Lung Cancer 137 (2019) 85–93

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Impact of EGFR-TKIs combined with PD-L1 antibody on the lung tissue of *EGFR*-driven tumor-bearing mice

Yijun Jia[a,1], Sha Zhao[a,1], Tao Jiang[a,1], Xuefei Li[b], Chao Zhao[b], Yiwei Liu[a], Ruoshuang Han[a], Meng Qiao[a], Sangtian Liu[a], Chunxia Su[a], Shengxiang Ren[a], Caicun Zhou[a,*]

[a] Department of Medical Oncology, Shanghai Pulmonary Hospital & Thoracic Cancer Institute, Tongji University School of Medicine, Shanghai, 200433, PR China
[b] Department of Lung Cancer and Immunology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, 200433, PR China

ARTICLE INFO

Keywords:
Lung cancer
EGFR-TKI
PD-L1
Pneumonitis

ABSTRACT

Objectives: EGFR-targeted tyrosine kinase inhibitors (TKIs) have been the standard treatment for non-small cell lung cancer patients with *EGFR* mutations. However, most patients eventually develop resistance. With the development of immune checkpoint inhibitors targeting the programmed cell death receptor/ligand 1 (PD-1/PD-L1), there is a growing interest in developing combination strategies. However, there are concerns that the combination of a PD-(L)1 inhibitor and EGFR-TKI may be associated with an increased risk of pneumonitis. Therefore, we utilized an established EGFR-driven tumor-bearing mouse model to investigate whether the combination would induce pneumonitis in mouse lung tissue.

Materials and Methods: Mice were treated with monotherapy or combined therapy of PD-L1 antibody and EGFR-TKIs including first-generation gefitinib and third-generation osimertinib. Bronchoalveolar lavage fluids (BALFs) and lung tissues were collected for analysis at the end of treatment.

Results and Conclusion: The osimertinib and anti-PD-L1 combined treatment group had the highest inflammation scores in pathologic grades of H&E staining of lung tissue and had the highest percentages of myeloperoxidase positive cells. However, combining gefitinib and anti-PD-L1 treatment appeared to not increase the level of pneumonitis in mice. Total cell counts, neutrophil counts and total protein concentration in BALFs were also significantly increased in the osimertinib and anti-PD-L1 combined treatment group. We next evaluated proinflammatory factors in BALFs. The levels of IFN-γ, IL-2, IL-5, TNF-α and IL-12p70 were increased in osimertinib and anti-PD-L1 combined treatment group. Comparison of different sequences of drug administration demonstrated that mice treated with osimertinib followed by PD-L1 antibody did not show evident lung inflammation. Our findings indicate that osimertinib, rather than gefitinib combined with anti-PD-L1 treatment could lead to lung injury in an *EGFR* mutated tumor-bearing mouse model. The sequence and timing of combining EGFR-TKI and PD-L1 antibody may influence the severity of pneumonitis.

1. Introduction

The development of EGFR tyrosine kinase inhibitors (TKIs) in advanced non-small cell lung cancer (NSCLC) patients with *EGFR* mutations represents one of the most significant advances in lung cancer management over the last several decades [1]. For years, first-generation EGFR-TKIs like gefitinib and erlotinib have been recommended as first-line therapy for patients with NSCLC harboring an *EGFR* mutation [2,3]. However, almost all patients will eventually develop resistance. The *EGFR* secondary *T790M* mutation appears to be one of the most common resistance mechanisms, accounting for 50–60% of resistance [4]. Osimertinib is a third-generation, irreversible EGFR-TKI that selectively inhibits both sensitizing *EGFR* mutation and *EGFR T790M* resistance mutation [5]. It has been approved for the treatment of patients with *T790M*-positive NSCLC who have acquired resistance to prior-line EGFR-TKIs, and also as first-line therapy for patients with activating *EGFR* mutations [6,7]. In spite of the superior efficacy and prolonged PFS of osimertinib, progression inevitably occurs, and thus

Abbreviations: TKIs, Targeted tyrosine kinase inhibitors; NSCLC, Non-small cell lung cancer; PD-1/PD-L1, Programmed cell death receptor/ligand 1; BALFs, Bronchoalveolar lavage fluids; MPO, Myeloperoxidase

* Corresponding author at: Department of Medical Oncology, Shanghai Pulmonary Hospital & Thoracic Cancer Institute, Tongji University School of Medicine, No. 507, Zheng Min Road, Shanghai, 200433, PR China.
E-mail address: drzhoucaicun@163.com (C. Zhou).

[1] These authors contributed equally to this paper.

https://doi.org/10.1016/j.lungcan.2019.09.016
Received 20 June 2019; Received in revised form 17 September 2019; Accepted 18 September 2019

novel therapeutic strategies are required.

Another achievement in the management of advanced NSCLC has been the development of immune checkpoint inhibitors targeting programmed death receptor/ligand 1 (PD-1/PD-L1) [8]. Previously, studies have reported that the activation of oncogenic *EGFR* pathway signaling induces PD-L1 expression, which may lead to a PD-L1 mediated tumor immune escape [9,10]. Moreover, in a preclinical study, the PD-1 antibody showed a significant anti-tumor effect in murine models harboring an *EGFR* mutation [9]. Based on these findings, there is a growing interest in exploring the possibility of combining PD-(L)1 blockade with EGFR-TKIs in clinic. Several clinical studies have been conducted regarding this combination therapy in EGFR-TKI naïve or pre-treated NSCLC patients with *EGFR* mutation [11–13].Although there seemed to be potential for enhanced activity, the combination of PD-(L)1 antibody and EGFR-TKI was reported as causing an unexpected high incidence of adverse events, resulting in the limitation of further investigation [14].

Pneumonitis is one of the most serious clinical problems during the treatment of immune checkpoint inhibitors [15]. A recent analysis demonstrated an increased risk of pneumonitis when patients received nivolumab in combination with EGFR-TKI compared with treatment with either drug alone [16].Moreover, a phase Ib clinical trial of concurrent durvalumab plus osimertinib (TATTON) was terminated due to high rates of interstitial lung disease(ILD) [17]. However, important uncertainties remain to be explained. The association between the incidence of pneumonitis and the sequence and timing of anti-PD-(L)1 antibody and EGFR-TKI has not been well accessed. A recent study demonstrated that sequential PD-(L)1 blockade followed later by osimertinib was related to a higher incidence of adverse events [18]. Meanwhile, the underlying mechanism associated with the high incidence of ILD remains uncertain. Despite unrelated mechanisms of action, both targeted therapy and immune checkpoint inhibitors can lead to changes in the immune environment [19,20]. Therefore, the interaction between EGFR-TKIs, immune checkpoint inhibitors and the immune system need to be explained. In this study, we utilized a genetically-engineered *EGFR*-driven mouse model to explore the effects of combined PD-L1 antibody and EGFR-TKI treatment in mouse lung tissues, aiming to provide implications to minimize the pulmonary toxicity of this combination.

2. Materials and methods

2.1. Animal model

All animal experiments were approved by the Institutional Animal Care and Use Committees at Shanghai Pulmonary Hospital. A doxycycline-inducible transgenic $EGFR^{L858R}$ mouse model was provided as a gift from Dr. Liang Chen of the National Institute of Biological Sciences. Orthotopic lung tumors were induced as previously described [21]. Briefly, activation of the mutant *EGFR* transgene was accomplished by feeding mice with doxycycline at a concentration of 0.5 g/L in 2% sucrose drinking water throughout the study. Age- and sex-matched transgenic mice were used in the experiments. FVB background mice without doxycycline induction of lung tumor were used as control (no tumor-bearing) mice.

2.2. Treatment

Osimertinib and gefitinib were purchased from Selleck and dissolved at a concentration of 5 mg/kg and 6.5 mg/kg in 1% DMSO, respectively, for *in vivo* use. Osimertinib or gefitinib was administered once daily by oral gavage. Anti-mouse PD-L1 mAb (InvivoMab anti-mousePD-L1, clone: 10 F.9G2, BioXcell) was given by intraperitoneal injection at 200ug/dose every three days. The vehicle (control) group was given 1% DMSO by oral gavage and/or isotype control IgG by intraperitoneal injection.

Schemes for the administration schedule are shown in Fig. 1B and Fig. 4A. Briefly, in the first cohort, mice were randomized into six groups with six mice per group: (a) vehicle control; (b) anti-PD-L1; (c) osimertinib; (d) osimertinib plus anti-PD-L1; (e) gefitinib; and (f) gefitinib plus anti-PD-L1. In the second cohort, mice were randomized into four groups with six mice per group: (a) osimertinib; (b) osimertinib followed by anti-PD-L1 one day later; (c) anti-PD-L1 followed by osimertinib one day later; and (d) concurrent osimertinib plus anti-PD-L1.

2.3. Bronchoalveolar lavage and cytokine measurement

To obtain bronchoalveolar lavage fluids (BALFs), 1 mL of PBS was injected into the trachea to inflate the lungs and later aspirated. Cell numbers in BALFs were automatically counted by using a Countess II FL Automated Cell Counter (Invitrogen, Thermo Fisher Scientific). Cells were then adhered to slides using a cytospin and stained with Diff-Quick stain (Yesean Biotech) for classification. BALF supernatants were separated by centrifugation and then stored at −80 °C until use. Total protein concentrations were determined by using the BCA Protein Assay Kit (Solarbio Life Science) before cytokine measurement. Levels of IFN-γ, IL-2, IL-5, TNF-α, GRO/KC and IL-12p70 in BALFs were measured using U-plex Biomarker Group Assays from Meso Scale Discovery according to the manufacturer's instructions. All samples were measured in duplicate.

2.4. Histology and immunohistochemistry

Lungs were inflated with 10% formalin and embedded in paraffin. Subsequently, 5-μm sections were cut for hematoxylin/eosin staining and immunohistochemistry. Pathologic scoring on H&E staining was performed by two independent investigators. To evaluate the degree of inflammation, the following criteria were used: 0 = no lung abnormality; 1 = presence of inflammation involving 25% of the lung parenchyma; 2 = lesions involving 25–50% of the lung; and 3 = lesions involving 50% of the lung. The sections were stained with anti-myeloperoxidase (MPO) antibody (Abcam, #ab9535) for IHC analysis. Quantification of MPO positive cells was examined in at least five random fields from each section.

2.5. Statistical analysis

Numerical results are presented as means ± SEM. Statistical analyses between two groups were performed using the unpaired student *t* test. One-way ANOVA was used for the multiple comparison among treatment groups. $p < 0.05$ was considered statistically significant. All data analyses were performed using GraphPad Prism (version 5.0, GraphPad Software, Inc.).

3. Results

3.1. Effects of concurrent EGFR-TKI and anti-PD-L1 treatment on lung inflammation

Tumor and normal lung tissue were distinguished by H&E stain (Fig. 1A). Tumor bearing mice were treated with EGFR-TKI or PD-L1 antibody alone or in combination as shown in Fig.1B. On day 9, mice were sacrificed and lungs were harvested for histological analysis to evaluate inflammatory degree. Compared with osimertinib alone, concurrent treatment of osimertinib plus PD-L1 antibody had significantly higher score for inflammation. However, combined treatment of

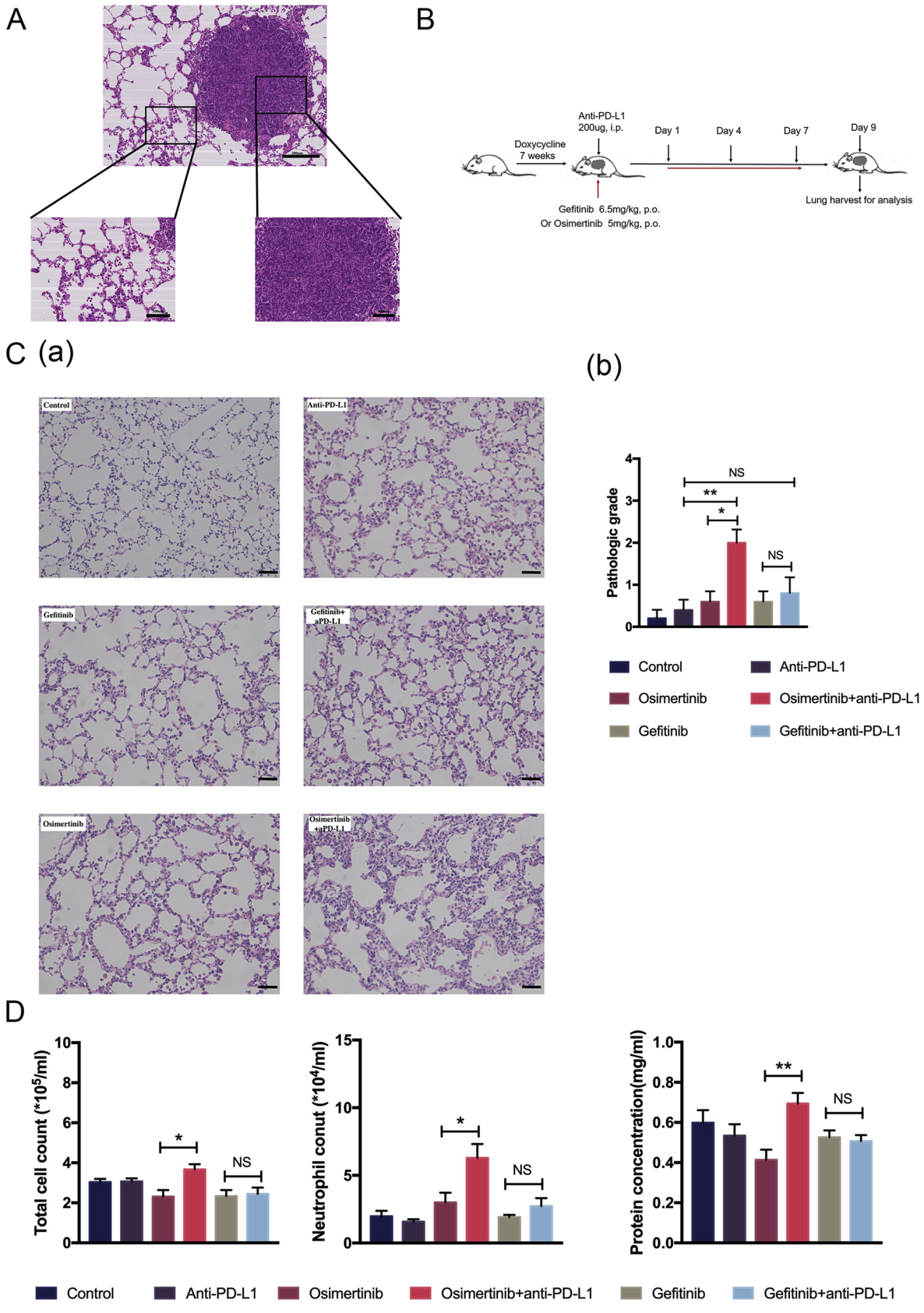

Fig. 1. Effects of concurrent EGFR-TKIs and anti-PD-L1 treatment on lung inflammation. A: Representative H&E staining of lung tumor and normal lung tissue (scale bars, upper 100 μm, lower 50 μm). B: Experimental scheme detailing the administration of gefitinib or osimertinib and PD-L1 antibody. C: Representative images of H &E stained of lung tissue from mice treated with different regimens (scale bars, 50 μm). (b): The pathologic grade of inflammation was quantified. *P* values were calculated using a one-way ANOVA. D:(a) BALFs harvested on day 9 were analyzed for total cell counts, neutrophil counts and protein concentrations. To compare differences between single-agent TKI and TKI plus anti-PD-1, unpaired student *t* test was used. Results were presented as mean ± SEM. N = 5–6 per group. *P < 0.05, **P < 0.01, ***P < 0.001, P value > 0.05 was indicated as no significance (NS).

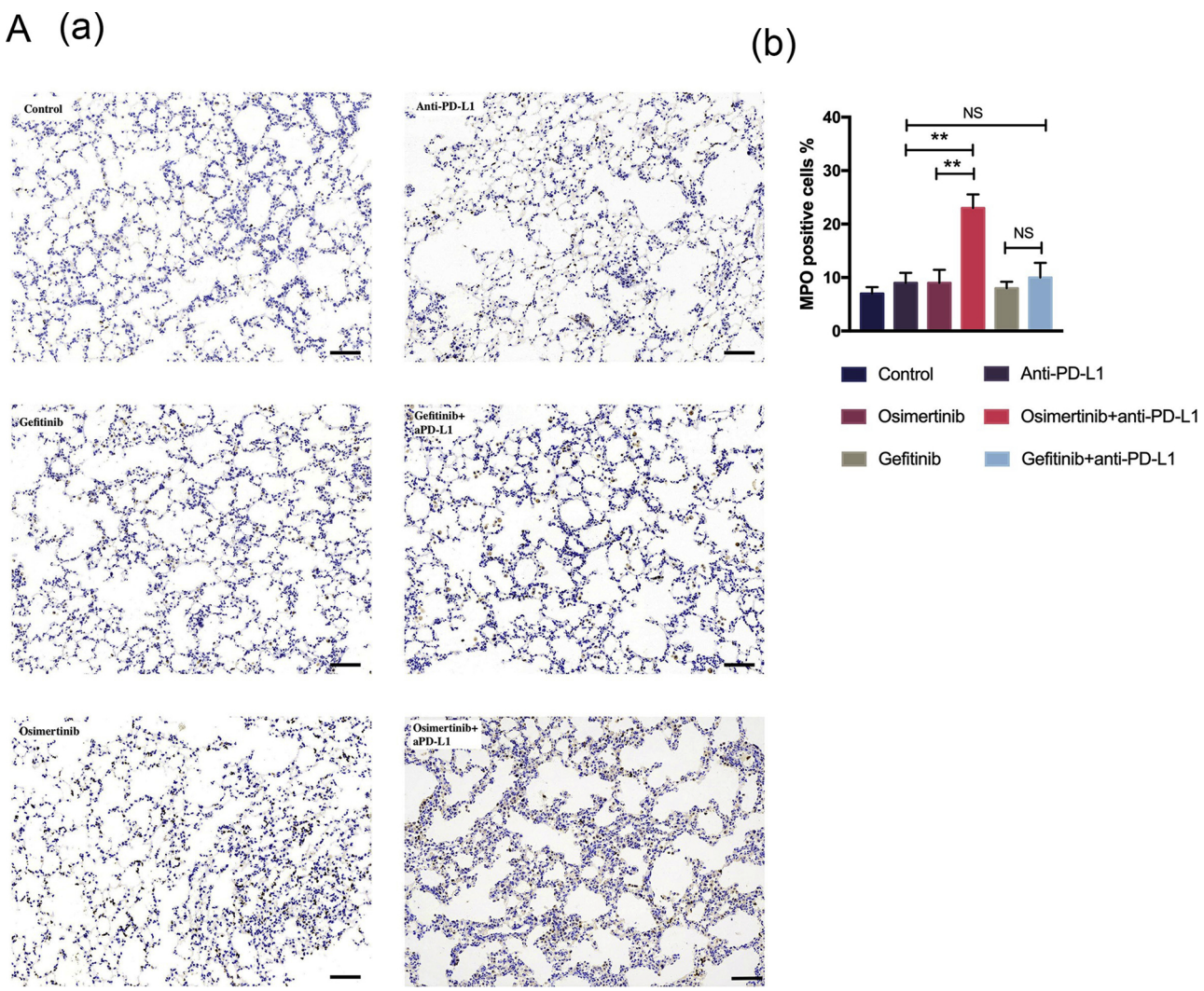

Fig. 2. A: Representative images of IHC staining for MPO in lung tissues of mice from different treatment groups (scale bars, 50 μm). B: Percentages of MPO-positive cells were quantified. Results were presented as mean ± SEM. N = 5–6 per group. *P* values were calculated using a one-way ANOVA. *P < 0.05, **P < 0.01, ***P < 0.001, P value > 0.05 was indicated as no significance (NS).

gefitinib and PD-L1 antibody did not seem to exacerbate lung injury compared with gefitinib alone (Fig. 1C). We next measured total cell counts, neutrophil counts and protein concentrations in bronchoalveolar lavage fluids. Similarly, the combined administration of osimertinib and PD-L1 antibody increased total cell counts, total number of neutrophils and protein concentrations in the BALFs, compared with single-agent osimertinib treatment. Meanwhile, changes were not observed for total cell counts, neutrophil counts and protein concentrations between gefitinib plus anti-PD-L1 and gefitinib alone (Fig. 1D).

Myeloperoxidase (MPO) is abundantly expressed in neutrophils and secreted during their activation, which has been considered as an important marker for inflammatory response [22]. Therefore, we further evaluated the expression of MPO in lung tissue to confirm the degree of pneumonitis. As expected, the percentages of MPO positive cells in the osimertinib and anti-PD-L1 combined treatment group were the highest among all groups (Fig. 2).

We next performed the same experiments on control mice which were FVB background mice without doxycycline induction of lung tumor. Mice were treated with osimertinib, gefitinib, PD-L1 antibody, osimertinib plus PD-L1 antibody, gefitinib plus PD-L1 antibody, or vehicle. Lungs were harvested for H&E and MPO staining on day 9. Combining gefitinib or osimertinib with anti-PD-L1 treatment appeared not to increase the level of pathologic grade of H&E staining (supplementary Fig. 1) and the percentage of MPO positive cells (supplementary Fig. 2) in mice without tumors.

3.2. EGFR-TKIs and anti-PD-L1 combined treatment changed cytokine levels in BALFs

Given that total cell counts, neutrophil counts and protein concentrations of BALFs were significantly increased in the osimertinib and PD-L1 antibody combined treated group, we next measured if proinflammatory cytokine (IFN-γ, IL-2, IL-5, TNF-α, GRO/KC and IL-12p70) production in BALFs was changed by combination treatment. The concentrations of IFN-γ, IL-2, IL-5, TNF-α and IL-12p70 were significantly higher in the osimertinib plus anti-PD-L1 treatment group compared with the single osimertinib treatment group. Similarly, the addition of PD-L1 antibody to gefitinib did not lead to changes of proinflammatory cytokines in BALFs (Fig. 3).

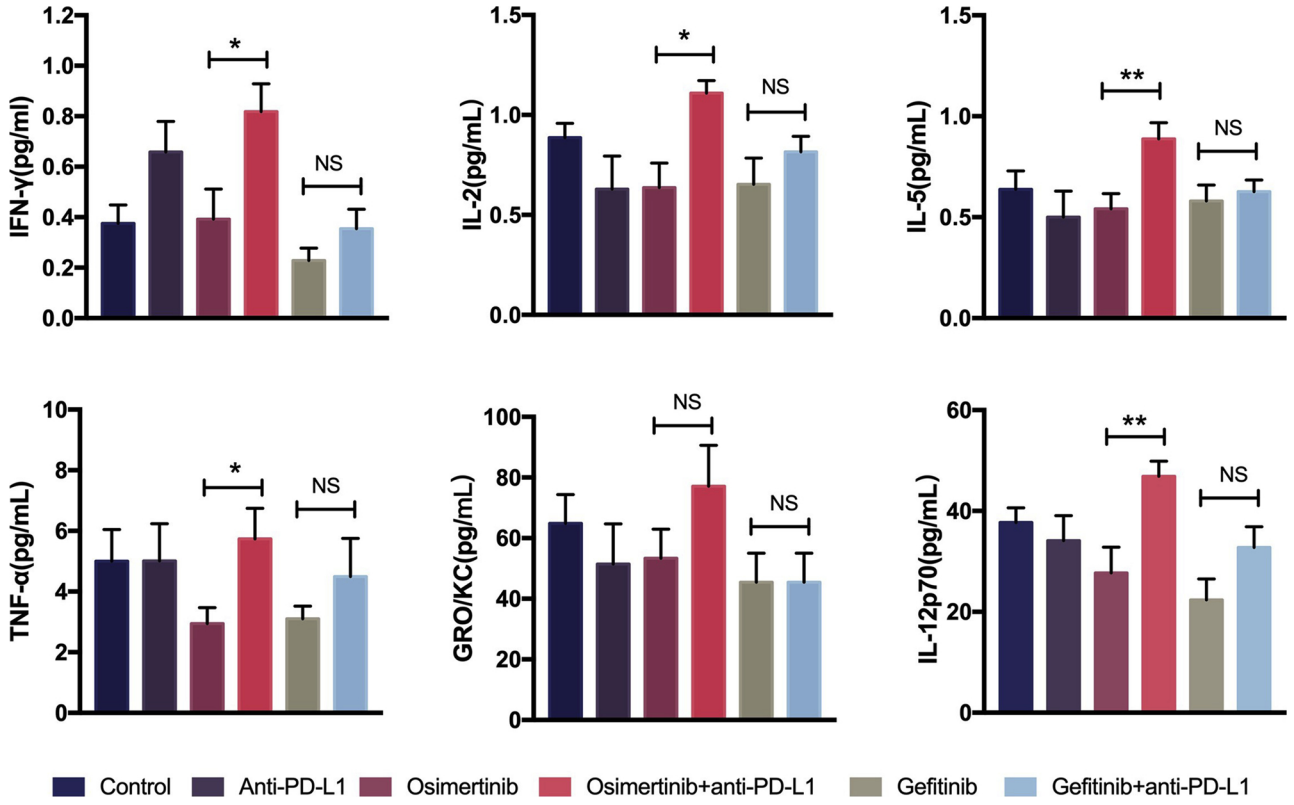

Fig. 3. EGFR-TKIs and anti-PD-L1 combined treatment changed cytokine levels in BALFs. The levels of IFN-γ, IL-2, IL-5, TNF-α, GRO/KC and IL-12p70 concentration in BALFs from different treatment groups were measured by MSD assays. Results were presented as mean ± SEM. N = 5–6 per group. To compare differences between single-agent TKI and TKI plus anti-PD-L1, unpaired student *t* test was used. *P < 0.05, **P < 0.01, ***P < 0.001, P value > 0.05 was indicated as no significance (NS).

3.3. Sequence of administration affected the occurrence of pneumonitis

To explore if there was potential association between pneumonitis and the sequence of drug use, tumor-bearing mice were treated with osimertinib alone, osimertinib concurrently, or sequentially combined with PD-L1 antibody as shown in Fig. 4A. Pathologic grades of H&E staining showed a more severe level of pneumonitis in the concurrent treatment group and in anti-PD-L1 followed by the osimertinib treatment group (Fig. 4B). Total cell counts, neutrophil counts and protein concentrations in BALFs were also increased in only these two groups only (Fig. 4C). We further used MPO immunohistochemical analysis to confirm the pneumonitis evaluation. As expected, the percentages of MPO positive cells were increased in both the concurrent use group and anti-PD-L1 followed by the osimertinib group. And treatment with osimertinib followed by anti-PD-L1 did not seem to exacerbate lung inflammation (Fig. 5).

We also evaluated whether the production of proinflammatory cytokines (IFN-γ, IL-2, IL-5, TNF-α, GRO/KC and IL-12p70) in BALFs was correlated with the sequence of drug administration. The concentrations of IFN-γ, IL-2, IL-5, TNF-α, and IL-12p70 in BALFs were elevated in the concurrent treatment group and in sequential anti-PD-L1 followed by the osimertinib group. Additionally, there was no significant changes of proinflammatory cytokines in mice treated with osimertinib followed by PD-L1 antibody compared with osimertinib alone (Fig. 6).

4. Discussion

With the increasing interest in developing EGFR-TKIs and PD-(L)1 antibody combined strategy for advanced NSCLCs with mutated *EGFR*, the elevated risk of adverse effects with the use of this combination is a significant concern that needs to be addressed. In this study, we demonstrated that the combined use of EGFR-TKI and PD-L1 antibody is associated with increased inflammation in the lung tissue of EGFR-driven tumor-bearing mice. More importantly, the occurrence of pneumonitis appears to be drug-specific, and also dependent on the sequence of combined treatment. Although this study is a pre-clinical study, our results provide implications to minimize pulmonary toxicity and to determine an optimal sequence for combinational strategies in advanced lung cancer patients with *EGFR* mutations.

In the TATTON study, the frequency of interstitial lung disease was 38% and 64% in patients with *EGFR*-mutant NSCLC who progressed during prior line EGFR-TKI treatment and patients with EGFR-TKI naïve respectively, which was much higher than patients that received single agent osimertinib (2.9%) or durvalumab (2%) [17,23]. Considering that the ILD issue has not been reported from combination studies of other EGFR-TKIs like gefitinib or erlotinib plus immune checkpoint inhibitors, osimertinib might be considered as responsible for a high incidence of ILD when combined with immune checkpoint inhibitors [11–13]. In a study performed on a naphthalene-induced acute pneumonitis mouse model, combined administration of second-generation EGFR-TKI afatinib and PD-1 antibody also showed no additional effect on the pathologic grade of lung tissue [24].Our study also showed that the group receiving osimertinib and anti-PD-L1 combined treatment had the highest lung pathologic score and also an increased inflammatory level in BALFs compared with the single agent treatment groups. However, combining gefitinib and anti-PD-L1 treatment did not seem to cause lung injury or increase the cell number and protein concentration in BALFs. Myeloperoxidase is mainly released by

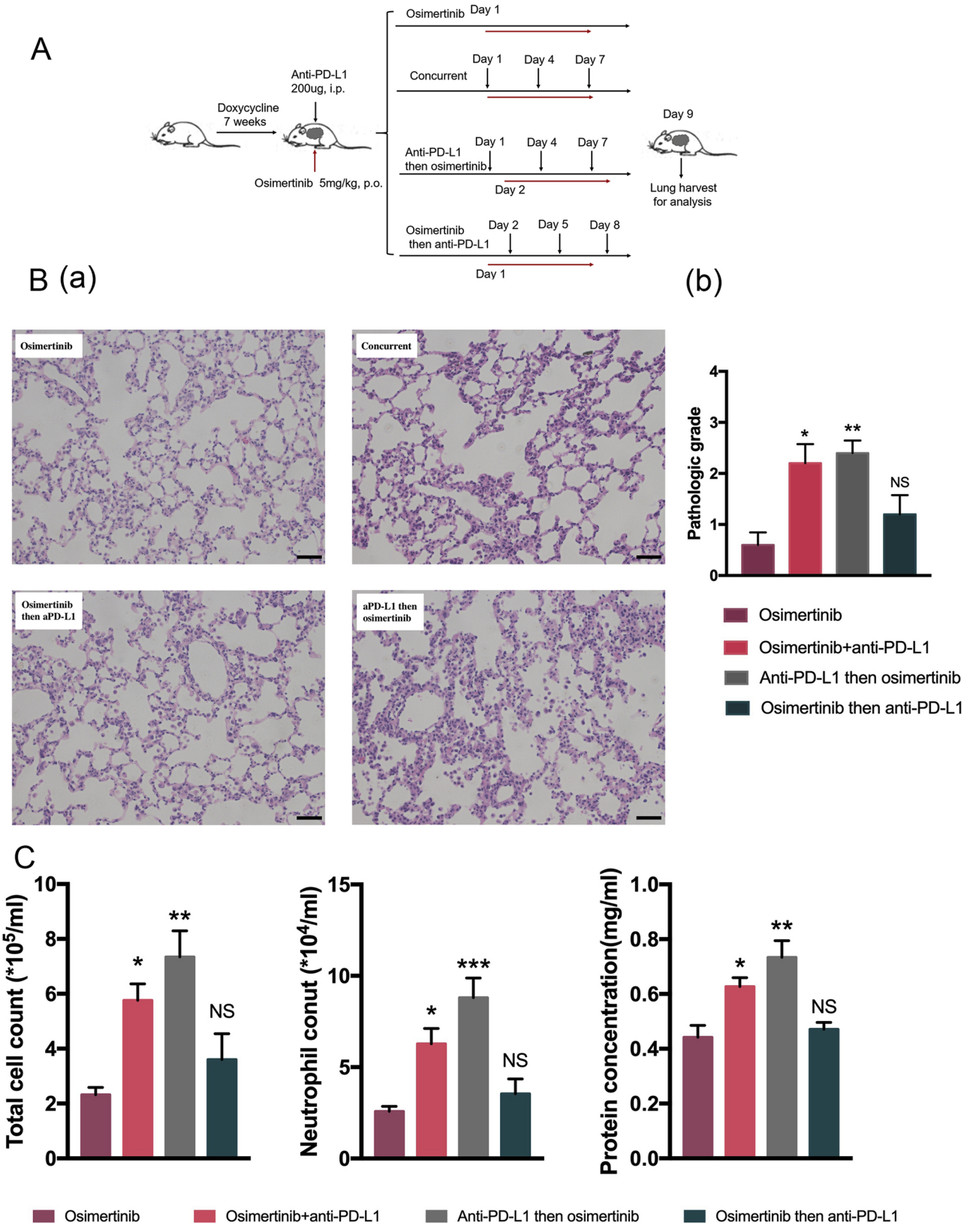

Fig. 4. Sequence of administration affected the occurrence of pneumonitis. A: Experimental scheme showing the different sequences of administration. B: (a) Representative images of H&E stained of lung tissue from mice treated with different regimens showed in Fig. 4. A (scale bars, 50 μm). (b): The pathologic grade of inflammation was quantified. C: BALFs harvested on day 9 were analyzed for total cell counts, neutrophil counts and protein concentration. Results were presented as mean ± SEM. N = 5–6 per group. *P* values were calculated using a one-way ANOVA. P < 0.05, **P < 0.01, ***P < 0.001, P value > 0.05 was indicated as no significance (NS) compared with osimertinib single-agent treatment group.

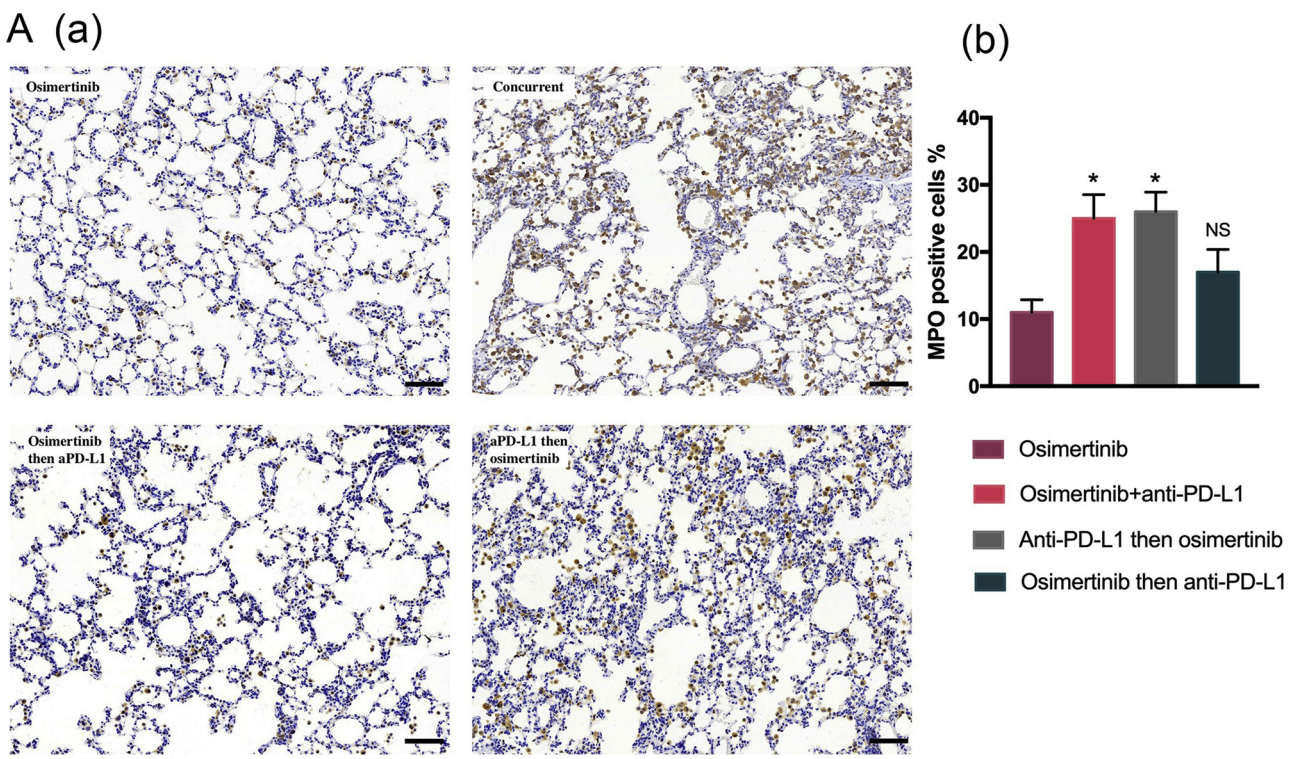

Fig. 5. A: Representative images of IHC staining for MPO in lung tissues of mice from different treatment groups showed in Fig. 4. A (scale bars, 50 μm). B: Percentages of MPO-positive cells were quantified. Results were presented as mean ± SEM. N = 5–6 per group. *P* values were calculated using a one-way ANOVA. *P < 0.05, **P < 0.01, ***P < 0.001, P value > 0.05 was indicated as no significance (NS) compared with osimertinib single-agent treatment group.

neutrophils and was reported to be used as an important marker to reflect the severity of lung injury [25]. Therefore, in this study we used the proportion of MPO positive cells to validate the level of pneumonitis, and the differences among groups were in accordance with the changes in lung inflammation score. Levels of proinflammatory cytokines, including IFN-γ, IL-2, IL-5, TNF-α and IL-12p70 were also higher in the combined treatment group than the osimertinib single-agent treatment group, indicating that the combined therapy promoted the release of inflammatory factors and exacerbate damage to normal lung tissues. However, the mechanisms underlying the synergistic toxicity of osimertinib and anti-PD(L)1 therapy is still unclear. We could infer from our results that unlike other EGFR-TKIs, osimertinib may have unexpected immunomodulatory effects. Future investigation is warranted to validate our theory.

Previous small-sample clinical studies showed that the incidence of ILD is much higher in EGFR-TKI naïve patients than patients who were previously treated with an EGFR-TKI [17,26], which suggested that there might be an association between the risk of ILD and line of therapy. Recently, a retrospective study showed that NSCLC patients harboring an *EGFR* mutation receiving an initial PD-(L)1 blockade followed by osimertinib treatment are associated with the most frequent immune-related adverse events [18]. Our study also compared the effects of different timing of therapy on lung tissue. Indeed, we did observe more severe lung inflammation in the osimertinib and PD-L1 antibody concurrent treatment group and also in the PD-L1 antibody followed by osimertinib group. Meanwhile we did not observe any signs of increased lung injury in mice treated with osimertinib followed by anti-PD-L1 therapy. The present results indicated that the specific sequence of therapy is an important determinant for the occurrence of pneumonitis. This may be due to the durable immune response of the PD-(L)1 antibody [27,28]. In contrast, EGFR-TKIs like osimertinib take effect in a short period of time [29]. Our previous study in tumor-bearing mouse models also demonstrated that the immunomodulatory effects of EGFR-TKIs appeared early, but some were temporary and disappeared as treatment went on [21]. Hence it is possible that initial osimertinib followed by anti-PD-L1 treatment would not cause an overlapping influence on the immune system.

In this study, we investigated the effect of EGFR-TKI combined with PD-L1 antibody in an *EGFR*-driven mouse model. There are several limitations regarding this work. The mechanisms of pneumonitis caused by combining osimertinib and anti-PD-L1 therapy still require further study. Our study explored only two kinds of EGFR-TKIs combined with a PD-L1 inhibitor, thus evaluations applying other EGFR-TKIs and also PD-1 inhibitors are needed to validate our findings. Finally, our conclusion was drawn based on a pre-clinical study using an animal model, and further validation in a clinical setting is warranted.

In conclusion, research relating to the combinational use of EGFR-TKIs and immune checkpoint inhibitors in the treatment of NSCLC with *EGFR* mutations is still at an early stage. Careful evaluation is required due to the potential for overlapping toxicities resulting from combination therapy. Our study may provide implications to minimize pulmonary side effects. Further efforts in a clinical setting are needed to assess different drugs, dosages and timing of administration associated with toxicities from this combination therapy.

Declaration of Competing Interest

The authors declare no competing conflicts of interest.

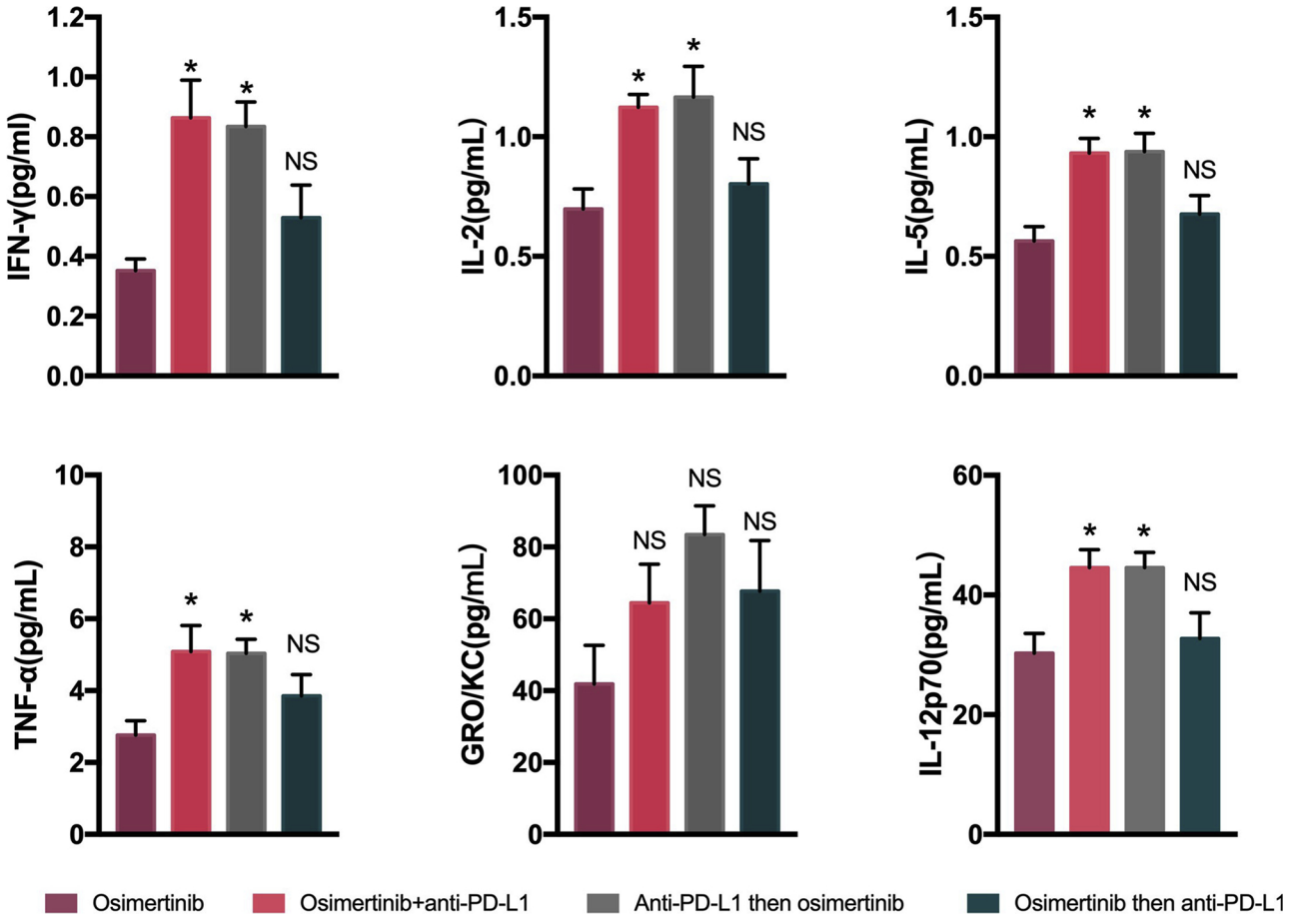

Fig. 6. The levels of IFN-γ, IL-2, IL-5, TNF-α, GRO/KC and IL-12p70 concentration in BALFs from different treatment groups showed in Fig. 4. A were measured by MSD assays. Results were presented as mean ± SEM. For the comparison among treatment groups, one-way ANOVA was performed. N = 5–6 per group. *P < 0.05, **P < 0.01, ***P < 0.001, P value > 0.05 was indicated as no significance (NS) compared with osimertinib single-agent treatment group.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China NO.81672286, NO. 81871865and NO.81874036, Ministry of Science and Technology of the People's Republic of China NO. 2016YFC0902302.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.09.016.

References

[1] T.S. Mok, Y.L. Wu, S. Thongprasert, C.H. Yang, D.T. Chu, N. Saijo, P. Sunpaweravong, B. Han, B. Margono, Y. Ichinose, Y. Nishiwaki, Y. Ohe, J.J. Yang, B. Chewaskulyong, H. Jiang, E.L. Duffield, C.L. Watkins, A.A. Armour, M. Fukuoka, Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma, N. Engl. J. Med. 361 (10) (2009) 947–957.

[2] C. Zhou, Y.L. Wu, G. Chen, J. Feng, X.Q. Liu, C. Wang, S. Zhang, J. Wang, S. Zhou, S. Ren, S. Lu, L. Zhang, C. Hu, C. Hu, Y. Luo, L. Chen, M. Ye, J. Huang, X. Zhi, Y. Zhang, Q. Xiu, J. Ma, L. Zhang, C. You, Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study, Lancet Oncol. 12 (8) (2011) 735–742.

[3] M. Maemondo, A. Inoue, K. Kobayashi, S. Sugawara, S. Oizumi, H. Isobe, A. Gemma, M. Harada, H. Yoshizawa, I. Kinoshita, Y. Fujita, S. Okinaga, H. Hirano, K. Yoshimori, T. Harada, T. Ogura, M. Ando, H. Miyazawa, T. Tanaka, Y. Saijo, K. Hagiwara, S. Morita, T. Nukiwa, Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR, N. Engl. J. Med. 362 (25) (2010) 2380–2388.

[4] C.K. Lee, S. Kim, J.S. Lee, J.E. Lee, S.M. Kim, I.S. Yang, H.R. Kim, J.H. Lee, S. Kim, B.C. Cho, Next-generation sequencing reveals novel resistance mechanisms and molecular heterogeneity in EGFR-mutant non-small cell lung cancer with acquired resistance to EGFR-TKIs, Lung Cancer 113 (2017) 106–114.

[5] P.A. Janne, J.C. Yang, D.W. Kim, D. Planchard, Y. Ohe, S.S. Ramalingam, M.J. Ahn, S.W. Kim, W.C. Su, L. Horn, D. Haggstrom, E. Felip, J.H. Kim, P. Frewer, M. Cantarini, K.H. Brown, P.A. Dickinson, S. Ghiorghiu, M. Ranson, AZD9291 in EGFR inhibitor-resistant non-small-cell lung cancer, N. Engl. J. Med. 372 (18) (2015) 1689–1699.

[6] J.C. Soria, Y. Ohe, J. Vansteenkiste, T. Reungwetwattana, B. Chewaskulyong, K.H. Lee, A. Dechaphunkul, F. Imamura, N. Nogami, T. Kurata, I. Okamoto, C. Zhou, B.C. Cho, Y. Cheng, E.K. Cho, P.J. Voon, D. Planchard, W.C. Su, J.E. Gray, S.M. Lee, R. Hodge, M. Marotti, Y. Rukazenkov, S.S. Ramalingam, Osimertinib in untreated EGFR-Mutated advanced non-small-Cell lung Cancer, N. Engl. J. Med. 378 (2) (2018) 113–125.

[7] T.S. Mok, Y.L. Wu, M.J. Ahn, M.C. Garassino, H.R. Kim, S.S. Ramalingam, F.A. Shepherd, Y. He, H. Akamatsu, W.S. Theelen, C.K. Lee, M. Sebastian, A. Templeton, H. Mann, M. Marotti, S. Ghiorghiu, V.A. Papadimitrakopoulou, Osimertinib or platinum-pemetrexed in EGFR T790M-Positive lung Cancer, N. Engl. J. Med. 376 (7) (2017) 629–640.

[8] J. Remon, N. Pardo, A. Martinez-Marti, S. Cedres, A. Navarro, A.M. Martinez de Castro, E. Felip, Immune-checkpoint inhibition in first-line treatment of advanced non-small cell lung cancer patients: current status and future approaches, Lung Cancer 106 (2017) 70–75.

[9] E.A. Akbay, S. Koyama, J. Carretero, A. Altabef, J.H. Tchaicha, C.L. Christensen, O.R. Mikse, A.D. Cherniack, E.M. Beauchamp, T.J. Pugh, M.D. Wilkerson, P.E. Fecci, M. Butaney, J.B. Reibel, M. Soucheray, T.J. Cohoon, P.A. Janne, M. Meyerson, D.N. Hayes, G.I. Shapiro, T. Shimamura, L.M. Sholl, S.J. Rodig, G.J. Freeman, P.S. Hammerman, G. Dranoff, K.K. Wong, Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors, Cancer Discov. 3 (12) (2013) 1355–1363.

[10] N. Chen, W. Fang, J. Zhan, S. Hong, Y. Tang, S. Kang, Y. Zhang, X. He, T. Zhou, T. Qin, Y. Huang, X. Yi, L. Zhang, Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-Driven NSCLC: implication for optional immune targeted therapy for NSCLC patients with EGFR mutation, J. Thorac. Oncol.

10 (6) (2015) 910–923.
[11] N.A. Rizvi, L.Q.M. Chow, H. Borghaei, Y. Shen, C. Harbison, S. Alaparthy, A.C. Chen, S.N. Gettinger, Safety and response with nivolumab (anti-PD-1; BMS-936558, ONO-4538) plus erlotinib in patients (pts) with epidermal growth factor receptor mutant (EGFR MT) advanced NSCLC, J. Clin. Oncol. 32 (15) (2014).
[12] D.L. Gibbons, L.Q. Chow, D.W. Kim, S.W. Kim, T. Yeh, X. Song, H. Jiang, R. Taylor, J. Karakunnel, B. Creelan, Efficacy, safety and tolerability of MEDI4736 (durvalumab D), a human IgG1 anti-programmed cell death-ligand-1 (PD-L1) antibody, combined with gefitinib (G): A phase I expansion in TKI-naive patients (pts) with EGFR mutant NSCLC, J. Thorac. Oncol. 11 (4) (2016) S79-S79.
[13] B.B.Y. Ma, C.M. Rudin, A. Cervantes, A. Dowlati, D. Costa, P. Schmid, R. Heist, V.M. Villaflor, I. Sarkar, M.A. Huseni, P. Foster, C. O'Hear, S. Gettinger, B. Besse, Preliminary safety and clinical activity of erlotinib plus atezolizumab from a Phase Ib study in advanced NSCLC, Ann. Oncol. 27 (2016).
[14] M.J. Ahn, J.M. Sun, S.H. Lee, J.S. Ahn, K. Park, EGFR TKI combination with immunotherapy in non-small cell lung cancer, Expert Opin. Drug Saf. 16 (4) (2017) 465–469.
[15] M. Porcu, P. De Silva, C. Solinas, A. Battaglia, M. Schena, M. Scartozzi, D. Bron, J. Suri, K. Willard-Gallo, D. Sangiolo, L. Saba, Immunotherapy associated pulmonary toxicity: biology behind clinical and radiological features, Cancers 11 (3) (2019) 305.
[16] Y. Oshima, T. Tanimoto, K. Yuji, A. Tojo, EGFR-TKI-Associated interstitial pneumonitis in nivolumab-treated patients with non-small cell lung Cancer, JAMA Oncol. 4 (8) (2018) 1112–1115.
[17] M.J. Ahn, J. Yang, H. Yu, H. Saka, S. Ramalingam, K. Goto, S.W. Kim, L. Yang, A. Walding, G.R. Oxnard, Osimertinib combined with durvalumab in EGFR-mutant non-small cell lung cancer: results from the TATTON phase Ib trial, J. Thorac. Oncol. 11 (4) (2016) S115-S115.
[18] A.J. Schoenfeld, K.C. Arbour, H. Rizvi, A.N. Iqbal, S.M. Gadgeel, J. Girshman, M.G. Kris, G.J. Riely, H.A. Yu, M.D. Hellmann, Severe immune-related adverse events are common with sequential PD-(L)1 blockade and osimertinib, Ann. Oncol. 30 (5) (2019) 839–844.
[19] F. Mascia, D.T. Schloemann, C. Cataisson, K.M. McKinnon, L. Krymskaya, K.M. Wolcott, S.H. Yuspa, Cell autonomous or systemic EGFR blockade alters the immune-environment in squamous cell carcinomas, Int. J. Cancer 139 (11) (2016) 2593–2597.
[20] J.A. Wargo, A. Reuben, Z.A. Cooper, K.S. Oh, R.J. Sullivan, Immune effects of chemotherapy, radiation, and targeted therapy and opportunities for combination with immunotherapy, Semin. Oncol. 42 (4) (2015) 601–616.
[21] Y. Jia, X. Li, T. Jiang, S. Zhao, C. Zhao, L. Zhang, X. Liu, J. Shi, M. Qiao, J. Luo, S. Liu, R. Han, C. Su, S. Ren, C. Zhou, EGFR-targeted therapy alters the tumor microenvironment in EGFR-driven lung tumors: implications for combination therapies, Int. J. Cancer (2019).
[22] D. Odobasic, A.R. Kitching, S.R. Holdsworth, Neutrophil-mediated regulation of innate and adaptive immunity: the role of Myeloperoxidase, J. Immunol. Res. 2016 (2016) 2349817.
[23] M.-J. Ahn, Combination of Osimertinib with Durvalumab in Epidermal Growth Factor Receptor-Mutant Non-Small Cell Lung Cancer: Is There Room for Reinvestigation? J. Thorac. Oncol. 14 (5) (2019) 766–767.
[24] N. Hamada, T. Yanagihara, K. Suzuki, S. Ogata-Suetsugu, E. Harada, H. Mikumo, M. Arimura-Omori, Y. Nakanishi, Treatment with a programmed cell death-1-specific antibody has little effect on afatinib- and naphthalene-induced acute pneumonitis in mice, Biochem. Biophys. Res. Commun. 491 (3) (2017) 656–661.
[25] F. Wang, Y. Luo, X. Tian, S. Ma, Y. Sun, C. You, Y. Gong, C. Xie, Impact of radiotherapy concurrent with Anti-PD-1 therapy on the lung tissue of tumor-bearing mice, Radiat. Res. 191 (3) (2019) 271–277.
[26] J. Chih-Hsin Yang, F.A. Shepherd, D.-W. Kim, G.-W. Lee, J.S. Lee, G.-C. Chang, S.S. Lee, Y.-F. Wei, Y.G. Lee, G. Laus, B. Collins, F. Pisetzky, L. Horn, Osimertinib plus durvalumab versus osimertinib monotherapy in EGFR T790M-Positive NSCLC following previous EGFR TKI therapy: CAURAL brief report, J. Thoracic Oncology 14 (5) (2019) 933–939.
[27] J.R. Brahmer, C.G. Drake, I. Wollner, J.D. Powderly, J. Picus, W.H. Sharfman, E. Stankevich, A. Pons, T.M. Salay, T.L. McMiller, M.M. Gilson, C. Wang, M. Selby, J.M. Taube, R. Anders, L. Chen, A.J. Korman, D.M. Pardoll, I. Lowy, S.L. Topalian, Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates, J. Clinical Oncology 28 (19) (2010) 3167–3175.
[28] E.J. Lipson, W.H. Sharfman, C.G. Drake, I. Wollner, J.M. Taube, R.A. Anders, H. Xu, S. Yao, A. Pons, L. Chen, D.M. Pardoll, J.R. Brahmer, S.L. Topalian, Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody, Clinical Cancer Res. 19 (2) (2013) 462–468.
[29] D.A. Cross, S.E. Ashton, S. Ghiorghiu, C. Eberlein, C.A. Nebhan, P.J. Spitzler, J.P. Orme, M.R. Finlay, R.A. Ward, M.J. Mellor, G. Hughes, A. Rahi, V.N. Jacobs, M. Red Brewer, E. Ichihara, J. Sun, H. Jin, P. Ballard, K. Al-Kadhimi, R. Rowlinson, T. Klinowska, G.H. Richmond, M. Cantarini, D.W. Kim, M.R. Ranson, W. Pao, AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer, Cancer Discov. 4 (9) (2014) 1046–1061.