

Real world treatment practice in patients with advanced melanoma in the nivolumab era: five novel Italian case reports and a literature review

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Abstract. – OBJECTIVE: The approval of the anti-PD1 antibody nivolumab has provided a significant therapeutic opportunity in the landscape of metastatic melanoma. In pivotal clinical trials, nivolumab improved clinical outcomes with a great safety profile. However, in real-world practice, the majority of the population with metastatic melanoma does not meet one or more eligibility criteria of pivotal trials, since they have an ECOG-PS ≥ 2 or active/untreated known brain metastases. Waiting for larger real-world studies that are currently lacking, but would be crucial to confirm the efficacy of nivolumab in challenging patients and to detect rare adverse events that could not be noticed in pivotal trials, this review collects both literature and unpublished case reports on nivolumab treatment in metastatic melanoma.

PATIENTS AND METHODS: Case reports, published from 2016 to February 2018, and five, unpublished case reports, representative of Italian clinical practice, were reported and potential issues that physicians could face with the use of nivolumab in the real world were discussed.

RESULTS: Among Italian cases, one patient had a huge retro-nuchal mass, which significantly decreased with few cycles of nivolumab; two patients were affected by cardiovascular comorbidities and one had brain metastasis; the last had a long history of disease, firstly diagnosed in 1997. A literature review was mainly focused on the experience in the management of rare immune adverse events related to treatment.

CONCLUSIONS: Nivolumab confirmed its efficacy and safety in real-world; the decision-making process on starting and scheduling the

treatment, even in the management of adverse events, should consider multiple factors related to both patient (i.e., BRAF status, ECOG PS, comorbidities) and disease (burden, metastasis).

Key Words:

Nivolumab, Metastatic melanoma, Case report, Anti-PD1, Real world, Immunotherapy, Brain metastasis, Clinical practice.

Introduction

The approval of immune checkpoint blocking antibodies has provided a significant therapeutic opportunity in the landscape of many cancers¹. These drugs – ipilimumab, a fully human IgG1 monoclonal antibody that targets CTLA4, nivolumab and pembrolizumab, humanized IgG4 monoclonal antibodies that target PD1 – can prevent the interaction between co-inhibitory molecules and their receptors, thereby boosting the body's natural defense against tumors^{2,3}. FDA has approved nivolumab for many indications, including advanced melanoma, advanced non-small cell lung cancer, advanced renal cell carcinoma, classical Hodgkin lymphoma, advanced squamous cell carcinoma of the head and neck, urothelial carcinoma, MSI-H or dMMR metastatic colorectal cancer, and hepatocellular carcinoma. The longest clinical experience on nivolumab

use has been achieved in the setting of metastatic melanoma that historically has been one of the first cancers treated with immunotherapy. Melanoma cells express co-inhibitory molecules within the tumor microenvironment to escape the immune system and hamper an effective tumor clearance^{4,5}. Therefore, it is the ideal disease to target with checkpoints inhibitors.

The current World Health Organization (WHO) estimates are that 132,000 melanomas occur each year around the world, resulting in 65,000 deaths annually⁶. Early diagnosis and resection cure almost 90% of cases of stage I melanoma⁷. By contrast, the prognosis for regional and distant metastatic melanoma (stages III and IV, respectively) is variable and generally poor, with 5-year survival rates for stage III of 13%-69% and as low as 6% in stage IV^{8,9}. An improvement of clinical outcomes in patients with metastatic melanoma has been reported with the introduction of checkpoint inhibitors, which seem are more effective and yet no less tolerable than control interventions¹⁰. A recent metanalysis indicates that targeting PD1 seems to offer greater efficacy than blocking CTLA4¹⁰. Indeed, in clinical trials, nivolumab showed a significant improvement in clinical outcomes, as compared with dacarbazine and ipilimumab¹¹⁻¹⁴. The safety profile of this drug was favorable, with pruritus, fatigue, diarrhea, and nausea as the most common adverse events¹¹⁻¹⁴.

However, in pivotal trials, patients were highly selected and only patients with ECOG-PS ≤ 1 , without active brain metastasis, ocular melanoma or autoimmune disease were included¹¹⁻¹³. In real-world practice, 55% of the total population with metastatic melanoma did not meet one or more eligibility criteria at first evaluation, and an ECOG-PS ≥ 2 or active/untreated known brain metastases accounted alone for 74% of non-eligibility cases¹⁵.

Furthermore, infrequent and rare adverse events could be more accurately described in a wider population than those included in pivotal trials, as in post-marketing surveillance and real-world study.

As per our knowledge, no data are currently available about real-world practice with nivolumab in metastatic melanoma. Therefore, in this minireview, we collected clinical case reports published from 2016 to February 2018 and 5 novel cases from Italian real-world experience, to better define the characteristics of patients who may have major benefits from nivolumab therapy.

Italian patients provided a written consent. Ethical requirements were fulfilled, accordingly to “Decreto Legge 196”, article 4 (2003) and all clinical cases were conducted according to current good clinical practices and local laws.

Case 1

In 2015, a 72-year-old woman was diagnosed of melanoma. The lesion was vascularized, dermo-hypodermically located at the retro-nuchal level, and *wild-type* for BRAF mutation. Baseline Fdg-PET/CT scan showed increased uptake in the retro-nuchal mass as well as in the omolateral nuchal and cervical lymph nodes, left upper pulmonary lobe, left pulmonary hilar and mediastinal lymph nodes. From May 2016 to June 2016, the patient received a first-line chemotherapy with carbo-platin plus paclitaxel for six courses, without obtaining any clinical response, whereas the burden of the lesion was progressively increasing (Figure 1A). In August 2016, when a second-line treatment with nivolumab (3 mg/kg, every two weeks) was started, the CT-scan described a 90 × 75 × 60 mm right nuchal mass infiltrating the adjacent muscles, a 60 × 60 × 40 mm left pulmonary lesion at the hilum without apparent cleavage plane with the pulmonary artery, and pathological lateral cervical and mediastinal lymph nodes.

LDH level was $\geq 5 \times$ Upper Limit Normal. After two doses of nivolumab size reduction of the main lesion was visible, becoming more evident in the next weeks; (Figure 1B) and lactate dehydrogenase (LDH) level returned within the normal range after the 5th cycle of therapy. The CT scan performed after 11 cycles of nivolumab, January 2017, showed a markedly reduction in size of the retro-nuchal lesion (45 × 30 mm) and the left pulmonary lesion (20 mm), and the cervical and mediastinal lymph-nodes returned within < 1 cm in short axis. Nivolumab was continued for further six months, when the patient reported pruritus (grade 2) at arms and trunk, with desquamation areas. Cetirizine treatment (10 mg twice daily) and emollient cream were prescribed and nivolumab was temporarily discontinued. The last radiological assessment (October 2017) confirmed the excellent clinical response, and the main lesion is no more visible (Figure 1C). Treatment is still ongoing without significant toxicities

Case 2

In 2016, an 80-years-old man with severe cardiac comorbidities was diagnosed of metastatic melanoma. Previously, in 2008, he underwent

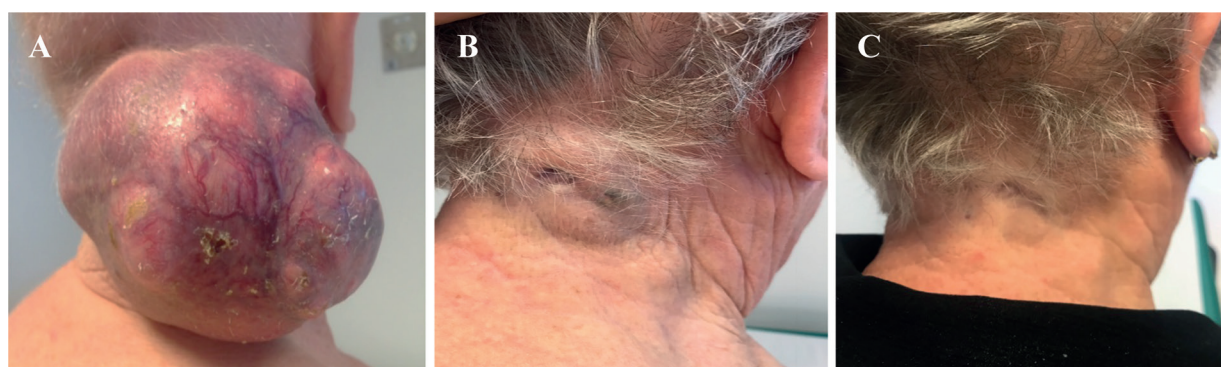


Figure 1. Main lesion size decreasing during nivolumab treatment. (A) Before treatment; (B) after two doses of nivolumab; (C) after few months of nivolumab.

anterior resection with colorectal anastomosis for rectal carcinoma, treated with neoadjuvant chemo-radiation; in 2015, he underwent coronary artery bypass graft surgery for hypertension and received an implantable cardioverter defibrillator. In addition, he took ASA, warfarin, digoxin, furosemide, atorvastatin, amiodarone, and carvedilol.

In January 2016, the CT scan showed a nodule 2.1×2.0 cm in the apical segment of the right lower lobe and 1.0 cm right hilar lymph node; the PET-CT analysis highlighted an uptake in the pulmonary nodule, but not in the hilar lymph node. Hyperactivity was also observed at the dermal-subcutaneous thickening in the left scapular region and in the right mammary region. In March 2016, the presence of multiple subcutaneous nodules in the shoulder and right sub-mammary was confirmed by soft tissue ultrasound analysis and the histopathological report referred as melanoma metastasis, with a V600K mutation in B-Raf gene. Considering the existing cardiovascular condition, a thorough cardiovascular evaluation and echocardiography were performed: the patient presented a dilated hypokinetic cardiomyopathy, mitral failure, ejection fraction at 38%, and congestive heart class III NIHA. In April 2016, in addition to previously observed pulmonary lesions (Figure 2A), the CT scan showed a further solid lesion (1.8×1.5 cm) in the pararenal adipose tissue and in the objective examination three subcutaneous nodules in the left axilla, left scapula and perineal area were detected. In May 2016, the patient started a therapy with nivolumab (3 mg/kg, every two weeks). At the first tumor assessment, CT scan showed complete regression of pulmonary metastasis (Figure 2B), significant regression of the posteri-

or pararenal metastatic localization, and complete regression of subcutaneous metastatic melanoma. The patient continued nivolumab, with excellent tolerance and a good clinical condition (ECOG PS=0). The cardiac evaluation confirmed that the pre-existing dilated hypokinetic cardiomyopathy and the severely reduced contractile function (EF 36%) were unchanged. No drug-related adverse events were noted. In August 2017, at the last tumour assessment, CT scan confirmed the complete regression of pulmonary, pararenal, and subcutaneous metastases. After the 14th cycle of nivolumab, asymptomatic hypothyroidism was reported (TSH level < 0.1 mU/ml, FT4 and FT3 in the normal range) and none therapy was prescribed. After the 18th cycle, the TSH value returned within the normal range (0.38 mU/ml, range 0.27-4.20). Currently, the patient's clinical status is excellent; he is still receiving therapy with nivolumab in complete remission, with a good tolerance after 33 cycles.

Case 3

In November 2011, a 72-year old man affected by hypertension and asymptomatic ischemic disease underwent surgery to excise a cutaneous pigmented lesion in the xiphoid region. The histopathological report referred as epithelioid nodular melanoma, not ulcerated (Breslow 2.35 mm; pT3a). The sentinel lymph node biopsy in the right axilla was positive for multiple sub-capsular metastases, which occupy 30% of parenchyma. However, the histological report after axillary dissection did not refer any lymph node as positive. In January 2015, several nodules of malignant melanoma in transit, with melanomatous cells in reticular derma and in the vascular-adipose tissue were detected. The histological examination indicated the presence

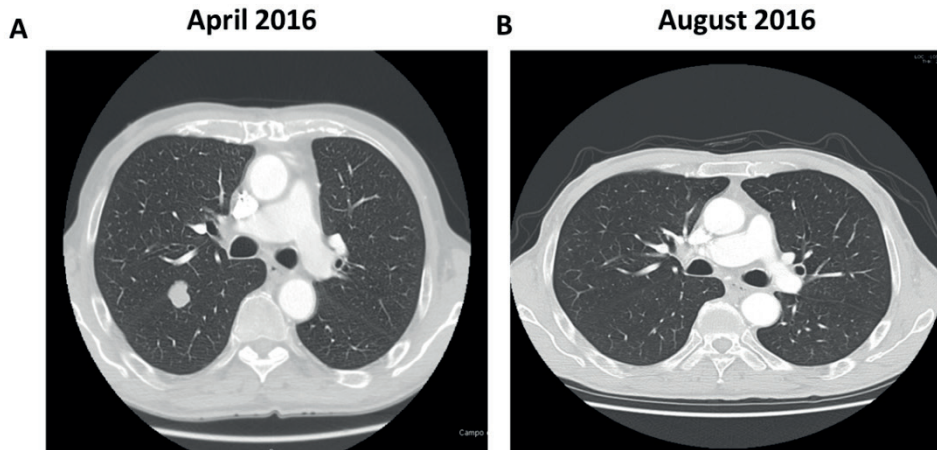


Figure 2. CT scan before nivolumab (*left panel*); CT scan, at the first tumour assessment, showed complete regression of the pulmonary metastatic disease (*right panel*).

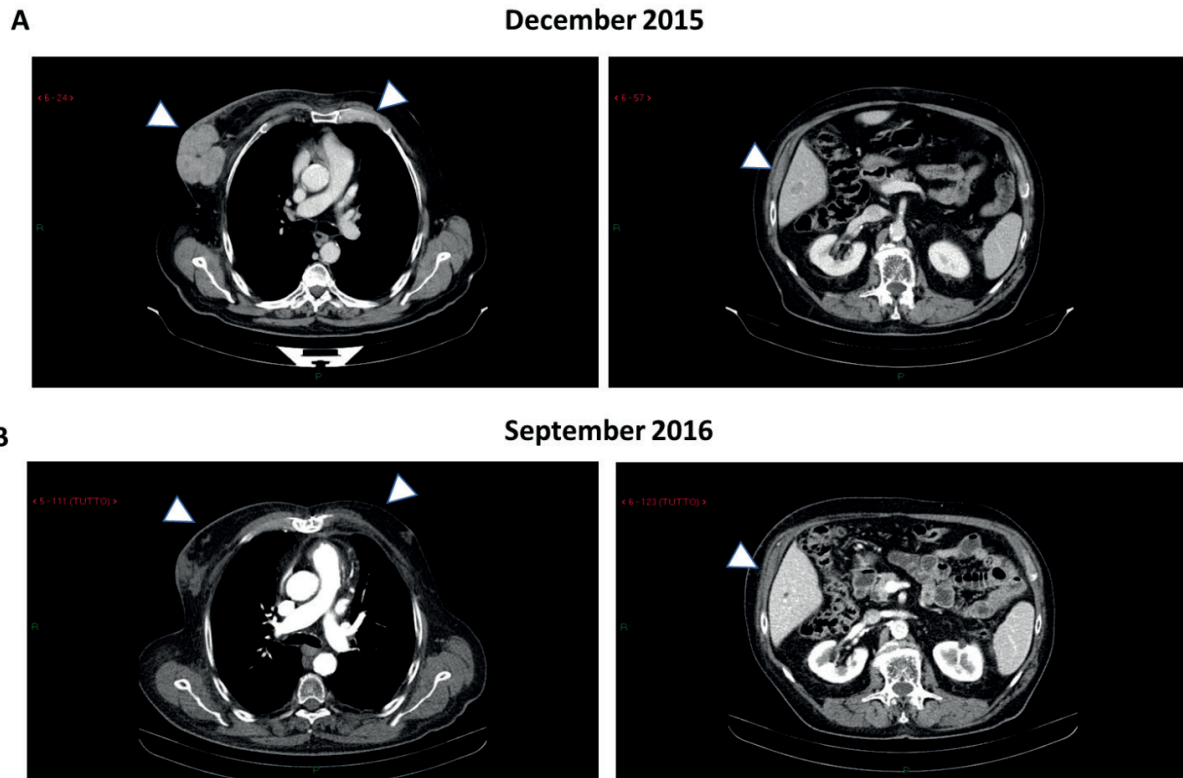


Figure 3. Progression o in mammary and hepatic lesions and a novel pre-pectoral lesion, at the third tumor assessment, during ipilimumab treatment (*upper panel*). Partial response in mammary and hepatic metastasis and the pre-pectoral lesion (*lower panel*).

of BRAF mutation V600E, while NRAS was *wild-type*; LDH level was within the normal range (531 U/L, range 313-618U/L). In March 2015, a PET-CT scan showed high metabolic activity at right mammary and left axillary region. Therefore, a treatment with ipilimumab (3 mg/kg q21 for 4 infusions) was started. The disease was

stable in the first two assessments, but at the third evaluation, a progression was reported: the right mammary lesion (from 5.3 cm to 7.8 cm) and the hepatic lesion (2.3 cm) in the VI segment increased their volumes, and a novel lesion was observed at pre-pectoral level (2.0 cm) (Figure 3A). LDH levels consistently increased (Figure 4).

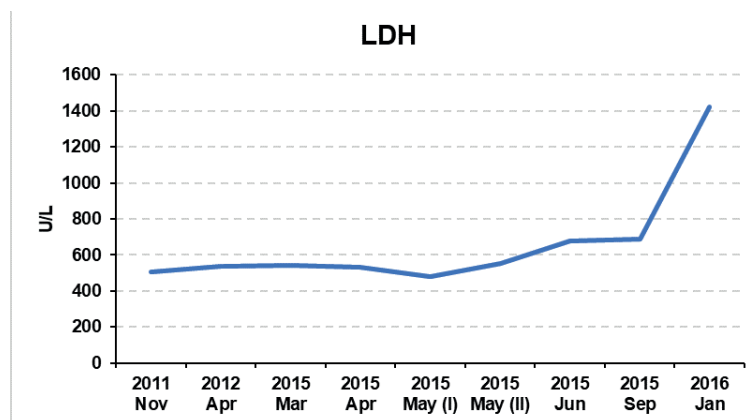


Figure 4. LDH level increased during the time.

In January 2016, the patient started a therapy with vemurafenib, a BRAF inhibitor (960 mg, twice daily). Considering the cardiovascular comorbidities, a thorough cardiological evaluation was performed: the sinus rhythm was normal (QTc interval 414 ms), with ejection fraction (EF) =58%. In March 2016, the patient experienced an acute coronary syndrome with STEMI, complicated by atrial fibrillation (cardiac toxicity of grade 3), which was treated with myocardial re-vascularization. After one month, the overall systolic function was good, with EF=59%; the sinus rhythm showed an abnormal ST interval and QTc=427 ms. In April 2016, vemurafenib was continued at lower dosing (480 mg, twice daily). In June 2016, when the QTc was 551 ms, and the EF=45%, vemurafenib was stopped for cardiac toxicity. In July 2016, the patient started a treatment with nivolumab (3 mg/kg, every two weeks). In September 2016, at the first radiological evaluation, a partial response was reported: the mammary lesion (from 7.8 cm to 4.5 cm) and the hepatic lesion (2.3 cm to 0.9 cm) were decreased and the lesion at pre-pectoral level was undetectable (Figure 3B). In the following radiological evaluations, the disease was stable. The treatment with nivolumab is currently ongoing after 15 months, with a stable disease and without any toxicity.

Case 4

In 2011, a 64-years old man underwent surgery to remove a cutaneous neoplasia from the left shoulder. The histopathological report referred as a superficial spreading melanoma, with vertical growth, and ulcerated (Clark IV, Breslow 3,59 mm, pT3b Nx). The margin status and senti-

nel lymph node were examined: the biopsy was positive for the presence of micrometastases of melanoma in the left axilla. Therefore, the left axillary lymph node dissection was performed: one lymph node out of 14 was positive, with a V600E mutation in the BRAF gene. The patient refused an adjuvant treatment with interferon and started the follow-up. In 2012, a CT scan revealed hepatic and splenic disease progression. From September 2012 to February 2013, the patient received vemurafenib, obtaining a complete response. However, in March 2013, disease progression at hepatic and splenic level was detected again. The patient started a treatment with ipilimumab for 4 cycles, obtaining a stable disease. In August 2013, CT scan detected one metastatic encephalic lesion, which was excised. The follow-up was continued, and, in May 2016, a progression of the encephalic lesion was observed and treated with gamma-knife radiotherapy. MRI and total body CT scan showed an ependymal nodule in the right caudate nucleus, identified as metastatic lesion, progression of splenic and adrenal lesions, and stable disease in the liver. In October 2016, the patient started nivolumab treatment (3 mg/kg, every two weeks) and all lesions, especially encephalic, adrenal and splenic, were progressively reduced. The treatment with nivolumab is currently ongoing after 25 cycles, the patient's clinical status is good, and he maintains a clinical response.

Case 5

In 1997, 39 years-old male patient affected by hypercholesterolemia and type 2 diabetes mellitus underwent the excision of dorsal melanoma, followed by radicalization. The histopathological

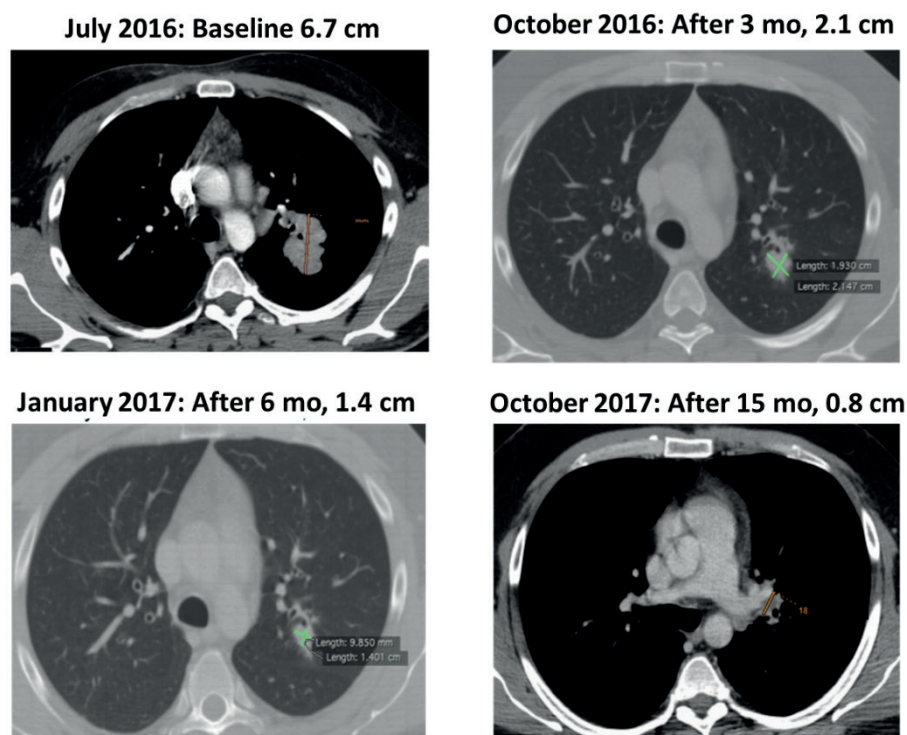


Figure 5. Tumor size progressive reduction during nivolumab treatment.

report referred as melanoma with a Breslow of 0.87 mm with peritumoral signs of regression (Stage I). The patient underwent clinical follow-up until June 2016, when he was admitted to the emergency room due to ataxia and headache. In the local hospital, the patient performed a cerebral MRI, which showed two lesions in the cerebellar hemisphere zone, with signs of recent bleeding and edema with mass effect. Immediately, the patient underwent surgery to remove these lesions completely, which resulted as melanoma metastasis. A dermatological and an ophthalmological examination, a gastroscopy, and colonoscopy were performed to exclude the presence of *de novo* melanoma. In July 2016, total body CT scan revealed only the presence of one metastatic lesion (6.7 cm) in left lung near to the ileus where it contacts the peri-bronchus structure. Although the patient had a unique metastasis in the lung and a good clinical condition, with an ECOG-PS=0, surgical resection was not feasible due to the position of lesion that would have made the procedure as difficult and would have drastically reduced the patient's quality of life. Therefore, the only therapeutic option was a systemic treatment, considering that BRAF was mutated (V600E) and the LDH level was normal. In July

2016, the patient started nivolumab treatment (3 mg/kg, every two weeks). After six cycles, the total body CT scan revealed the reduction of lung lesion (2.1 cm), considered as an unconfirmed partial response according to iRECIST. After three months of treatment, the patient performed a new radiological assessment that confirmed the partial response, according to iRECIST. The therapy with nivolumab is currently ongoing, and the patient performs a total body CT scan every three months as per guidelines in his setting. The last radiological assessment completed in October 2017 confirmed the partial response of lung lesion (0.8 cm) (Figure 5). During the treatment, the only adverse event was diarrhea of grade 1.

Literature Review

Case reports from literature are summarized in Table I. In these reports, safety issues of nivolumab treatment have been reported more frequently than efficacy; however, when present, clinical outcomes are usually favourable¹⁶⁻²⁴. Waiting for wide real-world studies, these case reports describe immune-related adverse events that have not been recorded in pivotal trials (i.e., intestinal perforation) for the limited number of patients enrolled that was not powered to detect so rare adverse events. Liver

immune-related injury was observed in a patient with malignant melanoma with multiple, after the first cycle of nivolumab¹⁶. He was initially treated with interferon and, then, with nivolumab. Clinical response was relevant at the metastasis site, but grade 4 aminotransferase elevation was observed. Liver histology revealed drug-induced injury that was treated with steroid half-pulse therapy followed by oral methylprednisolone. However, even after five months ALT level did not completely recover to the normal range. Nivolumab was discontinued due to persistent hepatitis, but the patient showed remission of his metastatic lung lesion for further five months¹⁶. Seronegative rheumatoid arthritis was reported in another patient with melanoma and metastatic lesions to multiple organs. After failure of vemurafenib and ipilimumab, the patient was treated with nivolumab for 20 months, with marked improvement. During treatment, the occurrence of polyarthritis and synovitis compromised her quality of life; she gained benefit and symptom control only from hydroxychloroquine 300 mg daily¹⁷. Cutaneous immune-related reactions were described in two case reports of bullous pemphigoid-like lesions^{18,19}. Bullous pemphigoid is the most common blistering skin disorder; it normally presents with an initial non-bullous phase of pruritus, followed by development of generalized or localized tense blisters filled with serous or haemorrhagic fluid. In both cases, a treatment with corticoids was resolute^{18,19}. A case of bilateral uveitis was reported after the third infusion of nivolumab in a patient with metastatic melanoma, affecting the lymph nodes and duodenum and harbouring a BRAF V600E mutation²⁰. The patient complained of sudden bilateral visual acuity impairment, confirmed by the ophthalmologic evaluation. Nivolumab was stopped, and a local treatment with a topic corticosteroid eye drops (sodium phosphate dexamethasone 0.1%) was not enough to reduce the visual acuity decline, that was controlled only with oral corticosteroid treatment (1 mg/kg, prednisone); a complete recovery was obtained after one month of systemic treatment. Treatment with nivolumab was re-initiated and corticosteroids were gradually decreased, without any further relapse of bilateral uveitis; corticosteroids were not completely stopped on nivolumab²⁰. An immunologic reaction may have a potential to influence intestinal perforation, but the mechanism of gastrointestinal perforation due to nivolumab is not understood. A patient with malignant melanoma in the anal canal and multiple metastases reported abdominal distension and

progressive diffuse abdominal pain after the third treatment with nivolumab: he had an intestinal perforation, requiring a surgical intervention. After surgery and medical treatment for sepsis, the patient completely recovered²¹. Nivolumab was active on small intestine metastases, without inducing any side effect²². This patient, after 8 cycles of nivolumab, showed depigmentation on the melanoma macula, likely due to a reduction in epidermal melanocytes, following the successful treatment with immunotherapy²². Among endocrinology related-dysfunction, hypophysitis and thyroid impairment are frequent. Okano et al²³ described a case of hypophysitis where a patient initially developed progressive fatigue and appetite loss, after sixth administration of nivolumab; laboratory data indicated eosinophilia and hyponatremia, and ACTH and cortisol levels were low. The patient was treated with hydrocortisone (20 mg/d), and the 7th administration of nivolumab was completed without exacerbating patient's general condition. A case of sarcoid-like granulomatous reaction induced by nivolumab was reported in 2016²⁴. After 10 months, the patient achieved a melanoma complete response, but he developed sarcoid-like granulomatous reaction in the mediastinal lymph node and skin, which resumed after nivolumab arrest; melanoma did not relapse after 12 months of follow-up.

Discussion

Italian case reports confirm efficacy and safety of nivolumab in patients who do not meet the inclusion criteria of a clinical trial, i.e., for brain metastasis, cardiovascular comorbidities, or elderly. In many cases, nivolumab has been successfully used as second-line therapy, after treatment with carbo-platin and taxanes, ipilimumab, and BRAF inhibitors, confirming that the objective response is not affected by prior BRAF inhibitor therapy or prior ipilimumab therapy²⁵. The efficacy and safety of nivolumab is even independent of the mutational status of BRAF, which is, conversely, determinant in the choice of targeted therapy with RAF and MEK inhibitors²⁵. According to international guidelines^{26,27}, patients with mutated BRAF can be treated with immunotherapy or targeted combined therapy. In long-term analysis, both therapeutic options have demonstrated a durable survival: the 3-year OS rate was 44% with BRAF and MEK inhibitors²⁸, 58% with combined nivolumab and ipilimumab.

Table I. Literature review of case reports from 2016 to February 2018.

	Patients	Efficacy	Safety
Matsubara 2018 ¹⁶	42-year-old man with c stage III malignant melanoma with multiple lung metastases	Significant decrease in the size of metastases	ALT elevation grade 4 and immune-related liver injury treated with steroid half-pulse therapy followed by oral methylprednisone
Haikal 2018 ¹⁷	65-year-old Caucasian female with Stage IV melanoma (BRAF-positive)	Marked improvement in metastasis in multiple organs	Symmetrical polyarthritis with synovitis and swelling of both Metacarpophalangeal Joints (MCPs) and (PIPs) Proximal Interphalangeal Joints bilaterally. Treatment with hydroxychloroquine with remarkable improvement
Anastasopoulou 2018 ¹⁸	48-year-old patient with melanoma	Not reported	Bullous pemphigoid-like skin lesions along with fever, arthralgia and overt eosinophilia. Treatment with corticosteroids
Naidoo 2016 ¹⁹	80-year-old male with metastatic melanoma	Complete remission	Bullous pemphigoid, treated with systemic corticosteroids
Theillac 2018 ²⁰	55-year-old man with metastatic melanoma, affecting the lymph nodes and duodenum. BRAF V600E.	Not reported	Bilateral granulomatous uveitis and unilateral Posterior retinal serous detachment after the third infusion. Treatment with both local and systemic corticosteroids
Yasuda 2017 ²¹	73-year-old man with melanoma in the anal canal with multiple metastases in the lungs, liver, and bones, thyroid gland, and subcutaneous tissue.	Not reported	Intestinal perforation, successfully resolved after surgical treatment
Yamamura 2017 ²²	66-year-old woman with metastatic melanoma, with small intestine lesions	After two cycles, reduction the lesion in the small intestine; after 8 cycles, dermoscopic changes	Not reported
Okano 2016 ²³	50-year-old man with metastatic melanoma on the right side of the lingual root, with cervical and mediastinal metastases.	Effective for the primary and mediastinal lesions	Hypophysitis at the 7th cycle, treated with hydrocortisone
Danlos 2016 ²⁴	57-year-old man with desmoplastic melanoma stage III	At 10 months durable complete response	Sarcoid-like granulomatous reaction in the mediastinal lymph node and skin

ab and 52% with nivolumab alone¹³. However, targeted therapies induced rapid responses in the majority of BRAF-mutant patients, but 50% of these responders developed resistance within approximately 13 months. In contrast, immunotherapies, particularly inhibitors of PD-1, induced responses, which tended to be durable, in 40-55% of patients²⁸.

Subgroup analyses of large clinical trials would help to identify which patient-centered factors are valuable in choice of first-line therapy: for instance, patients with low tumor burden could benefit of immunotherapies that can

continue for long time, with very long-lasting response and without side effects that compromise the quality of life or exacerbate existing comorbidities. In this series, two patients had pre-existing cardiovascular comorbidities and one of them had showed QT prolongation during treatment with vemurafenib. QT prolongation syndrome has been associated with the use of BRAF inhibitors in clinical studies, albeit with low frequency, and it should be early noticed and promptly managed²⁹. Cardiotoxicity with immunotherapy is rare, but multiple manifestations of immune-related cardiac syndromes have

been observed; a case series based on the experience of several institutions in the United States and Germany documented cases of autoimmune myocarditis, cardiomyopathy, heart failure, cardiac fibrosis and cardiac arrest. Therefore, the deterioration of heart function should be closely monitored, especially in patients with preexisting cardiac conditions^{30,31}.

There are few evidence about the efficacy of nivolumab in patients with active brain metastasis. In this cases series, one patient with brain metastasis gained clinical benefits from nivolumab therapy, despite the previous progression after excision and gamma-knife radiosurgery (GKRS). Nordmann et al³² analyzed the experience of their institution on concurrent treatment with PD-1 inhibitors and GKRS to enhance the treatment of metastatic melanoma: the combination showed some radiologic benefit in 13 out of 25 patients, and 2 radiologic pseudo-progressions, thus indicating that checkpoint inhibition may result in an accelerated response to GKRS. From a molecular point of view, the immune microenvironment in brain metastases may be an ideal target for immunotherapy, since it is active with a high density of tumor-infiltrating lymphocytes³³. A recent retrospective analysis³⁴ suggested that anti-PD-1 antibodies (both nivolumab and pembrolizumab) obtained an intracranial overall response in 21% of patients and the disease control in 56%, with a median overall survival of 9.9 months (95% CI 6.93-17.74).

Nivolumab treatment resulted as generally well tolerated, with the most frequent treatment-related adverse events as dermatologic, gastrointestinal, endocrine, hepatic, renal, and pulmonary toxicities³⁵. Most events have a low grade and are successfully managed with supportive care, as per well-established safety guidelines; grade 3 to 4 adverse events are normally resolved with dose delay or permanent discontinuation, with or without administration of systemic corticosteroids or other suppressive immune-modulating agents³⁵. Treatment-related adverse events leading to discontinuation were reported in 3% of patients (17 out 576) in pivotal trials, with the most common being colitis, increased alanine aminotransferase, increased lipase, and pneumonitis (two patients [0.3%] each); none instance of gastrointestinal perforation was reported³⁵.

A case of intestinal perforation after nivolumab treatment has been described in real world practice²², thus confirming the importance of post-marketing monitoring to identify rare ad-

verse events. The similar autoimmune response was associated with colitis that is more frequently observed and may lead to intestinal perforation. In phase I study, one serious adverse event of inflammatory colitis was observed in a patient³⁶.

Other immune reactions may interest numerous organs, including liver, skin, joints, endocrine system, as reported in case reports from literature¹⁶⁻²⁴. Physicians should be aware of potential immune-related risks and should promptly diagnose and treat these conditions. Immune-related toxicities are rare, but often challenging to be managed. Monitoring for these adverse reactions is advisable to early diagnose and treat them and to avoid delay in nivolumab treatment that may compromise the clinical outcomes on metastatic melanoma.

Unfortunately, not all patients experience a favorable response to anti-PD1 treatment and a better selection of patients is mandatory. Several biomarkers have been investigated, but no consensus has been reached yet. The high PD-L1 expression on melanoma was found predominantly in regions of abundant inflammation or TIL (tumor infiltrating lymphocytes) infiltrates, even in sanctuaries like brain metastases^{37,38}, but it failed to predict responses to nivolumab in metastatic melanoma¹². Recently, a functional method called “the ex vivo metastatic Lymph Node assay”, capable of assessing the reactivity of infiltrating immune effectors (T and NK cells) during stimulation with various immune checkpoint blockers and their combinations, was coupled with a paired blood and tumor immune profiling to correlate immune fingerprints with clinical parameters³⁸. Preliminary results indicated that PD-L1 expression on circulating T cells was relevant in the prediction of resistance to ipilimumab, alone or combination with IL-2 or GM-CSF and that detectable levels of CD137 on circulating CD8+ T cells tended to predict longer PFS for the anti-CTLA-4 + anti-PD-1 co-blockade³⁸. On the other hand, it will be very important to deeply investigate the mechanisms underlying the antitumor effect of nivolumab, from the *in vitro* to the clinical relevance, as reported in recent publication³⁹.

Conclusions

These clinical cases demonstrate how nivolumab has changed the natural history of metastatic melanoma, leading it to become a disease man-

ageable for a long time. In the therapeutic decision on metastatic melanoma, not only the BRAF status, but also disease characteristics, tumor burden, number of metastatic sites, LDH levels, and the performance status of the patient should be considered. Adverse events are often manageable and can be resolved within few weeks with delay or suspension of the therapy. Attention should be paid for rare immune-related toxicities that may be more challenging to both diagnose properly and treat efficiently.

Funding

Bristol-Myers Squibb S.r.l. funded editorial support, provided by Content Ed Net, with the helpful contribution in drafting the test by Elisa Sala, Ph.D, Medical Writer.

Acknowledgements

Authors wish to thank Dr Pasquale Vitale for his precious contribution.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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