Adjuvant treatment for stage III melanoma in the era of targeted medicine and immunotherapy

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Practice points

- Until recently, interferon alpha has been the only approved drug for the treatment of adjuvant melanoma, with unclear overall survival benefit.
- Ipilimumab, an anti-CTLA-4 antibody, approved in 2015 for adjuvant treatment of melanoma, is the first immune check-point inhibitor for adjuvant treatment in cancer.
- Anti-PD-1 treatments are currently in advanced clinical trials for adjuvant melanoma.
- MAPK inhibitors, either as monotherapy or in combination may prove beneficial for stage III melanoma patients bearing *BRAF* V600 mutation-positive melanoma.
- Case reports of neoadjuvant treatments with MAPK inhibitors in *BRAF* V600-mutation positive melanoma demonstrate profound regressions of lymph node metastases as well as pathologic responses.
- Neoadjuvant treatments with immune check point modulators and with MAPK inhibitors are being tested in clinical trials.

The accelerated development in the treatment of metastatic melanoma, both in molecular targeted therapy and immunotherapy, is already starting to impact on adjuvant therapy in stage III melanoma. Following the approval of ipilimumab for adjuvant therapy in melanoma, clinical trials assessing other checkpoint modulators and MAPK pathway inhibitors as adjuvant treatments for melanoma are currently ongoing. As results from these trials mature in the next few years, a change in the landscape of adjuvant treatment for melanoma is expected, resulting in new challenges in treatment decisions such as optimizing patients selection through predictive and prognostic biomarkers, and management of treatment related adverse events, in particular immune related toxicities.

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Malignant melanoma, the fifth most common cancer in men and the seventh in women, continues to rise steadily over the past decades, with estimated 73,870 new cases and 9940 deaths in the USA in 2015, according to the US Surveillance, Epidemiology, and End Results (SEER) registry. Although median age at diagnosis and age of death from melanoma are in the sixth and seventh decades respectively, melanoma affects individuals at young ages as well, consequently ranking third in terms of average years of life lost, after childhood malignancies and testicular cancer [1–3]. Patients with thick primary tumors (T4N0M0, American Joint Committee on Cancer [AJCC]) and patients with regional lymph node metastases (T1–4N1–3M0, AJCC stage III) have a relatively

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high risk of recurrent disease and death, despite optimal surgery, with reported 60–75% recurrence rates and 30–70% 5-year survival rates for these stages [4]. This group of high-risk patients necessitates effective adjuvant therapy (following definitive surgery) in order to reduce the risk of relapse and death.

After decades of therapeutical stagnancy, recent years are noted for accelerated development in the field of melanoma therapy, as breakthrough progress both in molecular targeted therapy and immunotherapy have revolutionized the management and treatment for this disease. The discoveries of the RAF oncogene and the pivotal role MAPK pathway plays in the tumorigenesis and evolution of melanoma, along with the identification of immune checkpoints as critical regulators of the delicate equilibrium between the cancerous melanoma cells and the immune system, have led to the development of a variety of drugs for metastatic melanoma, all with significant impact on overall survival. Based on results from large randomized control trials, between 2011 and 2015, no less than seven new drugs were approved by the US FDA for the treatment of metastatic melanoma [5–16]. This stands in sharp contrast to adjuvant therapy in stage III melanoma, where since 1995 and until very recently, no new drugs were approved in two decades.

Clearly, the next step in adjuvant therapy for melanoma will include incorporation of drugs that have improved overall survival in metastatic disease, in hope to prevent, or at least substantially delay recurrences when used as adjuvant therapy.

This review will focus on currently approved adjuvant treatments for stage III melanoma and will try and portray the changing landscape of adjuvant melanoma management in the era of targeted molecular therapy and immunotherapy.

History of adjuvant immunotherapy & interferon in melanoma

The positive effects of stimulation of the host immune system against melanoma have been known for more than four decades. Over these years multiple approaches to stimulate the immune system in order to reduce the risk of relapse in high risk melanoma have been tested. These approaches included nonspecific immune adjuvants (e.g., Bacillus Calmette–Guérin vaccine, levamisole), antigen incorporated vaccines (MAGE-3, gp-100), granulocyte–macrophage

colony-stimulating factor (GM-CSF) and others. Disappointingly, none of these treatments proved beneficial compared with observation or placebo for stage III disease, with the exception of IFN- α 2b (IFN- α) [17–23].

IFN- α , a part of the interferon cytokine family, was discovered in the late 1950s by Isaacs and Lindenmann and is a member of type I interferons produced in response to infectious agents. Type I interferons exert an immunomodulatory effect through promoting antigen presentation and natural killer cell functions while restraining proinflammatory response, and by activation of the adaptive immune system through priming high affinity antigen-specific T- and B-cell responses [24–27].

In metastatic melanoma, several clinical studies with IFN- α have reported single-agent objective response rates of 13–24%, comparable with other agents at the time, such as recombinant IL-2 and dacarbazine. Interestingly, in metastatic melanoma, IFN- α seemed to be an important component of drug combinations with IL-2 and chemotherapy regimens (chemobiotherapy), which appeared to have greater antitumor activity than either agent alone, producing in approximately 10% of patients' durable remissions. Nevertheless, neither IFN- α monotherapy nor any combination with IFN- α produced a statistically significant impact on overall survival in metastatic disease. Despite the lack of survival gain in metastatic disease, the comparable activity (in terms of response rates) observed with IFN- α served as a basis for multiple clinical studies with IFN- α in high-risk resected melanoma. These studies evaluated different doses of IFN- α (low-dose ≤ 3 million international units [MIU]/dose, intermediate-dose 5–10 MIU/dose, high-dose ≥ 10 MIU/dose), various subspecies of IFN- α (IFN- α -2a, IFN- α -2b, IFN- α -2c) and multiple scheduling of therapies [28–36].

While some studies with low or intermediate dose interferon as adjuvant therapy for high-risk resected melanoma have demonstrated improvement in relapse-free survival, none showed an improvement in overall survival and consequently are not recommended as adjuvant therapy.

High dose interferon regimen, which includes an induction of intravenous administration five-times weekly for 4 weeks of 20 MIU/m², followed by 48 weeks of subcutaneous maintenance interferon thrice weekly of 10 MIU per meter

square has been evaluated in several clinical trials.

The ECOG 1684 (E1684) was the first randomized trial to compare high-dose IFN- α with observation as adjuvant treatment in high-risk patients (stage IIB and III melanoma) in 287 patients. At a median follow-up of 6.9 years, statistically significant improvements in 5-year relapse-free survival (37% in the treatment arm vs 26% with observation) and 5-year overall survival (46% in the treatment arm vs 37% with observation) were observed. Based on these results, the FDA approved in 1995 the use of IFN- α for postsurgical adjuvant therapy of melanoma [37]. However, additional follow-up at 12.6 years did not demonstrate a survival difference between the two arms, although a relapse-free survival benefit was preserved [38,39]. Although speculations have been raised regarding potential competing causes of death (vascular events or others) in the study cohort that might have influenced on the reduction in overall survival benefit in the presence of long standing relapse-free survival, subsequent trials, unfortunately, have not been as clear as had been hoped to clarify this issue. The results of a following trial, the ECOG 1690 trial (E1690), a larger three-arm trial (608 patients) of high-dose and low-dose IFN- α versus observation in high-risk melanoma, confirmed a 5-year relapse-free benefit but did not demonstrate an overall survival advantage [40]. In contrast to the E1684, the E1690 trial was designed with slightly different eligibility criteria (patients with T4 primary lesions without clinical evidence of lymph node metastasis [T4cN0] were not required to undergo lymphadenectomy) but more importantly, a post-relapse salvage therapy crossover was allowed. The crossover may have confounded interpretation of the survival benefit in that trial, in part explaining the unusual median overall survival of 6 years in the observation arm in this trial compared with 2.8 years in the E1684 observation arm. The subsequent ECOG 1694 trial compared high-dose IFN- α for 1 year with an experimental vaccine GM2-KLH/QS-21(GM2) in 880 patients with resected high-risk melanoma. The trial was closed prematurely after interim analysis indicated inferiority in relapse-free survival and overall survival at 2 years with the GM2 vaccine compared with high-dose IFN- α . Although concerns have been raised regarding the adequacy of the control arm and a potential

GM2 vaccine detrimental effect on survival in that trial, a more recent Phase III EORTC trial (EORTC 18961) which compared GM2 vaccine with observation in 1314 patients with stage II melanoma, did not show a difference in relapse-free survival or overall survival between the two arms at 4 years [41,42].

Pegylated-IFN- α (Peg-IFN α) has also been approved by FDA in 2011 for adjuvant treatment in stage III melanoma, based on a single trial, the EORTC 18991, in which 1256 patients received Peg-IFN- α (6 μ g/kg per week for 8 weeks induction period, then 3 μ g/kg per week maintenance) for an intended duration of 5 years, or were followed with observation. Initial analysis at a median follow-up of 3.8 years, reported a statistical significant 4-year recurrence-free survival rate of 45.6% in the Peg-IFN- α arm compared with 38.9% in the observation arm. A subsequent analysis of longer follow-up at a median of 7.6 years showed a diminished benefit in 7-year recurrence-free survival (39.1% in the Peg-IFN- α arm vs 34.6% for observation) which was only marginally significant, without significant increase in distant metastasis-free survival or overall survival. Notably, although not pre-planned, a subgroup analysis demonstrated a sustained and increased benefit in patients with an ulcerated primary tumor and microscopic nodal involvement, raising a hypothesis that lower stage and ulceration might be predictive for IFN sensitivity [43,44]. The EORTC 18081 clinical trial (NCT01502696) is currently evaluating adjuvant Peg-IFN- α for 2 years versus observation in patients with stage II primary ulcerated melanoma (T2-4N0M0). The trial is designed to prospectively assess the efficacy of Peg-IFN- α in low burden ulcerated melanoma.

Meta-analyses have tried to identify the actual benefit of interferon in the adjuvant setting. In spite of wide clinical heterogeneity between included trials, a consistent statistically significant benefit in relapse-free survival has been confirmed, but evidence of overall benefit is still mixed, with the largest individual patient data meta-analysis in over 6000 melanoma patients concluding a small but statistically significant advantage of 2.8% absolute difference in 5-year survival [44–50].

Although approved as adjuvant therapy for melanoma both in the USA and in Europe, IFN- α is not widely regarded as standard of care, and there is general agreement that patients with low risk stage II melanoma (depth <4 mm, no

ulceration, low mitotic rate of $<1 \text{ mm}^2$) which usually carries an excellent prognosis with 80% or better overall survival rate, can be safely observed and do not warrant further treatment, while IFN- α is reserved for high-risk stage II and stage III patients. Still, a search for more effective treatments has been ongoing, and only recently, when practice changing therapies have demonstrated meaningful survival benefit for metastatic melanoma, that a hope for a similar efficacy in adjuvant therapy could be expected.

Ipilimumab: the first immune check point inhibitor in the adjuvant setting in cancer

CTLA-4 is a key negative regulator of T-cell activation.

Inhibition of negative regulation through blocking of the CTLA-4 receptor has been shown to potentiate T-cell activation and promote adaptive immunity stimulation [51].

Ipilimumab, a fully human monoclonal antibody that blocks CTLA-4, was the first agent with a statistical significant overall survival benefit in metastatic melanoma and was approved in 2011 for this indication following superior efficacy over dacarbazine and gp-100 peptide vaccine in two Phase III trials [5,6]. Since then, longer follow-up has demonstrated that a subset of patients that were alive at 3 years (21%) maintained a plateau in survival curve afterwards, suggesting a long-term survival benefit for patients who survive for 3 years [52].

In 2008, the EORTC has launched a Phase III trial (EORTC 18071) of adjuvant ipilimumab versus placebo after complete resection of high-risk stage IIIA (if N1a, at least one metastasis $>1 \text{ mm}$), stage IIIB or stage IIIC with no in-transit metastasis melanoma. In this trial, 951 patients were randomly assigned (1:1) to receive either ipilimumab (10 mg/kg) or placebo every 3 weeks for four doses, then every 3 months for a maximum of 3 years, or until disease recurrence or intolerable toxicity. At a median follow-up of 2.74 years, ipilimumab reduced the risk of recurrence by 25% versus placebo, with a median relapse-free survival of 26.1 months in the ipilimumab arm versus 17.1 months in the placebo arm and a 3-year recurrence-free survival of 46.5% for ipilimumab versus 34.8% placebo, both statistically significant. The benefit in relapse-free survival was observed across all subgroups irrespective of the number of nodes involved, although in *post hoc* subgroup analyses the relapse-free survival gain seemed

more prominent in patients with microscopic lymph node involvement than in patients with palpable nodes [53].

Subsequently, in October 2015, based on results from this trial, the FDA expanded the approval of ipilimumab in melanoma to include adjuvant treatment of patients with stage III melanoma, with pathologic involvement of regional lymph nodes $>1 \text{ mm}$ who have undergone complete lymphadenectomy.

Yet, despite unequivocal improvement in recurrence-free survival, concerns have been raised regarding the trial primary end point and safety profile. First, overall survival was not a primary end point for this trial, as had been for almost all adjuvant interferon trials in the past. As debate has been ongoing regarding the elusive benefit in survival for interferon, it is viewed by many that a significant survival benefit should be a part of any additional adjuvant therapy in melanoma. Sondak and McArthur [54] have also pointed out to the potential effect of crossover on the outcome, as patients in the placebo group which eventually relapse with metastatic disease would be expected to receive ipilimumab. If survival does not differ significantly between patients in the ipilimumab adjuvant arm and patients in the placebo arm (ipilimumab upon relapse) it would suggest a benefit for ipilimumab regardless of stage and thus would be more reasonable to reserve its use for a more advanced stage, rather than exposing disease free patients to the toxic effects of this drug. Second, the adverse event profile of ipilimumab in that trial was substantial and exceeded the expected toxicity profile based on previous studies with 10 mg/kg of ipilimumab in the metastatic setting, resulting in a median of four doses of ipilimumab per patient (out of 16). In total, 54% of the patients in the ipilimumab arm experienced a grade 3 or 4 adverse event and 52% of patients in the ipilimumab arm discontinued treatment because of such event, resulting in approximately 40% of patients discontinuing treatment by the end of the initial four cycles and before maintenance therapy. While most immune-related adverse events resolved within 4–6 weeks, the median time for immune-related endocrinopathies was 31 weeks, and 44% of patients with endocrinopathies remained on hormone replacement therapies. The trial also reported five drug-related fatalities in the ipilimumab arm (1%) which included three patients with colitis, one patients with myocarditis and one patient with

multiorgan failure with Guillain-Barre syndrome, all of which occurred prior to initiation of maintenance therapy. Quality of life, undoubtedly a major concern in adjuvant trials was not reported in the initial report and is expected.

Ongoing clinical trials with checkpoint inhibitors as adjuvant therapy

• Ipilimumab versus IFN- α

The ECOG 1609 is a Phase III trial currently comparing high-dose ipilimumab (10 mg/kg, as evaluated in the EORTC 18071) and low-dose ipilimumab (3 mg/kg) versus high-dose IFN- α . Enrollment includes patients with completely resected stage III (IIIB and IIIC) or stage IV (M1a, M1b) melanoma. This study will assess the relapse-free survival and overall survival with high-dose and low-dose ipilimumab compared with IFN- α as primary outcomes, as well as global quality of life and toxicity of adjuvant ipilimumab as secondary outcome measures. The study estimated enrollment is approximately 1500 patients with primary completion date in 2018 (NCT01274338).

• Ipilimumab versus nivolumab

Nivolumab is a PD-1 immune checkpoint inhibitor. PD-1 is a transmembrane protein expressed on hematopoietic cells, and is a major pathway exploited by tumors to suppress and evade the immune control. PD-1 is a member of the immunoglobulin superfamily (related to CD28 and CTLA-4) and is expressed on the surface of activated T cells. Under normal conditions PD-1 suppresses unwanted or excessive immune response, thus minimizing the risk of autoimmune reactions. The mechanism by which PD-1 suppresses T-cell responses is similar but distinct from that of CTLA-4. While CTLA-4 downregulates T-cell activation through ligation to the B7 receptors, PD-1 ligation through PD-L1 and PD-L2 down-modulates activated T cells functioning to limit immune effectors at the site of activation. Chronic or persistent stimulation, seen by various tumor types including melanoma, leads a state of T-cell energy or 'exhaustion', in which T cells lose function and proliferative capacity in a suppressive tumor microenvironment [55–60].

Nivolumab, an IgG inhibiting antibody that targets PD-1, was compared against chemotherapy in two large randomized trials, in treatment naive and in ipilimumab and BRAF inhibitor (if *BRAF* V600 positive) treated patients. Both

trials had shown superior efficacy for nivolumab over chemotherapy, with higher survival rates, longer durable objective responses and fewer side effects. A combination of nivolumab and ipilimumab was also compared with ipilimumab alone in the CheckMate-069 study, where an objective response rate of 60% was observed for the combination compared with 11% with ipilimumab alone in patients with *BRAF* wild-type melanoma, resulting in a 60% reduction in the risk of progression or death with the combination treatment. Nivolumab is approved as monotherapy and as combination therapy with ipilimumab for metastatic melanoma [13–15].

Following promising results in a Phase I study in high risk stage III melanoma treated with nivolumab and multi-peptide vaccine followed by nivolumab monotherapy (relapse-free survival 47.1 months; overall survival not reached), a Phase III randomized, placebo-controlled, double-blind study is currently comparing nivolumab with ipilimumab (at 10 mg/kg) as adjuvant therapy in patients with stage IIIB/C or stage IV melanoma (no evidence of disease) with high risk for recurrence (NCT02388906) [61].

• Pembrolizumab versus placebo

Pembrolizumab is another PD-1 immune checkpoint inhibitor. Pembrolizumab gained accelerated approval in the USA based on results from a Phase Ib trial (KEYNOTE-001) where ipilimumab-naïve and ipilimumab-refractory melanoma patients received pembrolizumab at 2 mg/kg or 10 mg/kg every 3 weeks. A 24% objective response rate and a 58% 12-month overall survival were reported for the 2 mg/kg arm, with clinical benefit observed in ipilimumab-naïve, ipilimumab-refractory patients, and in patients with *BRAF*-mutant tumors with and prior BRAF inhibitor treatment. Pembrolizumab at 10 mg/kg every 2 weeks or every 3 weeks was also compared with ipilimumab in a Phase III study, where increased response rates and 6-month progression-free survival were observed in patients treated with pembrolizumab compared with ipilimumab (~33% compared with 11.9% and 47% compared with 26.5%, respectively) [11,12]. The EORTC 1325 (NCT02362594) trial is currently comparing pembrolizumab at a fixed-dose (200 mg) every 21 days for up to 1 year versus placebo for high-risk stage III melanoma after complete resection. The primary outcomes are recurrence-free survival for all participants (up to 3 years from first

participant in) and recurrence-free survival for participants with PD-L1-positive tumor expression. The EORTC 1325 is designed to allow crossover and receive pembrolizumab in case of relapse, and will enable to assess whether early access to pembrolizumab (in adjuvant treatment) is more beneficial than late access in case of relapse, a potential effect of crossover on outcome that was a concern in the EORTC 18071 trial (adjuvant ipilimumab).

• Pembrolizumab versus high-dose interferon

The SWOG S1404 is a Phase III trial comparing high-dose IFN- α with pembrolizumab in patients with high risk resected melanoma (stages III and IV with no evidence of disease). Expected accrual is 1378 patients. Patients will be randomized in a 1:1 ratio to either high-dose IFN- α or pembrolizumab at 200 mg for 52 weeks. Primary outcomes include overall survival, relapse-free survival in PD-L1 positive and negative subgroups. Estimated primary completion date is 2020 (NCT02506153) [62].

MAPK pathway & melanoma

The RAS–RAF–MAPK signaling pathway has been recognized for many years as a critical regulator of growth and survival in a broad spectrum of human tumors [63]. In nonmalignant cells, ligand interaction with its receptor activates a G-protein of the RAS family, which serves as a mediator to activate the RAF serine/threonine kinases (BRAF and CRAF) thereby initiating MEK phosphorylation, in turn leading to ERK phosphorylation and activation, a critical step in cellular growth and survival. In melanoma, *BRAF* and *NRAS* mutation leading to constitutive activation of this pathway are frequently observed (in ~50 and 15% of tumors, respectively), resulting in uncontrolled cell proliferation and survival [64]. This ‘oncogene addiction’ of the mutated melanoma cells has made the MAPK pathway particularly attractive for the development of various small molecules inhibitors designed to target different steps in this pathway.

Vemurafenib and dabrafenib are potent inhibitors of the kinase domain of mutated *BRAF*. Vemurafenib, the first RAF inhibitor to demonstrate a significant benefit in *BRAF*-mutant metastatic melanoma, was compared with dacarbazine in a randomized Phase III trial [9]. In this trial, 675 patients with previously untreated metastatic melanoma with *BRAF* V600E mutation

were randomly assigned to receive either vemurafenib (960 mg orally twice daily) or dacarbazine (1000 mg/m² of body surface area intravenously every 3 weeks). At 6 months, the overall survival rate was 84% in the vemurafenib arm compared with 64% in the dacarbazine arm. A following final analysis reported a progression-free survival prolongation of 6.9 months compared with 1.6 months, and a median overall survival of 13.6 months compared with 9.7 months in favor of the vemurafenib treated arm. Treatment with vemurafenib or dabrafenib is associated with rapid and dramatic tumor responses in many *BRAF*-mutated melanoma patients, a benefit observed across all subtypes of *BRAF* V600 mutations [9,65]. These responses, however, are usually short lived, resulting in a median progression-free survival period of 6–8 months. Resistance to BRAF inhibitors frequently develops through reactivation of MAPK pathway, resulting in fatal drug resistant disease in most patients [7,66,67]. Combination therapy of RAF inhibitors and downstream MEK inhibitors (trametinib, cobimetinib) has been shown to yield higher response rates, longer progression-free survival and longer overall survival compared with RAF inhibition alone. The combination of dabrafenib (150 mg orally, twice daily) and trametinib (2 mg orally, once daily) was compared with vemurafenib in 704 patients with *BRAF* V600 metastatic melanoma. Response rates, progression-free survival and overall survival significantly favored the combination arm, with 64% objective response, 11.4 months progression-free survival and 72% 1-year survival rate for the combination compared with 51%, 7.3 months and 65% objective response, progression-free survival and 1-year survival rate in the vemurafenib arm [7]. Similar efficacy was reported in response rate (68 vs 45%) and median progression-free survival (9.9 vs 6.2 months) favoring the combination of vemurafenib (960 mg orally twice daily) and cobimetinib (60 mg orally once daily for 21 days of a 28-day cycle) compared with vemurafenib alone [8].

Case reports on preoperative use of RAF inhibitors in bulky or inoperable *BRAF* V600-positive stage III melanoma describe substantial tumor reduction enabling resection of bulky primary unresectable tumors, along with marked histopathological regression of tumor cells in previously viable sites [68–73]. The significant clinical activity observed with RAF and MEK kinase inhibitors has made this pathway inhibition attractive for adjuvant and neoadjuvant therapy.

Ongoing clinical trials with MAPK inhibitors as adjuvant therapy

• Vemurafenib versus placebo

The BRIM8 is a Phase III double-blind, placebo-controlled study of vemurafenib as adjuvant therapy in patients with surgically resected stage IIC or stage III *BRAF*-mutant melanoma at high risk for recurrence. Primary efficacy outcome measure is disease-free survival. Final data collection date for primary outcome is June 2016 (NCT01667419).

Dabrafenib & trametinib versus placebo

The COMBI-AD is a Phase III double-blind study of dabrafenib in combination with trametinib versus placebo in adjuvant treatment for stage III *BRAF*V600 mutation-positive melanoma after surgical resection. Primary efficacy outcome measure is disease-free survival. Final data collection date for primary outcome is October 2016 (NCT01682083).

Neoadjuvant approach in the era of targeted drugs & immunotherapy

Neoadjuvant therapy is a mainstream treatment for various solid tumors including breast, esophageal, bladder, rectal cancer and others. In these tumors, neoadjuvant treatment has been shown to improve local control, organ preservation and even survival [74–78]. As might be expected, neoadjuvant clinical trials in melanoma with chemotherapeutic agents such as temozolomide or biochemotherapy (combination of cisplatin, vinblastine, dacarbazine and IL-2 with or without low-dose IFN- α) failed to show a benefit and were consequently abandoned [79–81].

In a report by Moschos *et al.* in 2006, 4 weeks of neoadjuvant high-dose IFN- α was

investigated in a small group of 20 patients presenting with palpable regional lymph nodes metastases (stage IIIB or IIIC melanoma). High-dose IFN- α treatment was followed by complete lymphadenectomy and standard adjuvant IFN- α for 48 weeks. In the published results, 55% of patients demonstrated clinical regression of their palpable tumors after 4 weeks of high-dose IFN- α and 15% demonstrated complete pathologic response in post-treatment lymph node tissue [82].

Neoadjuvant treatment with high-dose ipilimumab (10 mg/kg intravenously every 3 weeks for two doses) was evaluated in a single-arm study comprising of 33 patients with surgically operable regionally advanced melanoma. Although not a primary end point, a 9% objective response and 64% stable disease were reported. All patients had histologically documented residual melanoma at definitive surgery following two doses of ipilimumab [83].

There are currently multiple trials evaluating immunotherapies and MAPK inhibitors as neoadjuvant therapy for melanoma (Table 1).

Conclusion & future perspective

Several Phase III trials in adjuvant melanoma reported mostly disappointing results in recent years. The AVAST-M compared the VEGF monoclonal antibody, bevacizumab (7.5 mg/kg every 3 weeks), with observation in high-risk (stages IIB, IIC and III) resected melanoma patients. In a preplanned interim analysis report with a median follow-up of 25 months, the primary end point of overall survival was not met. However, there was a statistically significant improvement in disease-free interval for patients treated with bevacizumab compared

Table 1. Neoadjuvant trials in melanoma.

Trial name	Design (trial ID)	Primary objective
Neoadjuvant combination biotherapy of pembrolizumab and high dose IFN- α	Phase I single arm (NCT02339324)	Safety
Neoadjuvant and adjuvant checkpoint blockade in patients with clinical stage III or oligometastatic stage IV melanoma	Phase II randomized (NCT02519322)	Pathologic response
Neoadjuvant combination therapy with ipilimumab and high dose IFN- α for melanoma	Phase II (NCT01608594)	Safety
Neoadjuvant vemurafenib and cobimetinib in melanoma: NEO-VC	Phase I single arm (NCT02303951)	Operability at 18 weeks
Study of neoadjuvant use of vemurafenib plus cobimetinib for <i>BRAF</i> -mutant melanoma with palpable lymph node metastases	Phase II single arm (NCT02036086)	Safety
Neoadjuvant dabrafenib plus trametinib for AJCC stage IIIB-C <i>BRAF</i> V600 mutant melanoma	Phase II single arm (NCT01972347)	Pathologic response at 12 weeks
Combi-Neo study for stage IIIB-C and oligometastatic stage IV melanoma	Phase II randomized (NCT02231775)	1-year relapse-free survival

with observation, with a one and 2-year disease-free interval of 77 and 59% compared with 70 and 57%, respectively. Longer follow-up is needed to assess the 5-year overall survival rate [84]. The E4697 trial compared GM-CSF with or without a peptide vaccination versus placebo in patients with complete surgical resection of locally advanced stage III or stage IV melanoma. Neither adjuvant GM-CSF nor peptide vaccination significantly improved relapse-free survival or overall survival in these patients [22]. Finally, the DERMA trial assessed the efficacy of MAGE-A3 cancer immunotherapeutic compared with placebo in stage IIIB/IIIC melanoma patients with macroscopic nodal involvement and whose tumor showed expression of the *MAGE-A3* gene. The study first co-primary end point of disease-free survival in the overall MAGE-A3 positive population was not met, and the second co-primary end point of disease-free survival in a gene signature population is still expected.

Nevertheless, the treatment of metastatic melanoma has been revolutionized in the past years. MAPK pathway inhibitors in *BRAF*-mutant melanoma and immune checkpoint modulators have impacted immensely on overall survival and quality of life of these patients. As a continuum of the benefit observed in advanced melanoma, for the first time in 20 years a new agent, ipilimumab, is approved for adjuvant therapy in stage III melanoma, after demonstrating a statistical significant 3-year recurrence-free survival advantage compared with placebo. Although hard to predict, based on therapeutic effects of other approved agents in advanced melanoma and the numerous ongoing clinical trials in stage III melanoma, it is reasonable to assume we can expect some of these agents to demonstrate clinical benefit for patients of this group as well.

For adjuvant immunotherapy in melanoma, the repertoire of immune check point modulators (costimulatory and coinhibitory molecules) is rapidly growing and new agents are currently being tested in clinical trials. For example, LAG3 and TIM3, are immune check modulators commonly co-expressed with PD-1 on tumor-specific cytotoxic T cells. Anti-LAG-3 and anti-TIM-3 are currently being tested in clinical trials (NCT01968109 and NCT02608268, respectively), with anti-PD-1 as dual bloc or as monotherapy. Certainly, as we understand more of the mechanisms underlying tumor-host

interaction, new challenges in adjuvant immunotherapy in melanoma would develop. These challenges will include (among others): optimizing patient selection – as more therapies hopefully become approved in the following years, prognostic and predictive biomarkers must be developed in order to differentiate between patients who will derive the most benefit from each treatment, without exposing unnecessary ineffective and toxic treatments for this population. Will ulcerated primary melanoma become a biomarker for IFN- α , or the expression of PD-L1 have an impact on relapse-free survival or overall survival in adjuvant anti-PD-1 treatment? An important issue dealt for many years and yet to be determined is the optimal duration of adjuvant treatment in melanoma, particularly in the face of treatment associated toxicity. Regimens durations currently range from 1 year (anti-PD-1 clinical trials) through 3 years (ipilimumab) to 5 years (pegylated IFN α), but tolerability reported in clinical trials is much lower, such as a median of four cycles out of 16 in the ipilimumab adjuvant trial and only a 15% 5-year completion rate in the Peg-IFN α EORTC trial. Probably most important and challenging would be the management of immune related adverse events in this healthy patient population. Clearly, in the interferon era, the benefit observed with therapy outweighed the risks of toxicity in many cases, in the absence of effective salvage treatments. In the ipilimumab trial, drug-related toxicity was substantial and exceeded the expected toxicity profile. It has been suggested that the lower tumor burden in the adjuvant patient population might result in a lesser suppressive tumor-targeted immune response. As more immunotherapies become available, toxicity is expected to be of considerable importance in adjuvant therapy.

For molecularly targeted therapy, the high response rates observed with MAPK pathway inhibitors in metastatic melanoma are expected to translate into positive outcomes in adjuvant clinical trials, as reports from neoadjuvant treatments in selected patients yielded profound clinical and pathological responses. As more insight is gained into the complex regulation of drug response and acquired resistance mechanisms, new strategies such as downstream ERK and PI3K/mTOR inhibition, might circumvent BRAF inhibition resistance.

In conclusion, the therapeutic renaissance experienced in metastatic melanoma will

undoubtedly influence the landscape of adjuvant stage III melanoma treatment and perhaps even of high risk stage II disease. With increase in drug armamentarium against melanoma, deeper insight into tumor host interaction and drug-resistance mechanisms, and with proper patient selection, we should expect future treatments for stage III melanoma to offer meaningful survival advantage and hopefully cure, for this high-risk population.

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