



## Case report

# Immunotherapy “Shock” a case series of PD-L1 100% and pembrolizumab first-line treatment



Paul Zarogoulidis <sup>a,\*</sup>, Evaggelia Athanasiou <sup>b</sup>, Theodora Tsiouda <sup>a</sup>,  
 Dimitrios Hatzibougias <sup>b</sup>, Haidong Huang <sup>c</sup>, Chong Bai <sup>c</sup>, Georgia Trakada <sup>d</sup>,  
 Lemonia Veletza <sup>d</sup>, Anastasios Kallianos <sup>d</sup>, Christoforos Kosmidis <sup>e</sup>, Nikolaos Barbetakis <sup>f</sup>,  
 Dimitrios Paliouras <sup>f</sup>, Aggeliki Rapti <sup>g</sup>, Dimitrios Drougas <sup>h</sup>,  
 Wolfgang Hohenforst-Schmidt <sup>i</sup>

<sup>a</sup> Pulmonary-Oncology Unit, “Theageneio” Anticancer Hospital, Thessaloniki, Greece

<sup>b</sup> Private Pathology Center, “Microdiagnostics”, Thessaloniki, Greece

<sup>c</sup> Department of Respiratory and Critical Care Medicine, Shanghai Hospital, Second Military Medical University, Shanghai, China

<sup>d</sup> Division of Pulmonology, Department of Clinical Therapeutics, National and Kapodistrian University of Athens School of Medicine, Alexandra Hospital, Athens, Greece

<sup>e</sup> Surgery Department, “Interbalkan” European Medical Center, Thessaloniki, Greece

<sup>f</sup> Thoracic Surgery Department, “Theageneio” Anticancer Hospital, Thessaloniki, Greece

<sup>g</sup> 2nd Pulmonary Department, “Sotiria” Hospital of Chest Diseases, Athens, Greece

<sup>h</sup> Private Scientigraphy Department, “Bioiatriki”, Thessaloniki, Greece

<sup>i</sup> Sana Clinic Group Franken, Department of Cardiology/Pulmonology/Intensive Care/Nephrology, “Hof” Clinics, University of Erlangen, Hof, Germany

## ARTICLE INFO

## Article history:

Received 18 August 2017

Received in revised form

18 August 2017

Accepted 19 August 2017

## Keywords:

Adenocarcinoma

Lung cancer

Immunotherapy

Nivolumab

Pembrolizumab

Programmed death-ligand

## ABSTRACT

In this decade a “bloom” of novel therapies has been observed for non-small cell lung cancer. We have new tools for the diagnosis of lung cancer and also we can re-biospy easier than before in different lesions and obtain tissue samples in order to investigate whether a patient can receive new targeted therapies. Immunotherapy has been well established previously for other forms of cancer, and nowadays it is also available for lung cancer. There are two immunotherapies for now nivolumab and pembrolizumab which can be administered as second line treatment, the second can also be administered as first-line if there is a programmed death-ligand 1  $\geq 50\%$  expression.

© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

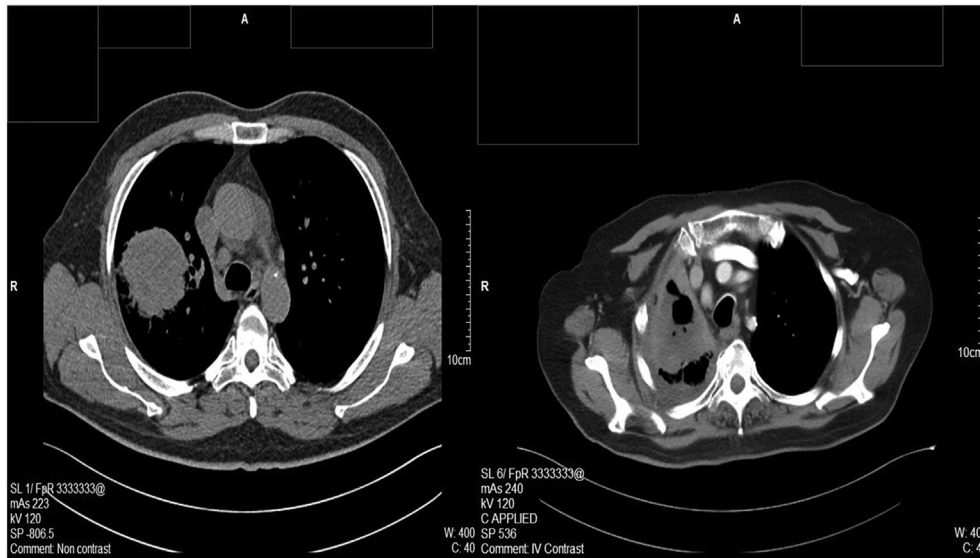
## 1. Introduction

Lung cancer is still diagnosed at a late stage although we have new diagnostic tools in our everyday clinical practice. Regarding the imaging methods we have the positron emission tomography-cat scan (PET-CT) for diagnosis and staging of lung cancer [1]. Regarding interventional methods we have the radial endobronchial ultrasound and the convex-probe endobronchial ultrasound (EBUS) which we have again for the diagnosis and staging of lung

cancer [2,3]. In the past decade targeted therapy was added to our clinical practice with tyrosine kinase inhibitors (TKIs), and in the last year immunotherapy was added in our everyday clinical practice. Regarding immunotherapy we have nivolumab as second line treatment and pembrolizumab as first line treatment if we have  $\geq 50\%$  programmed death-ligand 1 (PD-L1) and as second line if we have  $\geq 1\%$  [4,5]. There are different adverse effects between the targeted treatments and immunotherapy, in any case of course there are much less than the non-specific cytotoxic agents [6,7]. In order to investigate the PD-L1 expression we use the PD-L1 IHC 22C3 pharmDx (DAKO) which was developed in partnership with the pharmaceutical company producing the pembrolizumab drug. Nowadays there also other kits that can identify the PD-L1 expression and are being tested. However; as it has been

\* Corresponding author. Pulmonary Oncology Unit, “G. Papanikolaou” General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece.

E-mail address: [pzarog@hotmail.com](mailto:pzarog@hotmail.com) (P. Zarogoulidis).



**Fig. 1.** Left side the mass upon diagnosis and right side after 7 days of 200 mg pembrolizumab administration.

observed once again “tissue is the issue”, the tissue specimen has to be well prepared after biopsy and well preserved as it has been observed PD-L1 expression depends on the “slice” of the paraffin block. In the same tissue biopsy the expression might be low in a slice of the paraffin block, while in the next slice high.

## 2. Methods of programmed death-ligand 1 (PD-L1)

Immunohistochemistry for both antibodies was performed in 2- $\mu$ m tissues on positive charged slides.

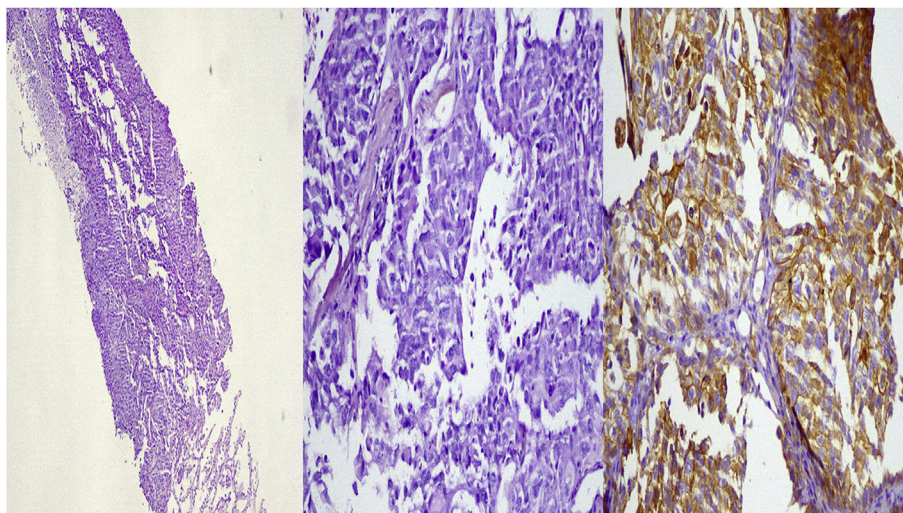
a) **Programmed death-ligand 1 (PD-L1)** [clone: CAL10 (RTU, CE, IVD), Biocare, CA, USA)

External positive controls were tonsil tissues placed on each slide. Immunohistochemistry was performed in Autostainer platform BOND Max™ (Leica Biosystems, Wetzlar, G). The antibody was in a ready-to-use form (RTU) and the incubation time was 20 minutes.

b) **Programmed death-ligand 1 (PD-L1)** PD-L1, 22C3 pharmDx™ Kit NSCLC. Companion diagnostic system Dako, Denmark, EU. This protocol is a complete automate stable procedure, performed in AutostainerLink48 Dako platform. Each staining run includes external positive cell line control.

## 3. Case 1

A 65 year old man was diagnosed with lung adenocarcinoma with fine needle biopsy 18G needle from a large mass on the right upper lobe (Fig. 1). Epidermal growth factor (EGFR) and anaplastic lymphoma kinase (ALK) mutations were negative. Programmed death-ligand 1 expression however was 100% with DAKO and 95% with the “BioCare” (Figs. 2 and 3). The patient was stage IV with bone metastasis and it was decided that he receives pembrolizumab 200 mg. After four days of administration the patient presented fever  $\geq 38$  °C and dyspnea. In the emergency room he had saturation 70%SpO<sub>2</sub> with FiO<sub>2</sub> 21%. Laboratory findings from its



**Fig. 2.** BioCare: left side X 40 magnification, middle X 200 magnification and right side X 400 magnification.

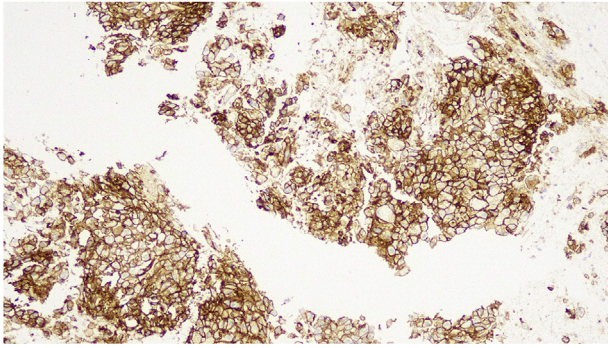


Fig. 3. DAKO: x 10 magnification.

blood were White Blood Count (WBC): 10.16 K/ $\mu$ L, Hemoglobin (HGB): 11.5 g/dL, Platelets (PLT): 201 K/ $\mu$ L, C-reactive protein (CRP):

1.30 mg/dL, Procalcitonin (PCT): 0.3 ng/mL and Erythrocyte Sedimentation Rate (ESR): 5 mm. The rest of his laboratory findings were within normal rates, we focus on these values because we want to distinguish whether he had an abscess and infection or just an abscess. Indeed, a new CT of the thorax revealed that the lung mass had become an abscess (Fig. 1). The patient also started to cough large quantities of thick sputum. Oxygen and supportive therapy for his vitals were initiated. The patient remained in the intensive care unit (ICU) until he was stable and pembrolizumab was again initiated, he remains under treatment and follow-up with 200 mg.

#### 4. Case 2

A 55 year old man was diagnosed with lung adenocarcinoma with a Pentax convex-probe endobronchial ultrasound (EBUS) right hemithorax paratracheal (Figs. 4–6). The patient was stage IV with bone metastasis and he did not have EGFR or ALK mutations,

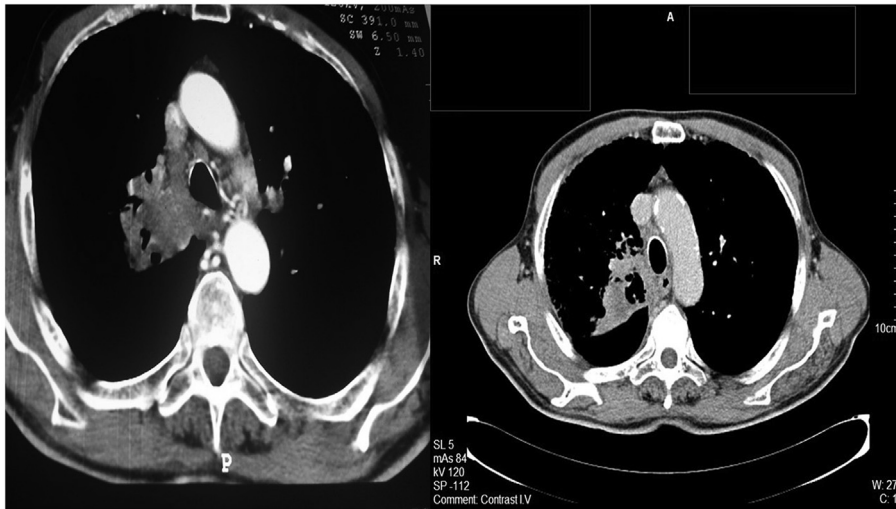


Fig. 4. Left side the mass upon diagnosis and right side after 7 days of pembrolizumab 200 mg administration.

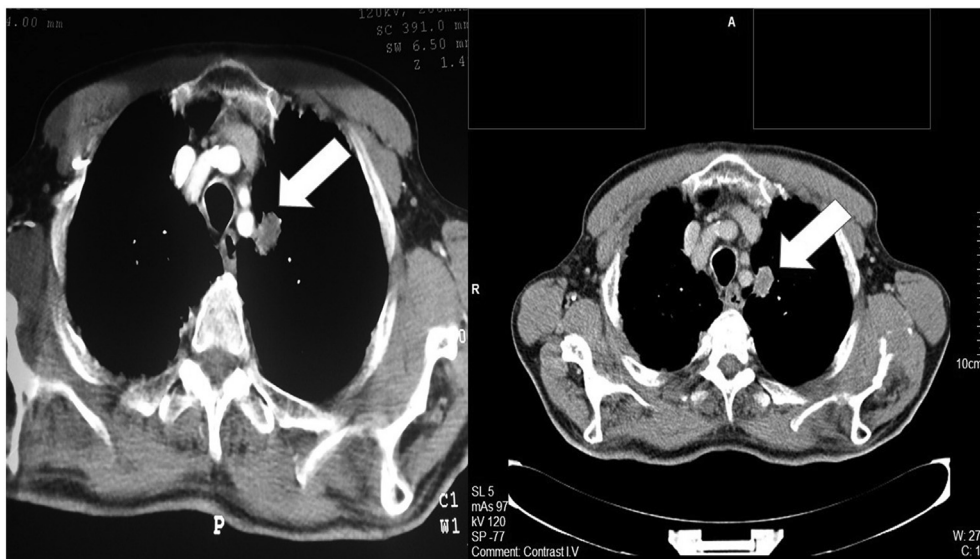


Fig. 5. Left side: lung metastasis of the left lung upon diagnosis and right side: the same lung metastasis of the left lung which did not change after the pembrolizumab administration.

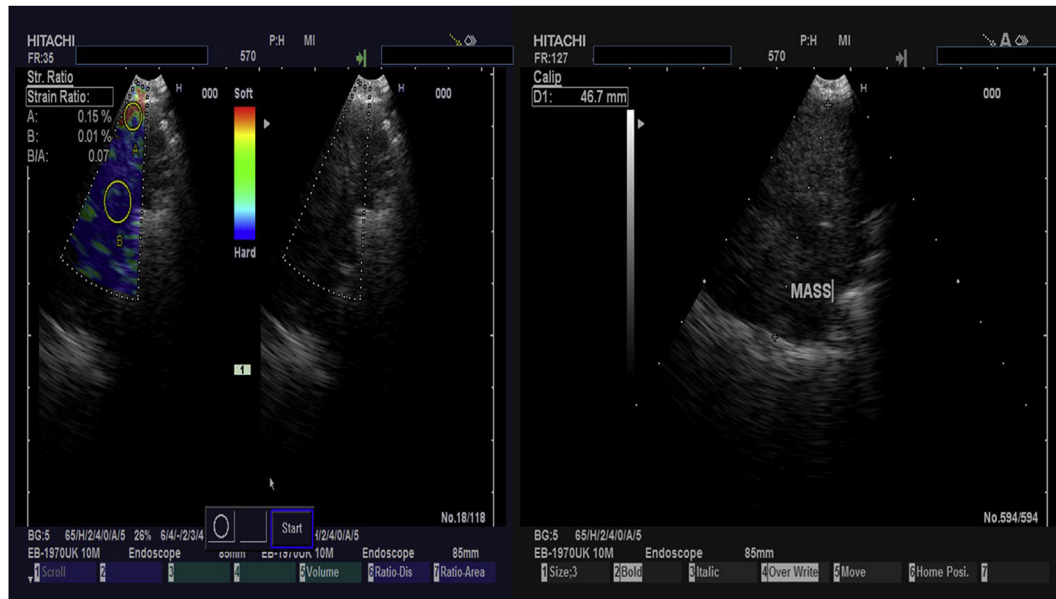


Fig. 6. Elastography upon diagnosis with a strain ratio of 0.07%.

however; he had programmed death-ligand 1 expression 100% with DAKO and 90% with BioCare (Figs. 7 and 8). Pembrolizumab was initiated with 200 mg as it is indicated. Fever was observed  $\geq 38$  °C after 2 days the laboratory findings indicated a mild infection and therefore antibiotics were initiated. Laboratory findings from its blood were White Blood Count (WBC): 18.36 K/ $\mu$ L, Hemoglobin (HGB): 14 g/dL, Platelets (PLT): 250 K/ $\mu$ L, C-reactive protein (CRP): 8 mg/dL, Procalcitonin (PCT): 5 ng/mL and Erythrocyte Sedimentation Rate (ESR): 30 mm. However; based on our previous experience we performed a new CT of the thorax and it was observed that the primary lesion was an abscess, however; regional lymph nodes remained the same. Moreover a previous lesion on the left lung remained the same (24mm) (Figs. 4–6). Upon his new treatment appointment the patient (21 days after the initial administration) had normal laboratory values without fever and therefore it was decided again to administer pembrolizumab

200 mg. The patient is on follow-up. Elastography was performed to this patient and a strain ratio of 0.07% was identified. This finding suggests as an initial finding that the lesion was a malignancy, however; nothing more. Elastography cannot be used to predict the efficiency of a therapy.

## 5. Discussion

Certainly the adverse effects of immunotherapy are less than the standard chemotherapy [8], however; immunotherapy adverse effects are being investigated [5]. Pembrolizumab demonstrated efficiency in first-line treatment for patients with programmed death-ligand 1 expression  $\geq 50\%$  vs chemotherapy, however; the same results were not observed with nivolumab [9]. This observation was attributed to the different patient selection [10]. In the case of a patient with both EGFR mutation and PD-L1  $\geq 50\%$  tkis are

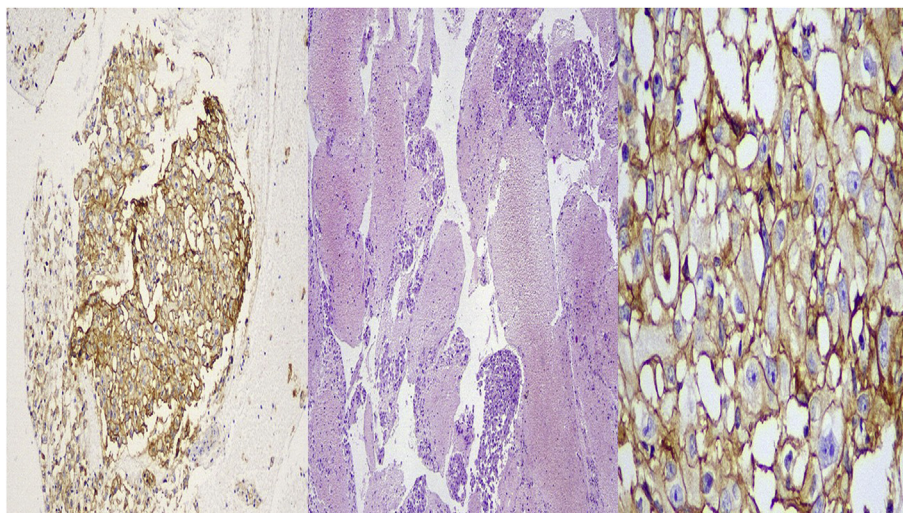


Fig. 7. BioCare: left side X 10 magnification, middle: X 40 magnification, right side: X 400 magnification.

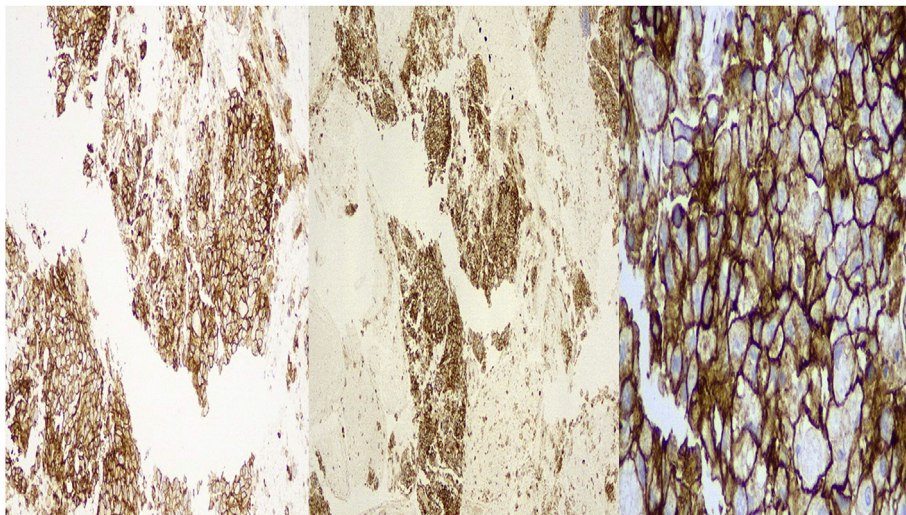


Fig. 8. DAKO: left side X 10 magnification, middle: X 40 magnification, right side: X 400 magnification.

currently advised [11]. Tyrosine kinase inhibitors have few and well controlled adverse effects [12–14]. These adverse effects are also well controlled for thertkis administered for mutation T790M [15,16]. The cases that we currently present are the first where PD-L1 = 100% with DAKO has been observed along with abscess which we attribute to the connection of pembrolizumab and the high expression of PD-L1. The adverse effects of immunotherapy have been previously well described, however; we have still many to observe [17–20]. We have observed small differences between the Bio Care kit clone CAL10 and the 22C3 pharmDX™ Kit, DAKO expression. These small differences we attribute them to the different tissue specimen (slice from the paraffin block), however; definitely we need more tissue specimens and patients to have a clear view on this subject. Probably different lesions of the same patient have different PD-L1 expression, therefore we observe different efficacy of the therapy in different lesions. Caution should be taken for these patients with such PD-L1 expression.

#### Conflict of interest

None to declare.

#### Acknowledgements

The authors would like to thank the director of the pathology department of “G. Papanikolaou” General Hospital, Dr. Liana Papaemmanouil for her valuable insights regarding the pathology techniques used. Moreover; EBUS biopsy and elastography was performed by Dr. Paul Zarogoulidis with a PENTAX EB-1970UK and HITACHI EUB-6500HV.

#### References

- [1] H.S. Kim, K.S. Lee, Y. Ohno, E.J. van Beek, J. Biederer, PET/CT versus MRI for diagnosis, staging, and follow-up of lung cancer, *J. Magn. Reson. Imaging JMIR* 42 (2) (2015) 247–260.
- [2] B. Zaric, V. Stojisic, V. Carapic, T. Kovacevic, G. Stojanovic, M. Panjkovic, I. Kioumis, K. Darwiche, K. Zarogoulidis, G. Stratakos, D. Tsavlis, W. Hohenforst-Schmidt, G. Pitsiou, A. Zissimopoulos, N. Sachpekidis, I. Karapantzos, C. Karapantzou, P. Zarogoulidis, B. Perin, Radial endobronchial ultrasound (EBUS) guided suction catheter-biopsy in histological diagnosis of peripheral pulmonary lesions, *J. Cancer* 7 (1) (2016) 7–13.
- [3] B. Zaric, V. Stojisic, T. Sarcev, G. Stojanovic, V. Carapic, B. Perin, P. Zarogoulidis, K. Darwiche, K. Tsakiridis, I. Karapantzos, G. Kesisis, I. Kougiountzi, N. Katsikogiannis, N. Machairiotis, A. Stylianaki, C.N. Foroulis, K. Zarogoulidis, Advanced bronchoscopic techniques in diagnosis and staging of lung cancer, *J. Thorac. Dis.* 5 (Suppl 4) (2013) S359–S370.
- [4] D.A. Goldstein, U. Bilal, V. Prasad, Pembrolizumab as first-line therapy in programmed death ligand 1-positive advanced lung cancer: is it as effective as we think it is? *Cancer* (2017) (Epub ahead of print).
- [5] E. Feld, L. Horn, Emerging role of nivolumab in the management of patients with non-small-cell lung cancer: current data and future perspectives, *OncoTargets Ther.* 10 (2017) 3697–3708.
- [6] P. Tomasini, S. Brosseau, J. Mazieres, J.P. Merlio, M. Beau-Faller, J. Mosser, M. Wislez, L. Ouafik, B. Besse, I. Rouquette, D. Debieuvre, F. Escande, V. Westeel, C. Audigier-Valette, P. Missy, A. Langlais, F. Morin, D. Moro-Sibilot, G. Zalcman, F. Barlesi, EGFR tyrosine kinase inhibitors versus chemotherapy in EGFR wild-type pre-treated advanced nonsmall cell lung cancer in daily practice, *Eur. Respir. J.* 50 (2) (2017).
- [7] E. Giroux Leprieur, C. Dumenil, C. Julie, V. Giraud, J. Dumoulin, S. Labrune, T. Chinet, Immunotherapy revolutionises non-small-cell lung cancer therapy: results, perspectives and new challenges, *Eur. J. Cancer* 78 (2017) 16–23.
- [8] C. Chouaid, D. Loirat, E. Clay, A. Millier, C. Godard, A. Fannan, L. Levy-Bachelot, E. Angevin, Cost analysis of adverse events associated with non-small cell lung cancer management in France, *Clin. Outcomes Res. CEOR* 9 (2017) 443–449.
- [9] M. Reck, D. Rodriguez-Abreu, A.G. Robinson, R. Hui, T. Csozsi, A. Fulop, M. Gottfried, N. Peled, A. Tafreshi, S. Cuffe, M. O'Brien, S. Rao, K. Hotta, M.A. Leiby, G.M. Lubiniecki, Y. Shentu, R. Rangwala, J.R. Brahmer, K.- Investigators, Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer, *N. Engl. J. Med.* 375 (19) (2016) 1823–1833.
- [10] Why first-line nivolumab is no better than chemo, *Cancer Discov.* (2017) (Epub ahead of print).
- [11] P. Zarogoulidis, P. Chinelis, C. Efthymiou, A. Athanasiadou, V. Mpikos, G. Papatsibas, V. Papadopoulos, E. Maragouli, H. Huang, G. Trakada, A. Kallianos, L. Veletza, W. Hohenforst-Schmidt, EGFR or PD-L1 decision for first line therapy in a case series of EGFR positive and PD-L1 >50, *Respir. Med. Case Rep.* 22 (2017) 7–10.
- [12] W. Hohenforst-Schmidt, P. Zarogoulidis, M. Steinheimer, N. Benhassen, T. Tsiouda, S. Baka, L. Yarmus, G. Stratakos, J. Organtzis, A. Pataka, K. Tsakiridis, I. Karapantzos, C. Karapantzou, K. Darwiche, A. Zissimopoulos, G. Pitsiou, K. Zarogoulidis, Y.G. Man, H. Rittger, Tyrosine kinase inhibitors for the elderly, *J. Cancer* 7 (6) (2016) 687–693.
- [13] K. Domvri, P. Zarogoulidis, K. Darwiche, R.F. Browning, Q. Li, J.F. Turner, I. Kioumis, D. Spyratos, K. Porpodis, A. Papaiwannou, T. Tsiouda, L. Freitag, K. Zarogoulidis, Molecular targeted drugs and biomarkers in NSCLC, the evolving role of individualized therapy, *J. Cancer* 4 (9) (2013) 736–754.
- [14] P. Zarogoulidis, S. Baka, S. Labaki, G. Lazaridis, G. Trakada, Targeted lung cancer treatments and eye metastasis, *Med. Hypothesis Discov. Innov. Ophthalmol.* 6 (1) (2017) 10–13.
- [15] P. Zarogoulidis, A. Rapti, C. Sardeli, P. Chinelis, A. Athanasiadou, K. Paraskevaidou, A. Kallianos, L. Veletza, G. Trakada, W. Hohenforst-Schmidt, H. Huang, Re-biopsy after relapse of targeted therapy. T790M after epidermal growth factor mutation, where and why based on a case series, *Respir. Med. Case Rep.* 21 (2017) 171–175.
- [16] P. Zarogoulidis, M. Gaga, H. Huang, K. Darwiche, A. Rapti, W. Hohenforst-Schmidt, Tissue is the issue and tissue competition. Re-biopsy for mutation T790: where and why? *Clin. Transl. Med.* 6 (1) (2017) 6.
- [17] P. Zarogoulidis, P. Chinelis, A. Athanasiadou, T. Tsiouda, G. Trakada, A. Kallianos, L. Veletza, D. Hatzibougias, E. Mihalopoulou, E. Goupou, C. Kosmidis, C. Sardeli, H. Huang, W. Hohenforst-Schmidt, Possible adverse

- effects of immunotherapy in non-small cell lung cancer; treatment and follow-up of three cases, *Respir. Med. Case Rep.* 22 (2017) 101–105.
- [18] R. Costa, R.B. Costa, S.M. Talamantes, I. Helenoswki, B.A. Carneiro, Y.K. Chae, W.J. Gradishar, R. Kurzrock, F.J. Giles, Analyses of selected safety endpoints in phase 1 and late-phase clinical trials of anti-PD-1 and PD-L1 inhibitors: prediction of immune-related toxicities, *Oncotarget* (2017) (Epub ahead of print).
- [19] S. Cousin, A. Italiano, Toxicity profiles of immunotherapy, *Pharmacol. Ther.* (2017) (Epub ahead of print).
- [20] F. Kroschinsky, F. Stolzel, S. von Bonin, G. Beutel, M. Kochanek, M. Kiehl, P. Schellongowski, H. Intensive Care in, G. Oncological Patients Collaborative, New drugs, new toxicities: severe side effects of modern targeted and immunotherapy of cancer and their management, *Crit. Care* 21 (1) (2017) 89.