Universiteit Antwerpen

This item is the archived peer-reviewed author-version of:

Nivolumab in melanoma

Reference:

Specenier Pol.- Nivolumab in melanoma
Expert review of anticancer therapy - ISSN 1473-7140 - Abingdon, Taylor & francis ltd, 16:12(2016), p. 1247-1261
Full text (Publisher's DOI): http://dx.doi.org/doi:10.1080/14737140.2016.1249856
To cite this reference: http://hdl.handle.net/10067/1402470151162165141

uantwerpen.be

Institutional repository IRUA

Taylor & Francis
Taylor & Francis Group

Expert Review of Anticancer Therapy

ISSN: 1473-7140 (Print) 1744-8328 (Online) Journal homepage: http://www.tandfonline.com/loi/iery20

Nivolumab in melanoma

Pol Specenier

To cite this article: Pol Specenier (2016): Nivolumab in melanoma, Expert Review of Anticancer Therapy, DOI: 10.1080/14737140.2016.1249856

To link to this article: http://dx.doi.org/10.1080/14737140.2016.1249856

Accepted author version posted online: 25 Oct 2016.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=iery20

Download by: [Cornell University Library] **Date:** 26 October 2016, At: 15:25

Publisher: Taylor & Francis

Journal: *Expert Review of Anticancer Therapy*

DOI: 10.1080/14737140.2016.1249856

Review

Nivolumab in melanoma

Pol Specenier
Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium
Department of Medical Oncology, Antwerp University Hospital, Wilrijkstraat 10, 2650 Edegem, Belgium
32 3 821401
pol.specenier@uza.be

Abstract

Introduction: The treatment of melanoma is evolving rapidly over the past few years.

Areas covered: We conducted a comprehensive review of the literature on the role of nivolumab in melanoma

Expert commentary: Nivolumab is approved by FDA and EMA for the treatment of patients with metastatic melanoma. Nivolumab is superior to chemotherapy and to ipilimumab in previously untreated patients and to chemotherapy in ipilimumab pre-treated patients. The addition ipilimumab to nivolumab is associated with a higher response rate and a better PFS, particularly in patients with PD-L1 negative tumors, albeit at the cost of an increase in grade 3-4 adverse event rate. Definitive survival data on this combination are pending and the selection of patients most likely to benefit from this combination and its pharmacoeconomics are to be elucidated. Prospectively validated predictive markers are lacking. Of particular interest are immune-related adverse events which should be managed according to published guidelines.

ACCEPTED MANUSCRIPT

Keywords

Ipilimumab, anti-PD1, melanoma, advanced, metastatic, unresectable, adjuvant, nivolumab, adverse events.

1. Introduction

The incidence of melanoma is increasing (1-4). About 200.000 new cases of cutaneous melanoma are diagnosed worldwide each year (1-4). The outcome depends on the stage at diagnosis (5-8). Until recently, overall, long term survival in patients with stage IV melanoma was lower than 10 %, although in some patients the disease may have a more indolent and protracted course (5-9). Objective response rates with standard chemotherapy were consistently lower than 20 % and no proven survival benefit was demonstrated in randomized phase III trials. Long term remissions were observed in highly selected patients with a toxic regimen of high-dose interleukin-2 (5;8;9). Ipilimumab is a monoclonal antibody (mAB) directed against the Cytotoxic T Lymphocyte Antigen-4 (CTLA-4) receptor which induces durable responses and long term survival in about 20 % of the patients (10). The checkpoint inhibitors nivolumab and pembrolizumab are even more active than ipilimumab (11-13). We reviewed the role of nivolumab (MDX-1106, BMS-936558/ONO-4538,Opdivo®, Ono Pharmaceutical Co., Ltd, Osaka, Japan, Bristol-Meyers Squibb [BMS], New York, USA).

Nivolumab is a high-affinity, fully human, Programmed Cell Death Ligand 1 (PD-L1)-specific, immunoglobulin G4 (IgG4) (S228P) mAB that inhibits the binding of PD-L1 to both Programmed Cell Death-1 (PD-1) and CD80 (14;15). Programmed death 1 protein is T-cell coinhibitory receptor which has two known ligands, PD-L1 (B7-H1) and Programmed Cell Death Ligand 2 (PD-L2)(B7-DC) (14;16-22). PD-L1 is selectively expressed on many tumors and on cells within the tumor microenvironment in response to inflammatory stimuli (14;23-26). Blockade of the interaction between PD-1 and PD-L1 potentiates immune responses in vitro and mediates preclinical antitumor activity (14;23;25;27). PD-L1 is the primary PD-1 ligand that is up-regulated in solid tumors, where it can inhibit cytokine production and the cytolytic activity of PD-1+, tumor-infiltrating CD4+ and CD8+ T cells (14;23;28-30). Nivolumab consists of four polypeptide chains: two identical heavy chains of 440 amino acids and two identical kappa light chains of 214 amino acids, which are linked through inter-chain disulfide bonds (31). Nivolumab is produced from cell culture using a Chinese hamster ovary (CHO) cell line that was transfected with an expression vector containing coding sequences for the heavy and light

ACCEPTED MANUSCRIPT

chains of the nivolumab IgG (14;31). Mice transgenic for human Ig loci were immunized with Chinese hamster ovary cell PD-1 transfectants and a PD-1/human IgG1 Fc fusion protein (15;32).

2. Phase I and II trials

2.1 Single agent nivolumab

Thirty-nine patients with advanced metastatic melanoma, colorectal cancer (CRC), castrate-resistant prostate cancer, non–small-cell lung cancer (NSCLC), or renal cell carcinoma (RCC) received a single intravenous infusion of nivolumab in dose-escalating six-patient cohorts as a 60-minute intravenous infusion at 0.3, 1, 3, or 10 mg/kg, followed by a 15-patient expansion cohort at 10 mg/kg (15). Patients with stable disease or evidence of lesional tumor regression at 8 and 12 weeks, no adverse events $\geq$ grade 3, and no evidence of human antihuman antibody at a 1:10 serum dilution received additional doses of nivolumab at weeks 12 and 16 and were then observed for 3 months and restaged. Those with continued clinical benefit could receive two more doses, spaced by 4 weeks. Each re-treatment phase was 16 weeks. Patients with objective responses were observed, with optional re-treatment on progression. Nivolumab was well tolerated, no dose limiting toxicities (DLTs) were observed after one dose, and a maximum tolerated dose (MTD) was not defined in this study (15). To further assess the safety, side-effect profile, and clinical-activity profile disease-specific cohorts were enrolled. In this expansion cohorts, nivolumab was administered on days 1, 15, and 29 of each 6-week cycle (14). Patients continued treatment for up to 16 cycles unless they had unacceptable toxic effects, disease progression, or withdrew consent. In clinically stable patients, treatment beyond initial disease progression was permitted until further progression was confirmed. Initially, 5 expansion cohorts (with 16 patients per cohort) were enrolled in parallel and received 10 mg per kilogram for the treatment of NSCLC, CRC, RCC, ovarian cancer and melanoma. On the basis of initial signals of activity, additional expansion cohorts (with up to 16 patients per cohort) were enrolled for the treatment of melanoma (at 1 and 3 mg per kilogram), NSCLC (divided into cohorts with squamous or nonsquamous subtypes and randomly assigned to receive 1, 3, or 10 mg per kilogram), pancreatic cancer, breast cancer, and gastric cancer (all at 10 mg per kilogram). A maximum tolerated dose was not reached. Serum levels of anti-PD-L1 antibody increased in a dose-dependent manner from 1 to 10 mg per kilogram in 131 patients who were evaluated (14).

ACCEPTED MANUSCRIPT

The serum half-life of nivolumab was estimated from population pharmacokinetics as approximately 15 days. PD-L1 receptor occupancy on CD3+ peripheral-blood mononuclear cells was assessed in 29 patients with melanoma at the end of one cycle of treatment, at doses of 1 to 10 mg per kilogram (14). The median PD-1-receptor occupancy by nivolumab prior to the fifth administration was 64 to 70% across all dose levels (31). Increasing doses up to 10.0 mg/kg did not substantially increase PD-1 receptor occupancy at the time point tested (31).

Following a 6.5-month hiatus for protocol amendment, additional melanoma cohorts randomly assigned to 0.1, 0.3, and 1.0 mg/kg were enrolled (33). In patients with melanoma receiving 0.1 or 0.3 mg/kg who had progressive disease, intrapatient dose escalation to 1.0 mg/kg was permitted. Ultimately 107 melanoma patients were treated (33). A retrospective analysis of overall survival (OS) was conducted after a minimum follow up of 14 months (34). In patients with melanoma, overall response rate (ORR) across doses was 30.8 % (95 % CI 22.3-40.5). Stable disease > 24 weeks was observed in 6.5 % (95 % CI 2.7-13.0). Median progression-free survival (PFS) was 3.7 months (95 % CI 1.9-9.1) and 1- and 2-year PFS rates were 36% (95% CI 27-46) and 27% (95% CI, 17-36), respectively. Median OS was 16.8 months (95 % CI 12.5-31.6). One- and 2-year survival rates were 62% (95% CI 53-72) and 43% (95% CI 32-53), respectively. In 33 patients with objective responses, the Kaplan-Meier estimated median duration of response was 2 years. Nineteen of 33 responses (58%) were ongoing at the time of this analysis (34). After a minimum follow up of 45 months, the 60-month OS rate was 34% (95% 25-43) and median OS was 17.3 months (95% CI 12.5-37.8) (35). Overall survival rates appeared to plateau at ~48 months, although further follow-up is needed (35).

In a phase I/II study, Weber et al (36) assessed the safety of nivolumab 3 mg/kg every 2 weeks for 24 weeks followed by 3 mg/kg every 12 weeks for up to 2 years, in 92 ipilimumab-refractory patients, including patients who experienced grade 3-4 drug-related toxicity to ipilimumab. The ORR for ipilimumab-refractory patients was 30% (95% CI 21-41). After a median follow-up of 16 months, median duration of response was 14.6 months, median PFS was 5.3 months, and median OS was 20.6 months. One- and 2-year survival rates were 68.4% and 31.2%, respectively. Ipilimumab-naïve and ipilimumab-refractory patients showed no significant difference in survival. The 21 patients with prior grade 3-4 toxicity to ipilimumab that was managed with steroids tolerated nivolumab well, with 62% (95% CI 38-82) having complete or partial responses or stabilized disease at 24 weeks. High numbers of myeloid-derived suppressor cells (MDSC) were associated with poor survival. Prior grade 3-4 immune-related adverse effects from ipilimumab were not indicative of nivolumab toxicities (36).

2.2 Nivolumab in combination with ipilimumab

Wolchock et al (37) treated successive cohorts of patients with escalating doses of intravenous nivolumab (0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg) and ipilimumab (1 mg/kg, 3 mg/kg, and 10

ACCEPTED MANUSCRIPT

mg/kg) administered concurrently every 3 weeks for four doses, followed by nivolumab alone every 3 weeks for four doses (concurrent-regimen group) (53 patients). The combined treatment was subsequently continued every 12 weeks for up to eight doses. In a sequenced regimen, 33 patients previously treated with ipilimumab received nivolumab (1 mg/kg or 3 mg/kg) every 2 weeks for up to 48 doses. In the concurrent-regimen group, the doses of nivolumab 1 mg/kg and ipilimumab 3 mg/kg were identified as the maximum doses that were associated with an acceptable level of adverse events. In the concurrent-regimen cohorts, ORR was 40% (95% CI 27-55) according to modified WHO criteria. In addition, 4 patients had an objective response according to immune-related response criteria and 2 had an unconfirmed partial response. In the sequenced-regimen cohorts, ORR was 20 % (95 % CI 8-39) (37).

Postow et al randomly assigned (11) (CheckMate 069), in a 2:1 ratio, 142 patients with previously untreated metastatic melanoma to receive ipilimumab, 3 mg/kg, combined with either nivolumab, 1 mg/kg, or placebo once every 3 weeks for four doses, followed by nivolumab, 3 mg/kg, every 2 weeks until the occurrence of disease progression or unacceptable toxic effects. The primary end point was the investigator-assessed confirmed ORR among patients with BRAF v600 wild-type tumors. The ORR was 61% in the group that received both ipilimumab and nivolumab (combination group) versus 11% in the group that received ipilimumab and placebo (ipilimumab-monotherapy group) ($p<0.001$), with complete responses reported in 22% of the patients in the combination group and no patients in the ipilimumab-monotherapy group (11). After a minimum follow up of 18 months, median PFS was not reached with the combination therapy and was 4.3 months with ipilimumab monotherapy (hazard ratio [HR]0.34; 95% CI 0.2-0.57; $p<0.0001$) (38). The 12- and 18-month PFS rates were 55.1% and 53.4%, respectively, for the combination, and 16.2% and 8.1%, respectively, for ipilimumab alone. At 18 months of follow-up, OS rates in BRAF wild-type patients were 73% for the combination versus 56% for ipilimumab alone, and median OS had not been reached in either group (HR 0.56; 95% CI 0.29-1.10; $p=0.089$). Median duration of response was not reached in either group. Grade 3-4 treatment-related adverse events were reported more frequently with the combination than with ipilimumab alone (55 % versus 22%), and led to discontinuation in 30% and 9% of patients, respectively (38). Patients who discontinued treatment due to drug toxicity derived an OS benefit similar to the overall population (39). The ORR in patients who discontinued nivolumab and ipilimumab was 68 %. After a median follow up of 18 months, median OS was not reached (NR) with the combination and was 11.2 months (95 % CI 2.2-NR) with ipilimumab alone (HR 0.24 [95 % CI 0.09-0.70]; $p = 0.004$). Eighteen-month OS was 79.5 % and 40.0 %, respectively (39). Similar results for ORR and PFS were observed in 33 patients with BRAF mutation-positive tumors (11).

2.3 Nivolumab in combination with vaccine

ACCEPTED MANUSCRIPT

Weber et al (40) assessed the safety and tolerability of nivolumab (1 mg/kg, 3 mg/kg, or 10 mg/kg) with (49 patients) or without (41 patients) the HLA-A*0201–restricted peptide vaccines gp100209-217 or MART-126-35 in patients with unresectable stage III or IV melanoma who were ipilimumab-naive and had experienced progression after at least one prior therapy (34 patients) or experienced progression after prior ipilimumab (56 patients). Treatment was administered every 2 weeks for 24 weeks, then every 12 weeks for up to 2 years. Nivolumab with vaccine was well tolerated and safe at all doses. The ORR according to RECIST 1.1 was 25% (95 % CI 16.6-35.8) for both ipilimumab-refractory and -naive patients. Median duration of response was not reached at a median of 8.1 months of follow-up. Progression-free survival rate at 24 weeks was 46 %. High pre-treatment NY-ESO-1 and MART-1-specific CD8+ T cells were associated with progression of disease. At week 12, increased peripheral-blood T regulatory cells and decreased antigen-specific T cells were associated with progression. PD-L1 tumor staining was associated with responses to nivolumab, but negative staining did not rule out a response.

Gibney et al (41) investigated nivolumab plus vaccine as adjuvant therapy in resected stage IIIC and IV melanoma patients. Thirty-three HLA-A*0201 positive patients with HMB-45-, NY-ESO-1-, and/or MART-1- positive resected tumors received nivolumab (1 mg/kg, 3 mg/kg, or 10 mg/kg i.v.) with a multi-peptide vaccine (gp100, MART-1, and NY-ESO-1 with Montanide ISA 51 VG) every 2 weeks for 12 doses followed by nivolumab maintenance every 12 weeks for 8 doses. Median follow-up was 32.1 months. Treatment was well tolerated. Maximum tolerated dose was not reached. Most common related adverse events were vaccine injection site reaction, fatigue, rash, pruritus, nausea, and arthralgias. Five related grade 3 adverse events (hypokalemia, rash, enteritis, and colitis [2]) were observed. Estimated median relapse-free survival (RFS) was 47.1 months; median OS was not reached (41).

3 Phase III trials

Efficacy data observed in randomized phase III trials with single agent nivolumab and nivolumab in combination with ipilimumab are summarized in table 1 (13;42;43).

CheckMate 037 (43) is an open-label, phase 3 trial, in which 272 patients with unresectable or metastatic melanoma, who had progressed after ipilimumab, or ipilimumab and a BRAF inhibitor if they were BRAFv600 mutation-positive, were randomly assigned 2:1 to receive nivolumab 3 mg/kg every 2 weeks or investigator's choice chemotherapy (dacarbazine 1000 mg/m² every 3 weeks or paclitaxel 175 mg/m² combined with carboplatin, area under the curve 6, every 3 weeks)(ICC) until progression or unacceptable toxic effects. Confirmed objective responses were reported in 31.7% (95% CI 23.5-40.8) of the patients with nivolumab versus 10·6% (95 % CI 3.5-23.1) of the patients in

ACCEPTED MANUSCRIPT

the ICC group. Grade 3-4 drug-related serious adverse events were observed in 5% of the nivolumab-treated patients and 9% of the patients in the ICC group (43).

Nivolumab is associated with significant improvements in OS and PFS, as compared with dacarbazine, among previously untreated patients with metastatic BRAF wild-type melanoma (42). Robert et al (42) randomly assigned 418 previously untreated patients who had metastatic BRAF wild-type melanoma to receive nivolumab, at a dose of 3 mg per kilogram of body weight every 2 weeks and dacarbazine-matched placebo every 3 weeks, or dacarbazine, at a dose of 1000 mg/m² every 3 weeks and nivolumab-matched placebo every 2 weeks (Checkmate 066). The primary end point was OS. At 1 year, the overall survival rate was 72.9% (95% CI 65.5-78.9) in the nivolumab group, as compared with 42.1% (95% CI 33.0-50.9) in the dacarbazine group (HR 0.42; 99.79% CI 0.25-0.73; p<0.001). The median PFS was 5.1 months in the nivolumab group versus 2.2 months in the dacarbazine group (HR 0.43; 95% CI 0.34-0.56; p<0.001). The objective response rate was 40.0% (95% CI 33.3-47.0) and 13.9% (95% CI 9.5-19.4), respectively, with an odds ratio (OR) of 4.06 (p<0.001). The survival benefit with nivolumab versus dacarbazine was observed across pre-specified subgroups, including subgroups defined by status regarding PD-L1. Common adverse events associated with nivolumab included fatigue, pruritus, and nausea. Grade 3-4 drug-related adverse events occurred in 11.7% of the patients treated with nivolumab and 17.6% of those treated with dacarbazine (42). Health-related quality of life (HRQoL) using EQ-5D, EORTC-QLQ-C30 and health care resource use (HCRU) (% days in hospital, number of healthcare visits) in Checkmate 066 were evaluated at baseline and at treatment cycles every 6 weeks (44). EQ-5D utility index score improvement was greater for nivolumab versus dacarbazine at week 7; a statistically significant improvement from baseline was noted for nivolumab from weeks 7-49. For EQ-5D VAS, statistically significant improvement from baseline was noted for nivolumab at week 25, 31, 37, 49, and 61. Statistically significant improvements occurred with nivolumab in some EORTC-QLQ-C30 domains in a longitudinal model. There were no significant differences between arms in visit number or hospital duration at any point, except that dacarbazine had fewer visits at weeks 31 and 37 and shorter hospital duration at week 43. Proportion of patients with hospital visits was similar for nivolumab and dacarbazine (11.0% versus 14.4%) (44).

Larkin et al (13) randomly assigned, in a 1:1:1 ratio, 945 previously untreated patients with unresectable stage III or IV melanoma to receive nivolumab, 3 mg/kg every 2 weeks plus ipilimumab-matched placebo), nivolumab 1 mg/kg every 3 weeks plus ipilimumab 3 mg/kg every 3 weeks for 4 doses, followed by nivolumab 3 mg/kg every 2 weeks for cycle 3 and beyond, or ipilimumab 3 mg/kg every 3 weeks for 4 doses plus nivolumab-matched placebo) (CheckMate 067). It has to be emphasized that the study was not designed for a formal statistical comparison between the nivolumab group and the nivolumab-plus-ipilimumab group. Data on OS (co-primary endpoint) are

ACCEPTED MANUSCRIPT

not yet mature and the study remains blinded with respect to OS as follow up of the patients is planned to continue until the specified number of events have occurred. The median PFS (co-primary endpoint) was 11.5 months (95% CI 8.9-16.7) with nivolumab plus ipilimumab, 2.9 months (95% CI 2.8-3.4) with ipilimumab (HR 0.42; 99.5% CI 0.31-0.57; p <0.001), and 6.9 months (95% CI 4.3-9.5) with nivolumab (HR for the comparison with ipilimumab, 0.57; 99.5% CI, 0.43 to 0.76; p<0.001). In patients with PD-L1 positive tumors, median PFS was 14.0 months both in the nivolumab-plus-ipilimumab group and in the nivolumab group. In patients with PD-L1-negative tumors, median PFS was 11.2 months (95% CI 8.0-NR] and 5.3 months (95% CI 2.8-7.1) with nivolumab alone. Treatment-related adverse events of grade $\geq$ 3 were reported in 16.3% of the patients treated with nivolumab, 55.0% of the patients treated with the combination, and 27.3% of those treated with ipilimumab alone. The most frequent reason for discontinuation was disease progression in the nivolumab and ipilimumab monotherapy groups (49.2% and 65.0%, respectively), and toxicity in the nivolumab-plus ipilimumab group (38.3%) (13). Updated efficacy and safety results from the CheckMate 067 study were recently presented (45) . At ≥18 months of follow-up, median PFS continued to be significantly longer for nivolumab plus ipilimumab (11.5 months; 95 % CI 8.9-16.7) and nivolumab (6.9 months; 95 % CI 4.3-9.5) versus ipilimumab (2.9 months; 95 % CI 2.8-3.4; p < 0.001), and was numerically longer for nivolumab plus ipilimumab versus ipilimumab alone (HR 0.76; 95 % CI 0.60-0.92). The ORR was 57.6 %, 43.7%, and 19 %, respectively. Median duration of response had not been reached in the combination arm, and was 22.3 months and 14.4 months with single agent nivolumab and ipilimumab, respectively. In patients with PD-L1 expression $\geq$ 5 %, median PFS was NR (95 % CI 9.7-NR) with nivolumab plus ipilimumab (HR versus nivolumab 0.87; 95 % CI 0.54-1.41), 22.0 months (95 % CI 8.9-NR) with nivolumab and 3.9 months (95 % CI 2.8-4.2) with ipilimumab. In patients with PD-L1 expression < 5 %, median PFS was 11.1 months with the combination (95 % CI 8.0-22.0; HR versus nivolumab alone 0.74 [95 % CI 0.58-0.96], 5.3 months (95 % CI 2.8-7.1) with nivolumab and 2.8 months (95 % CI 2.8-3.1) with ipilimumab. Median PFS was 15.5 months, 5.6 months, and 4.0 months in patients with BRAF mutant tumors, respectively, and 11.3 months, 7.1 months, and 2.8 months in patients with BRAF wild-type tumors, respectively (45). At the doses used in CheckMate 067, the combination of ipilimumab and nivolumab is associated with substantial toxicity. CheckMate 511 (CA209-511) is a multicenter, randomized, double blinded, phase IIIb/IV trial, comparing nivolumab 3 mg/kg in combination with ipilimumab 1 mg/kg with nivolumab 1 mg/kg in combination with ipilimumab 3 mg/kg in subjects with previously untreated, unresectable or metastatic melanoma with safety as primary endpoint (46). In a randomized phase II trial, the addition of sargramostim to ipilimumab in patients with unresectable melanoma was associated with an improved survival and decreased toxicity (47). NCT02339571 is a randomized phase II/III study comparing nivolumab plus ipilimumab plus sargramostim with nivolumab plus ipilimumab in patients with

ACCEPTED MANUSCRIPT

unresectable stage III or stage IV melanoma (48). CheckMate 238 is a phase 3, randomized, double-blind study of adjuvant immunotherapy with nivolumab versus ipilimumab after complete resection of stage IIIb/c or stage IV melanoma in subjects who are at high risk for recurrence. The primary endpoint is recurrence-free survival. Recruitment has been completed and follow up is ongoing (49-51).

4 Sequential administration of ipilimumab and nivolumab

Concurrent administration of the immune checkpoint inhibitors nivolumab and ipilimumab is associated with substantial toxicity. CheckMate 064 (52) assessed whether sequential administration of nivolumab followed by ipilimumab, or the reverse sequence, could improve safety without compromising efficacy. The study strongly suggests that nivolumab followed by ipilimumab is a more clinically beneficial option as compared with the reverse sequence, albeit with a higher frequency of adverse events. In this randomised, open-label, phase 2 study conducted at nine academic medical centres in the USA, 140 treatment-naive patients or patients who had progressed after no more than one previous systemic therapy, were randomly assigned (1:1) to induction with intravenous nivolumab 3 mg/kg every 2 weeks for six doses followed by a planned switch to intravenous ipilimumab 3 mg/kg every 3 weeks for four doses, or the reverse sequence. After induction, both groups received intravenous nivolumab 3 mg/kg every 2 weeks until progression or unacceptable toxicity. The primary endpoint was treatment-related grade 3-5 adverse events until the end of the induction period (week 25), analysed in the as-treated population. Secondary endpoints were the proportion of patients who achieved a response at week 25 and disease progression at weeks 13 and 25. Overall survival was a pre-specified exploratory endpoint. The frequencies of treatment-related grade 3-5 adverse events up to week 25 were similar in the nivolumab followed by ipilimumab group (50%; 95% CI 37.6-62.4) and in the ipilimumab followed by nivolumab group (43%; 31.1-55.3). The most common treatment-related grade 3-4 adverse events during the whole study period were colitis (15% in the nivolumab followed by ipilimumab group versus 20% in the reverse sequence group), increased lipase (15% versus 17%), and diarrhoea (12% versus 7%). No treatment-related deaths occurred. The proportion of patients with a response at week 25 was higher with nivolumab followed by ipilimumab than with the reverse sequence (41% [95% CI 29.4-53.8] versus 20% [95 % CI 11.4-31.3]). Progression was reported in 38% (95% CI 26.7-50.8) and 61% (95 % CI 49.0-72.8) of the patients at week 13, respectively, and in 38% (95 % CI 26.7-50.8) and 60% (95 % CI 47.6-71.5) of the patients at week 25, respectively. After a median follow-up of 19.8 months (interquartile range [IQR] 12.8-25.7), median OS was not reached in the nivolumab followed by ipilimumab group (95% CI 23.7-

ACCEPTED MANUSCRIPT

NR), whereas over a median follow-up of 14.7 months (IQR 5.6-23.9) in the ipilimumab followed by nivolumab group, median OS was 16.9 months (95% CI 9.2-26.5; HR 0.48 [95% CI 0·29-0·80]). A higher proportion of patients in the nivolumab followed by ipilimumab group achieved 12-month OS than in the ipilimumab followed by nivolumab group (76%; [95% CI 64-85] versus 54% [95% CI 42-65]) (52). Prospective data on the potential role of ipilimumab after failure of an anti-PD1 directed monoclonal antibody are lacking, although some activity has been observed in small series or ad hoc subgroup or retrospective analyses (50;51;53). Memorial Sloan Kettering Cancer Center is sponsoring a randomized, phase 2 study comparing ipilimumab with ipilimumab plus nivolumab in patients with stage III-IV melanoma who have progressed or relapsed on nivolumab or pembrolizumab (54).

5 Nivolumab in patients with brain metatastases

MelBase is a French multicentric clinical and biological cohort dedicated to the prospective follow-up of patients with unresectable stage III or stage IV melanoma. Thirty-nine out of 754 patients had brain metastases and were treated with anti-PD1 therapy. After a median follow-up of 5.6 months, ORR in evaluable patients was 15 % (3 % CR, 12 % PR) and the disease control rate was 33 %. Median OS and PFS were 5.5 months and 2.8 months, respectively (55).

The role of nivolumab as a single agent or in combination with ipilimumab in patients with melanoma brain metastases is being studied in CA209-322 (56) and NCT02374242 (57), and in CheckMate 204 (58), respectively.

6 Nivolumab and radiotherapy

Ahmed et al (59) retrospectively analyzed data from two prospective nivolumab protocols enrolling 160 patients with advanced resected and unresectable melanoma at H. Lee Moffitt Cancer Center and Research Institute, Tampa. Twenty-six patients with a total of 73 brain metastases treated over 30 sessions were identified. Radiation was administered before, during,and after nivolumab in 45%, 7%, and 48% of the lesions, respectively. All brain metastases were treated with stereotactic radiosurgery (SRS) in a single session except 12 metastases which were treated with fractionated stereotactic radiation therapy, nine of which were in the postoperative setting. One patient experienced grade 2 headaches following SRS with symptomatic relief with steroid treatment. No other treatment-related neurologic toxicities or scalp reactions were reported. Eight (11%) local brain metastasis failures with a ≥20% increase in volume were observed in 11 % of the lesions. In these lesions, hemorrhage was noted in 4, and edema was noted in 7. Kaplan-Meier estimates for local

ACCEPTED MANUSCRIPT

brain metastasis control following radiation at 6 and 12 months were 91% and 85%, respectively. In patients receiving nivolumab for unresected disease, median OS from the date of stereotactic radiation and from nivolumab initiation was 11.8 and 12.0 months, respectively. Median OS was not reached in patients treated in the resected setting. These retrospective data suggest that stereotactic radiation to brain metastases is well tolerated prior to, during, or after treatment with nivolumab. Brain metastasis control and OS compare favourably with current standard treatment. However, prospective validation is warranted (59). Radiation therapy controls local disease but also prompts the release of tumor-associated antigens and stress-related danger signals that primes T cells to promote tumor regression at unirradiated sites known as the abscopal effect (60;61).
Abscopal effects have been repeatedly reported when radiotherapy is combined with ipilimumab (62). The combination of nivolumab and radiotherapy in melanoma is tested in several prospective trials i.e. NCT02374242, NIRVANA, NCT02716948 (63-65).

7 Uveal melanoma

Javed et al (66) analyzed the expression of PD-L1 on tumor cells and PD-1 receptor on tumor infiltrating lymphocytes (TILs) in metastatic uveal melanoma and metastatic cutaneous melanoma. In total, 106 metastatic cutaneous melanoma and 18 metastatic uveal melanoma specimens were analyzed for PD-L1 expression, while 89 metastatic cutaneous melanoma and 17 metastatic uveal melanoma specimens were tested for PD-1 expression. Overall, 20.8 % of the metastatic cutaneous melanoma specimens expressed PD-L1 while none of the metastatic uveal melanoma specimens showed PD-L1 expression. PD-1 expression was seen in 76.4% and 53% of metastatic cutaneous melanoma and metastatic uveal melanoma specimens, respectively (66) .
Tsai et al (67) retrospectively analyzed a cohort of stage IV uveal melanoma treated with antibodies against PD-1 (pembrolizumab, nivolumab) or its ligand, PD-L1 (atezolizumab), and identified 58 patients across 9 academic institutions. Forty (69%) received pembrolizumab, 16 (28%) received nivolumab, and 2 (3%) received atezolizumab. Durable responses were rare and the ORR was 3% (95% CI 0-8.4). Stable disease greater than 6 months was observed in 7% of the patients. Median PFS was 2.7 months (95% CI 2.4-3.3) and median OS was 9.5 months (95% CI 5.5-15)(67).
The role of nivolumab plus ipilimumab in metastatic uveal melanoma is being studied in several prospective phase II trials (NCT01585194, NCT02626962) (68;69).

8 Mucosal and acral melanoma

ACCEPTED MANUSCRIPT

Clinically meaningful activity has been reported with PD1-blockade in patients with advanced mucosal and acral melanoma (70). Munhoz et al (70) conducted a multicenter retrospective study of patients with advanced mucosal or acral melanoma treated with nivolumab (n = 20) or pembrolizumab (n = 40). Sixty patients were identified (35 with mucosal melanoma and 25 with acral melanoma. Fifty-one patients had received prior therapy, including 77% prior ipilimumab. The ORR was 23% (95 % CI 10-40) in mucosal melanoma and 32 % (95 % CI 5-54) in acral melanoma. Median PFS was 3.9 and 4.1 months, respectively. Median OS for the entire cohort was 16.8 months (12.4 months in mucosal melanoma and 31.7 months in acral melanoma) (70).

Larkin et al (71) conducted a pooled data analysis of patients who received nivolumab monotherapy,3 mg/kg every 2 weeks, in CheckMate trials 003 and 038 (phase 1) and 037, 066, and 067 (phase 3). Among 889 treated patients, 86 (10%) had mucosal melanoma. At a median follow-up of 9.2 months (range 0.3–62.5), median PFS was 3.0 months (95% CI 2.2-5.4) for patients with mucosal melanoma and 5.1 months (95% CI 3.9-6.1) for all treated patients. The ORR rate was 23.3% (95% CI 14.8-33.6) for patients with mucosal melanoma and 35.9% (95% CI: 32.7-39.1) for all treated patients, with complete responses in ~6% of patients in both cohorts. Median duration of response was not reached for patients with mucosal melanoma and was 22.0 months (95% CI: 22.0-NR) for all treated patients. Among patients evaluable for PD-L1 status, 11/86 (12.8%) with mucosal melanoma and 221/889 (24.9%) of all treated patients had tumors with ≥5% PD-L1 expression and ORR in these patients was 45.5% and 51.1%, respectively. Among patients with <5% tumor PD-L1 expression, ORR was 20.9% and 35.9%, respectively. Grade 3–4 treatment-related adverse events occurred in 8.1% of patients with mucosal melanoma and in 12.1% of all treated patients with similar types of adverse events in both cohorts (71).

9. Response evaluation

Response Evaluation Criteria in Solid Tumors (RECIST) or World Health Organization (WHO) criteria, designed to detect the effects of cytotoxic agents, may not provide adequate tools for the assessment of immunotherapeutic agents (72;73). Novel immune-related response criteria (irRC) for the evaluation of antitumor responses with immunotherapeutic agents have been proposed (72;73). Median time to response in phase III trials varied between 2.1 and 2.78 months (with 95 % CI up to 1 year) (13;42;43), although rapid responses have been reported after one administration of nivolumab as a single agent or in combination with ipilimumab (74;75).

10. Adverse events

ACCEPTED MANUSCRIPT

In the pooled dataset of nivolumab 3 mg/kg as monotherapy across tumour types, the most frequent adverse reactions were fatigue (34%), rash (19%), pruritus (14%), diarrhoea (13%), nausea (13%), and decreased appetite (10%). The majority of adverse reactions were mild to moderate (Grade 1 or 2). In the pooled dataset of nivolumab in combination with ipilimumab in melanoma , the most frequent adverse reactions were rash (51%), fatigue (43%), diarrhoea (42%), pruritus (35%), nausea (25%), pyrexia (19%), decreased appetite (15%), hypothyroidism (15%), vomiting (14%), colitis (14%), abdominal pain (13%), arthralgia (11%), and headache (11%). The majority of adverse reactions were also mild to moderate (Grade 1 or 2). (31). Grade 3-5 adverse events reported in randomized phase III trials with nivolumab as a single agent or in combination with ipilimumab in melanoma patients are summarized in table 1 (13;42;43). In the pivotal Checkmate 067 trial, grade 3-5 adverse events occurred in 43.5 % of the patients treated with nivolumab alone and in 68.7 % of the patients treated with nivolumab in combination with ipilimumab. Treatment-related serious adverse events occurred in 16.3 % and 55.0 % of the patients, respectively, leading to discontinuation of treatment in 5.1 % and 29.4 % of the patients respectively. Adverse events of any grade reported in phase III trials in melanoma patients are summarized in table 2 (13;42;43). Of particular interest are immune-related adverse events (irAEs) including pneumonitis, hypophysitis and pitiutary dysfunction, hypo- or hyperthyroidy, and diabetes mellitus, which can be acute and fulminant (76-85) and which should be managed according to published guidelines (86-90). Grade 3 toxicities are generally managed by holding or stopping therapy, administering immunosuppressive drugs including corticosteroids until irAEs resolve to grade < 1 and then tapering over > 4 weeks.

The differential diagnosis of pulmonary changes during treatment with anti-PD1 mABs can be particularly challenging (91-95).

As virtually any organ can be effected, a high index of suspicion for irAEs is warranted in patients treated with anti-PD1 mABS. Zimmer et al and Hofmann et al (96;97) retrospectively analyzed in detail adverse events in 496 metastatic melanoma patients treated with pembrolizumab or nivolumab at 15 large skin centres in Germany and Switserland. Two hundred forty-two side-effects in 138 patients were reported, including vitiligo, lichen planus, pancreatitis, interstitial nephritis, myocarditis with cardiomyopathy, ventricular arrhythmia, myositis, seizures, polyneuropathy, meningo-(radiculitis), polyradiculitis, cardiac arrhythmia, asystolia, myasthenia gravis, Guillain-Barré syndrome, and paresis (96-99).

Dermatologic adverse events are among the frequently observed toxicities of anti-PD1 mABs and can have a substantial impact on quality of life (100-102).

Several cases of exacerbation of existing psoriasis or occurrence of de novo psoriasis, either as a single event or in combination with other irAEs, were reported after one to four administrations of

ACCEPTED MANUSCRIPT

single agent nivolumab for advanced melanoma (103-106). Other dermatologic adverse events include toxic epidermal necrolysis and bullous pemphigoid (107-109).

Neurological or muscular adverse events, including myasthenia gravis, chronic inflammatory demyelinating polyradiculoneuropathy, subacute multifocal central nervous system (CNS) demyelination, myocarditis, polymyositis, and rhabdomyolysis may occur early after the start of nivolumab, both as a single agent or in combination with ipilimumab (110-112). .

Hematological adverse events reported in patients treated with nivolumab include warm autoimmune hemolytic anemia ,bicytopenia (anemia and thrombocytopenia), and thrombocytopenia.(113-115). Several cases of severe acute interstitial (tubulo)nephritis were reported in patients treated with an anti-PD1 mAB, including nivolumab as a single agent or in combination with ipilimumab (116-118). Autopsy studies may identify clinically unapparent irAEs in patients treated with immunotherapy as demonstrated by Koelzer et al (119) who reported a comprehensive analysis of systemic irAE pathology based on the autopsy of a 35-year-old female patient with metastatic melanoma treated first with ipilimumab and then nivolumab . During therapy with ipilimumab, radiographic features of immune-related pneumonitis were noted. The autopsy examination established a sarcoid-like granulomatous reaction of the lung, pulmonary fibrosis and diffuse alveolar damage. A clinically unapparent but histologically striking systemic inflammation involving the heart, central nervous system, liver and bone marrow was identified (119).

11. Safety in particular populations

Prevalence of autoimmune comorbidities in newly diagnosed metastatic melanoma patients is high and increasing over time (120). Among 12,028 patients with newly-diagnosed metastatic melanoma patients extracted from MarketScan, a large US claims database, the prevalence rate for autoimmune disorders increased 1.7-fold from 17.1% in 2004 to 28.3% in 2014 ($p < 0.001$). The prevalence rates were lower in patients with non-metastatic melanoma (11.7% in 2004 to 19.8% in 2014) and in the general population (7.9% in 2004 to 9.2% in 2014), but the rates increased 1.7- and 1.2-fold over time, respectively. Additionally, female gender and presence of bone or gastrointestinal metastasis were found to be associated with higher risk of autoimmune disorders (120).

Autoimmune, particularly rheumatologic, disorders may occur or flare under anti-PD1 monoclonal antibodies (121). In patients with prior major irAEs with ipilimumab, recurrence of the same irAE with anti-PD1 treatment is rare, but new irAEs frequently occur. Menzies et al (121) retrospectively identified 119 advanced melanoma patients who had pre-existing autoimmune disorders and/or major irAEs requiring systemic immunosuppression with prior ipilimumab and who were subsequently treated with either pembrolizumab (n = 109) or nivolumab (n = 10).

ACCEPTED MANUSCRIPT

Of 52 pts with pre-existing autoimmune disorder, 15 (29%) had active symptoms at start of anti-PD1 treatment and 16 (31%) were on systemic immunosuppression. Twenty patients (38%) flared, including 7/13 with rheumatoid arthritis, 3/3 with polymyalgia rheumatica, 2/2 with Sjogren's syndrome, 1/2 with scleroderma, 2/2 with immune thrombocytopaenic purpura, 3/8 with psoriasis, 1/4 with Graves' disease, 0/6 with gastrointestinal (including 3 Crohn's disease) and 0/5 with neurological disorders. The ORR was 33%. Sixty seven patients had irAEs requiring systemic immunosuppression with prior ipilimumab, including 47 with ≥ grade 3 colitis (of whom 15 had been treated with infliximab), 2 with grade 4 hepatitis (1 had received antithymocyte globulin), and 9 with hypophysitis. All irAEs except hypophysitis had resolved at start of anti-PD1 treatment, except in 1 patient with arthritis, and 5 were on systemic immunosuppression. A recurrence of the same irAEs occurred in 2 patients (3 %)(arthritis, colitis) after the start of anti-PD1 treatment, but 23 (34%) developed new irAEs (19% ≥ grade 3), and 11 (16%) discontinued anti-PD1 therapy. There were no treatment related deaths. The ORR was 40% (121).

Maeda et al (122) successfully treated a 79-year-old melanoma patient who had pre-existing ocular myasthenia gravis and a continued small amount of corticosteroid. Grade 3 creatine phosphokinase elevation appeared after two doses of nivolumab, and the treatment was postponed until it improved to grade 1. After three doses of nivolumab, he experienced diplopia and facial muscle weakness which were consistent with an acute exacerbation of myasthenia gravis but the symptoms relieved without additional treatment for myasthenia gravis. He achieved shrinkage of metastasis after ten doses of nivolumab (122).

The safety of immune checkpoint inhibitors in organ transplant recipients is not well defined. Spain et al reported acute graft rejection in a kidney transplant recipient after treatment with nivolumab, after prior progression on ipilimumab (123).

12. Prognostic and predictive markers

Unfortunately, thus far prognostic or predictive markers for metastatic melanoma patients treated with nivolumab, which have been validated in a prospective study, are lacking (124-126).

PD-L1 tumor staining is associated with responses to nivolumab, but negative PD-L1 does not rule out a response (40). In the phase I trial with nivolumab with or without a peptide vaccine, ORR was 67% (eight of 12 patients) in the patients with the PD-L1 $\geq$ 5 % positive group whereas for patients with PD-L1< 5 % ORR was 19% (p = 0.004). At a cut-off of 1 %, ORR was 39 % and 23 %, respectively (40).

In CheckMate 066 (42), PD-L1 positivity was defined as at least 5% of tumor cells showing cell-surface PD-L1 staining of any intensity in a section containing at least 100 tumor cells that could be evaluated. Although the response rate in the nivolumab arm was higher in patients with PD-L1-

ACCEPTED MANUSCRIPT

positive tumors (52.7 % [95 % CI 40.8-64.3) than in patients with PD-L1-negative tumors (33.1 % [95 % CI 25.2-41.7]), nivolumab-treated patients had improved OS, as compared with dacarbazine-treated patients, regardless of PD-L1 status (unadjusted HR 0.30 [95 % CI 0.15-0.60] for patients with PD-L1 positive tumors, 0.48 [0.32-0.71]for patients with PD-L1-negative tumors), although the HR was numerically better in patients PD-L1-positive tumors. The same definition for PD-L1 positivity was used in CheckMate 037 (43). In patients treated with nivolumab, ORR was 43.6 % in patients with PD-L1-positive tumors and 20.3 % in patients with PD-L1-negative tumors.

Carbognin et al (127) extracted and cumulated ORR, by adopting a fixed and random-effect model with 95% confidence interval, from phase I-III trials investigating nivolumab, pembrolizumab and atezolizumab for advanced melanoma, non-small cell lung cancer (NSCLC) and genitourinary cancer. A significant interaction ($p<0.0001$) according to tumor PD-L1 expression was found in the overall sample. ORR was significantly higher in PD-L1 positive in comparison to PD-L1 negative patients for nivolumab and pembrolizumab. The absolute difference in ORR was 22.8 % for melanoma patients (127).

PD-L2 expression may enrich response to nivolumab in melanoma and PD-L2 expression may partially explain the efficacy of nivolumab in patients with low-to-no PD-L1 (128). Rodig et al (128) analyzed the association between PD-L1/PD-L2 expression and efficacy in CheckMate 064, a phase II study comparing nivolumab followed by ipilimumab (cohort A) versus ipilimumab followed by nivolumab (cohort B). In pre-treatment samples, 32/97 (33%) patients had PD-L1 expression ≥5% and 38/83 (46%) patients had PD-L2 expression ≥70%. No consistent change from baseline in PD-L1 or PD-L2 was observed in either cohort with treatment. The frequency of patients with PD-L2 expression ≥70% was higher among patients with PD-L1 ≥5% versus PD-L1 <5%: 63% (19/30) versus 36% (17/47). In cohort A, there was a greater mean tumor burden reduction and higher response rates at week 13 among patients with high versus low PD-L2 expression, even in patients with low-to-no PD-L1. No association between PD-L2 and efficacy was observed in cohort B (128).

Johnson et al (129) hypothesized that MHC-I/II expression is required for tumor antigen presentation and may predict anti-PD-1 therapy response in advanced melanoma. Across 60 melanoma cell lines, they found a bimodal expression patterns of MHC-II, while MHC-I expression was ubiquitous. In two independent cohorts of anti-PD-1-treated melanoma patients, MHC-II positivity on tumor cells was associated with therapeutic response, progression-free and overall survival, as well as CD4+ and CD8+ tumour infiltrate (129). High numbers of myeloid-derived suppressor cells (MDSC) were associated with poor survival in a phase I/II trial in patients who were treated with nivolumab after progression after prior ipilimumab (36).

In CheckMate 037 (43), ORR with nivolumab was 23.1 % in BRAFv600 mutation positive patients and 34.0 % in BRAF wild-type patients. A pooled retrospective analysis of 4 trials suggests that nivolumab

ACCEPTED MANUSCRIPT

has similar efficacy and safety outcomes in patients with BRAF wild-type or BRAF-mutant melanoma, regardless of prior treatment with a BRAF-inhibitor or ipilimumab (130).

A total of 440 patients, treated with nivolumab, at doses of 0.1, 0.3, 1.0, 3.0, or 10.0 mg/kg every 2 weeks, were included in the analysis. Three hundred and thirty-four patients were BRAF wild-type and 106 were positive for BRAF V600 mutation. The ORR in evaluable patients was 34.6% (95% CI 28.3-41.3) for BRAF wild-type patients versus 29.7% (95% CI 19.7-41.5) in the BRAF-mutant patients. The objective response rates did not seem to be affected by prior BRAF inhibitor therapy, prior ipilimumab therapy, or PD-L1 status of the tumor. The median duration of response was 14.8 months (95% CI 11.1-24.0) for wild-type BRAF and 11.2 months (95% CI 7.3-22.9) for the BRAF-mutant patients. Median time to objective response was 2.2 months in both patient groups. The incidence of treatment-related adverse events of any grade was 68.3% in the BRAF wild-type group and 58.5% in the BRAF-mutant group, with grade 3 or 4 adverse events in 11.7% and 2.8% of patients, respectively (130).

A retrospective analysis of irAEs in 148 (33 resected, 115 unresectable) melanoma patients treated with nivolumab plus peptide vaccine or nivolumab alone, administered every 2 weeks for 12 weeks suggests that cutaneous irAEs are associated with improved survival in melanoma patients treated with nivolumab (131). A 12-week landmark analysis, using a multivariate time-dependent Cox proportional hazard model assessed difference in OS in the presence or absence of irAEs. Immune-related adverse events of any grade were observed in 68.2% of patients. A statistically significantly better OS was noted among patients with any grade of irAE versus those without ($p \leq 0.001$), and OS benefit was noted in patients who reported three or more irAEs ($p \leq 0.001$). Subset analyses showed statistically significant OS differences with rash ($p = 0.001$; HR 0.42 [95% CI 0.24-0.74]) and vitiligo ($p = 0.012$; HR 0.18 [95% CI 0.036-0.94]). Rash and vitiligo correlated with statistically significant OS differences in patients with metastatic disease ($p = 0.004$ and $p = 0.028$, respectively). No significant survival differences were seen with other irAEs (endocrinopathies, colitis, or pneumonitis) (131).

In a retrospective anlysis of 337 patients treated with either pembrolizumab 2 mg/kg or 10 mg/kg every 3 weeks or nivolumab 3 mg/kg every 2 weeks, patients who had liver metastasis (OR 0.35, 95% CI] 0.21-0.59; $p < 0.0001$) and patients who received prior ipilimumab (OR 0.39, 95% CI 0.24-0.64; $p = 0.0002$) were less likely to respond to treatment. Patients with lung metastasis were more likely to respond (OR 1.56, 95% CI 0.99-2.51; $p = 0.06$). Odds ratio for elevated lactate dehydrogenase (LDH) was 0.64 (95 % CI 0.4-1.04; $p = 0.07$) (132). Nosrati et al (133) developed a clinical prediction scale for response to anti-PD1 in melanoma patients. A cohort of 337 patients treated between December 2011 and October 2013 at 4 academic centers with either pembrolizumab, 2 mg/kg or 10 mg/kg every 3 weeks or nivolumab, 3 mg/kg every 2 weeks, was retrospectively analyzed and divided into derivation (n = 223) and validation (n = 114) cohorts. The developed clinical prediction scale included

ACCEPTED MANUSCRIPT

4 prognostic factors that were significantly correlated with lower response: abnormal LDH (1 point), female sex (1 point), presence of liver metastasis (1 point) and previous ipilimumab treatment (1 point). The scale had an area under the receiver-operating curve (AUC) of 0.718 (95% CI 0.65-0.78) in predicting response to therapy. The predictive performance of the score was maintained in the validation cohort (AUC 0.74; 95% CI 0.66-0.83) and the goodness-to-fit model demonstrated good calibration. A score of 4 on the validation model was associated with 0% response to therapy while a score of 0 was associated with 100% response (133). Diem et al evaluated 66 consecutive patients with advanced/metastatic melanoma treated with nivolumab or pembrolizumab. After a median follow-up of 9 months, patients with an elevated baseline LDH had a significantly shorter OS compared with patients with normal LDH (6-month OS: 60.8% versus 81.6% and 12-month OS: 44.2% versus 71.5% [log-rank P=0.0292]). In patients with an elevated baseline LDH, the relative change during treatment was significantly associated with an objective response on the first scan and better OS (134). In an analysis by Loo et al (135) the proportion of "exhausted" antigen experienced T cells/Total CD8+ cells was an accurate predictor of response to anti-PD1 monotherapy but not to the combination of ipilimumab and nivolumab. Freshly isolated tumor samples were digested and tumor infiltrating lymphocytes (TILs) were extracted and stained with T cell myeloid and activation markers, and analyzed with 14 color FACS. Experienced T cells was calculated as the percentage of CD8+, PD-1+, CTLA-4+ cells divided by total CD8+ T cells in the tumor sample. Patients were treated with the combination therapy ipilimumab 3 mg/kg every 3 weeks x 3 and nivolumab 1 mg/kg every 3 weeks, or monotherapy of either nivolumab 3 mg/kg every 2 weeks or pembrolizumab 2 mg/kg every 3 weeks. Fifty-three patients were evaluable for both response and T cell phenotype (15 patients treated with the combination, 38 with monotherapy). For monotherapy, no responders had a proportion of "exhausted" antigen experienced T cells ≤20%, while 81% of the responders had a proportion of "exhausted" antigen experienced T cells >20%. For responders, median proportion of "exhausted" antigen experienced T cells was 40.3%, while for non-responders, median proportion "exhausted" antigen experienced T cells was 16%, p<0.0001. At this threshold, the negative predictive value (NPV) was 100% and the positive predictive value (PPV) was 81%. For patients treated with the combination, the threshold for response was much lower. Responses to the combination therapy were seen at all levels with NPV=50% and PPV=80%, with median "exhausted" antigen experienced T cells of 19.7% 7.8 % (p = 0.13) for responders and non-responders, respectively. In this analysis, the proportion of "exhausted" antigen experienced T cells is an accurate predictor of response to monotherapy but not combination checkpoint therapy (135).

Wong et al (136) analysed baseline parameters on 18-fluorodeoxyglucose (FDG) positron emission tomography (PET) and its association with clinical outcome in 90 evaluable patients treated with either ipilimumab (n = 51), an anti-PD1 monoclonal antiboy (n = 20) or ipilimumab followed by an

ACCEPTED MANUSCRIPT

anti-PD-1 antibody (n = 20). Baseline PET scans were assessed for maximum standardised uptake value (SUV max), whole body metabolic tumour volume (MTV), tumoral percentage of injected dose (%ID) and spleen to liver ratio (SLR). No significant associations between PET parameters and PFS were found for anti-PD1. However, considering all patients from date of first immunotherapy, SLR > 1.1 was associated with poor OS (HR 3.92; p = 0.003) and poor PFS after ipilimumab (136).
CA209-260 is an ongoing trial which explores the relationship Between Tumor Mutation Burden and Predicted Neo-antigen Burden in Patients With Advanced Melanoma or Bladder Cancer Treated With Nivolumab or Nivolumab Plus Ipilimumab (137). Genomic mutational load is associated with a favourable outcome in patients treated with anti-PD1/anti-PD-L1 therapy (138).
Hugo et al (139) analyzed the somatic mutanomes and transcriptomes of pretreatment melanoma biopsies. Overall high mutational loads associate with improved survival, and tumors from responding patients are enriched for mutations in the DNA repair gene BRCA2. Innately resistant tumors display a transcriptional signature (referred to as the IPRES, or innate anti-PD-1 resistance), indicating concurrent up-expression of genes involved in the regulation of mesenchymal transition, cell adhesion, extracellular matrix remodeling, angiogenesis, and wound healing. Mitogen-activated protein kinase (MAPK)-targeted therapy (MAPK inhibitor) induces similar signatures in melanoma, suggesting that a non-genomic form of MAPK inhibitor resistance mediates cross-resistance to anti-PD-1 therapy (139). Desmoplastic melanoma has been reported to have a higher mutational load than other sub-types of melanoma and patients with desmoplastic melanoma have higher response rates and favourable clinical outcomes to anti-PD/PDL1 therapy compared to other patients with advanced melanoma (140).

13. Pharmacoeconomics

Bohensky et al estimated the cost-effectiveness of single agent nivolumab compared with ipilimumab in BRAF wild-type melanoma from the Australian health system perspective. The estimated Incremental Cost-effectiveness Ratios (ICERs) were Australian Dollar (AUD) $35,748 (approx United States Dollar [USD] $27,883) per Year of Life Saved (YLS) and AUD $43,402 (approx USD $33,853)/ Quality Adjusted Life Year (QALY) (141;142), respectively. Bohensky et al (143) also calculated the cost-effectiveness of nivolumab plus ipilimumab compared to ipilimumab alone from the Australian health system perspective. The estimated ICERs were USD $37,684/YLS and USD$44,867/QALY, respectively. The company submitted a semi Markov survival model to the National Institute for Health and Care Excellence (NICE) in order to estimate the cost effectiveness of nivolumab in people with previously untreated advanced (unresectable, metastatic) melanoma (144). In the company's base-case analyses, nivolumab had an ICER of £23,583 per QALY gained compared with dacarbazine.

ACCEPTED MANUSCRIPT

Similarly, in BRAF mutation positive melanoma nivolumab dominated (that is, provided more QALYs at lower cost than) both dabrafenib and vemurafenib. Nivolumab was more costly and more effective than ipilimumab, with an ICER of £7346 per QALY gained (144). In contrast, nivolumab was considered not cost effective by the Irish National Centre for Pharmacoeconomics (NCPE) (145). The ICER versus ipilimumab was estimated at €101,282/QALY for BRAF wild-type patients, and at €76,540/QALY for BRAFv600-mutant patients. For BRAF mutant patients, the ICERs versus vemurafenib and dabrafenib were €29,018/QALY and €46,276/QALY, respectively. The probability of nivolumab being cost effective relative to ipilimumab at a willingness to pay (WTP) threshold of €45,000/QALY was estimated to be 0% in BRAF wild-type patients and 6% in BRAF-mutant patients (145).

Tartari et al (146) evaluated the potential theoretical worldwide budget impact of the anti-PD-1 agents nivolumab and pembrolizumab in patients with advanced melanoma.

Considering that nivolumab costs USD $28/mg and is administered at 3 mg/kg every 2 weeks, and taking into account the median PFS of 5.1 months and a median body weight of 70 kg, the cost/patient was estimated at USD $64,680. Based on the number of new cases worldwide according to World Health Organization (WHO) in 2012 (132,000) and a rate of patients with synchronous or metachronous disease of 20%, the potential worldwide annual cost for nivolumab could be 1.7 billion USD $.When given in combination, nivolumab and ipilimumab, are administered at 1 mg and 3 mg, respectively. Considering a cost/mg of ipilimumab of USD $158 and the PFS (11.5 months) obtained by this combination, the per patient cost can be estimated at USD $68,296 and the theoretical maximal worldwide annual cost for nivolumab plus ipilimumab at 1.8 billion USD $ (146).

14. Regulatory status

Nivolumab, administered as a single agent or in combination with ipilimumab, is approved by FDA and EMA for the treatment of patients with unresectable or metastatic melanoma (147;148).

15. Recommended dose

The recommended dose of nivolumab as a single agent is 3 mg/kg administered intravenously over 60 minutes every 2 weeks. When nivolumab is combined with ipilimumab, the recommended dose is 1 mg/kg nivolumab administered as an intravenous infusion over 60 minutes every 3 weeks for the first 4 doses in combination with 3 mg/kg ipilimumab administered intravenously over 90 minutes. This is then followed by a second phase in which 3 mg/kg nivolumab is administered as an intravenous infusion over 60 minutes every 2 weeks. Treatment with nivolumab, either as a

ACCEPTED MANUSCRIPT

monotherapy or in combination with ipilimumab, should be continued as long as clinical benefit is observed or until treatment is no longer tolerated by the patient (147;148).

16. Conclusions

The anti-PD1 directed mAB nivolumab were approved by FDA and EMA for patients with unresectable and metastatic melanoma, based on randomized phase III trials demonstrating superiority over single agent ipilimumab in previously untreated patients, and over chemotherapy both in untreated patients and after failure of ipilimumab. The addition ipilimumab to nivolumab is associated with a higher response rate and a better PFS, particularly in patients with PD-L1 negative tumors. However, the grade 3/4 toxicity rate with the combination quadruples. Thus far prognostic or predictive markers for metastatic melanoma patients treated with nivolumab, which have been validated in a prospective study, are lacking.

17. Expert commentary

The anti-PD1 directed antibody nivolumab is superior to chemotherapy and to single agent ipilimumab in previously untreated metastatic melanoma patients, and to chemotherapy after failure of ipilimumab. Data from the phase I trial with nivolumab suggest that responses are durable with about a third of the patients surviving at 5 years. Definitive OS data on the combination of nivolumab and ipilimumab are pending. Although CheckMate 067 was not designed for a formal comparison between nivolumab plus ipilimumab and nivolumab alone, the combination is associated with a higher response rate and a better PFS, particularly in patients with PD-L1 negative tumors, albeit at the cost of a steep increase in grade 3-4 adverse event rate. The optimal sequence of immunotherapy and the BRAF/MEK inhibitor combination in patients harbouring BRAFv600-mutated tumors is to be determined. In an ongoing Intergroup Trial sponsored by the National Cancer Institute (NCT02224781) patients with stage III-IV BRAFV600 melanoma are randomized between dabrafenib and trametinib followed by ipilimumab and nivolumab or ipilimumab and nivolumab followed by dabrafenib and trametinib (149). SECOMBIT is a 3-arms prospective, randomized phase II study evaluating the best sequential approach with ipilimumab/nivolumab and a combination of a BRAF-inhibitor (LGX818, Encorafenib) and a MEK inhibitor (MEK162) in patients with BRAF-mutant metastatic melanoma (150). NCT01940809 is a randomized phase I trial study of the side effects and

ACCEPTED MANUSCRIPT

optimal way to give combine ipilimumab with or without dabrafenib, trametinib and/or nivolumab (151).

Many issues remain to be resolved including when to stop treatment, biomarkers for choosing single agent or combination therapy, optimal schedule of ipilimumab in combination with anti-PD1 MAbs, optimal management of adverse events, role of immunotherapy in particular populations (brain metastasis, mucosal melanoma, uveal melanoma), role of other immunotherapeutic agents… Continued accrual into clinical trials should be encouraged.

18. Five year view

Hopefully, in five years, we will be able to better select patients most likely to benefit from the combination. Tumor infiltrating lymphocytes (152) and new immunotherapeutic agents directed at other targets, including TSR-022 (anti-TIM-3 [T cell immunoglobulin and mucin containing protein-3] antibody) (153), indoleamine 2,3-dioxygenase 1 (IDO1) (i.e. epacadostat (154), indoximod) (155), 4-1BB, OX40, CD27 (i.e. varlilumab) (156), and GITR, either as single agents or in combination with the currently available check point inhibitors are already under investigation or will be investigated in the near future.

The potential role of anti-PD1 directed mABs, either as monotherapy or in combination, in the (neo)adjuvant setting is another area of active investigation (49;157-159).

Funding

This paper was not funded

Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

ACCEPTED MANUSCRIPT

Reference List

** Article of interest

** Article of considerable interest

(1) http://seer.cancer.gov/statfacts/html/melan.html. 2016.
Ref Type: Online Source

(2) http://www.who.int/uv/faq/skincancer/en/index1.html. 2016.
Ref Type: Online Source

(3) Jemal A, Saraiya M, Patel P, Cherala SS, Barnholtz-Sloan J, Kim J et al. Recent trends in cutaneous melanoma incidence and death rates in the United States, 1992-2006. J Am Acad Dermatol 2011 November;65(5 Suppl 1):S17-S25.

(4) Weir HK, Marrett LD, Cokkinides V, Barnholtz-Sloan J, Patel P, Tai E et al. Melanoma in adolescents and young adults (ages 15-39 years): United States, 1999-2006. J Am Acad Dermatol 2011 November;65(5 Suppl 1):S38-S49.

(5) Dummer R, Hauschild A, Lindenblatt N, Pentheroudakis G, Keilholz U. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2015 September;26 Suppl 5:v126-v132.

(6) Eggermont AM, Spatz A, Robert C. Cutaneous melanoma. Lancet 2014 March 1;383(9919):816-27.

(7) Miller AJ, Mihm MC, Jr. Melanoma. N Engl J Med 2006 July 6;355(1):51-65.

(8) Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. N Engl J Med 2004 September 2;351(10):998-1012.

(9) Agarwala SS. Current systemic therapy for metastatic melanoma. Expert Rev Anticancer Ther 2009 May;9(5):587-95.

(10) Specenier P. Ipilimumab in melanoma. Expert Rev Anticancer Ther 2016 July 12.

(11) Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med 2015 May 21;372(21):2006-17.

(12) Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 2015 June 25;372(26):2521-32.

ACCEPTED MANUSCRIPT

(13) Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 2015 July 2;373(1):23-34.

(14) Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012 June 28;366(26):2455-65.

(15) Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 2010 July 1;28(19):3167-75.

(16) Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 1999 August;11(2):141-51.

(17) Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 2007 July;19(7):813-24.

(18) Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 1999 December;5(12):1365-9.

(19) Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 2000 October 2;192(7):1027-34.

(20) Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2001 March;2(3):261-8.

(21) Tseng SY, Otsuji M, Gorski K, Huang X, Slansky JE, Pai SI et al. B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. J Exp Med 2001 April 2;193(7):839-46.

(22) Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008;26:677-704.

(23) Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002 August;8(8):793-800.

(24) Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. Nat Med 2003 May;9(5):562-7.

(25) Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A 2002 September 17;99(19):12293-7.

(26) Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. Nat Rev Immunol 2008 June;8(6):467-77.

ACCEPTED MANUSCRIPT

(27) Fife BT, Pauken KE, Eagar TN, Obu T, Wu J, Tang Q et al. Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. Nat Immunol 2009 November;10(11):1185-92.

(28) Brahmer JR, Hammers H, Lipson EJ. Nivolumab: targeting PD-1 to bolster antitumor immunity. Future Oncol 2015;11(9):1307-26.

(29) Hino R, Kabashima K, Kato Y, Yagi H, Nakamura M, Honjo T et al. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. Cancer 2010 April 1;116(7):1757-66.

(30) Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res 2014 October 1;20(19):5064-74.

(31) http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/003985/WC500189767.pdf. 2016.
Ref Type: Online Source

(32) Wong RM, Scotland RR, Lau RL, Wang C, Korman AJ, Kast WM et al. Programmed death-1 blockade enhances expansion and functional capacity of human melanoma antigen-specific CTLs. Int Immunol 2007 October;19(10):1223-34.

(33) Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012 June 28;366(26):2443-54.

(34) Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol 2014 April 1;32(10):1020-30.

(35) Hodi FS, Kluger HM, Sznol M, Carvajal RD, Lawrence DP, Atkins MB et al. Durable, long-term survival in previously treated patients with advanced melanoma who received nivolumab monotherapy in a phase I trial . Presented at the 2016 AACR Annual Meeting CT001. 2016.
Ref Type: Abstract

*: of importance: long term follow-up of melanoma patients included in the phase I trial, demonstrating the durability of the responses

(36) Weber J, Gibney G, Kudchadkar R, Yu B, Cheng P, Martinez AJ et al. Phase I/II Study of Metastatic Melanoma Patients Treated with Nivolumab Who Had Progressed after Ipilimumab. Cancer Immunol Res 2016 April;4(4):345-53.

(37) Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 2013 July 11;369(2):122-33.

(38) Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D et al. Initial report of overall survival rates from a randomized phase II trial evaluating the combination of nivolumab and ipilimumab in patients with advanced elanoma. Presented at AACR Annual Meeting, April 17, 2016 . 2016.
Ref Type: Abstract

ACCEPTED MANUSCRIPT

(39) Hodi FS, Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann KF et al. Overall survival in patients with advanced melanoma who discontinued treatment with nivolumab plus ipilimumab due to toxicity in a phase II trial (CheckMate 069)**.** J.Clin.Oncol. [suppl], 9518. 2016.
Ref Type: Abstract

(40) Weber JS, Kudchadkar RR, Yu B, Gallenstein D, Horak CE, Inzunza HD et al. Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma. J Clin Oncol 2013 December 1;31(34):4311-8.

(41) Gibney GT, Kudchadkar RR, DeConti RC, Thebeau MS, Czupryn MP, Tetteh L et al. Safety, correlative markers, and clinical results of adjuvant nivolumab in combination with vaccine in resected high-risk metastatic melanoma. Clin Cancer Res 2015 February 15;21(4):712-20.

(42) Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015 January 22;372(4):320-30.

**: of considerable importance: pivotal phase 3 trial of nivolumab in previously untreated melanoma

(43) Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol 2015 April;16(4):375-84.

**: of considerable importance: pivotal phase 3 trial of nivolumab in patients who progressed after ipilimumab

(44) Long GV, Atkinson V, Ascierto PA, Robert C, Hassel JC, Rutkowski P et al. Effect of nivolumab on health-related quality of life in patients with treatment-naive advanced melanoma: results from the phase 3 checkmate 066 study. Ann Oncol 2016 July 12.

*: of importance: patient reported outcome data from the pivotal phase 3 trial.

(45) Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL et al. Updated results from a phase III trial of nivolumab combined with ipilimumab in treatment-naive patients with advanced menlanoma (CheckMate 067). J Clin.Oncol. 34[suppl]. 2016.
Ref Type: Abstract

**: of considerable importance: results of phase 3 trial comparing nivolumab either as a single agent or in combination with ipilimumab, with ipilimumab in untreated melanoma patients.

(46) https://clinicaltrials.gov/ct2/show/NCT02714218. 2016.
Ref Type: Online Source

(47) Hodi FS, Lee S, McDermott DF, Rao UN, Butterfield LH, Tarhini AA et al. Ipilimumab plus sargramostim vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. JAMA 2014 November 5;312(17):1744-53.

(48) https://clinicaltrials.gov/ct2/show/NCT02339571. 2016.
Ref Type: Online Source

(49) https://clinicaltrials.gov/ct2/show/NCT02388906. 2016.

ACCEPTED MANUSCRIPT

Ref Type: Online Source

(50) Atkinson V, Ascierto P, Long GV, Brady B, Dutriaux C, Maio M et al. Two-Year Survival and Safety Update in Patients with Treatment-Naïve Advanced Melanoms receiving Nivolumab or Dacarbazine in Checkmate 066. 2015. Presented at MEL Bridge and SMR 2015.
Ref Type: Slide

(51) Bowyer S, Prithviraj P, Lorigan P, Larkin J, McArthur G, Atkinson V et al. Efficacy and toxicity of treatment with the anti-CTLA-4 antibody ipilimumab in patients with metastatic melanoma after prior anti-PD-1 therapy. Br J Cancer 2016 May 10;114(10):1084-9.

(52) Weber JS, Gibney G, Sullivan RJ, Sosman JA, Slingluff CL, Jr., Lawrence DP et al. Sequential administration of nivolumab and ipilimumab with a planned switch in patients with advanced melanoma (CheckMate 064): an open-label, randomised, phase 2 trial. Lancet Oncol 2016 July;17(7):943-55.

(53) Jacobsoone-Ulrich A, Jamme P, Alkeraye S, Dzwiniel V, Faure E, Templier C et al. Ipilimumab in anti-PD1 refractory metastatic melanoma: a report of eight cases. Melanoma Res 2016 April;26(2):153-6.

(54) https://clinicaltrials.gov/ct2/show/NCT02731729. 2016.
Ref Type: Online Source

(55) Allayous C, Dalle S, Lacour JP, Mortier L, Dutriaux C, Saiag P et al. Immunotherapy-treated melanoma brain metastases within the French national cohort, MelBase. J Clin.Oncol. 34[suppl], 9556. 2016.
Ref Type: Abstract

(56) https://clinicaltrials.gov/ct2/show/NCT02621515. 2016.
Ref Type: Online Source

(57) https://clinicaltrials.gov/ct2/show/NCT02374242. 2016.
Ref Type: Online Source

(58) https://clinicaltrials.gov/ct2/show/NCT02320058. 2016.
Ref Type: Online Source

(59) Ahmed KA, Stallworth DG, Kim Y, Johnstone PA, Harrison LB, Caudell JJ et al. Clinical outcomes of melanoma brain metastases treated with stereotactic radiation and anti-PD-1 therapy. Ann Oncol 2016 March;27(3):434-41.

(60) Seyedin SN, Schoenhals JE, Lee DA, Cortez MA, Wang X, Niknam S et al. Strategies for combining immunotherapy with radiation for anticancer therapy. Immunotherapy 2015;7(9):967-80.

(61) Schoenhals JE, Seyedin SN, Tang C, Cortez MA, Niknam S, Tsouko E et al. Preclinical Rationale and Clinical Considerations for Radiotherapy Plus Immunotherapy: Going Beyond Local Control. Cancer J 2016 March;22(2):130-7.

(62) Chandra RA, Wilhite TJ, Balboni TA, Alexander BM, Spektor A, Ott PA et al. A systematic evaluation of abscopal responses following radiotherapy in patients with metastatic melanoma treated with ipilimumab. Oncoimmunology 2015 November;4(11):e1046028.

ACCEPTED MANUSCRIPT

(63) https://clinicaltrials.gov/ct2/show/NCT02716948. 2016.
Ref Type: Online Source

(64) https://clinicaltrials.gov/ct2/show/NCT02659540. 2016.
Ref Type: Online Source

(65) https://clinicaltrials.gov/ct2/show/NCT02799901. 2016.
Ref Type: Online Source

(66) Javed A, Arguello D, Johnston C, Gatalica.Z., Orloff MM, Mastrangelo MJ et al. Disparity in PD-L1 expression between metastatic uveal and cutaneous melanoma. J Clin.Oncol. 34[suppl]. 2016.
Ref Type: Abstract

(67) Tsai KK, Shoushtari AN, Munhoz RR, Eroglu Z, Piulats JM, Ott PA et al. Efficacy of programmed death receptor-1 (PD-1) blockade in metastatic uveal melanoma. J Clin.Oncol. 34[suppl]. 2016.
Ref Type: Abstract

(68) https://clinicaltrials.gov/ct2/show/NCT01585194. 2016.
Ref Type: Online Source

(69) https://clinicaltrials.gov/ct2/show/NCT02626962. 2016.
Ref Type: Online Source

(70) Munhoz RR, Shoushtari AN, Kuk D, Ott PA, Johnson DB, Tsai KK et al. Clinical activity of anti-programmed death-1 (PD-1) agents in acral and mucosal melanoma. J Clin.Oncol. 34[suppl]. 2016.
Ref Type: Abstract

(71) Larkin J, D'Angelo SP, Sosman JA, Lebbe C, Brady B, Neyns B et al. Efficacy and safety of Nivolumab monotherapy in the treatment of advanced mucosal melanoma. Pigment Cell Melanoma Res. Presented at the 2015 Society for Melanoma Research Congress, 789. 2015.
Ref Type: Abstract

(72) Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009 December 1;15(23):7412-20.

(73) Tuma RS. Immunotherapies in clinical trials: do they demand different evaluation tools? J Natl Cancer Inst 2011 May 18;103(10):780-1.

(74) Chapman PB, D'Angelo SP, Wolchok JD. Rapid eradication of a bulky melanoma mass with one dose of immunotherapy. N Engl J Med 2015 May 21;372(21):2073-4.

(75) Tsutsumi M, Asai J, Wada M, Takenaka H, Katoh N. Malignant melanoma showing a rapid response to nivolumab. Australas J Dermatol 2016 February;57(1):61-3.

(76) Abdel-Rahman O, ElHalawani H, Fouad M. Risk of endocrine complications in cancer patients treated with immune check point inhibitors: a meta-analysis. Future Oncol 2016 February;12(3):413-25.

ACCEPTED MANUSCRIPT

(77) McMillen B, Dhillon MS, Yong-Yow S. A rare case of thyroid storm. BMJ Case Rep 2016;2016.

(78) Miyoshi Y, Ogawa O, Oyama Y. Nivolumab, an Anti-Programmed Cell Death-1 Antibody, Induces Fulminant Type 1 Diabetes. Tohoku J Exp Med 2016;239(2):155-8.

(79) Narita T, Oiso N, Taketomo Y, Okahashi K, Yamauchi K, Sato M et al. Serological aggravation of autoimmune thyroid disease in two cases receiving nivolumab. J Dermatol 2016 February;43(2):210-4.

(80) Okamoto M, Okamoto M, Gotoh K, Masaki T, Ozeki Y, Ando H et al. Fulminant Type 1 Diabetes Mellitus with Anti-programmed Cell Death-1 Therapy. J Diabetes Investig 2016 April 18.

(81) Okano Y, Satoh T, Horiguchi K, Toyoda M, Osaki A, Matsumoto S et al. Nivolumab-induced hypophysitis in a patient with advanced malignant melanoma. Endocr J 2016 July 20.

(82) Tanaka R, Fujisawa Y, Maruyama H, Nakamura Y, Yoshino K, Ohtsuka M et al. Nivolumab-induced thyroid dysfunction. Jpn J Clin Oncol 2016 June;46(6):575-9.

(83) Teramoto Y, Nakamura Y, Asami Y, Imamura T, Takahira S, Nemoto M et al. Case of type 1 diabetes associated with less-dose nivolumab therapy in a melanoma patient. J Dermatol 2016 June 23.

(84) Watanabe S, Kimura H, Takato H, Waseda Y, Hara J, Sone T et al. Severe pneumonitis after nivolumab treatment in a patient with melanoma. Allergol Int 2016 May 12.

(85) Fujimura T, Kambayashi Y, Furudate S, Kakizaki A, Hidaka T, Haga T et al. Isolated adrenocorticotropic hormone deficiency possibly caused by nivolumab in a metastatic melanoma patient. J Dermatol 2016 July 21.

(86) Kahler KC, Hassel JC, Heinzerling L, Loquai C, Mossner R, Ugurel S et al. Management of side effects of immune checkpoint blockade by anti-CTLA-4 and anti-PD-1 antibodies in metastatic melanoma. J Dtsch Dermatol Ges 2016 July;14(7):662-81.

(87) Spain L, Diem S, Larkin J. Management of toxicities of immune checkpoint inhibitors. Cancer Treat Rev 2016 March;44:51-60.

(88) Weber JS, Postow M, Lao CD, Schadendorf D. Management of Adverse Events Following Treatment With Anti-Programmed Death-1 Agents. Oncologist 2016 July 8.

(89) Postow MA. Managing immune checkpoint-blocking antibody side effects. Am Soc Clin Oncol Educ Book 2015;76-83.

(90) Friedman CF, Proverbs-Singh TA, Postow MA. Treatment of the Immune-Related Adverse Effects of Immune Checkpoint Inhibitors: A Review. JAMA Oncol 2016 June 30.

*: of importance: guidelines on the treatment of immune-related adverse events in patients treated with checkpoint inhibitors.

(91) Carter CA, Browning R, Oronsky BT, Scicinski JJ, Brzezniak C. The Case of a Zebra That Was Misdiagnosed as a Horse: Pulmonary Tumor Thrombotic Microangiopathy, a New

ACCEPTED MANUSCRIPT

Paraneoplastic Syndrome, Mimicking PD-1-Induced Pneumonitis. Case Rep Oncol 2016 January;9(1):68-75.

(92) Fujita K, Terashima T, Mio T. Anti-PD1 Antibody Treatment and the Development of Acute Pulmonary Tuberculosis. J Thorac Oncol 2016 July 13.

(93) Montaudie H, Pradelli J, Passeron T, Lacour JP, Leroy S. Pulmonary sarcoid-like granulomatosis induced by nivolumab. Br J Dermatol 2016 June 13.

(94) Nakashima K, Naito T, Omori S, Yoshikawa S, Endo M, Kiyohara Y et al. Organizing Pneumonia Induced by Nivolumab in a Patient with Metastatic Melanoma. J Thorac Oncol 2016 March;11(3):432-3.

(95) Sano T, Uhara H, Mikoshiba Y, Kobayashi A, Uchiyama R, Tateishi K et al. Nivolumab-induced organizing pneumonia in a melanoma patient. Jpn J Clin Oncol 2016 March;46(3):270-2.

(96) Hofmann L, Forschner A, Loquai C, Goldinger SM, Zimmer L, Ugurel S et al. Cutaneous, gastrointestinal, hepatic, endocrine, and renal side-effects of anti-PD-1 therapy. Eur J Cancer 2016 June;60:190-209.

(97) Zimmer L, Goldinger SM, Hofmann L, Loquai C, Ugurel S, Thomas I et al. Neurological, respiratory, musculoskeletal, cardiac and ocular side-effects of anti-PD-1 therapy. Eur J Cancer 2016 June;60:210-25.

(98) Kimura T, Fukushima S, Miyashita A, Aoi J, Jinnin M, Kosaka T et al. Myasthenic crisis and polymyositis induced by one dose of nivolumab. Cancer Sci 2016 July;107(7):1055-8.

(99) Shirai T, Sano T, Kamijo F, Saito N, Miyake T, Kodaira M et al. Acetylcholine receptor binding antibody-associated myasthenia gravis and rhabdomyolysis induced by nivolumab in a patient with melanoma. Jpn J Clin Oncol 2016 January;46(1):86-8.

(100) Belum VR, Benhuri B, Postow MA, Hellmann MD, Lesokhin AM, Segal NH et al. Characterisation and management of dermatologic adverse events to agents targeting the PD-1 receptor. Eur J Cancer 2016 June;60:12-25.

(101) Hwang SJ, Carlos G, Wakade D, Byth K, Kong BY, Chou S et al. Cutaneous adverse events (AEs) of anti-programmed cell death (PD)-1 therapy in patients with metastatic melanoma: A single-institution cohort. J Am Acad Dermatol 2016 March;74(3):455-61.

(102) Abdel-Rahman O, ElHalawani H, Fouad M. Risk of cutaneous toxicities in patients with solid tumors treated with immune checkpoint inhibitors: a meta-analysis. Future Oncol 2015;11(17):2471-84.

(103) Kato Y, Otsuka A, Miyachi Y, Kabashima K. Exacerbation of psoriasis vulgaris during nivolumab for oral mucosal melanoma. J Eur Acad Dermatol Venereol 2015 September 21.

(104) Matsumura N, Ohtsuka M, Kikuchi N, Yamamoto T. Exacerbation of Psoriasis During Nivolumab Therapy for Metastatic Melanoma. Acta Derm Venereol 2016 February;96(2):259-60.

(105) Murata S, Kaneko S, Harada Y, Aoi N, Morita E. Case of de novo psoriasis possibly triggered by nivolumab. J Dermatol 2016 May 14.

ACCEPTED MANUSCRIPT

(106) Ohtsuka M, Miura T, Mori T, Ishikawa M, Yamamoto T. Occurrence of Psoriasiform Eruption During Nivolumab Therapy for Primary Oral Mucosal Melanoma. JAMA Dermatol 2015 July;151(7):797-9.

(107) Nayar N, Briscoe K, Fernandez PP. Toxic Epidermal Necrolysis-like Reaction With Severe Satellite Cell Necrosis Associated With Nivolumab in a Patient With Ipilimumab Refractory Metastatic Melanoma. J Immunother 2016 April;39(3):149-52.

(108) Jour G, Glitza IC, Ellis RM, Torres-Cabala CA, Tetzlaff MT, Li JY et al. Autoimmune dermatologic toxicities from immune checkpoint blockade with anti-PD-1 antibody therapy: a report on bullous skin eruptions. J Cutan Pathol 2016 August;43(8):688-96.

(109) Naidoo J, Schindler K, Querfeld C, Busam K, Cunningham J, Page DB et al. Autoimmune Bullous Skin Disorders with Immune Checkpoint Inhibitors Targeting PD-1 and PD-L1. Cancer Immunol Res 2016 May;4(5):383-9.

(110) Maurice C, Schneider R, Kiehl TR, Bavi P, Roehrl MH, Mason WP et al. Subacute CNS Demyelination after Treatment with Nivolumab for Melanoma. Cancer Immunol Res 2015 December;3(12):1299-302.

(111) Tanaka R, Maruyama H, Tomidokoro Y, Yanagiha K, Hirabayashi T, Ishii A et al. Nivolumab-induced chronic inflammatory demyelinating polyradiculoneuropathy mimicking rapid-onset Guillain-Barre syndrome: a case report. Jpn J Clin Oncol 2016 July 5.

(112) Bilen MA, Subudhi SK, Gao J, Tannir NM, Tu SM, Sharma P. Acute rhabdomyolysis with severe polymyositis following ipilimumab-nivolumab treatment in a cancer patient with elevated anti-striated muscle antibody. J Immunother Cancer 2016;4:36.

(113) Solomon LR. Thrombocytopenia due to low-dose colchicine therapy: A possible drug interaction with nivolumab and implications for supportive care. Acta Oncol 2015;54(8):1235-7.

(114) Kong BY, Micklethwaite KP, Swaminathan S, Kefford RF, Carlino MS. Autoimmune hemolytic anemia induced by anti-PD-1 therapy in metastatic melanoma. Melanoma Res 2016 April;26(2):202-4.

(115) Inadomi K, Kumagai H, Arita S, Tsuruta N, Takayoshi K, Mishima K et al. Bi-cytopenia possibly induced by anti-PD-1 antibody for primary malignant melanoma of the esophagus: A case report. Medicine (Baltimore) 2016 July;95(29):e4283.

(116) Shirali AC, Perazella MA, Gettinger S. Association of Acute Interstitial Nephritis With Programmed Cell Death 1 Inhibitor Therapy in Lung Cancer Patients. Am J Kidney Dis 2016 April 21.

(117) Vandiver JW, Singer Z, Harshberger C. Severe Hyponatremia and Immune Nephritis Following an Initial Infusion of Nivolumab. Target Oncol 2016 March 4.

(118) Murakami N, Borges TJ, Yamashita M, Riella LV. Severe acute interstitial nephritis after combination immune-checkpoint inhibitor therapy for metastatic melanoma. Clin Kidney J 2016 June;9(3):411-7.

ACCEPTED MANUSCRIPT

(119) Koelzer VH, Rothschild SI, Zihler D, Wicki A, Willi B, Willi N et al. Systemic inflammation in a melanoma patient treated with immune checkpoint inhibitors-an autopsy study. J Immunother Cancer 2016;4:13.

(120) Ma Q, Shilkrut M, Li M, Barber BL, Zhao Z. Prevalence of autoimmune comorbidities in patients with metastatic melanoma in the US. J Clin.Oncol. 34[suppl]. 2016.
Ref Type: Abstract

(121) Menzies AM, Johnson DB, Ramanujam S, Atkinson V, Wong ANM, Park JJ et al. Anti-PD1 therapy in patients with advanced melanoma and preexisting autoimune disorders or major toxicity with ipilimumab. J Clin.Oncol. 34[suppl]. 2016.
Ref Type: Abstract

(122) Maeda O, Yokota K, Atsuta N, Katsuno M, Akiyama M, Ando Y. Nivolumab for the treatment of malignant melanoma in a patient with pre-existing myasthenia gravis. Nagoya J Med Sci 2016 February;78(1):119-22.

(123) Spain L, Higgins R, Gopalakrishnan K, Turajlic S, Gore M, Larkin J. Acute renal allograft rejection after immune checkpoint inhibitor therapy for metastatic melanoma. Ann Oncol 2016 June;27(6):1135-7.

(124) Friedman CF, Postow MA. Emerging Tissue and Blood-Based Biomarkers that may Predict Response to Immune Checkpoint Inhibition. Curr Oncol Rep 2016 April;18(4):21.

(125) Mahoney KM, Atkins MB. Prognostic and predictive markers for the new immunotherapies. Oncology (Williston Park) 2014 November;28 Suppl 3:39-48.

(126) Festino L, Botti G, Lorigan P, Masucci GV, Hipp JD, Horak CE et al. Cancer Treatment with Anti-PD-1/PD-L1 Agents: Is PD-L1 Expression a Biomarker for Patient Selection? Drugs 2016 June;76(9):925-45.

(127) Carbognin L, Pilotto S, Milella M, Vaccaro V, Brunelli M, Calio A et al. Differential Activity of Nivolumab, Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity Analysis of Trials in Melanoma, Lung and Genitourinary Cancers. PLoS One 2015;10(6):e0130142.

(128) Rodig SJ, Gjini E, Desai K, Jin C, Horak C, Ruisi M et al. Association of programmed death-ligand 1 (PD-L1) and 2 (PD-L2) expression with nivolumab efficacy in advanced melanoma. Presented at AACR Annual Meeting, April 19, 2016 , CT133. 2016.
Ref Type: Abstract

(129) Johnson DB, Estrada MV, Salgado R, Sanchez V, Doxie DB, Opalenik SR et al. Melanoma-specific MHC-II expression represents a tumour-autonomous phenotype and predicts response to anti-PD-1/PD-L1 therapy. Nat Commun 2016;7:10582.

(130) Larkin J, Lao CD, Urba WJ, McDermott DF, Horak C, Jiang J et al. Efficacy and Safety of Nivolumab in Patients With BRAF V600 Mutant and BRAF Wild-Type Advanced Melanoma: A Pooled Analysis of 4 Clinical Trials. JAMA Oncol 2015 July;1(4):433-40.

(131) Freeman-Keller M, Kim Y, Cronin H, Richards A, Gibney G, Weber JS. Nivolumab in Resected and Unresectable Metastatic Melanoma: Characteristics of Immune-Related Adverse Events and Association with Outcomes. Clin Cancer Res 2016 February 15;22(4):886-94.

ACCEPTED MANUSCRIPT

(132) Goldinger SM, Tsai KK, Tumeh P, Hamid O, Nosrati A, Loo K et al. Correlation between metastatic site and response to anti-Programmed Death-1 (PD-1) agents in melanoma. J Clin.Oncol. 34[suppl]. 2016.
Ref Type: Abstract

(133) Nosrati A, Goldinger SM, Tsai KK, Loo K, Tumeh P, Hamid O et al. Derivation and validation of a prediction scale for response to PD-1 monotherapy. J.Clin.Oncol. 34[suppl], 9514. 2016.
Ref Type: Abstract

(134) Diem S, Kasenda B, Spain L, Martin-Liberal J, Marconcini R, Gore M et al. Serum lactate dehydrogenase as an early marker for outcome in patients treated with anti-PD-1 therapy in metastatic melanoma. Br J Cancer 2016 February 2;114(3):256-61.

(135) Loo K, Tsai KK, Pauli M, Munoz Sandoval P, Alvarado M, Algazi AP et al. Novel T cell exhaustion marker to predict monotherapy PD-1 compared to combination CTLA-4 and PD-1 response in melanoma. J Clin.Oncol. 34[suppl], 9520. 2016.
Ref Type: Abstract

(136) Wong ANM, Callahan J, Beresford J, Herschtal A, Fullerton S, Milne D et al. Spleen to liver ratio (SLR): Novel PET imaging biomarker for prediction of overall survival after ipilimumab and anti-PD1 in patients with metastatic melanoma. J Clin.Oncol. 34[suppl], 9523. 2016.
Ref Type: Abstract

(137) https://ClinicalTrials.gov/show/NCT02553642. 2016.
Ref Type: Online Source

(138) Johnson DB, Frampton GM, Rioth MJ, Yusko E, Ennis R, Fabrizio D et al. Hybrid capture-based next-generation sequencing (HC NGS) in melanoma to identify markers of response to anti-PD-1/PD-L1. J.Clin.Oncol. 34[suppl], 105 (abstract). 2016.
Ref Type: Abstract

(139) Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell 2016 March 24;165(1):35-44.

(140) Eroglu Z, Kim DW, Johnson DB, Algazi AP, Munhoz RR, Liniker E et al. Response to anti-PD1/PDL1 therapy in patients with metastatic desmoplastic melanoma. J.Clin.Oncol. 33[suppl], 9011 (abstract). 2016.
Ref Type: Abstract

(141) Bohensky M, Pasupathi K, Gorelik A, Kim H, Harrison JP, Liew D. Cost-Effectiveness of nivolumab versus ipilimumab in patients with BRAF mutation negative unresectable advanced melanoma (BRAF-AM) in Australia. Pigment Cell Melanoma Res. Presented at the 2015 Society for Melanoma Research Congress, 757. 2015.
Ref Type: Abstract

(142) Bohensky M, Pasupathi K, Gorelik A, Kim H, Harrison JP, Liew D. A Cost Effectiveness Analysis of Nivolumab Compared to Ipilimumab for the Treatment of Braf Wild-Type Advanced Melanoma in Australia. Value Health 2015 November;18(7):A340.

ACCEPTED MANUSCRIPT

(143) Bohensky M, Kim H, Gorelik A, Liew D. Cost-effectiveness of nivolumab in combination with ipilimumab in patients with unresectable advanced melanoma in Australia. Ann.Oncol. 26[suppl 9], ix103-ix106. 2015.
Ref Type: Abstract

(144) https://www.nice.org.uk/Guidance/TA384. 2016.
Ref Type: Online Source

(145) http://www.ncpe.ie/wp-content/uploads/2015/07/Summary-for-website-nivolumab-for-metastatic-melanoma.pdf. 2016.
Ref Type: Online Source

(146) Tartari F, Santoni M, Burattini L, Mazzanti P, Onofri A, Berardi R. Economic sustainability of anti-PD-1 agents nivolumab and pembrolizumab in cancer patients: Recent insights and future challenges. Cancer Treat Rev 2016 July;48:20-4.

(147) http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/003985/WC500189765.pdf. 2016.
Ref Type: Online Source

(148) https://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.Overview&DrugName=OPDIVO. 2016.
Ref Type: Online Source

(149) https://clinicaltrials.gov/ct2/show/NCT02224781. 2016.
Ref Type: Online Source

(150) https://clinicaltrials.gov/ct2/show/NCT02631447. 2016.
Ref Type: Online Source

(151) https://clinicaltrials.gov/ct2/show/NCT01940809. 2016.
Ref Type: Online Source

(152) https://ClinicalTrials.gov/show/NCT01993719. 2016.
Ref Type: Online Source

(153) https://clinicaltrials.gov/ct2/show/NCT02817633. 2016.
Ref Type: Online Source

(154) https://ClinicalTrials.gov/show/NCT02327078. 2016.
Ref Type: Online Source

(155) https://clinicaltrials.gov/ct2/show/NCT02073123. 2016.
Ref Type: Online Source

(156) https://clinicaltrials.gov/ct2/show/NCT02335918. 2016.
Ref Type: Online Source

(157) https://clinicaltrials.gov/ct2/show/NCT02519322. 2016.
Ref Type: Online Source

(158) https://clinicaltrials.gov/ct2/show/NCT02656706. 2016.

ACCEPTED MANUSCRIPT

Ref Type: Online Source

(159) https://clinicaltrials.gov/ct2/show/NCT02437279. 2016.
Ref Type: Online Source

ACCEPTED MANUSCRIPT