Thoracic Cancer

Open Access

Thoracic Cancer ISSN 1759-7706

BRIEF REPORT

Dynamics of blood neutrophil-related indices during nivolumab treatment may be associated with response to salvage chemotherapy for non-small cell lung cancer: A hypothesis-generating study

Hiroshi Soda[1], Daiki Ogawara[1], Yuichi Fukuda[1], Hiromi Tomono[1], Daisuke Okuno[1], Seiko Koga[2], Hirokazu Taniguchi[3], Masataka Yoshida[1], Tatsuhiko Harada[1], Asuka Umemura[1], Hiroyuki Yamaguchi[3] & Hiroshi Mukae[3]

1 Department of Respiratory Medicine, Sasebo City General Hospital, Nagasaki, Japan
2 Department of Pharmacy, Sasebo City General Hospital, Nagasaki, Japan
3 Department of Respiratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Keywords
Immune checkpoint inhibitor; neutrophil-to-lymphocyte ratio; salvage chemotherapy.

Correspondence
Hiroshi Soda, Department of Respiratory Medicine, Sasebo City General Hospital, 9-3 Hirase, Sasebo, Nagasaki 857-8511, Japan.
Tel: +81 956 24 1515
Fax: +81 956 22 4641
Email: h-souda@hospital.sasebo.nagasaki.jp

Received: 1 November 2018;
Accepted: 28 November 2018.

doi: 10.1111/1759-7714.12952

Thoracic Cancer **10** (2019) 341–346

Abstract

Several recent studies have shown that salvage chemotherapy following PD-1 blockade produces high antitumor activity in some patients with non-small lung cancer (NSCLC). However, the underlying synergistic mechanisms remain uncertain. The blood neutrophil-to-lymphocyte ratio (NLR) and absolute neutrophil count (ANC) can reflect the number of circulating myeloid-derived suppressor cells and tumor-associated neutrophils. The immunosuppressive status of the tumor microenvironment could be monitored by the time-series patterns of NLR and ANC. The dynamics of NLR and ANC during nivolumab treatment were retrospectively explored in 15 patients: 8 patients receiving subsequent salvage chemotherapy (2 groups: 3 non-responders and 5 responders), and 7 responders to nivolumab alone (2 groups: 4 partial response and 3 complete response). The dynamics of NLR and ANC during nivolumab differed among these four groups (NLR $P = 0.045$, ANC $P = 0.067$). NLR and ANC during nivolumab treatment increased over time in non-responders to salvage chemotherapy, with an inverse relationship between drug response and NLR or ANC at four to six weeks among the four groups. We hypothesize that the early dynamics of NLR and ANC during nivolumab may be associated with the late efficacy of subsequent salvage chemotherapy. Further studies involving a large cohort are needed to confirm these findings, which could provide insight into the role of myeloid immunosuppressor cells in combination PD-1 blockade and chemotherapy.

Introduction

The combination of immunotherapy and chemotherapy, including salvage chemotherapy following PD-1 inhibitor therapy, is a promising treatment for patients with non-small cell lung cancer (NSCLC).[1–3] We recently reported that salvage chemotherapy following nivolumab increased the response in some patients with NSCLC, regardless of tumor PD-L1 status.[4] In addition, the presence of minimal PD-1 expression on tumor-infiltrating lymphocytes (TILs) before treatment may be associated with improved response to salvage chemotherapy.[4] However, we did not clarify the mechanisms that created these synergistic effects during nivolumab treatment. Several studies have shown that the neutrophil-to-lymphocyte ratio (NLR) and the absolute neutrophil count (ANC) in the blood can predict the efficacy of PD-1 inhibitors in patients with NSCLC.[5–10] NLR and ANC have also been reported to reflect the number of circulating myeloid-derived suppressor cells (MDSCs) and tumor-associated neutrophils,[11–15] which inhibit the function of antitumor T cells.[16,17] The

immunosuppressive status in the tumor microenvironment could be monitored using the dynamics of NLR and ANC. Thus, the time-series patterns of NLR and ANC during nivolumab treatment were retrospectively explored in lung cancer patients who received subsequent salvage chemotherapy to find an immunological clue to design future studies.

Methods

This single-institute, exploratory study was performed retrospectively, following our previously published study.[4] In brief, patients with recurrent or advanced NSCLC underwent salvage chemotherapy following nivolumab treatment between April 2016 and December 2017. Patients were excluded from the analysis if their lung cancer harbored *EGFR* mutations or *ALK* rearrangements, if the patients had documented infection, or if they had any other neoplasm requiring treatment. As controls, patients who achieved a complete response (CR) or a partial response (PR) to nivolumab alone were also selected. Patients received 3 mg/kg of nivolumab every two weeks. All patients were followed-up to October 2018. The best tumor responses were assessed according to Response Evaluation Criteria in Solid Tumors version 1.1. In the present study, responders to salvage chemotherapy were defined as those with CR or PR, while non-responders were those with stable disease (SD) or progressive disease. An automated hematology analyzer, the Sysmex XE-5000 (Sysmex Corporation, Kobe, Japan), was used to evaluate the circulating leukocyte count. NLR was calculated as the ANC divided by the absolute lymphocyte count.

In the present study, four groups were analyzed according to drug response: non-responders and responders to salvage chemotherapy following nivolumab therapy, and patients with PR and CR to nivolumab alone. The differences in the time-series patterns of NLR and ANC among the groups were evaluated by repeated-measures analysis of variance. The ANC values were log-transformed for normalization. The Jonckheere–Terpstra test was used to evaluate the relationship between drug response and the neutrophil-related index. A two-tailed P value < 0.05 was considered significant. Statistical analyses were performed using R, version 3.5.1 (The R Foundation, Vienna, Austria). The institutional review board of Sasebo City General Hospital approved the study protocol.

Results

A total of 37 patients received nivolumab, including salvage chemotherapy, during the study period. As of October 2018, 8 patients (3 CR, 4 PR, and 1 SD) were still being treated with nivolumab. The other 19 patients underwent best supportive care following nivolumab treatment. The remaining 10 patients received salvage chemotherapy following nivolumab treatment; two were excluded because of the presence of concurrent cancer or *EGFR* mutation. Thus, 8 patients treated with salvage chemotherapy (3 non-responders and 5 responders) were analyzed. Coincidentally, the 8 patients who received salvage chemotherapy did not show any objective response to the preceding nivolumab treatment. As mentioned above, 7 patients who responded to nivolumab alone (3 CR and 4 PR) were also analyzed. The patients' characteristics are summarized in Table 1.

The dynamics of NLR during nivolumab differed significantly among the four groups ($P = 0.045$) (Fig 1a). NLR tended to increase over time in non-responders, while it was sustained in responders, with decreasing NLR in patients with PR and CR. ANC showed a similar trend over time ($P = 0.067$) (Fig 1b). A significant inverse relationship was observed between drug response and six-week NLR ($P = 0.048$), and between drug response and six-week ANC ($P = 0.044$) (Fig 2a). The four-week NLR ($P = 0.021$) and the four-week ANC ($P = 0.071$) showed a similar relationship (Fig 2b).

Discussion

This explorative, hypothesis-generating study provided the following important findings and suggestions. The dynamics of NLR and ANC during nivolumab treatment differed among the patients receiving nivolumab alone and salvage chemotherapy. An inverse relationship was observed between drug response and these neutrophil-related indices at four to six weeks after the initiation of nivolumab.

The dynamics of NLR and ANC during nivolumab treatment may be associated with the efficacy of the subsequent salvage chemotherapy. Many studies have shown that low baseline NLR and ANC predict the efficacy of nivolumab therapy in various cancers.[6,18–20] More recent studies have shown that an early decrease in NLR during treatment with PD-1 inhibitors predicts its efficacy.[5–10] NLR and ANC have been reported to correlate with the number of circulating MDSCs and tumor-associated neutrophils,[11–15] which suppress the function of antitumor T cells.[16,17] Circulating MDSC levels were inversely associated with response rate and survival after treatment with nivolumab in cancer patients.[21,22] In tumor-bearing mouse models, the chemokine-related substances that recruit neutrophils and MDSCs into the tumor suppressed the antitumor activity of PD-1 blockade.[23,24]

In contrast, our previous studies showed that the presence of a few PD-1$^+$ TILs was associated with the response to salvage chemotherapy, as well as nivolumab treatment.[4,24,25] However, we did not clarify why preceding

Table 1 Patient characteristics (n = 15)

Variable	Non-responders to salvage Cx (n = 3)	Responders to salvage Cx (n = 5)	Patients with PR to Nivo (n = 4)	Patients with CR to Nivo (n = 3)
Age, years				
Range	64–73	62–75	58–66	70–76
Gender				
Male	2	4	4	3
Female	1	1	0	0
ECOG PS				
0	0	1	1	0
1	3	4	3	3
Smoking status				
Former smoker	3	5	4	3
Never-smoker	0	0	0	0
Histologic subtype				
Adenocarcinoma	0	2	2	1
Squamous cell carcinoma	2	2	2	1
Others	1	1	0	1
Stage				
III	0	1	1	0
IV	2	3	2	0
Recurrent	1	1	1	3
Tumor PD-L1 expression				
≥ 50%	1	1	0	1
1–49%	1	1	2	1
< 1%	1	2	0	0
Not available	0	1	2	1
Line of nivolumab				
Second-line	2	1	1	0
Third-line	1	2	1	2
Fourth-line	0	2	2	1
Best response to nivolumab				
CR	0	0	0	3
PR	0	0	4	0
SD	0	1	0	0
PD	3	4	0	0
Regimen of salvage Cx				
S-1	3	2	Not done	Not done
Carboplatin + nab-paclitaxel	0	2	Not done	Not done
Docetaxel + ramucirumab	0	1	Not done	Not done
Best response to salvage Cx				
CR	0	0	Not done	Not done
PR	0	5	Not done	Not done
SD	2	0	Not done	Not done
PD	1	0	Not done	Not done

CR, complete response; Cx, chemotherapy; ECOG PS, Eastern Cooperative Oncology Group performance status; Nivo, nivolumab; PD, progressive disease; PR, partial response; SD, stable disease.

nivolumab therapy showed less antitumor activity in the responders to salvage chemotherapy. A recent study showed that the neutrophil lineage was the most abundant immune cell type within NSCLC tissues, where neutrophil content was inversely correlated with the content of $CD8^+$ T cells.[26] In addition, disease progression during first-line chemotherapy for NSCLC was reported to correlate with high levels of circulating MDSCs.[27] Combining these findings with the results of our previous studies,[4,25,28] we hypothesize that both the exhaustion levels of cytotoxic T cells and interference with myeloid cells may affect the efficacy of nivolumab and subsequent salvage chemotherapy (Fig 3).

The combination of PD-1 blockade and chemotherapy has shown clinical benefits in patients with advanced NSCLC as first-line treatment.[1,29] However, the complete

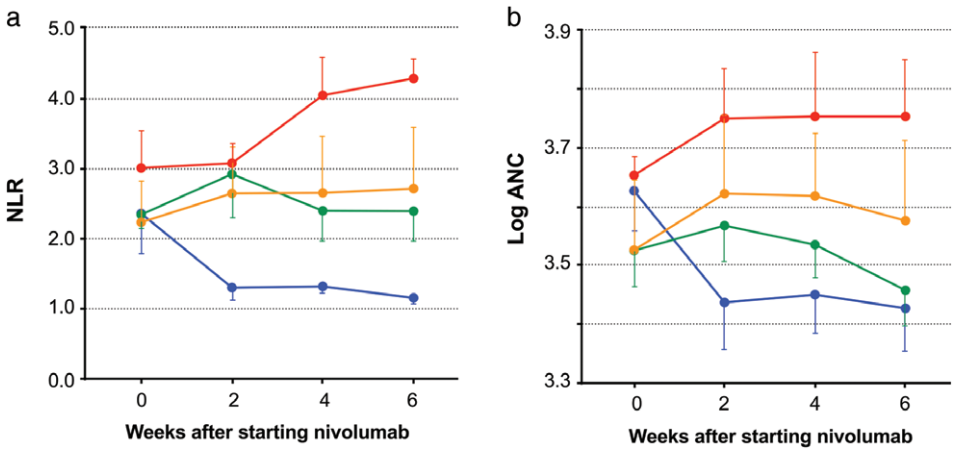

Figure 1 (**a**) The dynamics of the neutrophil-to-lymphocyte ratio (NLR) among the four groups: non-responders and responders to salvage chemotherapy following nivolumab therapy, and patients with a partial response (PR) and a complete response (CR) to nivolumab alone. The vertical bars indicate standard errors. The dynamics of NLR are significantly different among the four groups ($P = 0.045$) (——) Non-responders, (——) Responders, (——) Patients with PR, and (——) Patients with CR. (**b**) The dynamics of the absolute neutrophil count (ANC). The values of ANC were log-transformed for normalization. The dynamics of log ANC over time show a similar trend ($P = 0.067$) (——) Non-responders, (——) Responders, (——) Patients with PR, and (——) Patients with CR.

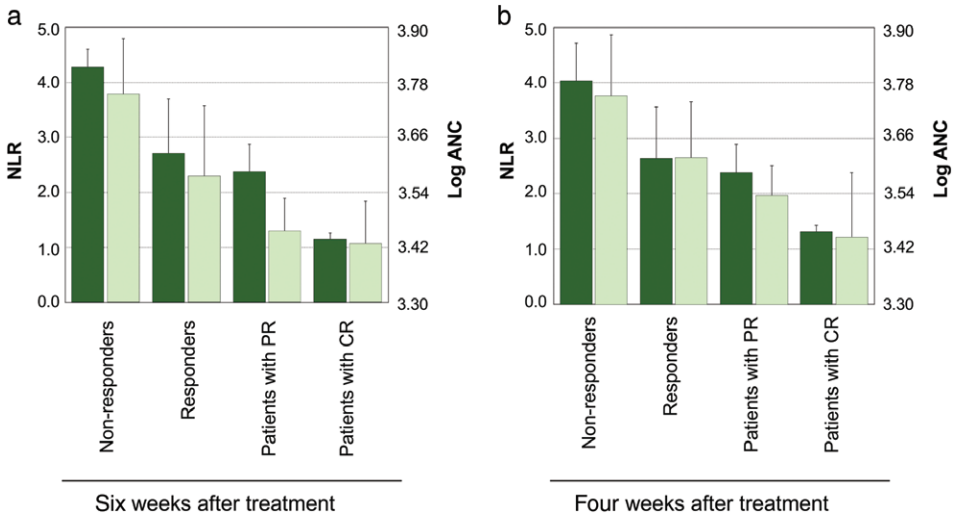

Figure 2 The relationships of drug response with the neutrophil-to-lymphocyte ratio (NLR) and the absolute neutrophil account (ANC) among the four groups: non-responders and responders to salvage chemotherapy following nivolumab therapy, and patients with a partial response (PR) and a complete response (CR) to nivolumab alone. The vertical bars indicate standard errors. The values of ANC were log-transformed for normalization. The relationships between drug-response and the (**a**) six-week NLR ($P = 0.048$) and six-week ANC ($P = 0.044$) and (**b**) the four-week NLR ($P = 0.021$) and four-week ANC ($P = 0.071$) show a similar relationship. (■) NLR, and (□) ANC.

response rates were not necessarily high, therefore further immunotherapeutic strategies are needed to cure lung cancer. Myeloid immunosuppressor cells are considered a major obstacle to cancer immunotherapy.[30] Many clinical trials with agents targeting myeloid immunosuppressor cells are ongoing.[31] For example, the nuclear hormone liver-X receptor agonist that depletes MDSCs enhances the antitumor activity of tumor-antigen specific T cells as monotherapy or in combination with PD-1 blockade in mice and cancer patients.[32] The dynamics of blood NLR and ANC might become a surrogate biomarker to identify patients who could benefit from immunotherapy targeting the myeloid immunosuppressor cells.

The present explorative study has several limitations, including a small population of heterogenous patients and the lack of immunohistochemical evaluation. The present findings are potentially subject to selection bias and should be interpreted cautiously.

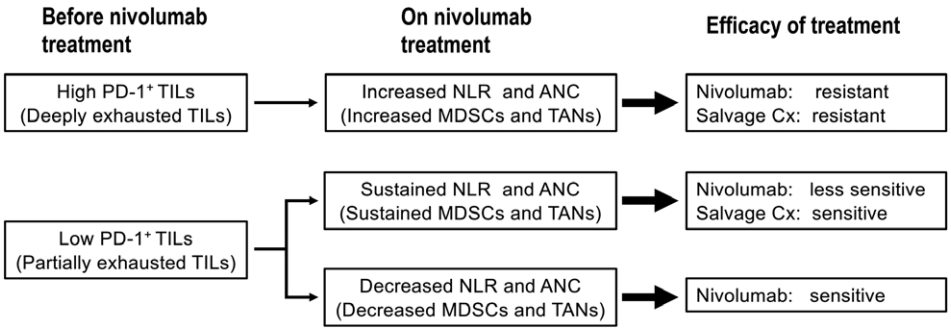

Figure 3 A hypothetical model of the possible underlying mechanisms of nivolumab and subsequent salvage chemotherapy, based on the results of our previous and present studies.[4,25,28] PD-1 expression levels on tumor-infiltrating lymphocytes (TILs) probably indicate the exhaustion of lymphocytes. The dynamics of the neutrophil-to-lymphocyte ratio (NLR) and absolute neutrophil count (ANC) during nivolumab treatment possibly reflect the number of myeloid-derived suppressor cells (MDSCs) and tumor-associated neutrophils (TANs) in the tumor. These combinations may contribute to the efficacy of each therapy. Cx, chemotherapy.

In conclusion, the present findings create a hypothesis that the early dynamics of NLR and ANC during nivolumab treatment may be associated with the late efficacy of subsequent salvage chemotherapy following nivolumab treatment. Further studies involving a large cohort are required to verify our hypothesis, which could shed light on the role of myeloid immunosuppressor cells when the combination of PD-1 blockade and chemotherapy is used.

Disclosure

No authors report any conflicts of interest.

References

1 Gandhi L, Rodríguez-Abreu D, Gadgeel S *et al.* Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med* 2018; **378**: 2078–92.
2 Park SE, Lee SH, Ahn JS, Ahn MJ, Park K, Sun JM. Increased response rates to salvage chemotherapy administered after PD-1/PD-L1 inhibitors in patients with non-small cell lung cancer. *J Thorac Oncol* 2018; **13**: 106–11.
3 Schvartsman G, Peng A, Bis G *et al.* Response rates to single-agent chemotherapy after exposure to immune checkpoint inhibitors in advanced non-small cell lung cancer. *Lung Cancer* 2017; **112**: 90–5.
4 Ogawara D, Soda H, Tomono H *et al.* Presence of few PD-1-expressing tumor-infiltrating immune cells is a potential predictor of improved response to salvage chemotherapy following nivolumab for non-small cell lung cancer: An exploratory case series. *Thorac Cancer* 2018; **9**: 1305–11.
5 Ameratunga M, Chénard-Poirier M, Moreno Candilejo I *et al.* Neutrophil-lymphocyte ratio dynamics of patients with advanced solid tumours on phase I trials of PD-1/PD-L1 inhibitors. *Eur J Cancer* 2018; **89**: 56–63.
6 Zer A, Sung MR, Walia P *et al.* Correlation of neutrophil to lymphocyte ratio and absolute neutrophil count with outcomes with PD-1 axis inhibitors in patients with advanced non-small-cell lung cancer. *Clin Lung Cancer* 2018; **19**: 426–34.
7 Suh KJ, Kim SH, Kim YJ *et al.* Post-treatment neutrophil-to-lymphocyte ratio at week 6 is prognostic in patients with advanced non-small cell lung cancers treated with anti-PD-1 antibody. *Cancer Immunol Immunother* 2018; **67**: 459–70.
8 Kiriu T, Yamamoto M, Nagano T *et al.* The time-series behavior of neutrophil-to-lymphocyte ratio is useful as a predictive marker in non-small cell lung cancer. *PLoS One* 2018; **13**: e0193018.
9 Takeda T, Takeuchi M, Saitoh M, Takeda S. Neutrophil-to-lymphocyte ratio after four weeks of nivolumab administration as a predictive marker in patients with pretreated non-small-cell lung cancer. *Thorac Cancer* 2018; **9**: 1291–9.
10 Nakaya A, Kurata T, Yoshioka H *et al.* Neutrophil-to-lymphocyte ratio as an early marker of outcomes in patients with advanced non-small-cell lung cancer treated with nivolumab. *Int J Clin Oncol* 2018; **23**: 634–40.
11 Chen MF, Tsai MS, Chen WC, Chen PT. Predictive value of the pretreatment neutrophil-to-lymphocyte ratio in head and neck squamous cell carcinoma. *J Clin Med* 2018; **7**: e294.
12 Gonda K, Shibata M, Ohtake T *et al.* Myeloid-derived suppressor cells are increased and correlated with type 2 immune responses, malnutrition, inflammation, and poor prognosis in patients with breast cancer. *Oncol Lett* 2017; **14**: 1766–74.
13 Basu A, Kollengode KA, Rafatnia A, Manoli H, Danenberg G, Chakravartty E. Relationship between neutrophil lymphocyte ratio (NLR) and MDSC concentration in localized and metastatic castration resistant prostate cancer (mCRPC) patients. *J Clin Oncol* 2018; **36** (6 Suppl.): Abstract 338.
14 Hiramatsu S, Tanaka H, Nishimura J *et al.* Neutrophils in primary gastric tumors are correlated with neutrophil infiltration in tumor-draining lymph nodes and the

systemic inflammatory response. *BMC Immunol* 2018; **19**: e13.
15 Riemann D, Cwikowski M, Turzer S *et al.* Blood immune cell biomarkers in lung cancer. *Clin Exp Immunol* 2018. https://doi.org/10.1111/cei.13219
16 Millrud CR, Bergenfelz C, Leandersson K. On the origin of myeloid-derived suppressor cells. *Oncotarget* 2017; **8**: 3649–65.
17 Aarts CEM, Kuijpers TW. Neutrophils as myeloid-derived suppressor cells. *Eur J Clin Invest* 2018; **48**: e12989.
18 Sacdalan DB, Lucero JA, Sacdalan DL. Prognostic utility of baseline neutrophil-to-lymphocyte ratio in patients receiving immune checkpoint inhibitors: A review and meta-analysis. *Onco Targets Ther* 2018; **11**: 955–65.
19 Tan Q, Liu S, Liang C, Han X, Shi Y. Pretreatment hematological markers predict clinical outcome in cancer patients receiving immune checkpoint inhibitors: A meta-analysis. *Thorac Cancer* 2018; **9**: 1220–30.
20 Mezquita L, Auclin E, Ferrara R *et al.* Association of the lung immune prognostic index with immune checkpoint inhibitor outcomes in patients with advanced non-small cell lung cancer. *JAMA Oncol* 2018; **4**: 351–7.
21 Delaunay M, Guibert N, Lusque A *et al.* Baseline circulating myeloid-derived suppressor cells and response to PD-1 inhibitor in non-small cell lung cancer patients. *J Clin Oncol* 2018; **36**(5 Suppl.): Abstract 145.
22 Weber J, Gibney G, Kudchadkar R *et al.* Phase I/II study of metastatic melanoma patients treated with nivolumab who had progressed after ipilimumab. *Cancer Immunol Res* 2016; **4**: 345–53.
23 Akbay EA, Koyama S, Liu Y *et al.* Interleukin-17A promotes lung tumor progression through neutrophil attraction to tumor sites and mediating resistance to PD-1 blockade. *J Thorac Oncol* 2017; **12**: 1268–79.
24 Najjar YG, Rayman P, Jia X *et al.* Myeloid-derived suppressor cell subset accumulation in renal cell carcinoma parenchyma is associated with intratumoral expression of IL1β, IL8, CXCL5, and Mip-1α. *Clin Cancer Res* 2017; **23**: 2346–55.
25 Suyama T, Fukuda Y, Soda H *et al.* Successful treatment with nivolumab for lung cancer with low expression of PD-L1 and prominent tumor-infiltrating B cells and immunoglobulin G. *Thorac Cancer* 2018; **9**: 750–3.
26 Kargl J, Busch SE, Yang GH *et al.* Neutrophils dominate the immune cell composition in non-small cell lung cancer. *Nat Commun* 2017; **8**: e14381.
27 Koinis F, Vetsika EK, Aggouraki D *et al.* Effect of first-line treatment on myeloid-derived suppressor cells' subpopulations in the peripheral blood of patients with non-small cell lung cancer. *J Thorac Oncol* 2016; **11**: 1263–72.
28 Okamura K, Fukuda Y, Soda H *et al.* Pulmonary pleomorphic carcinoma with few PD-1-positive immune cells and regulatory T cells that showed a complete response to nivolumab. *Thorac Cancer* 2018; **9**: 193–6.
29 Paz-Ares L, Luft A, Vicente D *et al.* Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. *N Engl J Med* 2018; **379**: 2040–51.
30 Awad RM, De Vlaeminck Y, Maebe J, Goyvaerts C, Breckpot K. Turn back the TIMe: Targeting tumor infiltrating myeloid cells to revert cancer progression. *Front Immunol* 2018; **9**: e1977.
31 Liu Y, Wei G, Cheng WA *et al.* Targeting myeloid-derived suppressor cells for cancer immunotherapy. *Cancer Immunol Immunother* 2018; **67**: 1181–95.
32 Tavazoie MF, Pollack I, Tanqueco R *et al.* LXR/ApoE activation restricts innate immune suppression in cancer. *Cell* 2018; **172**: 825–40.

