Case report

Anti PD-1 immunotherapy related interstitial lung disease presenting as respiratory failure - A review with case series

Padmastuti Akella, Sundaravadivel Loganathan, Vishal Jindal, Jamal Akhtar, Amos Lal*

Department of Medicine, Saint Vincent Hospital, 123 Summer Street, Worcester, MA, 01608, United States

ABSTRACT

Introduction: Lung cancer is one of the most common cancers in the world and it is the leading cause of cancer-related deaths, among men and women, in the United States [1]. In advanced non-small cell lung cancers, immune checkpoint inhibitors such as programmed cell death protein-1 inhibitors (PD-1 inhibitors) have become second-line therapy and have revolutionized the management in selective cases conferring better overall response rates and progression free survival.

Methods: We present a case series and review of literature emphasizing this immune-related adverse events (irAEs) in patients with metastatic non-small cell lung cancer and esophageal cancer who were treated with Nivolumab as a second line therapeutic option.

Results: PD-1 inhibitors such as Nivolumab and Pembrolizumab, have shown a stable regression of various malignancies, such as metastatic melanoma, renal cell carcinoma and metastatic non-small cell lung cancer. We describe 2 cases of such immune related adverse effects associated with immune check point inhibitors with recovery in one of the patients. Steroid therapy has been the cornerstone for treatment for such immune related adverse effects. Importance has also been laid on the typical radiographic patterns of pneumonitis and interstitial lung disease associated with immunotherapy.

Conclusions: We attempt to raise awareness, discuss early management strategies and hypothesize an association between the incidence and development of these adverse events in cancer patients treated with anti-PD-1 immunotherapeutic agents.

1. Introduction

Lung cancer is one of the most common cancers in the world and it is the leading cause of cancer-related deaths, among men and women, in the United States [1]. Platinum-based chemotherapy, subsequently followed by second-line cytotoxic chemotherapy, is the standard treatment for most patients with advanced non-small cell lung cancer (NSCLC) [2]. In advanced non-small cell lung cancers, immune checkpoint inhibitors have become second-line therapy and have revolutionized the management in selective cases conferring better overall response rates and progression free survival [3].

Immune checkpoint inhibitors such as programmed cell death protein-1 inhibitors (PD-1 inhibitors) are a form of passive immunotherapy [4]. While active immunotherapy strategies aim to enhance the host's anti-tumor immune responses, passive immunotherapy has used adoptive cell transfer (ACT) strategies to fight against a variety of solid and hematological malignancies [5]. In ACT, there is a transfer of tumor targeted mono-antibodies and donor T cells, to identify and destroy tumor cells whilst leaving normal host cells healthy and ensuring a specific immune response that directly goes into effector phase [6].

PD-1 is a negative costimulatory receptor expressed primarily on the surface of activated T cells and it mediates immunosuppression. PD-1 functions primarily in peripheral tissues, where T cells may encounter the immunosuppressive PD-1 ligands (PD-L1 AND PD-L2), which are expressed by tumor cells, stromal cells, or both. Inhibition of the interaction between PD-1 and PD-L1 can enhance T-cell responses in vitro and mediate antitumor activity [2]. Nivolumab (Opdivo© Bristol-Myers Squibb) is a fully human IgG4 monoclonal antibody that targets PD-1.

PD-1 inhibitors such as, Nivolumab and Pembrolizumab (Keytruda© Merck Sharp & Dohme Corp), have shown a stable regression of various malignancies, such as metastatic melanoma, renal cell carcinoma and metastatic non-small cell lung cancer (NSCLC) [5]. Nivolumab was first approved in Japan for treatment of metastatic melanoma in 2014 [7]. However, phase III trials in Japan (Checkmate 017 and Checkmate 057) studying Nivolumab in comparison to docetaxel, have shown to have treatment related immune adverse events such as pneumonitis and ILD, in the treatment of advanced squamous-cell non-small-cell lung cancer (NSCLC) [8,9].

Immune checkpoint inhibitors (ICIs) aid with enhancing antitumor activities, and as a byproduct can also stimulate the immune system resulting in immune related adverse events (irAEs). These irAEs in lung can be in the form of pneumonitis as highlighted in our case or sarcoidosis like reaction [10]. A higher incidence of pneumonitis among patients with NSCLC on immunotherapy can be explained on the...
understanding that these patients are more prone to develop drug-related lung toxic effects. This is contributed by their smoking history, damaged underlying lung parenchyma, chronic obstructive pulmonary disease and pulmonary fibrosis [11–14]. Existing tumor burden in the lung may also be a contributing factor limiting the tolerance of lung tissue to exogenous stress and injury [11]. A combined effect of all these risk factors increase the overall potential of NSCLC patients to develop grade 3 or higher pneumonitis.

With our cases and review of literature we highlight this immune-related adverse events (irAEs) in patients with metastatic non-small cell lung cancer and esophageal cancer who were treated with Nivolumab as a second line therapeutic option. We attempt to raise awareness, discuss early management strategies and hypothesize an association between the incidence and development of these adverse events in cancer patients treated with anti-PD-1 immunotherapeutic agents.

2. Case description 1

An 80-year-old female was treated for a large left upper lobe mass measuring 7.8 cm × 5.9 cm on a computed tomogram (CT) of chest diagnosed as metastatic non-small cell lung carcinoma (NSCLC) (Fig. 1A). The metastatic NSCLC, at the time of diagnosis, involved left hilar, left supraclavicular, left retropectoral/axillary lymph nodes, along with left hepatic, right adrenal and portal metastatic disease. Her past medical history included Chronic Obstructive Pulmonary Disease (COPD) with FEV1 being 51%, the FEV1/FVC was 65%. Other pertinent medical history included pulmonary hypertension, erosive gastritis, hypothyroidism and a history of left-sided breast cancer which was an invasive ductal carcinoma, well to moderately differentiated (Estrogen Receptor+ (ER+), Progestin Receptor+ (PR+), Herceptin Receptor- (HER2-)) discovered concurrently upon the diagnosis of her lung cancer. The patient was a former smoker with smoking history of fifty pack years and had quit smoking two years prior to diagnosis of lung cancer. Her BRCA 1 and 2 (Breast Cancer 1 and 2) gene testing was negative. The patient denied curative surgery for her breast cancer at the time of diagnosis and only agreed to endocrine therapy to treat her breast cancer. She was started on Anastrozole 1mg tablets daily. After patient’s first diagnostic positron emission tomography (PET) scan she was found to have interval progression of her active malignancy along with her metastases. This interval progression was an increase in the size and metabolic activity of the lung tumor and all previous metastases reported. Her initial treatment plan constituted three cycles of pemetrexed and Carboplatin. However, after the interval increase in tumor activity, she received three more cycles of Pemetrexed and
Carboplatin. PET/CT repeated in ten months to monitor chemotherapy response, showed improvement in her disease after the six cycles of Pemetrexed and Carboplatin with a decrease in the size of the left upper lobe lung mass to 5.5 cm × 4.3 cm, which was previously 7.8 cm × 5.9 cm (Fig. 1B). Her subsequent PET/CT scan in 6 months showed an interval increase in the size and metabolic activity of the left upper lobe mass measuring 6 cm and additional adjacent spiculation indicating tumor infiltration (Fig. 1C). Given her disease progression, decision was made to treat her with second line systemic therapy with a drug called Nivolumab (a PD-1 inhibitor). Her PET/CT scan after six cycles of Nivolumab showed a significant decrease in size and metabolic activity of the upper lobe mass measuring 2.4 cm × 2.8 cm and further improvement in subsequent PET/CT scans (Fig. 1D). The rest of her metastatic deposits in the liver, lymph nodes and adrenal gland also showed continued response to the drug with stable metabolic activity. However, ten months after the initiation of treatment with Nivolumab, her PET scan showed a new left upper lobe mass with increased fluoro-deoxyglucose (FDG) avidity. By this time, the patient had completed her eighteen cycles of Nivolumab.

Subsequently, the patient was hospitalized due to increased shortness of breath. It was initially attributed to congestive heart failure and atrial fibrillation. She was treated with diuretics with little alleviation of symptoms. Due to suboptimal response to the diuretic therapy, patient underwent CT of the chest to rule out other parenchymal pathologies which showed interval development of extensive bilateral peribronchovascular ground-glass opacities likely inflammatory in nature as compared to the CT scan before completion of treatment with Nivolumab (Fig. 1E). There was also associated traction bronchiectasis and left sided lung volume loss. At this time patient was considered to have the dreaded complication of interstitial pneumonitis, secondary to Nivolumab therapy and she was started on steroid therapy with intravenous methylprednisolone. She was discharged home on a tapering dose of oral prednisone. Due to her persistent hypoxic respiratory failure, patient became oxygen-dependent, requiring 2 L of oxygen via nasal cannula at all times. A follow up PET scan 3 months later showed further disease progression, mainly involving the left upper lobe, with increase in the size and activity of the tumor. Systemic therapy was started with a weekly Docetaxel as palliative treatment. She had four cycles of the treatment and has subsequently started developing diarrhea as a side effect of chemotherapy. She completed a prednisone steroid taper in July 2017 for a total of four weeks and her follow up CT chest showed remarkable improvement in the lung infiltrates (Fig. 1F).
Table 1

Occurrence of ILD/Pneumonitis in landmark studies of Immune check point Inhibitors in NSCLC.

| Study | Study description | Phase of study | Sample size | ILD/Pneumonitis cases, Any | Grade 3,4 Treatment related adverse events |
|-------|-------------------|----------------|-------------|--------------------------|------------------------------------------|
| Borghaei et al. | Checkmate 057 [8] Advanced NSCLC treated with Nivolumab | 3               | 19          | 4                        | 10%                                      |
| Brahmer et al. | Checkmate 017 [9] Advanced NSCLC treated with Nivolumab | 3               | 20%         | 9.2                     | 7%                                       |
| Rizvi et al. | Checkmate 063 [17] Advanced NSCLC treated with Nivolumab | 2               | 14.5%       | 8.2                     | 6% 4 17%                                |
| Garon et al. | Keynote 001 [2] Advanced NSCLC treated with pembrolizumab | 1               | 19.4%       | 12                      | 495 18 9 9.5%                           |
| Fehrenbacher et al. | [18] Advanced NSCLC treated with atezolizumab | 2               | 15          | 12.6                    | None 11%                                |
| Antonia et al. | [19] Advanced NSCLC treated with durvalumab and tremelimumab | 3               | NR          | 102                     | 5 36%                                   |

3. Case description 2

A 77-year-old male was treated for a metastatic esophageal adenocarcinoma with liver metastases. The patient was diagnosed in December 2015 when he presented with symptoms of progressive dysphagia to solids, odynophagia, unintentional weight loss of ten pounds in four weeks and generalized malaise to his primary care physician’s office. The patient underwent an esophagogastroduodenoscopy (EGD) and biopsies were obtained of the friable plaque like lesion at the distal esophagus. The histopathology was consistent with poorly differentiated adenocarcinoma with focal signet ring cell features. The patient then had a CT scan of the chest, and abdomen and PET scan done in December 2015, which showed increased metabolic activity in the thickened distal esophagus related to esophageal cancer (Fig. 2A). The CT scan of the chest done as a part of initial work up was unremarkable for any parenchymal lung disease (Fig. 2B). A 0.8 cm lesion in the segment 7 of the liver was also noted. To further characterize the liver lesion, the patient underwent a contrast CT scan of the abdomen which revealed three metastatic lesions in the liver. The patient’s disease was hence, deemed to be Stage IV and required palliative chemotherapy. Patient was started on systemic chemotherapy with Taxol and Carboplatin in February 2016 and received total of five cycles over the next four months. Restaging scans showed stable disease with no progression. The patient returned with complaints of dysphagia to solid foods 6 months after completion of initial chemotherapy. He underwent repeat PET/CT scan at the time which showed an enlarging hypermetabolic left gastric node and tracer uptake in the distal esophagus (Fig. 2C). A new 8 mm pulmonary nodule in the lingula was also noted. Given the progression of disease, the patient was started on a new immunotherapy drug called Ramucirumab (Crymaza©, Eli Lilly, USA). It is a fully human monoclonal antibody directed at the Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) given every other week for treatment of recurrent metastatic distal Gastro-esophageal junction cancers. The patient received 18 total cycles of Ramucirumab over the next 7 months. The patient was symptom free for a month thereafter, before he presented to his gastroenterologist once again with symptoms of dysphagia and underwent a repeat endoscopy which revealed a malignant stricture at the distal esophagus and a balloon dilatation was performed. The biopsy from the endoscopy revealed recurrent adenocarcinoma of the esophagus. His PET/CT scan done 6 months later revealed persistent activity with thickening of the distal esophagus and previously reported left gastric node was calcified and metabolically active once again (Fig. 2D). Given these findings, the patient was then counselled about Nivolumab (Opdivo© Bristol-Myers Squibb), as an alternative systemic immunotherapy. The patient received 2 cycles of Nivolumab over the next 2 months following which he presented to the hospital with increased shortness of breath and cough. The CT Chest revealed multifocal patchy, consolidative pneumonia along with architectural distortion and ground glass opacities suggesting acute interstitial pneumonitis (Fig. 2E and F). In view of this radiological finding, he was initiated on intravenous methylprednisolone 80mg every 8 hours and tapered to oral prednisone at the time of discharge from the hospital. Over the next few days patient had progressively worsening of his hypoxic respiratory failure requiring increasing amounts of oxygen. Due to grave prognosis, detailed discussion regarding goals of care was held. The patient and family decided to pursue palliation with hospice care. The patient passed away due to progressive respiratory failure over the next 2 weeks.

4. Discussion

The treatment of advanced NSCLC with PD-1 pathway inhibitors has been shown to be efficacious in cancers such as metastatic melanoma, non-small cell lung cancer, bladder cancer, head & neck tumors and lymphoma [15,16]. However, noteworthy are some of the side effects of these medications, which can be serious and even fatal. It is therefore of
utmost importance that clinicians are made aware of the characteristics of immun-related adverse events (irAEs) associated with these drugs [5].

The review of literature highlighting major landmark studies and immunotherapy associated interstitial lung disease and pneumonitis is highlighted in Table 1.

Review of literature with summary of recent reports on Anti-PD-1 induced pneumonitis/interstitial lung disease is shown in Table 2.

Thus far, most of the studies on immune-related adverse events have mentioned that Nivolumab has caused skin, gastrointestinal tract, endocrine, liver, lung and kidney adverse events [5,26]. In the skin, it has been shown to most commonly cause pruritus and rash while the most common endocrine disorder reported was hypothyroidism [5,26]. Diarrhea was the most frequent irAE in patients treated with Nivolumab and its incidence was between 10 and 13% [5]. The incidence of colitis and pancreatitis were rare.

Pneumonitis has been reported as one of the most common lung related immunological adverse events with phase 1 studies, meta-analyses, and case reports reporting radiological patterns of OP (organizing pneumonia), NSIP (non-specific interstitial pneumonia) and interstitial pneumonitis [4,27]. American Thoracic Society and European Respiratory Society have classified typical patterns of immunotherapy induced pneumonitis, elaborated in Table 3 [28–31].

One of the earliest Phase 1 studies to document the safety profiles, activity and immune adverse effects of anti-PD-1 antibody therapies in cancer was published by Topalian et al., in 2012. They underscored that drug-related grade 3-4 toxic effects such as pneumonitis were observed with radiological abnormalities showing progressive diffuse infiltrates and responded to cessation of treatment with initiation of glucocorticoids [27]. Pillai et al. in their systematic analysis comparing the toxicity profiles of PD-1 inhibitors showed a twofold rise in the rate of pneumonitis in patients treated with PD-1 inhibitors [32]. They reported a 4% complication rate of developing pneumonitis in 3284 patients who received PD-1 inhibitors and 9% developed diarrhea of any grade [32].

It is worth mentioning that there is strong data suggesting cumulative response rates and durability of responses have improved after treatment with immunotherapy in metastatic NSCLC [27]. There is also data suggesting that initiating glucocorticoid therapy and cessation of treatment immediately after recognition of PD-1 inhibitor-related pneumonitis, has affected survival, recovery and further retreatment with systemic chemotherapy in patients [33]. In the 2012 study by Topalian et al., the clinical response rate was 18% among patients with NSCLC and durability of response for 20 of 31 responses lasted 1 year or more in patients with 1 year or more of follow-up [27]. Most patients (17/20; 85%) with PD-1 inhibitor-related pneumonitis treated with corticosteroids for a median time of 6.1 weeks (oral prednisone taper) did very well [33]. A third of them were able to restart their Nivolumab therapy. Given these findings, cessation of treatment after recognizing the adverse events and initiating corticosteroid therapy is the cornerstone to positively affecting patient outcomes.

Nishino et al., in 2016 published a one of a kind study, characterizing the CT findings of 20 patients treated with a PD-1 inhibitor and the development pneumonitis in patients. Their study revealed that COP (cryptogenic organizing pneumonia) was the overall most common radiographic pattern of pneumonitis seen on Chest CT, followed by NSIP (non-specific interstitial pneumonia) and AIP/ARDS pattern in few patients [33]. In these patients, there was a clear predilection towards involvement of lower lung lobes like our patient and showed multifocal distribution, also seen in our patients (Figs. 1E and 2E/F). Furthermore, among specific CT findings, GGOs (ground-glass opacities), reticular opacities and consolidation were the most commonly noted, as seen in our patient 1 who had a development of extensive bilateral peribronchovascular ground-glass opacities (Fig. 1E) [33].

5. Conclusion

The importance of identifying immune related adverse events early is to diagnose and manage a clinical entity which has shown through studies to be highly susceptible to successful clinical and radiographic improvements with timely corticosteroid treatments. Significance of reporting such complications is pertinent since the medication that is used for the treatment of lung cancer can be a trigger for worsening shortness of breath and respiratory failure. It could be a challenging situation for the treating physicians to strike a balance with symptom management and achieve better survival benefit. Early recognition, post-marketing research and diligent clinical documentation is necessary to highlight the side effects and adverse events associated with these novel agents. As a novel mechanism to improve early detection and diagnosis of immune-related adverse events, we can turn to methods such as genomic sequencing and RNA sequencing as identifying predictive biomarkers for anti-PD-1 drug responses [26]. Finally,
treatment with such drugs could cause a destabilization of our immune system and making patients more prone to adverse immunological effects. The above mentioned approaches can help identify those patients who may be at higher risk of immune-related adverse events.

Authors’ contribution

Pdamastuti Akella: Study Design + Manuscript draft + Critical review of manuscript. 

Sundaravadiel Loganathan: Literature review + Data collection + Critical review of manuscript. 

Vishal Jindal: Literature review + Data Collection + Image selection + Critical review of manuscript. 

Jamal Akhtar: Literature review + Data collection + Critical review of manuscript. 

Amos Lal: Inception of idea + Critical review of manuscript + Image review and Selection.

Compliance with ethical standards

Conflict of statement

All authors claim no conflict of statement.

Source of funding

This manuscript and work involved required no external funding.

Ethics approval and informed consent

Not applicable.

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2017, Ca - Cancer J. Clin. 67 (1) (2017) 7–30.

[2] E.B. Garon, N.A. Rizvi, R. Hui, N. Leighl, A.S. Balmanoukian, J.P. Eder, et al., Pembrolizumab for the treatment of non-small-cell lung cancer, N. Engl. J. Med. 372 (21) (2015) 2018–2028.

[3] R.S. Herbst, P. Baas, D.W. Kim, E. Felip, J.L. Perez-Gracia, Y.J. Han, et al., Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial, Lancet 387 (10027) (2016) 1540–1550.

[4] V. Lenov, C. Templier, J.B. Faivre, A. Scherpereel, C. Foumier, L. Mortier, et al., Pembrolizumab-induced pneumonitis, ERJ Open. Res. 3 (2) (2017).

[5] P.F. Wang, Y. Chen, S.Y. Song, T.J. Wang, W.J. Ji, S.W. Li, et al., Immune-related adverse events associated with anti-PD-1/PD-L1 treatment for malignancies: a meta-analysis, Front. Pharmacol. 8 (2017) 730.

[6] M.E. Keir, M.J. Butte, G.J. Freeman, A.H. Sharpe, PD-1 and its ligands in tolerance and immunity, Annu. Rev. Immunol. 26 (2008) 677–704.

[7] S. Kitano, K. Tatsuno, J. Ishibe, T. Shimauchi, T. Fujiyama, T. Ito, et al., Nivolumab-induced organizing pneumonia in a melanoma patient, Jpn. J. Clin. Oncol. 46 (3) (2016) 270–272.

[8] K. Nakashima, T. Naito, S. Omori, S. Yoshikawa, M. Endo, Y. Kiyohara, et al., Organizing pneumonia induced by nivolumab in a patient with metastatic mela-noma, J. Thorac. Oncol. 11 (3) (2016) 432–433.

[9] V.H. Koelzer, S.I. Rothschild, D. Zihler, A. Wicki, B. Willi, N. Willi, et al., Systemic inflammation in a melanoma patient treated with immune checkpoint inhibitors—an autopsy study, J. Immunother Cancer 4 (2016) 13.

[10] S. Watanabe, H. Kimura, H. Takato, Y. Waseda, J. Hara, T. Sone, et al., Severe pneumonitis after nivolumab treatment in a patient with melanoma, Allergol. Int. 65 (4) (2016) 487–489.

[11] V. Gounant, S. Brosseau, C. Naltet, M.A. Opsomer, M. Antoine, C. Danel, et al., Nivolumab-induced organizing pneumonitis in a patient with lung sarcomatoid carcinoma, Lung Canc. 99 (2016) 162–165.

[12] P. Fragkou, M. Souli, M. Theohari, C. Koutsopoulou, S. Loukides, A. Koumarianou, A case of organizing pneumonia (OP) associated with Pembrolizumab, Drug Target Insights 10 (2016) 9–12.

[13] M. Nishino, L.M. Sholl, F.S. Hodi, H. Hatabu, N.H. Ramaiya, Anti-PD-1-Related pneumonitis during cancer immunotherapy, N. Engl. J. Med. 373 (3) (2015) 288–290.

[14] S.I. Topalian, F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D.F. McDermott, et al., Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, N. Engl. J. Med. 366 (26) (2012) 2443–2454.

[15] C.A. Powell, Pulmonary infiltrates in a patient with advanced melanoma, J. Clin. Oncol. 35 (7) (2017) 705–708.

[16] W.D. Travis, U. Costabel, D.M. Hansell, T.E. King Jr., D.A. Lynch, A.G. Nicholson, et al., An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias, Am. J. Respir. Crit. Care Med. 188 (6) (2013) 733–748.

[17] C.K. Toh, E.H. Wong, W.T. Lim, S.S. Leong, K.W. Fong, J. Wee, et al., The impact of smoking status on the behavior and survival outcome of patients with advanced non-small cell lung cancer: a retrospective analysis, Chest 126 (6) (2004) 1750–1756.

[18] D. Bours, K. Hatzakis, H. Labrakis, K. Zelbezoglou, Association of malignancy with diseases causing interstitial pulmonary changes, Chest 121 (4) (2002) 1278–1289.

[19] L.M. Goussens, Z. Werh, Inflammation and cancer, Nature 420 (6917) (2002) 863–867.

[20] X. Wang, Z. Bao, X. Zhang, F. Li, T. Lai, C. Cao, et al., Effectiveness and safety of PD-1/PD-L1 inhibitors in the treatment of solid tumors: a systematic review and meta-analysis, Oncotarget 8 (35) (2017) 59901–59914.

[21] H.O. Alsaah, S. Sau, R. Alzahrani, K. Tastipari, K. Bhise, S.K. Kashaw, et al., PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome, Front. Pharmacol. 8 (2017) 561.

[22] M. Nishino, L.M. Sholl, F.S. Hodi, H. Hatabu, N.H. Ramaiya, Anti-PD-1 Related pneumonitis in previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial, Lancet 387 (10030) (2016) 1837–1846.

[23] V.H. Koelzer, S.I. Rothschild, D. Zihler, A. Wicki, B. Willi, N. Willi, et al., Systemic inflammation in a melanoma patient treated with immune checkpoint inhibitors—an autopsy study, J. Immunother Cancer 4 (2016) 13.