma, 9 and 13 genes were detected as significantly mutated genes in patients with and without ILD, respectively (Table S1 in Supplementary Information). *EGFR*, *KRAS*, *TP53* and *RBI* were commonly detected in both groups (patients with and without ILD). The frequencies of *KRAS* and *TP53* in patients with ILD were significantly higher than those in patients without ILD. Conversely, patients with ILD showed low frequency in *EGFR* mutations compared with that in patients without ILD. These observations were consistent with previous reports.<sup>13</sup> Meanwhile, no significant difference was found between patients with and without ILD in frequency of mutations in genes relevant to pulmonary surfactant system (*NKX2-1/TTF1*, *SFTPA1*, *SFTPA2*, *SFTPB* and *SFTPC*) that has been reportedly associated with ILD (data not shown).<sup>13</sup>

## DISCUSSION

Reports about TMB characterization in patients with NSCLC and ILD are limited. We compared TMB in patients with NSCLC and concomitant ILD with those without ILD. Concomitant ILD was significantly associated with low TMB in patients with squamous cell carcinoma. In addition, small proportion (11.1%) of patients with squamous cell carcinoma and concomitant ILD



**Figure 2** (A) Analysis of the association between TMB and ILD in patients with adenocarcinoma (with ILD,  $n = 35$ ; without ILD,  $n = 251$ ).  $P = 0.0504$ . (B) Analysis of the association between TMB and ILD in patients with adenocarcinoma not harbouring driver mutation (with ILD,  $n = 28$ ; without ILD,  $n = 132$ ).  $P = 0.3960$ . ILD, interstitial lung disease; TMB, tumour mutation burden.

was classified into high TMB group (TMB  $\geq 10$  mutations/Mb). Our results were contrary to the findings of previous studies that reported that advanced NSCLC with ILD may be sensitive to immune checkpoint inhibitors.<sup>2-4</sup> Our findings indicate that immune checkpoint inhibitors should not be routinely used in patients with NSCLC and ILD based on the expectations of high TMB, which cannot be measured in clinical settings. Furthermore, these patients could be at risk of developing severe pneumonitis caused by immune checkpoint inhibitors.

We tried to find the cause of ‘low’ TMB observed in patients with squamous cell carcinoma and concomitant ILD. Generally, the TMB value of the patients harbouring driver mutations tends to be low.<sup>14</sup> However, we could not identify appropriate driver mutations specific to

**Table 3** Univariate and multivariate analyses of TMB value (A) in patients with adenocarcinoma ( $n = 286$ ) and (B) in patients with adenocarcinoma not harbouring driver mutation ( $n = 160$ )

| (A)   |  |  |
|---|--|--|
| Variable                                    | Univariate analysis<br><i>P</i> -value | Multivariate analysis<br><i>P</i> -value |
| Sex (female vs male)                        | <0.0001                                | 0.1166                                   |
| Age ( $\leq 75$ vs $> 75$ years)            | 0.8911                                 | 0.3462                                   |
| Pack-years ( $< 20$ or $\geq 20$ )          | <0.0001                                | <0.0001                                  |
| Pathological staging (I vs II vs III vs IV) | 0.1973                                 | 0.5517                                   |
| Existence of ILD (yes vs no)                | 0.0504                                 | 0.1744                                   |
| (B)   |  |  |
| Variable                                    | Univariate analysis<br><i>P</i> -value | Multivariate analysis<br><i>P</i> -value |
| Sex (female vs male)                        | <0.0001                                | 0.2904                                   |
| Age ( $\leq 75$ vs $> 75$ years)            | 0.2026                                 | 0.4774                                   |
| Pack-years ( $< 20$ or $\geq 20$ )          | <0.0001                                | 0.0024                                   |
| Pathological staging (I vs II vs III vs IV) | 0.0338                                 | 0.7805                                   |
| Existence of ILD (yes vs no)                | 0.3960                                 | 0.1506                                   |

ILD, interstitial lung disease; TMB, tumour mutation burden.

patients with squamous cell carcinoma and concomitant ILD in this study (Table S1 in Supplementary Information). Driver mutations in such patients have also not been reported in other studies. Therefore, further studies are necessary to identify mutations relevant to the mechanisms of carcinogenesis in patients with squamous cell carcinoma and ILD by more extensive exploration of intergenic regions and introns using whole-genome sequencing instead of whole-exome sequencing that was used in this study.

This study revealed no difference in the TMB value of adenocarcinoma between patients with and without ILD. Moreover, the proportion of high TMB (TMB  $\geq 10$  mutations/Mb) in patients with adenocarcinoma with ILD was the same as that in patients without ILD. Recently, the medium single-nucleotide variant rates in adenocarcinoma with UIP were reportedly significantly higher than those without UIP.<sup>13</sup> However, TMB data were not shown. Therefore, the results of this study, in which the TMB value of adenocarcinoma with ILD, which was different from the expected high TMB value,<sup>2-4</sup> was the same as that in patients without ILD, could be valuable.

Several limitations of the present study should be considered. First, the present study was a single centre, retrospective study; however, the present study evaluated TMB values in as many as 367 patients with NSCLC with ILD. Second, all patients with recurrent NSCLC who had ILD in the present study did not receive immune checkpoint inhibitors. Therefore, we could not evaluate their efficacy. However, the present

study revealed that patients with NSCLC and ILD should not routinely receive immune checkpoint inhibitors because of the expectation of high TMB. This is because immune checkpoint inhibitors may cause high risk of the acute exacerbation of ILD.

In conclusion, our data suggest that squamous cell carcinoma and adenocarcinoma with ILD do not have high TMB. Immune checkpoint inhibitors should not be routinely used in patients with NSCLC and ILD based on the expectation of high TMB, considering the risk of severe pneumonitis.

**Author contributions:** Conceptualization: Har.K., T.T. Data curation: Har.K., M.S., M.E. Formal analysis: Har.K., M.S., T.T. Investigation: Har.K. Methodology: Har.K., M.S. Project administration: Har.K., Tat.N., Hay.K., Hid.K., T.M., M.I., M.E., Tak.N., M.K., K.U., K.O., K.Y., Y.O., T.T. Writing—original draft: Har.K., M.S. Writing—review and editing: Har.K., M.S., Tat.N., Hay.K., Hid.K., T.M., M.I., M.E., Tak.N., M.K., K.U., K.O., K.Y., Y.O., T.T.

**Abbreviations:** ILD, interstitial lung disease; NSCLC, non-small cell lung cancer; TMB, tumour mutation burden; TNM, tumour, node and metastasis; UIP, usual interstitial pneumonia.

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### Supplementary Information

Additional supplementary information can be accessed via the *html* version of this article at the publisher's website.

**Table S1.** Significantly mutated genes in lung squamous cell carcinoma patients with and without interstitial lung disease.

**Table S2.** Significantly mutated genes in lung adenocarcinoma patients with and without interstitial lung disease.