Anti-VEGF Antibody Protects against Alveolar Exudate Leakage Caused by Vascular Hyperpermeability, Resulting in Mitigation of Pneumonitis Induced by Immunotherapy

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ABSTRACT

Immune-related pneumonitis is an important toxicity associated with checkpoint inhibitor therapy with anti–PD-1 or anti–PD-L1 antibodies, often necessitating discontinuation of treatment. Development of methods to mitigate checkpoint inhibitor–related pneumonitis is required.

The contributions of PD-L1, PD-L2, and VEGF to the pathogenesis of pneumonitis were examined in an IL2- plus IL18-induced mouse pneumonitis model (IL pneumonitis model). Furthermore, the incidences of pneumonitis were retrospectively examined in patients with non–small cell lung cancer treated with the anti–PD-L1 mAb atezolizumab plus chemotherapy, with or without the anti-VEGF mAb bevacizumab, in the phase III IMpower150 trial. PD-1 signal blockade by anti–PD-L1 and anti–PD-L2 antibodies aggravated pneumonitis in the IL pneumonitis model. An anti-VEGF antibody prevented PD-1 signal blockade from aggravating pneumonitis in this model. PD-1 signal blockade induced interstitial T-cell infiltration in the lungs, but VEGF blockade did not affect this T-cell infiltration. The anti-VEGF antibody protected against vascular-to-alveolar leakage of protein and fluid due to PD-1 signal blockade in a murine model. In the IMpower150 trial, incidence rates of pneumonitis of any grade were 4.3% in the group without bevacizumab and 2.8% in the group with bevacizumab. In patients with pneumonitis, outcomes of “Not recovered/Not resolved” were reported for 29.4% in the group without bevacizumab compared with 9.1% in the group with bevacizumab. Our findings suggest that anti-VEGF antibodies in combination with checkpoint inhibitors may be a treatment method that can control checkpoint inhibitor–related pneumonitis.

Introduction

Programmed cell death 1 receptor (PD-1) on T cells plays a major role in suppressing the host’s antitumor immune response by interaction with PD-L1 or PD-L2 on tumor cells, macrophages or dendritic cells, and treatment with anti–PD-1 or anti–PD-L1 antibodies generally has high clinical benefit in patients with cancer (1). However, the use of anti–PD-1 or anti–PD-L1 antibodies is often associated with potentially fatal immune-related pneumonitis, an important autoimmune toxicity with significant morbidity and mortality, often necessitating discontinuation of therapy (2, 3). Meta-analyses of published clinical trials of PD-1 and PD-L1 inhibitor therapy for patients with non–small cell lung cancer (NSCLC) show that the incidence of pneumonitis of any grade is significantly higher in patients treated with PD-1 inhibitors than in patients treated with PD-L1 inhibitors (4, 5), indicating that the appropriate clinical selection of these agents may be one of the options for reducing the risk of pneumonitis in NSCLC; however, even when using a PD-L1 inhibitor, the risk of pneumonitis remains. Therefore, the development of a method for controlling pneumonitis is required.

VEGF is an angiogenic factor (6) and also a potent inducer of vascular permeability (7, 8). It has been reported that VEGF plays a significant role in the development of several lung disorders, including chronic obstructive pulmonary disease, pulmonary hypertension, and acute lung injury (9). However, the role of VEGF in checkpoint inhibitor–related pneumonitis has not been investigated.

In this study, we used an IL2- plus IL18-induced mouse pneumonitis model (10) to investigate the roles of PD-L1, PD-L2, and VEGF in pneumonitis, and we investigated whether an anti-VEGF antibody has a mitigation effect on checkpoint inhibitor–related pneumonitis in the phase III IMpower150 trial.

Materials and Methods

Mice

Female 6-week-old C57BL/6 mice were obtained from Japan SLC. All animals were allowed to acclimatize and recover from shipping-related stress for 1 week prior to the study. The animals were allowed free access to chlorinated water and irradiated food, and were kept under a controlled light–dark cycle (12–12 hours). All animal procedures were approved by the Institutional Animal Care and Use Committee at Chugai Pharmaceutical Co., Ltd., and conformed to the Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources (ILAR).

Mouse pneumonitis model

Mice were treated once a day with an intraperitoneal injection of 10,000 IU/head rhIL2 (PeproTech) and 1 μg/head rmIL18 (R&D Systems). These cytokines were suspended in 200 μL of sterile PBS,

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and mice treated with 200 µL PBS were used as controls. Anti-mouse PD-L1 antibody (10F.9G2; BioLegend), anti-mouse PD-L2 antibody (TY25; BioLegend), anti-mouse VEGF antibody (B20–4.1.1; gifted from Genentech) or Rat IgG (MP Biomedicals) was administered intraperitoneally to the mice at a dose of 10 mg/kg three times from the day before cytokine treatment. Wet lung weight and moist rale were measured after 5 days of the treatment.

Flow cytometric analysis

For analysis of lung-infiltrating inflammatory cells, lung tissue was excised from treated mice after 5 days of the treatment, and single-cell suspensions were obtained by mincing lung tissues and homogenizing them by disruption and digestion with a gentleMACS Dissociator and a Lung Dissociation Kit for mice (Miltenyi Biotec). Single-cell suspensions were incubated with anti-Fcγ receptor antibodies (Tonbo Biosciences) and the fixable viability dye FVD506 or FVD780 (eBioscience) at room temperature for 5 minutes, and stained with the following mAbs: mouse CD45 (30-F11), CD4 (RM4–5), CD8α (53–6.7), NK1.1 (PK136), CD11c (HL3), CD103 (M290), CD11b (M1/70), Gr-1 (RB6–8C5), F4/80 (T45–2342), PD-L1 (MIH5), and PD-L2 (TY25) from BioLegend or BD Biosciences (Franklin Lakes). The appropriate conjugated isotype-matched immunoglobulin G (IgG) was used as the control for each. Cells were analyzed using an LSRFortessa X-20 cell analyzer (BD Biosciences) and FlowJo 10 software (Tree Star).

Bronchoalveolar lavage fluid

Lung tissue was excised from treated mice after 4 days of treatment. The trachea was surgically exposed and intubated with a syringe catheter. The lungs were subjected to lavage with 0.5 mL PBS three times. Bronchoalveolar lavage fluid (BALF) was obtained from each mouse and cells in BALF were pelleted by centrifugation (500 × *g*, 5 minutes). The supernatants were stored at −80°C for later measurements.

Histologic examination

Lung tissue was excised from treated mice after 5 days of treatment. After opening of the thorax, the lungs were immediately fixed by intratracheal instillation of 10% neutral buffered formalin. After gross examination, the excised tissues were placed in 10% formalin. Sections (4-µm thickness) were cut from paraffin-embedded tissues. Deparaffinized sections were stained with hematoxylin and eosin (HE).

Immunoassays

Concentrations of mouse SP-D were measured with a Quantikine ELISA kit (R&D Systems). VEGF was quantified by using a Quantikine ELISA kit (R&D Systems). Albumin was quantified by using a specific ELISA kit (Bethyl Laboratories).

Clinical study design

The IMpower150 trial (NCT02366143) was a randomized phase III study that evaluated the safety and efficacy of atezolizumab in combination with carboplatin + paclitaxel with or without bevacizumab compared with treatment with carboplatin + paclitaxel + bevacizumab in metastatic NSCLC (11). The IMpower150 study was done in full accordance with the guidelines for Good Clinical Practice and the Declaration of Helsinki, and all patients gave their written informed consent. We performed the human investigations after approval by the research ethics committee of Chugai Pharmaceutical Co., Ltd. Participants were randomized in a 1:1:1 ratio to the ACP arm (atezolizumab + carboplatin + paclitaxel), the ABCP arm (atezolizumab + carboplatin + paclitaxel + bevacizumab), and the BCP arm (carboplatin + paclitaxel + bevacizumab).

Statistical analysis

To evaluate statistical significance in the mouse model experiments, data was analyzed with the Wilcoxon test. For two groups, $P < 0.05$ was considered to indicate a significant difference. For multiple groups, P values were adjusted by the Holm−Bonferroni method (12) by using JMP version 10 software (SAS Institute).

Results

PD-1 signal blockade aggravated pneumonitis and VEGF blockade improved the pneumonitis in the immune-related pneumonitis model

BALF of patients with checkpoint inhibitor–related pneumonitis often suggests lymphocytic inflammation of the alveoli, and BALF cytology often presents an increased proportion of lymphocytes (13). Therefore, to investigate immune-related mechanisms for pneumonitis aggravated by PD-1 signal blockade, we examined the effects of anti-PD-L1 and anti-PD-L2 antibodies in an IL2- plus IL18-induced murine pneumonitis model with symptoms of interstitial lymphocyte infiltration in the lungs. The wet lung weight was significantly higher in the group with dual PD-L1/PD-L2 blockade compared with that in the IL2 plus IL18 alone group (**Fig. 1A**). Serum pulmonary surfactant

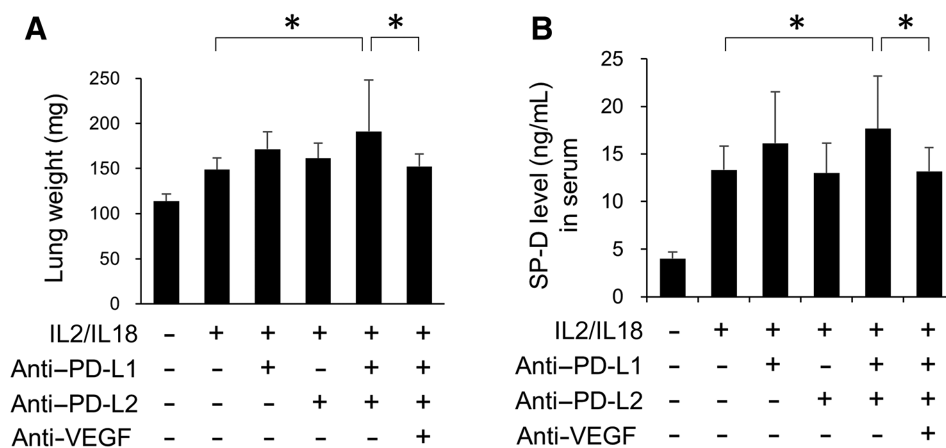

Figure 1.
Effect of PD-L1/PD-L2/VEGF blockade on lung weight and SP-D level in mice with lung damage induced by IL2 plus IL18. **A,** Lung weights. Data are shown as the mean + SD (n = 13–18/group). Statistical analysis used Wilcoxon rank sum test. *, P < 0.05. **B,** Levels of pulmonary surfactant protein-D (SP-D) in serum. Data are shown as the mean + SD (n = 10–15/group). Statistical analysis used Wilcoxon rank sum test. *, P < 0.05.

protein-D (SP-D) is a useful clinical biomarker for the diagnosis and management of pneumonitis and it also increases in patients with checkpoint inhibitor–related pneumonitis (14–16). In the current model, the serum SP-D level was significantly higher in the group with dual PD-L1/PD-L2 blockade compared with that in the IL2 plus IL18 alone group (**Fig. 1B**).

Next, to investigate the relation between VEGF signaling and checkpoint inhibitor–related pneumonitis, we examined the effect of VEGF blockade in the IL2- plus IL18-induced pneumonitis model. The wet lung weight was significantly lower in the VEGF blockade group compared with the group with dual PD-L1/PD-L2 blockade (**Fig. 1A**). The level of SP-D in serum was also significantly decreased by the addition of VEGF blockade (**Fig. 1B**).

PD-L1 and PD-L2 blockade induced interstitial T-cell infiltration in the lungs, but VEGF blockade did not prevent T cells from infiltrating

To investigate the mechanism of action through which dual PD-L1/PD-L2 blockade aggravated pneumonitis in the IL2- plus IL18-induced pneumonitis murine model, we analyzed the expressions of PD-L1 and PD-L2 in lung tissues of mice. In normal lung tissue, PD-L1 and PD-L2 were expressed most strongly on $CD103^-$ $CD11c^+$ dendritic cells (DC; **Fig. 2**). IL2 plus IL18 treatment upregulated PD-L1 expression on $CD103^-$ DCs, $CD103^+$ DCs, $CD11b^+$ $Gr1^{high}$ neutrophils, and $F4/80^+$ $CD11c^-$ macrophages. In contrast, IL2 plus IL18 treatment did not upregulate PD-L2 expression on these cells (**Fig. 2**).

Next, we analyzed the inflammatory condition of the lung tissue. Histological evaluation of mice treated with IL2 + IL18 + anti–PD-L1 antibody + anti-PD-L2 antibody revealed that the alveolar wall and interstitium had large interstitial infiltration of mononuclear cells and granulocytes (**Fig. 3**).

Flow cytometry analysis revealed that IL2 plus IL18 significantly increased $CD3^-$ $NK1.1^+$ NK cells, neutrophils, and macrophages in the lungs and significantly decreased $CD8\alpha^+$ T cells, while addition of dual PD-L1/PD-L2 blockade significantly increased $CD8\alpha^+$ T cells and $CD4^+$ T cells (**Fig. 4**). However, additional treatment with anti-VEGF antibody did not change the number of inflammatory cells in the lung tissues (**Fig. 4**).

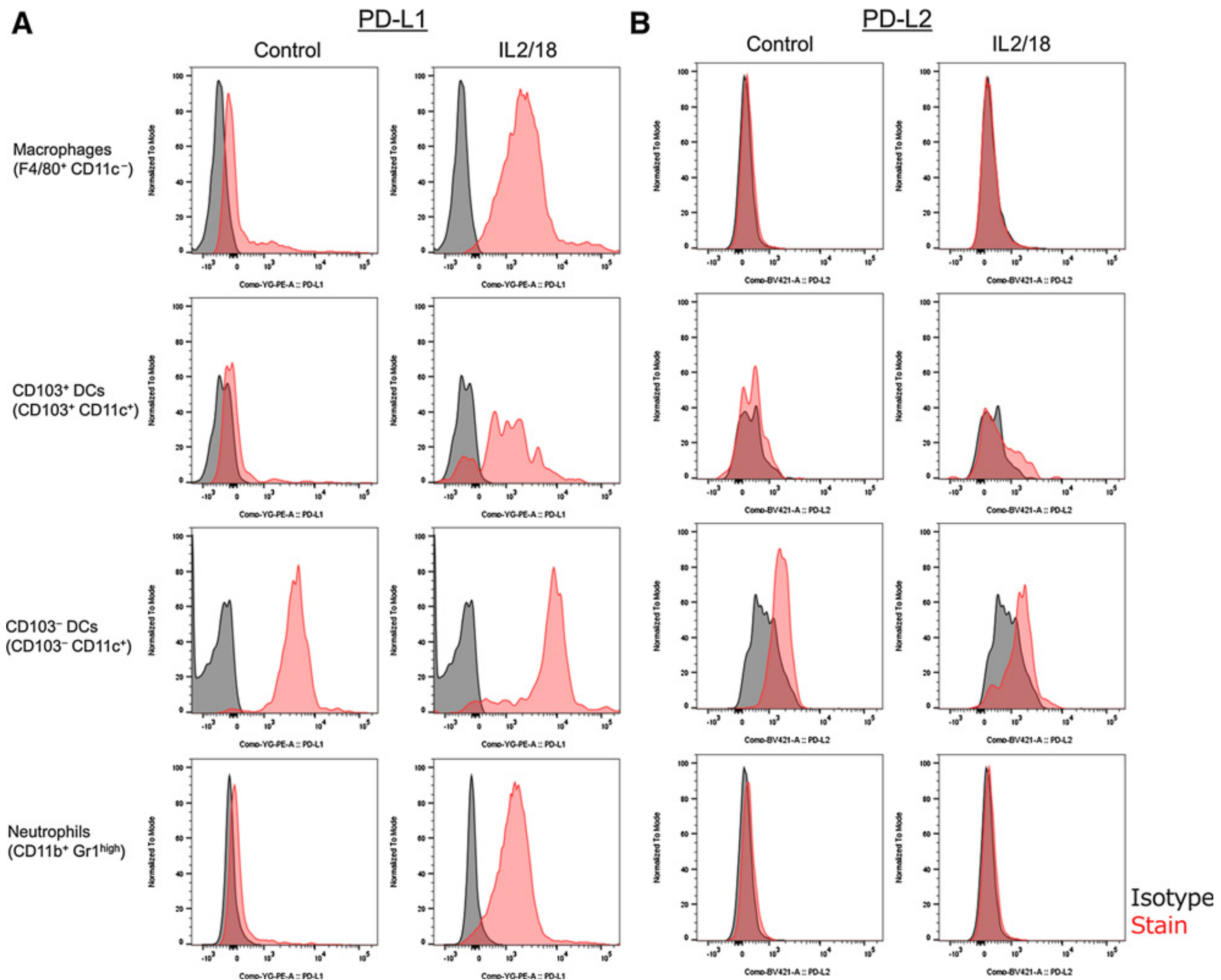

Figure 2.

Expression of PD-L1 or PD-L2 on inflammatory cells in mouse lung tissue. PD-L1 expression (**A**) and PD-L2 expression (**B**) in mouse lung tissues in the control group and the IL2- plus IL18-treated group. Cells were determined by flow cytometric analysis. DCs, dendritic cells.

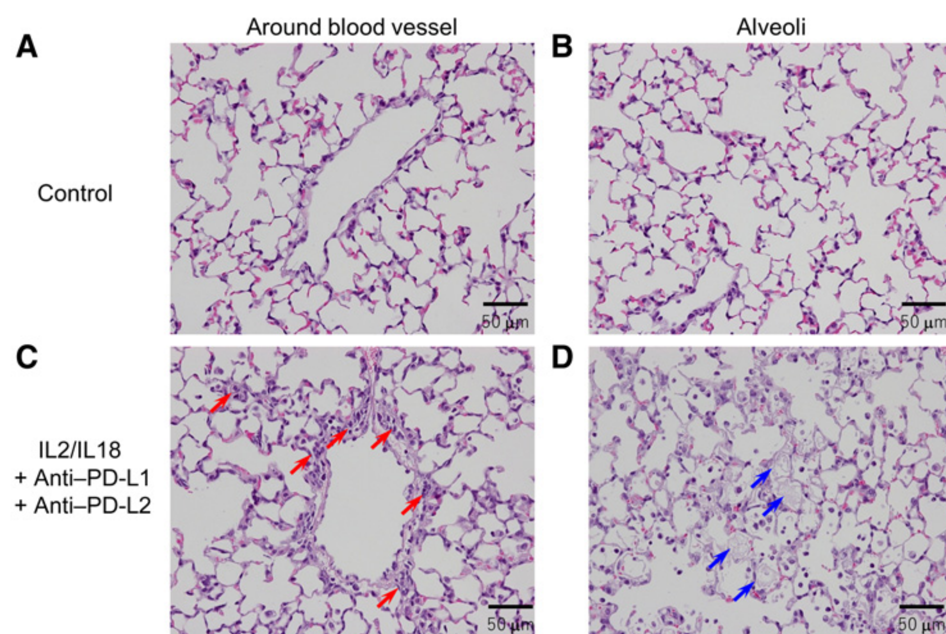

Figure 3.
Histopathologic examination of lung tissue stained with HE. **A,** Around blood vessel in lung tissue of a mouse in the control group. No lesions. **B,** Alveoli in lung tissue of a mouse in the control group. No lesions. **C,** Around blood vessel in lung tissue of a mouse treated with IL2 + IL18 + anti–PD-L1 antibody + anti–PD-L2 antibody. Red arrows: infiltration of mononuclear cells and granulocytes. **D,** Alveoli in lung tissue of a mouse treated with IL2 + IL18 + anti–PD-L1 antibody + anti–PD-L2 antibody. Blue arrows: exudate.

Anti-VEGF antibody protected against alveolar exudate leakage due to dual PD-L1/PD-L2 blockade

Although treatment with the anti-VEGF antibody prevented the increases in wet lung weight and serum SP-D level—an index useful for the evaluation of pneumonitis—it did not change inflammatory cell infiltration into lung tissues. A characteristic of this immune-related pneumonitis model is a moist rale caused by air mixing with fluid exudate in the bronchial tubes. A moist rale is also a typical clinical finding in patients with checkpoint inhibitor–related pneumonitis. The mice with symptoms of moist rale showed that alveolar exudate formed in the lung tissue (**Fig. 3**). Moist rale occurred in the group with PD-L1 blockade and in the group with dual PD-L1/PD-L2 blockade, but it did not occur in the group with PD-L1 + PD-L2 + VEGF blockade (**Fig. 5A**).

To ascertain whether VEGF blockade protects against vascular-to-alveolar leakage in this model, we measured the albumin concentration in BALF. The albumin concentration in the group with dual PD-L1/PD-L2 blockade was significantly higher than in the IL2 plus IL18 group and in the PD-L1 blockade group (**Fig. 5B**). Addition of VEGF blockade to the dual PD-L1/PD-L2 blockade treatment significantly decreased the albumin concentration in BALF (**Fig. 5B**).

Because VEGF blockade protected against vascular-to-alveolar leakage, we evaluated the expression of VEGF in lung tissues, expecting to see an increase. Unexpectedly, IL2 plus IL18 significantly decreased VEGF levels in the lung tissue and dual PD-L1/PD-L2 blockade did not change these decreased VEGF levels (**Fig. 5C**).

Bevacizumab reduced the incidence of checkpoint inhibitor–related pneumonitis and improved the rate of recovery from pneumonitis in patients with NSCLC

We showed that VEGF blockade prevented immune-related pneumonitis from aggravating in the murine model. Therefore, to investigate whether VEGF modulates the risk of immune-related pneumonitis in patients with NSCLC treated with immune checkpoint blockade therapy, we compared the status of pneumonitis between the atezolizumab + carboplatin + paclitaxel (ACP group) and the atezolizumab + bevacizumab + carboplatin + paclitaxel (ABCP group) in the phase 3 IMpower150 trial. Incidence rates of pneumonitis of any grade were 4.3% in the ACP group and 2.8% in the ABCP group (**Fig. 6**). Incidence of symptomatic grade 2–4 pneumonitis was 3.8% in the ACP group and 2.0% in the ABCP group (**Table 1**). Rates of "not recovered/not resolved" outcomes in patients with pneumonitis were 29.4% in the ACP group and 9.1% in the ABCP group (**Fig. 6**). Among patients with pneumonitis, 5.9% in the ACP group and 27.3% in the ABCP group did not use steroids (**Table 2**).

Discussion

We demonstrated that dual treatment with anti–PD-L1 antibody plus anti-PD-L2 antibody worsened the index for evaluation of pneumonitis in an IL2- plus IL18-induced murine pneumonitis model. Because pneumonitis does not develop in PD-$1^{-/-}$ C57BL/6 mice but is accelerated by PD-1 deficiency in the MRL background which has the risk of the autoimmune damage in lung (17), the aggravation of pneumonitis due to dual PD-L1/PD-L2 blockade may need a preexisting inflammatory immune microenvironment in the lungs. In patients with NSCLC, preexisting pulmonary fibrosis is reported to be a risk factor for anti–PD-1–related pneumonitis, supporting this hypothesis (18, 19). It has been reported that PD-L2 plays a role in the homeostasis of inflammation in the lungs (20). Our data showed that PD-L2 was expressed on DCs in murine lung tissue in a steady state and that that expression continued without a change even under the inflammatory conditions induced by IL2 plus IL18. Expression of PD-L1, on the other hand, was upregulated under the IL2- plus IL18-induced inflammatory conditions. These results suggest that not only PD-L2 but PD-L1 also may be active in the lungs under inflammatory conditions. In fact, we demonstrated that not only dual PD-L1/PD-L2 blockade but also blockade of PD-L1 alone caused symptoms such as moist rale under the IL2- plus IL18-induced inflammatory conditions. Although there is a higher incidence of pneumonitis with the use of PD-1 inhibitors than with the use of PD-L1 inhibitors (4), it is necessary to be prepared for pneumonitis also when using PD-L1 inhibitors.

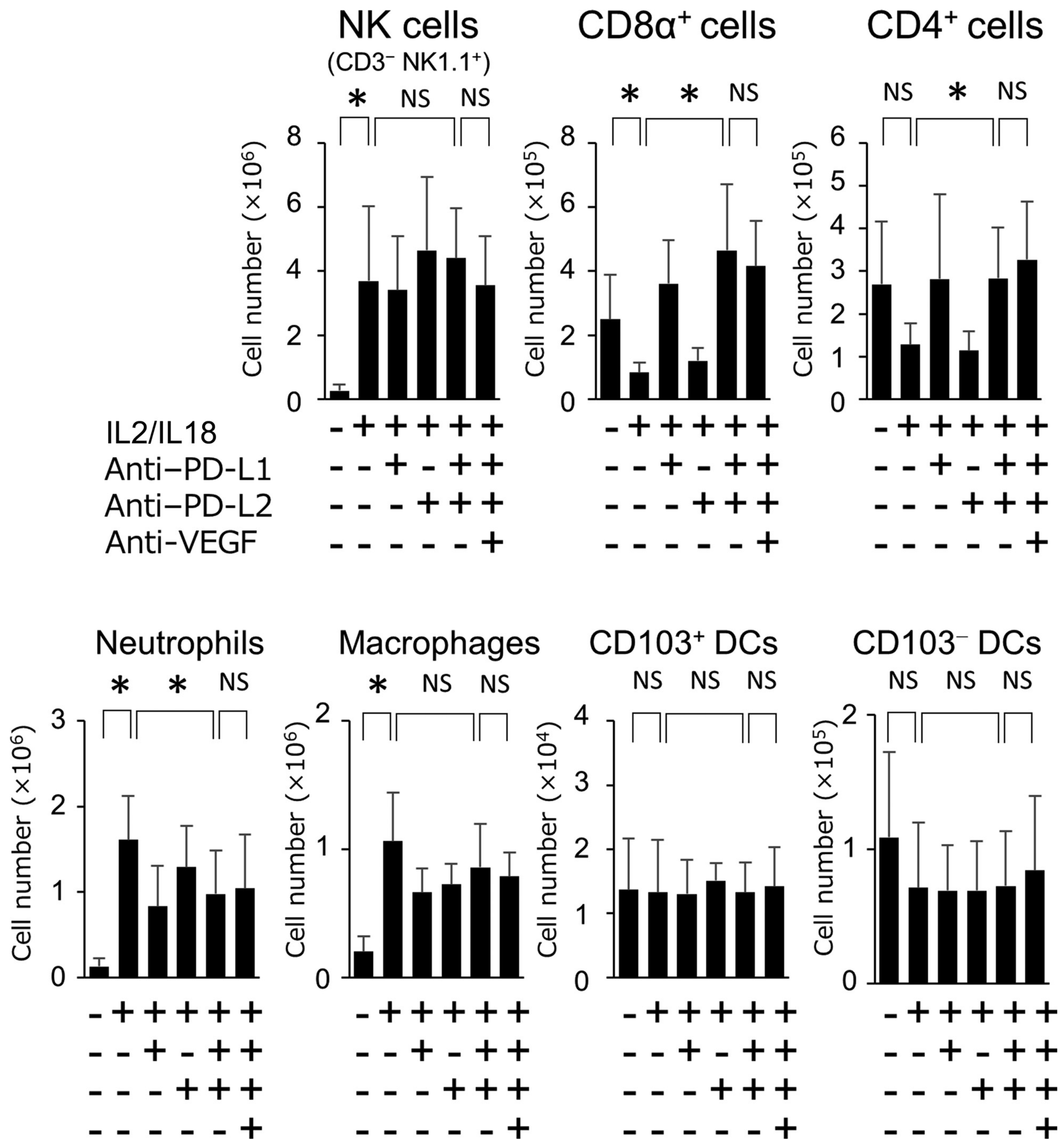

Figure 4.

Infiltration of inflammatory cells into lung tissue of treated mice. Number of NK cells (NK1.1$^+$), CD8α^+ cells, CD4$^+$ cells, neutrophils (CD11b$^+$ Gr-1^{high}), macrophages (F4/80$^+$), CD103$^+$ DCs (CD103$^+$ CD11c$^+$), and CD103$^-$ DCs (CD103$^-$ CD11c$^+$) in lung tissues after the indicated treatment ($n = 7$–12/group). Statistical analysis used Wilcoxon rank sum test. *, $P < 0.05$; NS, not significant. Data are shown as the mean + SD.

With regard to the role of VEGF in checkpoint inhibitor–related pneumonitis, we demonstrated that anti-VEGF antibody could control symptoms of checkpoint inhibitor–related pneumonitis in the murine model, especially the vascular-to-alveolar leakage and moist rale due to the leakage. Although moist rales/crackles are not very commonly manifested symptoms in the patients with immune checkpoint inhibitor-related pneumonitis, they has been listed as one of the clinical findings suspecting the onset of severe pneumonitis recommending a chest CT (21, 22). Furthermore, we demonstrated that in patients with NSCLC, the incidence of symptomatic pneumonitis induced by atezolizumab plus chemotherapy was lower in the group also treated with bevacizumab than in the group without bevacizumab. And even in the

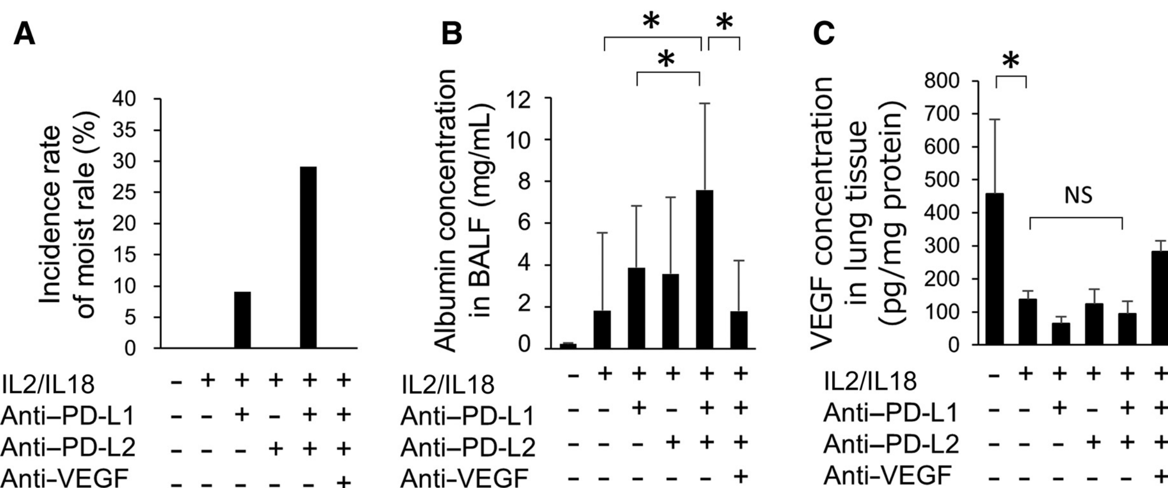

Figure 5.

Effect of anti-VEGF antibody on alveolar exudate leakage due to dual PD-L1/PD-L2 blockade. **A,** Incidence of moist rale ($n = 18$–24/group). **B,** Albumin concentration in BALF ($n = 11$–16/group). Data are shown as the mean + SD. Statistical analysis used Wilcoxon rank sum test and the Holm–Bonferroni method. *, $P < 0.05$; NS, not significant. **C,** VEGF concentration in lung tissues ($n = 6$/group). Data are shown as the mean + SD. Statistical analysis used Wilcoxon rank sum test and the Holm–Bonferroni method. *, $P < 0.05$; NS, not significant.

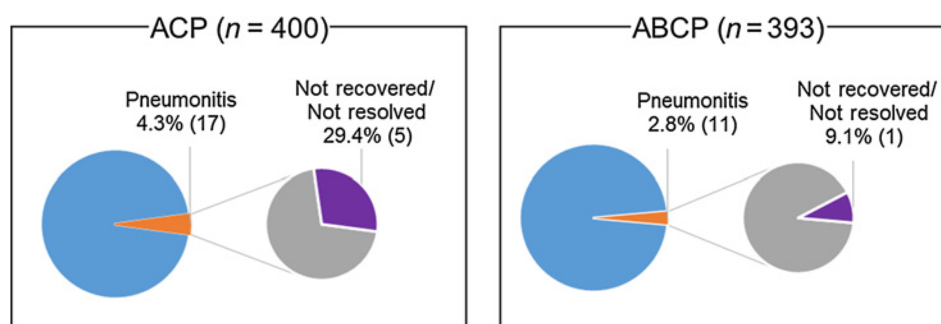

Figure 6.

Incidence rates of pneumonitis and outcome rates of patients with pneumonitis in the phase III IMpower150 trial. Incidence rates of pneumonitis and rates of "Not recovered/Not resolved" outcomes in patients with pneumonitis in the atezolizumab + carboplatin + paclitaxel group (ACP, $n = 400$) and in the atezolizumab + bevacizumab + carboplatin + paclitaxel group (ABCP, $n = 393$).

broadly defined pneumonitis, including interstitial lung disease, pulmonary fibrosis and etc., this trend does not change (23). Therefore, it is suggested that checkpoint inhibitor–related pneumonitis occurs when two conditions are both met: presence of intrinsic VEGF and aggravation of preexisting inflammation caused by PD-1 signal blockade. On the other hand, our results showed that the level of VEGF expression in lung tissues decreased in the IL2 + IL18 + anti-PD-L1/PD-L2 antibody-induced pneumonitis model. Although the lungs have the highest level of VEGF gene expression among all normal tissues (24), VEGF levels in BALF from patients with acute lung injury are reported to be lower than levels in normal BALF, and a possible explanation for these decreased levels of VEGF may be dilution resulting from alveolar flooding (25). Therefore, the same phenomenon may be happening in our pneumonitis model, and our study demonstrated that despite this lowered level of VEGF, it is still sufficient and critical for the massive alveolar exudate leakage seen in the immune-related pneumonitis model. Because increase in immune cell infiltration caused by PD-1 signal blockade was not affected by co-administration of anti-VEGF antibody, it may not be considered as a fundamental treatment measure to cure immune-related pneumonitis at least from the results using current mouse pneumonitis model. However, as there has been little information about the precise roles of infiltrated immune cells in immune checkpoint inhibitor–related pneumonitis, further studies are required to understand the contribution of each type of immune cells to the development of each symptom.

Table 1. Severity of pneumonitis in the IMpower150 trial.

	Grade	ACP ($n = 400$)	ABCP ($n = 393$)	BCP ($n = 394$)
Pneumonitis	Any grade	17 (4.3%)	11 (2.8%)	3 (0.8%)
	4	2 (0.5%)	1 (0.3%)	0
	3	6 (1.5%)	4 (1.0%)	1 (0.3%)
	2	7 (1.8%)	3 (0.8%)	2 (0.5%)
	1	2 (0.5%)	3 (0.8%)	0

Table 2. Steroid use in patients with pneumonitis in the IMpower150 trial.

	Use	ACP ($n = 17$)	ABCP ($n = 11$)	BCP ($n = 3$)
Steroid	No	1 (5.9%)	3 (27.3%)	1 (33.3%)
	Yes	16 (94.1%)	8 (72.7%)	2 (66.7%)

Although checkpoint inhibitor–related pneumonitis is one of the most serious immune-related adverse events, no preventive measures have been established. Although steroid therapy is an effective treatment, it carries the risk of suppressing the antitumor effect of checkpoint inhibitors (26). We demonstrated that among patients with NSCLC, the clinical outcome of pneumonitis induced by atezolizumab plus chemotherapy was better in patients treated with bevacizumab than in those without bevacizumab. Furthermore, the percentage of patients with pneumonitis who used steroids was lower in the group treated with bevacizumab than in the group without. Anti-VEGF antibodies such as bevacizumab are reported to have additive or synergistic effects when administered in combination with anti-PD-L1 antibodies (such as atezolizumab) in NSCLC, renal cell carcinoma, and hepatocellular carcinoma (11, 27, 28). Anti-VEGF antibodies plus checkpoint inhibitors may be a treatment method that can control at least a part of checkpoint inhibitor–related pneumonitis without loss of therapeutic effects. On the other hands, mechanism of checkpoint inhibitor–related pneumonitis is not clear and there is a limit what we can learn from our research in an IL2- plus IL18-induced murine pneumonitis model and the particular group of patients. Further study will be needed to clarify the clinically relevant mitigation effects of bevacizumab on immune-related pneumonitis.

In conclusion, we have shown that an anti-VEGF antibody protected against vascular-to-alveolar leakage of protein and fluid, resulting in mitigation of checkpoint inhibitor–related pneumonitis in a murine model. Our findings suggest that bevacizumab may be a meaningful therapeutic option for patients receiving checkpoint inhibitors, not only in terms of enhancing their antitumor effect but also in terms of reducing some side effects.

Authors' Disclosures

T. Iwai M. Sugimoto, K. Yorozu, M. Kurasawa, and O. Kondoh are an employee of Chugai Pharmaceutical Co., Ltd. No disclosures were reported by the other authors.

Authors' Contributions

T. Iwai: Conceptualization, data curation, formal analysis, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **M. Sugimoto:** Conceptualization, formal analysis, supervision, validation, methodology, writing–original draft, project administration, writing–review and editing. **H. Patel:** Formal analysis, validation, investigation, visualization, methodology. **K. Yorozu:** Formal analysis, validation, investigation, visualization, methodology. **M. Kurasawa:** Validation, investigation, visualization, methodology. **O. Kondoh:** Conceptualization, resources, supervision, funding acquisition, project administration, writing–review and editing.

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References

1. Okazaki T, Chikuma S, Iwai Y, Fagarasan S, Honjo T. A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. Nat Immunol 2013;14:1212–8.
2. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung cancer. N Engl J Med 2015;373:1627–39.
3. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet North Am Ed 2017;389:255–65.
4. Khunger M, Rakshit S, Pasupuleti V, Hernandez AV, Mazzone P, Stevenson J, et al. Incidence of pneumonitis with use of programmed death 1 and programmed death-ligand 1 inhibitors in non-small cell lung cancer: a systematic review and meta-analysis of trials. Chest 2017;152: 271–81.
5. Shankar B, Naidoo J. PD-1 and PD-L1 inhibitor toxicities in non-small cell lung cancer. J Thorac Dis 2018;10:S4034–S7.
6. Ferrara N. VEGF: an update on biological and therapeutic aspects. Curr Opin Biotechnol 2000;11:617–24.
7. Dvorak HF, Nagy JA, Berse B, Brown LF, Yeo K-T, Yeo T-K, et al. Vascular permeability factor, fibrin, and the pathogenesis of tumor stroma formationa. Ann N Y Acad Sci 1992;667:101–11.
8. Collins PD, Connolly DT, Williams TJ. Characterization of the increase in vascular permeability induced by vascular permeability factor in vivo. Br J Pharmacol 1993;109:195–9.
9. Voelkel NF, Vandivier RW, Tuder RM. Vascular endothelial growth factor in the lung. Am J Physiol-Lung Cell Mol Physiol 2006;290:L209–L21.
10. Okamoto M, Kato S, Oizumi K, Kinoshita M, Inoue Y, Hoshino K, et al. Interleukin 18 (IL-18) in synergy with IL-2 induces lethal lung injury in mice: a potential role for cytokines, chemokines, and natural killer cells in the pathogenesis of interstitial pneumonia. Blood 2002;99:1289–98.
11. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N Engl J Med 2018;378:2288–301.
12. Holm S. A simple sequentially rejective multiple test procedure. Scand J Statist 1979;6:65–70.
13. Cadranel J, Canellas A, Matton L, Darrason M, Parrot A, Naccache J-M, et al. Pulmonary complications of immune checkpoint inhibitors in patients with nonsmall cell lung cancer. Eur Respir Rev 2019;28:190058.
14. Ashinuma H, Mizuno S, Yoshida Y, Shingyoji M. A case of long-term survival after checkpoint inhibitor pneumonitis in a patient with squamous cell lung cancer. Case Rep Oncol Med 2019;2019:4836404.
15. Watanabe S, Kimura H, Takato H, Waseda Y, Hara J, Sone T, et al. Severe pneumonitis after nivolumab treatment in a patient with melanoma. Allergol Int 2016;65:487–9.
16. Kimura A, Sakai D, Kudo T, Nishida N, Katou A, Inagaki C, et al. Nivolumab-induced interstitial lung disease in a patient with gastric cancer. Oxf Med Case Rep 2019;2019:omz007.
17. Wang J, Okazaki I-M, Yoshida T, Chikuma S, Kato Y, Nakaki F, et al. PD-1 deficiency results in the development of fatal myocarditis in MRL mice. Int Immunol 2010;22:443–52.
18. Shibaki R, Murakami S, Matsumoto Y, Yoshida T, Goto Y, Kanda S, et al. Association of immune-related pneumonitis with the presence of preexisting interstitial lung disease in patients with non-small lung cancer receiving anti-programmed cell death 1 antibody. Cancer Immunol Immunother 2020;69: 15–22.
19. Yamaguchi T, Shimizu J, Hasegawa T, Horio Y, Inaba Y, Yatabe Y, et al. Pre-existing pulmonary fibrosis is a risk factor for anti-PD-1-related pneumonitis in patients with non-small cell lung cancer: a retrospective analysis. Lung Cancer 2018;125:212–7.
20. Heigener DF, Reck M. Advanced non-small cell lung cancer: the role of PD-L1 inhibitors. J Thorac Dis 2018;10:S1468–s73.

21. Bukamur H, Katz H, Alsharedi M, Alkrekshi A, Shweihat YR, Munn NJ. Immune checkpoint inhibitor-related pulmonary toxicity: focus on nivolumab. South Med J 2020;113:600–5.
22. Durrechou Q, Domblides C, Sionneau B, Lefort F, Quivy A, Ravaud A, et al. Management of immune checkpoint inhibitor toxicities. Cancer Manag Res 2020;12:9139–58.
23. Reck M, Wehler T, Orlandi F, Nogami N, Barone C, Moro-Sibilot D, et al. Safety and patient-reported outcomes of atezolizumab plus chemotherapy with or without bevacizumab versus bevacizumab plus chemotherapy in non-small-cell lung cancer. J Clin Oncol 2020;38:2530–42.
24. Kaner RJ, Ladetto JV, Singh R, Fukuda N, Matthay MA, Crystal RG. Lung overexpression of the vascular endothelial growth factor gene induces pulmonary edema. Am J Respir Cell Mol Biol 2000;22:657–64.
25. Ware LB, Kaner RJ, Crystal RG, Schane R, Trivedi NN, McAuley D, et al. VEGF levels in the alveolar compartment do not distinguish between ARDS and hydrostatic pulmonary oedema. Eur Respir J 2005;26:101–5.
26. Tokunaga A, Sugiyama D, Maeda Y, Warner AB, Panageas KS, Ito S, et al. Selective inhibition of low-affinity memory CD8(+) T cells by corticosteroids. J Exp Med 2019;216:2701–13.
27. Rini BI, Powles T, Atkins MB, Escudier B, McDermott DF, Suarez C, et al. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial. Lancet 2019;393:2404–15.
28. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim T-Y, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med 2020;382:1894–905.