HUMAN VACCINES & IMMUNOTHERAPEUTICS
2022, VOL. 18, NO. 3, e1971015 (11 pages)
https://doi.org/10.1080/21645515.2021.1971015

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Neoadjuvant immune checkpoint inhibitors in high-risk stage III melanoma

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ABSTRACT

The success of immunotherapy and targeted therapy for metastatic melanoma has generated considerable interest in the adjuvant setting, even though high-risk stage III melanoma (with or without in-transit metastases) still holds a substantial probability of relapse, despite surgical resection and available adjuvant treatments. Based on preclinical and clinical trials in resectable melanoma, immune checkpoint inhibitors can enhance anti-tumor immunity by activating antigen-specific T cells found in the primary site. These tumor-reactive T cells continue to exert their anti-tumor effects on remaining neoplastic cells after resection of the primary tumor, potentially preventing relapses from reoccurring. Several trials in the neoadjuvant setting have been conducted for melanoma patients using checkpoint inhibitors with promising early data, showing an improvement of operability and clinical outcomes. Hence, in this study, we review and discuss the available published and ongoing clinical trials to explore the scientific background behind immunotherapy in the neoadjuvant context.

ARTICLE HISTORY
Received 18 May 2021
Revised 3 August 2021
Accepted 15 August 2021

KEYWORDS
Melanoma; immune-checkpoint inhibitors; neoadjuvant treatment

Introduction

Melanoma patients with palpable locoregional nodes (stage IIIB/C as for 7th AJCC Cancer Staging edition and stage IIIB/C/D disease as for the last 8th edition) have a particularly poor prognosis. The 5- to 10-year overall survival (OS) rates for clinical stage IIIB, IIIC, and IIID are in fact 83–77%, 69–60%, and 32–24%, respectively.[1] In this group of patients, IFN-α, the only agent approved as adjuvant therapy in melanoma, showed a reproducible impact on disease free survival (DFS) but an inconsistent improvement in OS in several randomized trials and meta-analyses.[2] In the largest meta-analysis, the optimal duration of therapy or dose effect was not identified.[3–5]

In the advent of immune-checkpoint inhibitors within the adjuvant setting (both anti-CTLA4 and anti-PD1 antibodies), the prognosis of this high-risk subset of patients has improved with a relapse-free survival (RFS) at 4-years of 52% with nivolumab versus 42% with ipilimumab in CheckMate 238 study.[6] Similarly, in stages IIIB and IIIC the RFS at 3-years was 66% and 54% with pembrolizumab versus 47% and 32% for placebo, respectively, in the Keynote 054 trial.[7] Based on the results of these two latter registrative trials, nivolumab and pembrolizumab have currently been approved by both the FDA and EMA for the adjuvant treatment of patients with stage III melanoma.

Despite the impact of checkpoint inhibitors in reducing the risk of relapse and, at least for ipilimumab (too early for anti-PD1 inhibitors) the risk of death, about half of the patients with high-risk stage III disease still relapse at 4–5 years from surgery.[6–8] In retrospective analyses of patients treated with adjuvant anti-PD1, the majority of relapses (76%) occurred early and during the year of treatment after a median of 3.2 months where the site of relapse was distant in 50% of patients.[9] Furthermore, distant metastases were more frequent in those patients with macroscopic versus microscopic nodal disease at baseline (p = .04), thus confirming the negative impact of clinical stage III on prognosis.

Recognized effects of neoadjuvant therapy, according to established experiences in other solid tumors, include the reduction in tumor burden of local bulky disease, in order to facilitate surgery and the early treatment of microscopic systemic metastatic foci.[10] The information acquired from the pathological response can moreover guide the choice of adjuvant therapy within the individual patient and explore the possible mechanism of resistance and predictive biomarkers of outcome. On the other hand, one of the potential risks associated with neoadjuvant therapy is the delay in the surgical approach of locally advanced but still resectable disease that could convert, in case of lack of treatment activity, into unresectable disease. Moreover, the possible onset of severe long-term toxic effects from neoadjuvant therapy particularly reported with immune-checkpoint inhibitors, could further delay surgical intervention or increase surgical complications.

With the need to standardize neoadjuvant clinical trial methodology, the International Neoadjuvant Melanoma Consortium (INMC) was established in 2016.[11] A pool of international experts in medical oncology, surgical oncology, pathology, radiation oncology, radiology, and translational research developed recommendations for investigating neoadjuvant therapy in melanoma with the aim to facilitate and accelerate clinical and translational research. The joint efforts to identify surrogate endpoints able to predict long-term clinical outcomes such as pathological response and RFS would hopefully accelerate the approval pathway of neoadjuvant schedules in these very high-risk early stage melanoma patients.

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In the present review, we aim to provide an overview of current literature regarding immune checkpoint inhibitors in the neoadjuvant treatment of high risk resected melanoma with recent acquisitions on the predictive value of certain biomarkers evaluated in single trials.

Rationale of checkpoint inhibitors in the neoadjuvant setting

Preclinical studies seem to show that the neoadjuvant approach with checkpoint inhibitors can be associated with enhanced survival and antigen-specific T cell responses compared to adjuvant treatment.[12–14] Through the use of two models of spontaneous metastatic triple-negative breast cancer (orthotopic 4T1.2 and E0771 tumors), significant greater efficacy was demonstrated for neoadjuvant versus adjuvant immunotherapies using four different approaches: complete Treg depletion, anti-CD25, or anti-PD1 alone or in combination with anti-CD137. The reasons behind the greater efficacy of neoadjuvant immunotherapy could be traced back to its greater ability to increase the number of proliferating gp70 tumor-specific T cells with effectory/memory phenotype and its capability to produce IFNγ and TNF in the peripheral blood early after treatment. In general, various mechanisms have been hypothesized to explain the link to this phenomenon. Two of these could be the release of tumor-specific antigens from killing tumor cells as a form of vaccination to further prime and expand tumor-specific T cells at the site of primary tumor or directly after their release into the periphery blood. Besides the perspective of quantity, neoadjuvant immunotherapy may also affect the quality of tumor-specific T cells in regard to the adjuvant setting. In the same study, the authors observed a high proportion of gp70 tumor-specific CD8 + T cells persisting in the blood of long-term survivors (<70 days after tumor inoculation), displaying an effector/memory phenotype in the blood as well as across various organs, proliferative activity, and IFNγ and TNF production.

The neoadjuvant setting, defined as the context of loco-regional disease, is further associated with a lower likelihood of immune evasion and a higher chance to induce a stronger immune response compared to the metastatic setting.[12] Tolerogenic DCs and suppressor T lymphocytes are however present in melanoma at all stages of disease progression through the mechanism underlying tumor-associated immune suppression that may inhibit the immune response to the tumor possibly justifying the poor results of anti-melanoma vaccine strategies despite the induction of systemic immunity.[15]

The first clinical experiences with neoadjuvant ipilimumab plus nivolumab compared to the same regimen in the adjuvant setting (OpACIN trial) showed that pre-operative therapy results in expanding more tumor resident T cell clones in the peripheral blood than in the post-operative one.[16,17]

Checkpoint inhibitors and neoadjuvant trials in melanoma

Several trials with checkpoint inhibitors tested different schedules in terms of doses of single agents (alone or in combination) and duration of treatments in the neoadjuvant plus/minus adjuvant setting (Table 1).

Anti CTLA-4 inhibitors

Ipilimumab is a recombinant human monoclonal antibody that binds cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) and blocks the interaction of CTLA-4 with its ligands, CD80 and CD86. This was the first checkpoint inhibitor to receive FDA approval in 2011 for metastatic or locally advanced/unresectable melanoma.[18] Moreover, ipilimumab was the first immune checkpoint inhibitor to be approved (only by FDA) at the "high" dose of 10 mg/kg for the adjuvant treatment of stage III melanoma upon the results of EORTC1871 trial.[19] The high rate of severe immune-related toxicities together with the first results with the more efficacious and lesser toxic anti-PD1 antibodies prevented however its approval by the EMA.

Building on the results of ipilimumab in the unresectable/metastatic setting, Tarhini et al.[20] evaluated the safety and toxicity of two courses of ipilimumab (10 mg/kg) every three weeks before and after surgery in patients with stage IIIB, IIIC, and IV. The aim of this study was to evaluate the role of ipilimumab in modulating the expression of cellular immunosuppression markers in the tumor microenvironment and in the blood. Thirty-five patients were enrolled, where 8 patients (24%) had progression of disease (PD) and 5 (15%) had minimal residual disease at pathological assessment. A total of 21 patients (64%) had stable disease (SD). Median follow-up was 17.6 months with progression free survival (PFS) of 10.8 months. According to the primary endpoint, no grade 4–5 toxicity was reported as the more frequent G3 toxicity was diarrhea/colitis (14%). Ipilimumab in the neoadjuvant setting proved to have a significant immunomodulating role showing a significant decrease in circulating myeloid derived suppressor cells (MDSC) with a greater decrease correlating with better PFS (p = .03) and an increase in tumor infiltration by full activated effector T cells. An increase of circulating regulatory T cells (Treg) was also demonstrated which was unexpectedly associated with improved PFS consequently questioning the functional status of circulating Treg being the opposite change found in the tumor microenvironment.[20]

In 2018, Tarhini et al.[21] conducted a second trial investigating safety and efficacy of combination immunotherapy with ipilimumab and concurrent high-dose interferon-alpha2b (IFN). A total of 28 evaluable patients with locally or regionally advanced melanoma were randomized to receive ipilimumab at 3 or 10 mg/kg for two doses, plus high-dose IFN (20 MU/m^2/day, 5 days/week for 4 weeks, followed by 10 MU/m^2/day subcutaneously 3 days/week), prior to definitive resection. After surgery, ipilimumab was continued for up to at least four doses, while high-dose IFN was resumed with the same subcutaneous regimen for 46 additional weeks. This was a small trial but they reported a preoperative radiologic response rate of 36% and a pathological complete response (pCR) rate of 32%. They observed that higher T-cell clonality in the primary tumor and/or loco-regional disease post-treatment was associated with an increase in RFS. Combination therapy was well tolerated with no delays in planned surgery, but the 10 mg/kg dose of ipilimumab was associated with more immune-related adverse events (irAEs).[21]

Table 1. Neoadjuvant immunotherapy trials with published results

	Trial Name Registry Number	Phase	Treatment	Size N	Primary Endpoint (s)	Secondary Endpoint(s)	pCR	mPR	Radiographic Response
IPI	Tahrini A, 2014	I/II	Neoadj IPI 10mg/kg for 2 courses then adj IPI for 2 courses+IFNa	35	Safety, Biomarkers	PFS	0%	n/a	9%
	Tahrini A, 2018 NCT01608594	I/II	Neoad IPI 3 mg/kg or 10 mg/kg for 2 doses + high dose IFN, then adj IPI for 4 doses + high dose IFN (46 wks)	28	Safety	Efficacy	32%	39%	29% with 3 mg/kg vs. 43% with 10 mg/kg
PEMBRO	Huang AC, 2019 NCT02434354	I	Neoadj PEM (1 dose), then adj PEM (1 yr)	30	Safety	Biomarkers, efficacy	19%	30%	n/a
ICI COMNINATION	OpACIN Blank CU2018/ ESMO2020 NCT02437279	I	Arm A: adj NIVO + IPI (12 wks) Arm B: neoadj NIVO + IPI (6 wks), then adj NIVO + IPI (6 wks)	20	Biomarkers, safety, feasibility	RFS, safety	Arm B: 33%	Arm B: 78%	Arm B: 50%
	Amaria RN, 2018 ("MD Anderson study") NCT02519322	II	Arm A: neoadj NIVO (6 wks), then adj NIVO (26 wks) Arm B: neoadj NIVO + IPI (6 wks), then adj NIVO (26 wks) Arm C: neoadj NIVO + RELA (4 wks), then adj NIVO + RELA (40 wks)	53	pRR	Biomarkers, cRR, RFS, OS, safety	Arm A: 25% Arm B: 45% Arm C: 59%	Arm A: n/a Arm B: n/a Arm C: 66%	Arm A: 25% Arm B: 73% Arm C: 57%
	OpACIN-neo Rozeman EA2019/ ESMO2020 NCT02977052	II	Arms A and B: neoadj NIVO + IPI (6 wks) (2 different regimens) Arm C: neoadj NIVO followed by neoadj IPI (6 wks)	110	Safety, cRR, pRR, RFS	Safety, biomarkers, RFS	Arm A: 47% Arm B: 57% Arm C: 23%	Arm A: 80% Arm B: 77% Arm C: 65%	Arm A: 63% Arm B: 57% Arm C: 35%
	CA209-8HF Cocorocchio E. ESMO2020	II	neoadj NIVO+IPI (12 wks), then adj NIVO (24 wks)	23	pCR	Safety, biomarkers, RFS.QoL, microbioma	53%	78%	n/a
Oncolytic – therapy + ICIs	Dummer R. NCT02211131	II	Neoadj T-VEC 6 doses (12 wks)	150	RFS	pCR, ORR, OS, Safety	22,8%	n/a	n/a
	Neo-C-Nivo NCT03618641	II	NIVO + CMP-001	34	mPR, safety	RFS, OS, ORR	50%	n/a	43%

Neoadjuvant trials with ipilimumab proved that the approach was feasible confirming historical data of radiological response rate in metastatic disease with the agent and an interesting rate of pathological response. The immune-related toxicity (higher for "high" dose of ipilimumab) did not seem to impact the timing of planned surgery even if the advent of anti-PD1 antibodies led to evaluate these more active and less toxic agents both alone or in combination with ipilimumab.

Anti-P1 inhibitors

The next category of checkpoint inhibitors developed and tested in melanoma, targets the programmed cell death 1 (PD-1) receptor. PD-1 is present on T-cells and its ligand PD-L1 is found on tumor cells and responding immune cells. The binding of PD-1 to its ligand causes downregulation of the T cell response. Anti-PD-1 and anti-PD-L1 antibodies work by blocking this interaction, effectively boosting the immune response to tumor cells.[22] Nivolumab and pembrolizumab are both agents that target the PD-1 pathway and were approved for use in advanced melanoma in 2015 while in the adjuvant setting as far as nivolumab is concerned was in 2017 and pembrolizumab in 2019.[6,7,23,24]

Efficacy and safety of neoadjuvant pembrolizumab monotherapy was assessed in patients with resectable stage IIIB/C or stage IV melanoma by Huang et al. in a phase I trial. A total of 29 patients were enrolled to receive a single dose of pembrolizumab (200 mg IV) followed by radical surgery 3 weeks later; adjuvant pembrolizumab therapy at the same dose was continued for up to 1 year. The pathological response was assessed at the 3-week resection time point in 27 patients, with an overall pathological response rate of 30% (8 out of 27 patients of whom 5 had a pCR and 3 a major complete response). The pathological response showed a relevant prognostic value and all 8 patients remained recurrence free at a median follow up of 25 months post-surgical intervention, whereas patients without a significant pathological response had a higher risk of recurrence (10/19 patients recurred, 7 with metastatic disease). In addition, the DFS and OS rate at 2 years was 63% and 93%, respectively.[25] Moreover, tumor infiltrating lymphocyte (TIL) infiltration after the single neoadjuvant dose was associated with both clinical and pathologic response. In particular, the percentage of patients with brisk TILs (defined as lymphocytes diffusely infiltrating the invasive component of the tumor) increased after treatment, with a significant improvement in 1-y RFS (89% vs. 27% in non-brisk TIL patients), and all patients with pCR or near-pCR had brisk TILs at the time of surgical resection. Treatment was well tolerated, with grade 3 adverse events reported by six patients and no grade 4 adverse events or delay in surgical management due to toxicity.[26] The results of this study well compared with 2-years RFS of 68% in the Keynote 054 trial of adjuvant pembrolizumab 200 mg flat dose for 1 year after surgery in patients with stage IIIA-B-C melanoma (no patients with stage IV and no evidence of disease were included).[27] The small sample size, the design (phase 1 study) and the number of cycles in the neoadjuvant setting (only one) made it difficult to evaluate the adjunctive role of pembrolizumab as neoadjuvant therapy.

Anti CTLA-4 + anti-PD1 combinations

After the success of nivolumab and ipilimumab combination in the metastatic melanoma setting, various studies are currently evaluating different approaches with the same combination in the neoadjuvant setting.

With the aim to determine the immune efficacy, safety, and feasibility of giving checkpoint inhibitor combination as a neoadjuvant plus adjuvant treatment compared with adjuvant only, Blank et al.[28] reported the results of the OpACIN trial (NCT02437279), a small randomized Phase Ib trial. Twenty melanoma patients with palpable nodal disease (stage IIIB-C, AJCC 7th edition) were randomized to receive the combination of ipilimumab "full dose" 3 mg/kg and nivolumab 1 mg/kg, either as four courses of adjuvant therapy alone or two courses of neoadjuvant therapy followed by two courses of postoperative adjuvant treatment. In the neoadjuvant arm, all patients underwent complete lymph node dissection after at least one course of neoadjuvant therapy although only one patient completed all four intended courses of treatment. One patient in the adjuvant arm discontinued therapy due to disease progression. Notably, no delay or complications in surgery were reported, even though 90% of patients developed grade 3–4 irAEs (mostly within the first 12 weeks of treatment) in both arms, a higher rate than that observed of about 60% in advanced disease with the same schedule and likely related to a more competent immune system in patients with a lower tumor burden (localized disease).[24] Neoadjuvant combination immunotherapy led to a pathological tumor response in 7/9 (78%) evaluable patients confirming its high activity also in early disease: three patients achieved a pCR, three others patients a 'near' pCR, (≤10% viable tumor cells), and one patient experienced a partial pathologic response (pPR) (≤50% viable tumor cells). After a median follow up of 4 years none of the patients who obtained a pathological response relapsed while the only two patients without a pathology response had a relapse of disease.[17] Notably, as also subsequently confirmed by other studies, the radiological evaluation using the RECIST criteria highly underestimated the pathological responses with pCR or near pCR evaluated as radiological partial response or stable disease. These aspects will be further commented in the next paragraph.

A further phase II trial, conducted by Amaria et al.[13] compared the results of neoadjuvant nivolumab 3 mg/kg monotherapy for 4 courses with 3 courses of combination ipilimumab 3 mg/kg and nivolumab 1 mg/kg, in 23 patients with resectable clinical stage III or oligometastatic stage IV melanoma.[13] After surgery, adjuvant treatment with nivolumab 3 mg/kg for 13 doses were offered to patients. The planned accrual for this trial was 40 patients, but the trial ended early due to early disease progression in the monotherapy arm and high rates of grade 3 AEs in the combination arm. In particular, while all patients in the combination cohort underwent definitive surgical resection, two patients receiving neoadjuvant nivolumab therapy were unable to undergo surgery due to the development of synchronous metastatic disease as well as local progression. Combined treatment with neoadjuvant ipilimumab "full dose" 3 mg/kg and nivolumab 1 mg/kg achieved

higher response rates, compared with single-agent nivolumab (radiological ORR, 73% vs. 25%; pCR, 45% vs. 25%), but confirmed to be associated with high rate of severe adverse events (73% vs. 8% grade 3 irAEs). Despite the higher toxicities observed, combination treatment with checkpoint inhibitors in the neoadjuvant setting was, however, found to be highly efficacious, supporting the rationale for further studies exploring lesser toxic schedules of the combination immunotherapy.

Furthermore, a reduced-dose regimen of the combination was evaluated in the phase II OpACIN-neo trial with the aim to preserve a high response rate while minimizing the toxicity.[16] Eighty-six patients with resectable stage III melanoma were randomized to receive: arm A, two cycles of ipilimumab "full dose" 3 mg/kg + nivolumab 1 mg/kg; arm B, two cycles of ipilimumab "light dose" 1 mg/kg + nivolumab 3 mg/kg and arm C, two cycles of ipilimumab alone 3 mg/kg followed by two cycles of nivolumab alone 3 mg/kg. After neoadjuvant treatment, lymph node dissection was planned and no adjuvant treatment was considered. The co-primary endpoints of the study were both the proportion of patients with grade 3–4 immune-related toxicity within the first 12 weeks and the radiological and pathological response at 6 weeks. Arm C of sequential ipilimumab and nivolumab prematurely ended due to high incidence of severe adverse events with 5 cases of grade 3 colitis (one requiring a colectomy) and one case of grade 4 polyneuropathy. Immune-related grade 3–5 toxicities in arm A, B, and C were reported by 43%, 27%, and 54% of patients, respectively. High grade toxicities were more prevalent in female than male patients (51.4% vs. 32.7%, $p = .081$) while there were no differences observed between older and younger patients (60 years vs. ≥ 60 years: 35.3% vs. 44.2%, $p = .41$) (17). No correlation between the development of irAEs and pathological response was found. As observed in previous trials, a high pathological response rate to neoadjuvant combination immunotherapy was reported (74% through the three arms) and compared to sequence with the pCR at 47, 57, and 23% respectively, for the three arms. Again, radiological response in accordance with the RECIST criteria underestimated the pathological response for each arm with a global overall radiological response of 52% versus a pathological response rate of 74%. Neoadjuvant immunotherapy globally confirmed the data from the OpACIN trial but in a larger cohort of patients with a median RFS and event free survival (EFS) that were not reached in any of the three groups despite the fact that follow up data are still immature. Overall, the trial confirmed the durability of the response to checkpoint inhibitors with an estimated RFS at 2 years of 84% for the entire population, 97% for patients with a pathological response and only 36% for those who did not achieve a response ($p.< 0.001$).[16,17] The combination of ipilimumab "light" dose 1 mg/kg plus nivolumab 3 mg/kg seemed to be the optimal dose strategy with a good safety/activity ratio and, upon confirmation with more mature data, this schedule should be evaluated against adjuvant therapies in randomized phase 3 trials. Results from the phase 3b/4 CheckMate 511 trial in advanced disease comparing two different schedules of ipilimumab (3 or 1 mg/kg) in combination with nivolumab (1 or 3 mg/kg, respectively) followed by nivolumab 480 mg flat dose every 4 weeks demonstrate an improved safety profile and, although the study was not designed and powered to test a non inferiority in terms of efficacy, descriptive analyses showed similar results in terms of PFS and OS.[29] The role of 1 year adjuvant ipilimumab "light dose" 1 mg/kg every 6 weeks plus nivolumab 240 mg flat dose every 2 weeks compared to nivolumab alone 480 mg flat dose every 4 weeks in improving RFS and OS in patients with stage IIIB-D and IV with no evidence of disease, was explored in CheckMate 915 trial.[30] The trial failed to demonstrate an advantage for the combination versus nivolumab alone but the under-dosage of ipilimumab was the possible cause. More mature data from the neoadjuvant trial employing the combination at full demonstrated active dose would help in elucidating the role of the combination in early stage "high risk" melanoma.

The currently ongoing expansion cohort OpACIN-neo /PRADO trial (NCT 02977052) was designed to confirm the high pathological rate and safety of the combination of ipilimumab 1 mg/kg and nivolumab 3 mg/kg (arm B) but also to explore two challenging questions. On the one hand, the possibility to spare lymph node dissection in patients with pCR or near-pCR upon the results of MeMaloc substudy of OpACIN-neo trial that showed that the pathological response in the largest lymph node (index node) represented the entire lymph node bed.[31] On the other hand, the study will explore the role of adjuvant therapy (nivolumab or dabrafenib and trametinib for BRAF mutated patients) only in case of pathological no response.[32] In the first analysis the trial confirmed a high pathological response rate and safety of arm B of the OpACIN-neo with 50% pCR and 22% of grade 3–4 irAEs in the first 12 weeks. Total lymph node dissection (TLND) was spared for 60% of patients (59 out of 99 patients) with a reduced surgical morbidity for patients undergoing index lymph-node (ILN) only surgery (41 vs. 81% for patients with TLND). At the ESMO virtual meeting 2020, results from the 24-week Health Related Quality of Life confirm better scores for patients undergoing ILN procedure only.[33] Longer follow up will help to confirm whether the pathological response can be used as a surrogate outcome marker for RFS and OS and if the pathological response might guide the oncologist in deciding whether or not to add the adjuvant treatment.

Anti-LAG-3

Neoadjuvant trial data demonstrates that achieving a pCR correlates with improved RFS and OS. As discussed so far, combination immunotherapy with either high or low dose ipilimumab and nivolumab regimens produces a high pCR rate but with grade 3–4 toxicity rate of 20–90%. The goal for neoadjuvant trials to provide novel active therapies/combinations, with a lower toxicity profile, represents an important tool in drug development.

The third distinct checkpoint inhibitor under investigation, is relatlimab, a human LAG-3-blocking antibody that restores effector function of exhausted T cells. LAG-3 and PD-1 are distinct and often co-expressed on tumor-infiltrating lymphocytes and contribute to tumor-mediated T-cell exhaustion.[34,35] Initial efficacy of relatlimab in combination with nivolumab in

metastatic melanoma patients progressed after prior immunotherapy was reported in a phase I/II trial showing ORR of 16% and DCR of 45% with a favorable toxicity profile.[36,37] Recently, the activity of relatlimab in combination with nivolumab in untreated patients with resectable clinical stage III or oligometastatic stage IV melanoma has been analyzed in an open label phase II trial (NCT02519322). Updated initial results showed how this combination compared to other neoadjuvant regimens, produces similar efficacy but reduced toxicity. No treatment-related grade 3/4 or surgical delays due to treatment-related toxicity were noted in the neoadjuvant setting. With a median follow up of 16.2 months, neoadjuvant relatlimab plus nivolumab achieved high rates of pCR (59%) and major pathologic response (pCR + near pCR: 66%). Patients with major pathologic response have improved RFS compared to those without MPR with no relapses observed to date.[38]

Oncolytic immunotherapy

Recent advances in cancer immunotherapy are providing new strategies promoting local response in melanoma patients. For instance, intralesional therapies aim to regress the treated lesion and offer the potential for improved local efficacy, reducing toxicities by allowing delivery of an increased concentration of a drug at the injection site while reducing systemic exposure.[39]

Oncolytic viruses, wild-type and modified live viruses, are novel cancer treatments that may selectively infect or replicate within and lyse tumor cells, without harming normal tissues.[40–42] In regard to their ability to self-amplify, oncolytic viruses are unlike any other form of intralesional therapy. Oncolytic viruses can also activate anti-tumor immunity by triggering immune responses following the release of proinflammatory cytokines and tumor-derived antigens, leading to the possibility of durable responses. Talimogene laherparepvec (T-VEC) is the first oncolytic virus based on a genetically modified herpes simplex virus (HSV) type 1 designed to selectively replicate in and lyse tumor cells while promoting regional and systemic antitumour immunity. It was approved by the FDA for the treatment of advanced melanoma based on the results of the OPTiM trial.[43]
This phase 3 trial was the first to demonstrate a promising clinical benefit with an oncolytic immunotherapy in any cancer and the largest randomized controlled trial investigating a therapeutic option in unresectable stage IIIB/C melanoma. The clinical benefit of single agent T-VEC was also analyzed in the neoadjuvant setting in a phase II trial, where 150 patients with stage IIIB/C and IVM1a resectable melanoma, were randomized to receive either six doses of neoadjuvant T-VEC for up to 12 weeks, followed by resection or immediate surgery. Dummer R et al, showed an improvement in RFS and OS among the patients in the T-VEC arm, with 22.8% of pCR and 40.8% of the disease control rate (DCR).[44]

The most common AEs in T-VEC arm were flu-like symptoms; among G3 AEs, two cases of cellulitis and one case each of anembryonic gestation, cholecystitis, device occlusion, influenza, and wound infection, were reported. At 3 years follow-up, the OS rate was 83.2% and 71.6%, in T-VEC and surgery alone arms, respectively (HR: 0.54, 80% CI: 0.36–0.83; p = .061); the RFS rate was 46.5% with T-VEC plus surgery compared with 31% with surgery alone (HR: 0.67; 80% CI: 0.51–0.88, p = .043). The median OS at 3 years was not reached in both arms.[45]

A second type of intratumoral treatment involves the use of CMP-001, a virus-like particle utilizing a CpG-A oligonucleotide that activates tumor-associated plasmacytoid dendritic cells (pDC) via TLR9 and infiltrates the tumor microenvironment by a subsequent induction of both innate and adaptive anti-tumor immune responses. The efficacy of CMP-001 has been considered in Neo-C-Nivo phase II trial, that evaluates the effects of neoadjuvant intra-tumoral CMP-001 in combination with nivolumab in patients with stage IIIB/C/D treatment-naïve resectable melanoma, with an accessible tumor for biopsy and CMP-001 injection.[46]

At the final analysis presented at SITC 2020, no dose limiting toxicities or G4/5 trAEs were observed; the most frequent G3 AE was hypertension (9.7%), arthralgia (3.2%), colitis (3.2%), hypophosphatemia (3.2%), and injection site infection in 3.2% of patients. Radiological responses were seen in 43%, with a pCR in 50% of patients, while SD and PD were 30% and 27%, respectively. The RFS at 1 year was 90% in all pathological responders, with a median RFS not reached in pathological responders versus 5 months in nonpathological responders.[47]

International Neoadjuvant Melanoma Consortium

Neoadjuvant therapy is now an active area of research for melanoma patients and several completed and ongoing trials using contemporary targeted and/or immunotherapies are in light with disparate designs, endpoints, and analyses. Therefore, considering the promising early results shown and the need to tailor the ideal patient population, duration of treatment, and toxicity of neoadjuvant systemic therapy to balance the potential risks of this investigational approach, the 2016 International Neoadjuvant Melanoma Consortium (INMC) has developed recommendations for investigating neoadjuvant therapy with the goal to facilitate and accelerate neoadjuvant research in melanoma.[11]

With the aim to analyze the relationship between pathologic response and clinical outcomes with neoadjuvant anti-PD1-based immunotherapy and BRAF/MEK inhibitors, namely dabrafenib and trametinib, a pooled analysis on 192 patients (141 treated with immunotherapy and 51 with targeted therapy) from six clinical studies was conducted.[48] The INMC narrowed the inclusion criteria as follows: RECIST measurable disease; surgically resectable disease; stage III disease with nodal metastases; surgery currently performed after neoadjuvant treatment. A pCR was obtained in 40% of patients (47% with target therapy and 33% with immunotherapy overall considered, p = .017). Among patients treated with immunotherapy, a pCR and a pCR/near pCR however occurred in 43% and 61% of those who received combined anti-CTLA4 and anti-PD1 versus 20% and 26% of those who received anti-PD1 monotherapy, respectively. The pathological response overall confirmed to be correlated with RFS with a 2-year rate of 89% for pCR versus 50% for no pCR (p < .001). The OS at 2 years had also improved for patients with pCR versus no pCR (95% versus 83%, p = .027).

Analyzing the data for the kind of treatment received, patients who obtained a pCR, near pCR or pPR with immunotherapy had a 2-year RFS of 96%, no patient with a pCR yet experienced a relapse of disease, the latter for patients treated with target therapy was only 79% with almost 10% of patients who died at 2 years. Interestingly, while attaining a pCR proved to be particularly important with target therapy (no near pCR was obtained and pPR had similar outcomes as pathological no response (pNR), the „depht„ of response to immunotherapy seemed to have less impact on outcome because patients with near pCR or pPR with immunotherapy had similar 2-year RFS as those with pCR (100%, 94%, and 96%, respectively). This latter evidence seems to suggest that, in contrast to target therapy, any kind of response to immunotherapy might be a surrogate marker for long-term efficacy due to the chance of a protracted duration of immunological surveillance as observed from the clinical experience in metastatic disease for patients in response even after checkpoints discontinuation for various causes including toxicity.[49] Finally, the RFS curves of patients treated with neoadjuvant immunotherapy and target therapy in the pooled analyses seem to resemble those of patients treated with the same approaches as first line-therapy for metastatic disease with a superiority for BRAF/MEK inhibitors within the first 12 months and a subsequent crossing of the curves in favor of anti-PD1 and anti-CTLA4/anti-PD1 combination.[50] Patients treated with immunotherapy had overall superior RFS than patients treated with target therapy (1- and 2-year RFS of 75% vs. 78% and 75% vs. 47%, respectively, $p = .003$) with a separation of the curves around 1-year and a plateau for immunotherapy that seems to begin at about 9 months from surgery compared to a progressive decline of the curve for target therapy. Superiority of RFS with immunotherapy compared to target therapy was also confirmed at the multivariable analysis including sex, age, AJCC stage and sum of diameter of largest nodes (HR = 0.55, $p = .037$).

As previously reported for OpACIN and OpACIN-neo studies (but also for neoadjuvant trials with target therapy), the radiological response according to the RECIST criteria had a modest concordance with pathological response. Interestingly, after neoadjuvant immunotherapy, all patients with a radiological CR as well as over 80% of patients with a radiological PR had a pCR/near pCR, similar to the 38% of patients with radiological SD. Patients treated with immunotherapy who had a radiological CR or PR had excellent 2-year RFS rates (100% and 96%, respectively) compared to patients with SD or progressive disease (PD) (62% and 29%, respectively; $p < .001$), while the curves for target therapy clearly separate outcomes between CR, PR and SD (60% vs. 44% vs. 20%, respectively). It is important to note however that radiological response proved to be able to discriminate the different outcomes of patients who obtained a pNR with immunotherapy. Patients with RECIST PR and pNR fared in fact better than those with SD/PD and pNR (2-year RFS 100% vs. 20 and 27%, respectively, $p = .045$) similar to what was seen in the neoadjuvant trial with interferon alfa-2b.[51] Various factors may have played a role in generating the deeper discrepancies found in immunotherapy trials compared to target therapy trials in regards to the relationship between radiological and pathological response. First of all, while the assessment of pathological response was centrally reviewed by the pathologist experienced in evaluating response after neoadjuvant checkpoint inhibitors therapy in stage III melanoma using the Neoadjuvant Melanoma Consortium scoring system (at least for OpACIN – neo trial), tumor radiological response by CT before surgery was assessed by the radiologist at the individual sites without central review, consequently reducing the quality and comparison of data. Other explanations may include the different extent of disease within stage III and the possible different kinetics of tumor response (that generally depends by tumor burden) and host response that can have led to slower responses not captured by the CT scan but confirmed by pathological examination. Furthermore, RECIST criteria version 1.1 were utilized to assess the response in neoadjuvant trials, and it is now recognized that modified criteria can better capture the objective change in tumor size upon immunotherapy.[52] The possibility of early radiological and clinical picture of pseudoprogression or stability of disease linked to immune cell infiltration is in fact an option and the timing of response assessment could be decisive. Finally, an artificial intelligence based evaluation of the tumor on the pre-treatment contrast-enhanced CT imaging data would allow to develop and validate a noninvasive machine learning biomarkers (radiomics biomarlers) capable of distinguishing between immunotherapy responding and non-responding patients.[53]

Despite the proportion of patients who recurred after surgery and the patterns of recurrence were similar regardless of the neoadjuvant treatment employed (globally 41% locoregional versus 59% at distance), the incidence of brain metastases was significantly higher for patients treated with target therapy compared to immunotherapy (59% vs. 13%, $p = .005$).

With all the limitations linked to the heterogeneity of the study included in terms of schedules and patient characteristics, the pooled analyses of the Consortium confirmed that pathological response to neoadjuvant treatment (either immunotherapy and target therapy) correlates with improved RFS and OS and should be considered a new benchmark for an accelerated approval path in high-risk early-stage melanoma. Immunotherapy, and in particular combination therapy with anti-CTLA4 and anti-PD1 nevertheless appeared more active than target therapy where the extent of pathological response proved not to be critical for survival outcomes. The achievement of either a pCR or near pCR with immunotherapy is moreover associated with excellent survival and the major pathological response (i.e. combined pCR and near pCR) should be considered a surrogate marker for long-term outcomes with neoadjuvant checkpoint inhibitors in contrast to target therapy where only pCR should be the standard endpoint.

Neoadjuvant immunotherapy with checkpoint inhibitors and predictive factors of pathological response

Considering the promising role of pathological response as a surrogate marker for RFS, the identification of baseline clinical or biological markers potentially predictive of response remain an urgent clinical need. This is particularly important in the neoadjuvant setting to address patients toward the highest effective treatments but also to spare ineffective and toxic ones.

Table 2. Ongoing neoadjuvant immunotherapy trials.

Parameter	NCT03769155 (Phase 1)	NCT02519322 (Phase 2)	NCT03698019 (Phase 2)	NCT04013854 (Phase 2)	NCT02977052 (Phase 2, extension cohort)	NCT04495010 (Phase 2)
Studies	CA209-8N4 IRB00104273 NCI-2018-01229 Winship4400-18	2015–0041 NCI-2015-01520	NCI-2018-02107 S1801	CA209-74X UPCC 02619	OpACIN-neo CA209-701 M16OPN 2016–001984-35	CheckMate 7 UA 2020–000070-16
Design	PEP or PEP+Nivo or PEP+IPI or PEP+NIVO+IPI	NIVO or NIVO+IPI or NIVO+REL	Adj PEM or Neoadj + Adj PEM	Neoadj NIVO → Adj NIVO or NIVO+IPI (determined by pathological response)	NIVO+IPI	Neoadj NIVO+IPI → Adj NIVO or Neoadj NIVO+IPI → Adj NIVO or Observ vs adj NIVO
Population	Stage IIIB-D	Stage IIIB-IV	High-risk, Stage III/IV	Stage III	Stage III	Stage IIIB-D
Enrollment (estimated)	36	53	500	60	100–110	657
Primary endpoint	Biomarkers (CD8+ T cells)	Pathological RR	EFS	RFS	Safety, RR, RFS	EFS
Status (primary completion date)	Recruiting (Dec 2021)	Recruiting (Aug 2020)	Recruiting (Sep 2022)	Recruiting (Aug 2026)	Active, not recruiting (Jan 2020)	Not yet recruiting (Feb 2024)

adj, adjuvant; cRR, clinical response rate; EFS, event free survival; IPI, ipilimumab; neoadj, neoadjuvant; NIVO, nivolumab; OS, overall survival; PEM, pembrolizumab; PEP, pepinemab; RR, response rate; RFS, recurrence-free survival.

Table 3. Ongoing neoadjuvant immunotherapy trials plus other agents.

Parameter	NCT02303951 (Phase 2)	NCT03554083 (Phase 2)	NCT02858921 (Phase 2)	NCT03259425 (Phase2)	NCT03618641 (Phase 2)
Studies	NEO-VC EADO_VC_NEO_1	NeoACTIVATE MC1776 NCI-2018-01018	NeoTrio MIA2015/176	Neo-NivoHF10 HCI1023-46	17–169
Design	VEM+COBI (cohort 1) VEM+COBI+ATEZO (chort 2)	COBI+ATEZO Or VEM+COBI+ATEZO	DB+TRAM + Concurrent o sequential PEM or PEM	NIVO+HF10 (oncolytic virus)	NIVO+CMP-001
Population	Stage IIIC/IV	High-risk, Stage III	Stage IIIB/C With BRAF mut	Stage IIIB/C or IVM1a	Stage IIIB-D with Clinically LN disease
Enrollment	90 (estimated)	30 (estimated)	60 (estimated)	7 (actual)	34 (actual)
Primary endpoint	Resectability rate	pCR, RFS	Pathological RR	Pathological response	Major pathological RR
Status (primary completion date)	Recruiting (Feb 2021)	Recruiting (Jun 2023)	Recruiting (Nov 2020)	Active, not recruiting (Sep 2018)	Active, not recruiting (Aug 2020)

adj, adjuvant; ATEZO, atezolizumab; cRR, clinical response rate; COBI, cobimetinib; DB, dabrafenib; EFS, event free survival; IPI, ipilimumab; neoadj, neoadjuvant; NIVO, nivolumab; OS, overall survival; PEM, pembrolizumab; PEP, pepinemab; RR, response rate; RFS, recurrence-free survival; TRAM, trametinib; VEM, vemurafenib.

In the OpACIN trial, baseline interferon–gamma (IFN-γ) gene signature expression was associated with absence of relapse.[28] NanoString spatial microscopy analyses on baseline tumor biopsies showed that low CD3, β_2 microglobulin and PD-L1 expression (these latter two known to be upregulated from IFN-γ exposure) were strongly associated with relapse after both neoadjuvant or adjuvant ipilimumab plus nivolumab. Even if its role needs to be confirmed in a larger series of patients, low RNA expression of the IFN-γ signature was also associated with relapse independent of the setting of ipilimumab and nivolumab treatment while none of the patients with high or intermediate expression had relapsed.

In line with these results, an explorative biomarker analysis from the OpACIN-neo trial showed that IFN-γ signature (based on gene expression analysis with NanoString nCounter) was associated with relapse status with no relapse of disease among the patients with a high or intermediate IFN-γ signature.[17] However, in the present study, the discriminatory power of the IFN-γ signature was not concordant between the different techniques used (RNA sequencing and NanoString gene-expression) and the association with pathological response was less potent than the association with relapse status. An analysis of the entire T cell receptor repertoire in tumor samples at baseline demonstrated that a reduced T cell tumor infiltrate and a lower productive T cell clonality within the tumor was associated with relapse after receiving ipilimumab and nivolumab. A post-hoc exploratory analyses of OpACIN neo trial showed instead that no significant association could be found between demographic or clinical (radiological tumor burden, ulceration, PD-L1 expression) characteristics at baseline and pathologic response.[16]

Furthermore, even though a higher toxicity with checkpoint inhibitors in patients with earlier stages of disease was postulated due to lower systemic immune suppression, no significant associations were found between maximum grade of irAEs and response in the three study arms.[12]

An analysis exploring the association between specific biomarkers at baseline and pathological response of pooled data from OpACIN-neo and OpACIN neoadjuvant trials was recently reported.[17] High tumor mutational burden (TMB) and high IFN-γ related gene expression signature score (IFN-γ score) were associated with pathological response and low risk of relapse. In fact, patients with high IFN-γ score/high TMB achieved a pRR in 100% of cases, those with high IFN-γ score/low TMB or low IFN-γ score/high TMB in 91% and 88% of cases, respectively, as well as those with low IFN-γ score/low TMB in only 39% of cases. The corresponding elapse-free survival at 2 years was as low as 49.5% in the group with low IFN-γ score/low TMB compared to 83.3%, 93.8% and 100% for patients with low IFN-γ score/high TMB, high IFN-γ score/low TMB, and high IFN-γ score/high TMB, respectively (p = .0018). These results seem to suggest that it is possible to identify subgroups of patients with low probability to benefit from ipilimumab plus nivolumab and for which new approaches might be tested sparing unnecessary toxicities even if confirmatory analyses need to be carried out. The evidence from patients treated with neoadjuvant immunotherapy are not in line with those that emerged from the analyses of patients treated with adjuvant dabrafenib and trametinib versus placebo in the registrative COMBI-AD trial where IFN-γ expression but not TMB was predictive for RFS.[54] Patients with high TMB had in fact less noticeable benefit with targeted therapy compared to placebo especially if they have an IFN-γ signature lower than the median.

Finally, the IFN-γ score and TMB are under investigation as biomarkers of response to neoadjuvant anti-PD1 monotherapy to identify those patients to whom unnecessary toxicities are spared. In an ongoing phase 1b trial (DOMINI trial; NCT04133948) such a strategy is being tested in patients with a high IFN-γ score.

Current conclusions and future developments

Neoadjuvant therapy is the new test bench for immune-checkpoint inhibitors in melanoma. The combination of anti-CTLA4 and anti-PD1 antibodies (ipilimumab and nivolumab) proved to be associated with a higher rate of complete responses compared to the same agents alone. Shorter and less toxic neoadjuvant schedules and the role of adjuvant treatment still remain to be completely defined. The efficacy of neoadjuvant immunotherapy and the prognostic role of pCR are already raising important questions such as the real curative role and the need for surgery on the lymph node basin. Several neoadjuvant trials with immunotherapy are currently ongoing, exploring new immunomodulatory antibodies and combinations with target therapy and strategies of "personalized" adjuvant schedules depending on pathological response (Tables 2 and 3). Even though the results on the identification of predictive biomarkers of response remain promising, they are still elusive and require further validation.

In the absence of mature data of relapse-free, overall survival and of a direct comparison between immunotherapy and target therapy and considering the clinical design of available trials (only small size phase I/II clinical trials with different duration of neoadjuvant and adjuvant schedules and short follow up), no definitive conclusions on the superiority of which approach over another can be drawn, and neoadjuvant treatment is still to be considered an investigational approach.

Disclosure of potential conflicts of interest

Dr. Virginia Ferraresi declares honoraria received for Scientific Advisory Boards from Bristol Myers Squibb (BMS), Novartis and MSD and for speaker engagement from BMS, Novartis, Pierre-Fabre, and MSD. Dr. Sabrina Vari declares non conflicts of interest.

References

1. Gershenwald JE, Scolyer RA. Melanoma staging: American Joint Committee on Cancer (AJCC) 8th edition and beyond. Ann Surg Oncol. 2018;25(8):2105–2110.
2. Creagan ET, Dalton RJ, Ahmann DL, Jung SH, Morton RF, Langdon RM Jr, Kugler J, Rodrigue LJ. Randomized, surgical adjuvant clinical trial of recombinant interferon alfa-2a in selected patients with malignant melanoma. J Clin Oncol. 1995;2776–83. doi:10.1200/JCO.1995.13.11.2776
3. Ascierto PA, Gogas HJ, Grob JJ, Algarra SM, Mohr P, Hansson J, Hauschild A. Adjuvant interferon alfa in malignant melanoma: an interdisciplinary and multinational expert review. Crit Rev Oncol Hematol. 2013;85(2):149–61. doi:10.1016/j.critrevonc.2012.07.004.
4. Kirkwood JM, Manola J, Ibrahim J, Sondak V, Ernstoff MS. A pooled analysis of eastern cooperative oncology group and intergroup trials of adjuvant high-dose interferon for melanoma. Cil Cancer Res. 2004;10(5):1670–77. doi:10.1158/1078-0432.CCR-1103-3.
5. Malczewski A, Marshall A, Payne MJ, Mao L, Bafaloukos D, Si L, Pectasides D, Fountzilas G, Guo J, Gogas H, et al. Intravenous high-dose interferon with or without maintenance treatment in melanoma at high risk of recurrence: meta-analysis of three trials. Cancer Med. 2016 Jan;5(1):17–23.
6. Ascierto PA, Del Vecchio M, Mandalá M, Gogas H, Arance AM, Dalle S, Cowey CL, Schenker M, Grob -J-J, Chiarion-Sileni V, et al. Adjuvant nivolumab versus ipilimumab in resected stage IIIB-C and stage IV melanoma (CheckMate 238): 4-year results from a multicentre, double-blind, randomised, controlled, phase 3 trial. Lancet Oncol. 2020;21(11):1465–77. doi:10.1016/S1470-2045(20)30494-0.
7. Eggermont AMM, Blank CU, Mandala M, Long GV, Atkinson VG, Dalle S, Haydon A, Lichinitser M, Khattak A, Carlino MS, et al. Prognostic and predictive value of AJCC-8 staging in the phase III EORTC1325/KEYNOTE-054 trial of pembrolizumab vs placebo in resected high-risk stage III melanoma. Eur J Cancer. 2019;116:148–57. doi:10.1016/j.ejca.2019.05.020.
8. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, Hamid O, Robert C, Ascierto PA, Richards JM, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. Lancet Oncol. 2015;16(5):522–30. doi:10.1016/S1470-2045(15)70122-1.

9. Owen CN, Shoushtari AN, Chauhan D, Palmieri DJ, Lee B, Rohaan MW, Mangana J, Atkinson V, Zaman F, Young A, et al. Management of early melanoma recurrence despite adjuvant anti-PD-1 antibody therapy. Ann Oncol. 2020;31(8):1075–82. doi:10.1016/j.annonc.2020.04.471.
10. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, Bonnefoi H, Cameron D, Gianni L, Valagussa P, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2019;384 (9938):164–72. doi:10.1016/S0140-6736(13)62422-8.
11. Amaria RN, Menzies AM, Burton EM, Scolyer RA, Tetzlaff MT, Antdbacka R, Ariyan C, Bassett R, Carter B, Daud A, et al. Neoadjuvant systemic therapy in melanoma: recommendations of the International Neoadjuvant Melanoma Consortium. Lancet Oncol. 2019;20(7):e378–89. doi:10.1016/S1470-2045(19)30332-8.
12. Lui VK, Karpuchas J, Dent PB, McCulloch PB, Blajchman MA. Cellular immunocompetence in melanoma: effect of extent of disease and immunotherapy. Br J Cancer. 1975;32(3):323–30. doi:10.1038/bjc.1975.230.
13. Amaria RN, Reddy SM, Tawbi HA, Davies MA, Ross MI, Glitza IC, Cormier JN, Lewis C, Hwu W-J, Hanna E, et al. Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. Nat Med. 2018;24(11):1649–54. doi:10.1038/s41591-018-0197-1.
14. Amaria RN, Reddy SM, Tawbi HA, Davies MA, Ross MI, Glitza IC, Cormier JN, Lewis C, Hwu WJ, Hanna E, et al. Publisher correction: neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. Nat Med. 2018;24(12):1942. doi:10.1038/s41591-018-0252-y.
15. Polak ME, Borthwick NJ, Gabriel FG, Johnson P, Higgins B, Hurren J, McCormick D, Jager MJ, Cree IA. Mechanisms of local immunosuppression in cutaneous melanoma. Br J Cancer. 2007;96 (12):1879–87. doi:10.1038/sj.bjc.6603763.
16. Rozeman EA, Menzies AM, Van Akkooi ACJ, Adhikari C, Bierman C, van de Wiel BA, Scolyer RA, Krijgsman O, Sikorska K, Eriksson H, et al. Identification of the optimal combination dosing schedule of neoadjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma (OpACIN-neo): a multicentre, phase 2, randomised, controlled trial. Lancet Oncol. 2019;20 (7):948–60. doi:10.1016/S1470-2045(19)30151-2.
17. Rozeman EA, Hoefsmit EP, Reijers ILM, Saw RPM, Versluis JM, Krijgsman O, Dimitriadis P, Sikorska K, van de Wiel BA, Eriksson H, et al. Survival and biomarker analyses from the OpACIN-neo and OpACIN neoadjuvant immunotherapy trials in stage III melanoma. Nat Med. 2021;27(2):256–63. doi:10.1038/s41591-020-01211-7.
18. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–723. doi:10.1056/NEJMoa1003466.
19. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, Hamid O, Robert C, Ascierto PA, Richards JM, et al. Prolonged survival in stage III melanoma with Ipilimumab adjuvant therapy. Med N Engl J. 2016;375 (19):1845–55. Erratum in: N Engl J Med. 2018., p. 379(22):2185. doi:10.1056/NEJMoa1611299.
20. Tarhini AA, Edington H, Butterfield LH, Lin Y, Shuai Y, Tawbi H, Sander C, Yin Y, Holtzman M, Johnson J, et al. Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab. PLoS One. 2014;9(2):e87705. doi:10.1371/journal.pone.0087705.
21. Tarhini A, Lin Y, Lin H, Rahman Z, Vallabhaneni P, Mendiratta P, Pingpank JF, Holtzman MP, Yusko EC, Rytlewski JA, et al. Neoadjuvant ipilimumab (3 mg/kg or 10 mg/kg) and high dose IFN-α2b in locally/regionally advanced melanoma: safety, efficacy and impact on T-cell repertoire. J Immunother Cancer. 2018;6 (1):112. doi:10.1186/s40425-018-0428-5.
22. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. J Clin Oncol. 2015;33(17):1974-1982. doi:10.1200/JCO.2014.59.4358.
23. Schachter J, Ribas A, Long GV, Arance A, Grob -J-J, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet. 2017;390(10105):1853-1862. doi:10.1016/S0140-6736(17)31601-X.
24. Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob -J-J, Rutkowski P, Cowey CL, Lao CD, Schadendorf D, Wagstaff J, Dummer R, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol. 2018;19 (11):1480–1492. doi:10.1016/S1470-2045(18)30700-9.
25. Huang AC, Orlowski RJ, Xu X, Mick R, George SM, Yan PK, Manne S, Kraya AA, Wubbenhorst B, Dorfman L, et al. A single dose of neoadjuvant PD-1 blockade predicts clinical outcomes in resectable melanoma. Nat Med. 2019;25(3):454–61. doi:10.1038/s41591-019-0357-y.
26. Huang AC, Xu X, Orlowski RJ, Sangeeth MG, Chilukuri L, Kozlov A, Carberry M, Giles L, McGettigan S, Kreider K, et al. Safety, activity, and biomarkers for neoadjuvant anti-PD-1 therapy in melanoma. Abstract CT181. Clin Trials. 2018;2018:78.
27. Eggermont AMM, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, Haydon A, Lichinitser M, Khattak A, Carlino MS, et al. Adjuvant pembrolizumab versus placebo in resected stage III melanoma. N Engl J Med. 2018;378(19):1789–801. doi:10.1056/NEJMoa1802357.
28. Blank CU, Versluis JM, Reijers ILM, Sikorska K, van Houdt WJ, van Thienen JV, Adriaansz S, Mallo HA, Van Tinteren H, van de Wiel BA, et al. 3-years relapse-free survival (RFS), overall survival (OS) and long-term toxicity of (neo)adjuvant Ipilimumab (IPI) + Nivolumab (NIVO) in macroscopic stage III melanoma (OPACIN TRIAL). Ann Oncol. 2019;30(Suppl. 5):v533–v563. doi:10.1093/annonc/mdz255.003.
29. Lebbé C, Meyer N, Mortier L, Marquez-Rodas I, Robert C, Rutkowski P, Menzies AM, Eigentler T, Ascierto PA, Smylie M, et al. Two dosing regimens of nivolumab plus ipilimumab for advanced melanoma: 3-year results of CheckMate 511. ASCO. Presentation number 9516; 2021.
30. Long GV, Schadendorf D, Del Vecchio M, Larkin J, Atkinson V, Schenker M, Pigozzo J, Gogas HJ, Dalle S, Meyer N, et al. Adjuvant therapy with nivolumab (NIVO) combined with ipilimumab (IPI) vs NIVO alone in patients (pts) with resected stage IIIB-D/IV melanoma (CheckMate 915). AACR. Abstract CT004; 2021.
31. Schermers B, Franke V, Rozeman EA, van de Wiel BA, Bruining A, Wouters MW, van Houdt WJ, ten Haken B, Muller SH, Bierman C, et al. Surgical removal of the index node marked using magnetic seed localization to assess response to neoadjuvant immunotherapy in patients with stage III melanoma. Br J Surg. 2019;106 (5):519–22. doi:10.1002/bjs.11168.
32. Blank CU, Reijers ILM, Pennington T, Versluis JM, Saw RP, Rozeman EA, Kapiteijn E, Van Der Veldt AAM, Suijkerbuijk K, Hospers G, et al. First safety and efficacy results of PRADO: a phase II study of personalized response-driven surgery and adjuvant therapy after neoadjuvant ipilimumab (IPI) and nivolumab (NIVO) in resectable stage III melanoma. J Clin Oncol. 2020;38 (15_suppl):10002–10002. s.l.: Clinical trial information: NCT02977052. doi:10.1200/JCO.2020.38.15_suppl.10002.
33. Van Den Heuvel NMJ, Reijers ILM, Versluis JM, Rozeman AE, Jóźwiak K, Blommers KH, Saw RPM, Suijkerbuijk KPM, Kapiteijn E, Van der Veldt AAM, et al. 1085P-health-related quality of life in stage III melanoma patients treated. Ann Oncol. 2020. 31:S737. Clinical trial information OpACIN-neo /PRADO extension cohort NCT02977052.

34. Woo SR, Turnis ME, Goldberg MG, Bankoti J, Selby M, Nirschl CJ, Bettini ML, Gravano DM, Vogel P, Liu CL, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res. 2012;72:917–927.
35. Anderson AC, Joller N, Kuchrooet VK. Lag-3, tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. Immunity. 2016;44(5):989–1004. doi:10.1016/j.immuni.2016.05.001.
36. Ascierto PA, Melero I, Bhatia S,Bono P, Sanborn RE, Lipson EJ, Callahan MK, Gajewski T, Gomez-Roca CA, Hodi FS, et al. Initial efficacy of anti-lymphocyte activation gene-3 (Anti–LAG-3; BMS986016) in combination with nivolumab (Nivo) in pts with melanoma (MEL) previously treated with anti–PD-1/PD-L1 therapy. J Clin Oncol. 2017;35(15_suppl):9520. doi:10.1200/JCO.2017.35.15_suppl.9520.
37. Ascierto PA, Bono P, Bhatia S,Melero I, NyakasI MS, Svane M, Larkin J, Gomez-Roca C, Schadendorf D, Dummer R, et al. Efficacy of BMS-986016, a monoclonal antibody that targets lymphocyte activation gene-3 (LAG-3), in combination with nivolumab in pts with melanoma who progressed during prior anti–PD-1/PD-L1 therapy in all-comer and biomarker-enriched populations. ESMO, Abstract LBA18; 2017.
38. Amaria RN, Postow M, Tetzlaff MT,Ross MI, Glitza IC, McQuade JL, Wong MKK, Gershenwald JE, Goepfert R, Keung EZY, et al. Neoadjuvant AND adjuvant nivolumab with anti-lag3 antibody relatlimab for patients with resectable clinical stage III melanoma. ASCO; 2021.
39. Tronnier M, Mitteldorf C. Treating advanced melanoma: current insights and opportunities. Cancer Manag Res. 2014;6:349–56. doi:10.2147/CMAR.S49494.
40. Parato KA, Senger D, Forsyth PA, Bell JC. Recent progress in the battle between oncolytic viruses and tumours. Nat Rev Cancer. 2005;5(12):965–76. 26. doi:10.1038/nrc1750.
41. Forbes NE, Krishnan R, Diallo JS. Pharmacological modulation of antitumor immunity induced by oncolytic viruses. Front Oncol. 2014;4(191):27. doi:10.3389/fonc.2014.00191.
42. Russell SJ, Peng KW, Bell JC. Oncolytic virotherapy. Nat Biotechnol. 2012;30(7):658–70. doi:10.1038/nbt.2287.
43. Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, Delman KA, Spitler LE, Puzanov I, Agarwala SS,et al. Talimogene laherparepvec improves durable response. Clin Oncol. 2015;33(25):2780–88.
44. Dummer R, Gyorki D, Hyngstrom J,Berger AC, Conry RM, Demidov LV, Sharma A, Treichel S, Faries MB, Ross MI. Improved Recurrence-Free Survival (RFS) from a randomized phase 2 study of Neoadjuvant (Neo) Talimogene Laherparepvec (T-VEC) Plus Surgery (Surg) vs Surg for resectable stage IIIB-IVM1a Melanoma (MEL). Presented At: The 16th International Congress of the Society for Melanoma Research. Salt Lake City, UT-2019.
45. Dummer R, Gyorki D, Hyngstrom J, Berger A, Conry R, Demidov L, Chan E, Radcliffe H-S, Faries M, Ross M, et al. 3- year results of the phase 2 randomized trial for Talimogene Laherparepvec (T-VEC) Neoadjuvant treatment plus surgery vs Surgery in patients with resectable stage IIIB-IVM1a Melanoma. J Immunother Cancer. 2020. doi:10.1136/jitc-2020-SITC2020.0432.
46. Davar D, Karunamurthy A, Hartman D,De Blasio R, Chauvin JM, Ding Q, Pagliano O, Rose A, Kirkwood J and Zarour H. Phase II trial of Neoadjuvant Nivolumab (Nivo) and Intra-Tumoral (it) CMP-001 in high-risk resectable melanoma (Neo-C-Nivo): preliminary results. Presented At SITC 2019. J ImmunoTher Cancer. 2020;8.
47. Davar D, Karunamurthy A, Hartman D, DeBlasio R, Chauvin J-M, Ding Q, Pagliano O, Rose A, Kirkwood J, Zarour H, et al. Phase II trial of Neoadjuvant Nivolumab (Nivo) and intra-tumoral (it) CMP-001 in high-risk resectable melanoma (Neo-C-Nivo): final results. J Immunother Cancer. 2020;8(Suppl 3):A330–A330. doi:10.1136/jitc-2020-SITC2020.0303.
48. Menzies AM, Amaria RN, Rozeman EA, Huang AC, Tetzlaff MT, van de Wiel BA, Lo S, Tarhini AA, Burton EM, Pennington TE, et al. Pathological response and survival with neoadjuvant therapy in melanoma: a pooled analysis from the International Neoadjuvant Melanoma Consortium. Nat Med. 2021;27(2):301–09. doi:10.1038/s41591-020-01188-3.
49. Schadendorf D, Wolchok JD, Hodi FS, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob -J-J, Cowey CL, Lao CD, Chesney J, et al. Efficacy and safety outcomes in patients with advanced melanoma who discontinued treatment with nivolumab and ipilimumab because of adverse events: a pooled analysis of randomized phase II and III trials. J Clin Oncol. 2017;35(34):3807–14. doi:10.1200/JCO.2017.73.2289.
50. Ugurel S, Röhmel J, Ascierto PA, Becker JC, Flaherty KT, Grob JJ, Hauschild A, Larkin J, Livingstone E, Long GV, et al. Survival of patients with advanced metastatic melanoma: the impact of MAP kinase pathway inhibition and immune checkpoint inhibition - update 2019. Eur J Cancer. 2020;130:126–38. doi:10.1016/j.ejca.2020.02.021.
51. Moschos SJ, Edington HD, Land SR, Rao UN, Jukic D, Shipe-Spotloe J, Kirkwood JM. Neoadjuvant treatment of regional stage IIIB melanoma with high-dose interferon alfa-2b induces objective tumor regression in association with modulation of tumor infiltrating host cellular immune responses. Oncol J Clin. 2006;24(19):3164–71. doi:10.1200/JCO.2005.05.2498.
52. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, Lin NU, Litière S, Dancey J, et al; RECIST working group. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol. 2019 May; 20(5): e242. Erratum in: Lancet Oncol. PMID: 28271869; PMCID: PMC5648544. 2017, p. 18(3):e143-e152.
53. Trebeschi S, Drago SG, Birkbak NJ, Kurilova I, Călin AM, Delli Pizzi A, Lalezari F, Lambregts DMJ, Rohaan MW, Parmar C, et al. Predicting response to cancer immunotherapy using noninvasive radiomic biomarkers. Ann Oncol. 2019;30(6):998–1004. doi:10.1093/annonc/mdz108.
54. Dummer R, Brase JC, Garrett J, Campbell CD, Gasal E, Squires M, Gusenleitner D, Santinami M, Atkinson V, Mandalà M, et al. Adjuvant dabrafenib plus trametinib versus placebo in patients with resected, BRAFV600-mutant, stage III melanoma (COMBI-AD): exploratory biomarker analyses from a randomised, phase 3 trial. Lancet Oncol. 2020;21(3):358–72. doi:10.1016/S1470-2045(20)30062-0.