Neoadjuvant treatment for melanoma: current challenges and future perspectives

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Practice points

- Patients with potentially resectable advanced disease have historically had poor outcomes, with 5-year survival rates of 40–78%.
- HDI and ipilimumab are both approved for treatment of melanoma in the adjuvant setting.
- IFN- α is the only agent that has been shown and confirmed to improve survival in the adjuvant setting.
- Biochemotherapy refers to the use of chemotherapy with immune activating agents such as IFN- α and IL-2.
- Chemotherapy and biochemotherapy have been tested in the neoadjuvant setting, and have shown similar outcomes as when used in the metastatic setting.
- Neoadjuvant IFN- α improved disease-free and overall survival in patients with clinical response, though these results did not reach statistical significance.
- Ipilimumab has promising activity in the neoadjuvant setting, and favorably alters the tumor microenvironment.
- T-VEC, a herpes simplex type I-derived oncolytic immunotherapy that is injected directly into melanoma lesions, has been approved for use in patients with unresectable melanoma, and is being assessed in the neoadjuvant setting.
- Combination therapies with immune checkpoint inhibitors and immune stimulating agents, such as pembrolizumab and HDI or ipilimumab and HDI, are currently under way.
- Trials of *BRAF* inhibitors alone and in combination with immunotherapy in the neoadjuvant setting are underway.

There will be an estimated 76,100 new cases of melanoma diagnosed in 2015 and 9710 deaths. Patients with stage I/II disease have excellent outcomes, and the treatment landscape for patients with metastatic disease has been transformed by the approval of several immune checkpoint inhibitors and molecular targeted therapies. Patients with stage III disease, however, continue to have very limited options, as the only agent shown to improve survival in the adjuvant setting is high-dose IFN- α . Neoadjuvant trials of chemotherapy and chemobiotherapy have not been successful, and while neoadjuvant ipilimumab and high-dose interferon have shown promise in small trials, neither agent has been approved. Current trials are testing immune therapy and targeted therapy combinations in the neoadjuvant setting.

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The approval of multiple new agents has shifted the treatment paradigm for inoperable recurrent and metastatic melanoma, but patients with loco-regionally advanced melanoma that is resectable continue to have poor outcomes. Half of patients with lymph node metastases, satellite lesions or in

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transit metastases (i.e., locoregional disease) will have recurrence and die of metastatic disease [1]. The 5-year survival for patients with stage I/II is 97% [2], but the prognosis for patients with stage IV melanoma has historically been poor, with 1-year survival rates ranging from 62 to 33%, depending on the location of metastases [2]. The recent approval of multiple targeted and immunotherapies is rapidly improving outcomes; for example, in a recently published Phase III study, 1-year survival with pembrolizumab was 74.1%, 68.4% with ipilimumab dosed every 2 weeks and 58.2% with ipilimumab dosed every 3 weeks [3]. In patients with *BRAF*-mutant melanoma, 1-year overall survival (OS) is 74% with dabrafenib plus trametinib, and 68% with dabrafenib alone [4]. Similarly, 9-month overall survival is superior with vemurafenib and cobimetinib compared with vemurafenib with placebo (81 vs 73%; $p = 0.046$; hazard ratio [HR]: 0.65) [5]. Patients with stage III melanoma have 5-year survival rates of 78, 59 and 40% for stage IIIA, IIIB and IIIC, respectively [2]. The standard of care for patients with advanced locoregional disease for the past 20 years has been surgery followed by adjuvant high-dose interferon therapy. In October 2015, the US FDA approved adjuvant ipilimumab for use in patients with stage III, based on results from the Phase III EORTC 18071 trial, in which adjuvant ipilimumab at 10 mg/kg reduced the risk of recurrence by 25%, compared with placebo (HR: 0.75; 95% CI: 0.64–0.90; $p < 0.002$) [6]. Data on overall survival with adjuvant ipilimumab are not yet available. Because no adjuvant therapy other than IFN- α [7] has been shown to improve the overall survival of this patient population, novel approaches to the assessment of new agents and combinations for management of stage III melanoma are urgently needed.

Neoadjuvant therapy has potential advantages over standard postoperative adjuvant therapy in patients with locally advanced disease. In several malignancies, including breast, bladder and esophageal cancer, neoadjuvant chemotherapy improves survival outcomes compared with surgery alone [8–10]. Furthermore, neoadjuvant treatment may shrink tumors, making them more readily resectable, and thus confer better outcomes on this patient population. The advantages of neoadjuvant treatment are multifold: early systemic treatment may decrease the risk of metastasis, can potentially eradicate micrometastatic disease and facilitate surgical resection by reducing the local as well as distant microscopic extent

of the tumor. Finally and perhaps most important to the development of neoadjuvant therapy, obtaining tumor samples before and after treatment permits the *in vivo* assessment of tumor response, and affords us some understanding of immunologic mechanisms of tumor response and modulation of the tumor microenvironment.

Neoadjuvant chemotherapy

Shah *et al.* conducted a Phase II trial of neoadjuvant temozolomide (TMZ) in patients with potentially resectable stage III or IV melanoma [11]. Patients were treated with the extended dosing regimen, which consists of 75 mg/m²/day of TMZ by mouth for 6 weeks, followed by 2 weeks off. The trial's primary goal was to determine the objective response rate (ORR) in comparison to that reported with treatment with TMZ in the metastatic setting, to see if responses with neoadjuvant treatment were higher. Most patients received one cycle or less of TMZ, with treatment discontinued due to progression of disease. Of 22 enrolled patients, 19 were assessable for response; 14 of these had stage III and five had stage IV melanoma. ORR was 16% (one partial response and two complete responses); notably, all responders had stage III disease. Four patients with stable disease (SD) underwent complete excision and remained free of disease. A total of 12 patients had progression of disease, six of whom were rendered free of disease with surgery, although five ultimately recurred. All went on to receive systemic therapy. Of concern, the clinical activity of temozolomide was not different from that observed with treatment in the metastatic setting [11,12], making it unlikely that this agent would be of benefit as a single agent in the neoadjuvant setting.

Expression of MGMT, an enzyme that repairs guanine methylation caused by TMZ, may be a mechanism of drug resistance to TMZ. In the neoadjuvant TMZ study, four pretreatment tumor samples and five post-treatment samples were analyzed for MGMT promoter methylation. Two of these were from patients with CR, and the remainder from patients with progressive disease (PD). The MGMT promoter was unmethylated in all six samples, suggesting that the gene was not methylation-silenced. This study sample size is very small but argues that epigenetic silencing of MGMT may not be required for response to TMZ. Similar findings were noted in other studies [13,14], although in a

more recent study, MGMT promoter methylation was found to be associated with increased response rate and prolonged PFS (in metastatic disease) [15].

Neoadjuvant biochemotherapy

Biochemotherapy (BCT) refers to the use of combinations of cytotoxic chemotherapeutic agents with immunotherapies generally including IFN and IL-2. The various regimens of biochemotherapy have been reported to achieve response rates of about 50% in the metastatic disease setting [16]. Approximately half of patients with complete responses have had durable responses, suggesting that such combinations may have a goal of survival improvement in a subset of patients where complete response of the disease can be achieved [17,18]. On this basis, biochemotherapy was studied in the adjuvant and neoadjuvant settings (Table 1).

A Phase II study of neoadjuvant cisplatin, vinblastine, dacarbazine, IL-2 and IFN- α was conducted for melanoma patients with local-regional metastases (stage III, with nodal, satellite/in-transit metastases and/or local recurrence) [18]. Of 65 patients enrolled, 64 were eligible for response and toxicity assessment. Patients received two to four cycles of biochemotherapy before undergoing surgery, and patients with tumor regression after neoadjuvant BCT received two additional courses postoperatively. Of 28 patients (44%) who had a partial response, four (6.5%) had a pathologic complete response (pCR), resulting in an ORR of 50%. Although there was no correlation between pretreatment tumor burden and response ($p = 0.34$), patients who achieved a pCR had a significantly lower tumor burden than patients in other response categories ($p = 0.02$). In the aforementioned

Table 1. Completed neoadjuvant trials in potentially resectable melanoma.

Study (year)	Design	Primary objective	Treatment used	Main findings	Ref.
Chemotherapy and biochemotherapy					
Shah <i>et al.</i> (2010)	Single arm Phase II	Objective response rate	TMZ	Only patients with stage III disease responded Response rates similar to TMZ in metastatic setting	[11]
Buzaid <i>et al.</i> (1998)	Single arm Phase II	Clinical and histological response	Cisplatin, vinblastine, dacarbazine, IL-2, IFN- α	IL-2 and IFN- α did not improve on ORR or pCR compared with chemotherapy alone [†]	[18]
Gibbs <i>et al.</i> (2002)	Single arm Phase II	Safety and clinical response	Cisplatin, vinblastine, dacarbazine, IL-2, IFN- α	Persistence of positive lymph nodes associated with worse outcomes and median PFS of 7 months [†]	[19]
Lewis <i>et al.</i> (2006)	Multicenter Phase II	RFS, OS	Cisplatin, vinblastine, dacarbazine, IL-2, IFN- α	Lower relapse rate and improved survival for patients with a microscopically positive SLN compared with patients with clinically detected gross positive LN	[20]
Immunotherapy					
Moschos <i>et al.</i> (2006)	Single arm Phase II	Clinical response, pCR	High-dose IFN- α	DFS and OS were longer in responders [†] Increased CD11c ⁺ and CD86 ⁺ tumor infiltrating cells noted Downregulated STAT3, upregulated STAT1 and TAP2	[21]
Tarhini <i>et al.</i> (2014)	Single arm Phase I/II	Biomarker data, safety	Ipilimumab	Median PFS 11 months Increased Treg correlated with improved PFS Decreased MDSC, increased T-memory cells and TILs Activated CD4 and CD8 antigen-specific T cells	[22]

[†]These regimens were abandoned because biochemotherapy did not give a survival benefit in the metastatic setting.

DFS: Disease-free survival; LN: Lymph node; MDSC: Myeloid derived suppressor cell; ORR: Objective response rate; OS: Overall survival; pCR: Pathologic complete response; PFS: Progression-free survival; RFS: Recurrence-free survival; SLN: Sentinel lymph node; TIL: Tumor-infiltrating lymphocyte; TMZ: Temozolomide.

neoadjuvant temozolomide study, only patients with stage III (and no patients with stage IV disease) responded [11], suggesting that lower tumor burden and earlier stage of disease progression may be an important parameter for neoadjuvant treatment. Of concern, the addition of IL-2 and interferon gave ORR that are similar to what has been reported in a trials of chemotherapy with cisplatin, vinblastine and dacarbazine (ORR: 48%), as was pCR (10%) [23]. One possible reason is that the cytotoxicity of chemotherapy may negate the immune stimulating and immune modulating effects of immune therapy.

In a second study of neoadjuvant BCT in patients with stage III melanoma, 48 patients received two cycles of cisplatin, vinblastine, dacarbazine, IL-2 and IFN- α (the same regimen described in the prior cited study) with treatment cycles given before and after dissection of involved lymph nodes. Of 36 patients with measurable disease, 14 patients (38.9%) had clinical antitumor response, including one CR (2.8%) and 13 PR (36.1%). At a median follow-up of 31 months, 31 patients (64.6%) were without disease progression, and 79.2% were alive. Not surprisingly, the number of positive lymph nodes at the time of surgery was predictive of both PFS and OS, and the persistence of positive lymph nodes was associated with a poor outcomes, and median PFS of 7 months. These authors noted, as did the previously described study, that clinical and pathologic responses to BCT were predictive of survival outcomes. Overall, survival impact was of uncertain significance as this study population was heterogeneous in terms of prior treatment, extent of disease and prior surgical resections [19].

Although the response rates to BCT in both Phase II neoadjuvant trials were comparable, BCT did not demonstrate a survival advantage when compared with chemotherapy alone in the metastatic setting [24]. Reasons for this may include the potential immunosuppressive effect that chemotherapy has upon the anti-tumor immune system, which may have mitigated the positive immunomodulating effects of biotherapy with IL-2 and IFN- α . The use of attenuated dosages of both IL-2 and IFN- α may also be at issue. Unfortunately, none of these studies reported immunologic studies that might allow us to understand whether there was any modulation of the tumor microenvironment

or the circulating immune response after treatment. The ability to conduct mechanistic studies of tumor tissue and blood is one of the unique assets of neoadjuvant study design, and might have elucidated the effects of treatment upon the tumor parenchyma, and circulating immune parameters – and thereby have provided us a more sound rationale for moving forward with these regimens.

A larger Phase II study of neoadjuvant biochemotherapy for patients with stage III melanoma was conducted with the purpose of evaluating neoadjuvant biochemotherapy in a multicenter patient population [20]. Patients received two cycles of neoadjuvant biochemotherapy with cisplatin, dacarbazine, vinblastine, IL-2 and IFN- α , followed by complete regional lymphadenectomy and two postoperative cycles of treatment. Of 92 eligible patients, 50 (54%) had measurable disease. Two patients (4%) had a complete response and 11 patients (22%) had a partial response. ORR was 26%. Half of patients had stable disease and four (8%) had progressive disease. At median follow-up of 40.4 months, 58 patients (64%) remained free of disease. This study was different from the aforementioned trial in that a larger number of patients with positive SLN were enrolled (38 vs 12.5%) [19]. However, this study did not show a significant difference in RFS or OS for patients with a positive SLN compared with those who had clinically apparent disease. This highlights the difficulty that is encountered in attempting to interpret these studies; patients with stage III melanoma have a wide range of potential outcomes that is altered by the presence of ulceration of the primary lesion [25] and the tumor burden in the involved nodal basin. Since these neoadjuvant studies were performed, a recent Phase III adjuvant trial of standard high-dose IFN- α -2b (HDI) compared with BCT showed superior RFS with BCT (4.0 vs 1.9 years; HR: 0.75), but no difference in 5-year OS [26] as noted above. Overall, toxicity was worse in the BCT arm. One can only speculate whether the paucity of complete responses observed with BCT may be the basis of this lack of any survival benefit of the regimen.

Neoadjuvant immunotherapy

• HDI

Adjuvant HDI is the only treatment that has consistently been shown to improve RFS in

melanoma patients at high risk of recurrence (high-risk primary [T4 stage IIB] melanoma) and those with regional nodal involvement (N1–3, stage III) [26–29]. HDI has been evaluated in multiple Phase III trials attempting to delay or prevent subsequent metastasis and death, and has been evaluated in three Phase III US Intergroup trials (Eastern Cooperative Oncology Group; E-1684, E-1690, E-1694 [27–29]) for patients with high-risk primary melanomas or regional nodal involvement; this regimen has been associated with relapse-free survival (RFS) benefit in every study reported, and with OS benefit in two studies (E-1684 [29] and E-1694 [26,28]). More recently, adjuvant ipilimumab was approved for use in patients with stage III melanoma [6]. This was based on results from the Phase III EORTC 18071 trial, in which adjuvant ipilimumab at 10 mg/kg was shown to reduce the risk of recurrence by 25% versus placebo (HR: 0.75; 95% CI: 0.64–0.90; $p < 0.002$) [6]. To note, 10 mg/kg is higher than the 3 mg/kg ipilimumab dose that is approved in the metastatic setting. Data on OS with adjuvant ipilimumab are not yet available. ECOG led E-1609 (NCT01274338) is an ongoing randomized Phase III trial to evaluate adjuvant ipilimumab versus HDI in patients with resected high-risk melanoma. Patients are randomized to receive high-dose ipilimumab (10 mg/kg) or low-dose ipilimumab (3 mg/kg) and both arms are compared against the standard of HDI.

In a carefully immunologically assessed study, 20 patients with palpable regional lymph node metastases (stage IIIB and IIIC) or with recurrent regional lymphadenopathy underwent 4 weeks of induction HDI therapy, then complete lymphadenectomy followed by standard maintenance subcutaneous HDI for 48 weeks [21]. A total of 17 patients underwent biopsies at baseline and after 4 weeks of HDI in order to evaluate the pathologic response to therapy in addition to immunologic and histologic correlates of tumor response. One patient had clinical CR after 4 weeks of HDI treatment, and ten patients had clinical PR. Objective clinical response rate was 55%. Three patients (15%) had pCR, and residual microscopic disease in a single lymph node was found in two patients (10%) who had either clinical CR or PR. In total, 13 patients had pathologic evidence of macroscopic residual nodal disease after induction. At a median follow-up of 18.5 months, ten patients had no evidence of disease, seven

patients had died of metastatic disease, and three patients with metastatic disease were still alive. Median disease-free survival was 32 months for responders versus 10 months for nonresponders. Disease-free survival and OS were longer among patients with clinical response compared with nonresponders, but these results did not reach statistical significance (Table 1).

Tissue for IHC analysis of pre- and post-treatment biopsies was available for nine responders and eight nonresponders (17 total). After HDI induction, a significant increase in the number of CD11c⁺ and CD86⁺ tumor infiltrating cells was noted ($p = 0.047$ and $p = 0.074$, respectively), which probably represented a dendritic cell population. While a significant increase in the CD4⁺ cell infiltrate was not noted, nor was a significant increase in endotumoral CD11c⁺ and CD3⁺ cells noted in responders versus nonresponders, this may have been due to the small sample size. Based on immunohistochemistry (IHC), HDI did not differentially alter the immune cell infiltrates of clinical responders versus nonresponders, nor did HDI appear to alter angiogenesis, HLA expression of melanoma or mononuclear cells, proliferation, or apoptosis of melanoma cells. Overall, this suggested that the primary antitumor mechanism of HDI is an indirect immunomodulatory mechanism [21].

Subsequent studies of tissue obtained in this study aimed to discern the impact of HDI on the balance of pSTAT1 and pSTAT3 [30]. STAT1 plays a prominent role in the effector immune response, whereas STAT3 is implicated in tumor progression and downregulation of the response to type I IFNs. Increased STAT1/STAT3 ratios before treatment correlated with increased OS ($p = 0.032$), although individual STAT1 and STAT3 levels did not correlate with overall survival. HDI was shown to downregulate pSTAT3 and total STAT3 levels in tumor cells and lymphocytes, whereas it upregulated pSTAT1. This suggests that HDI's mechanism of action is at least in part due to positive immune modulation. Furthermore, HDI augmented the pSTAT1/pSTAT3 ratio in both melanoma cells ($p = 0.005$) and lymphocytes ($p = 0.002$). HDI also prominently increased TAP2 expression on melanoma cells and infiltrating lymphoid cells, although TAP1 and MCH class I/II expression were not affected. Both TAP1 and TAP2 are required for MHC class I antigen presenting function, although no effect upon TAP1 was observed in this study [30].

Ipilimumab

A single-arm Phase II neoadjuvant study was performed in patients with stage IIIB-C melanoma testing the role of high dose ipilimumab (10 mg/kg iv. every 3 weeks × four doses total), bracketing definitive surgery (Table 1) [22]. Tissue was obtained at baseline and at definitive surgery, and serum/PBMC were collected at baseline, at week 6, and months 3, 6, 9 and 12 as well as at progression. Among 29 evaluable patients, median PFS was 15.5 months. Among 27 patients with available samples, a significant increase in circulating Tregs (CD4⁺CD25⁺Foxp3⁺) was noted in reference to baseline at week 6 ($p = 0.02$). Increased Treg were associated with improved PFS ($p = 0.034$). While this appears counterintuitive, one potential basis for this may be found in the various subsets of Treg that are now being dissected and have diverse functions in relation to the antitumor and autoimmune responses [31]. A significant decrease in all populations of circulating myeloid derived suppressor cells (MDSC) was noted, most notably among the DC in the monocytic gate (HLA-DR⁺/low, CD14⁺; $p < 0.0001$), a key MDSC subset [32]. Furthermore, a greater decrease in circulating monocytic MDSC was associated with improved PFS ($p = 0.03$). Circulating CD4 and CD8 antigen-specific T cells (gp-100, MART-1, NY-ESO-1 peptides) were increased after treatment, and this was notably in the absence of vaccine. In 24 patients with available tumor samples, there was a significant increase in CD8⁺ tumor-infiltrating lymphocytes (TIL; $p = 0.02$) and T memory cells ($p = 0.03$) [22]. Taken together, these data suggest that ipilimumab favorably alters the immune infiltrate of the tumor microenvironment, and increases melanoma antigen-specific T-cell responses.

Pembrolizumab

NCT02306850 is an actively recruiting neoadjuvant open label Phase II trial of pembrolizumab for unresectable stage III and IV melanoma, with the rationale that PD-1 inhibition may render tumors resectable (Table 2). Patients will receive a flat dose of pembrolizumab 200 mg every 3 weeks for at least 24 weeks, and up to 2 years. To be eligible, patients can have failed prior immunotherapy or targeted therapy, or may have received no systemic therapy prior to enrollment. In addition, the patient must have unresectable stage III or IV melanoma that could be amenable to curative resection if

the site(s) decreased in size by up to 50%, as per the investigator. The primary and secondary outcomes are resectability rate at 24 weeks and response by RECIST criteria at 24 weeks, respectively.

Future directions: combined modality immunotherapy

In the largest analysis of OS to date for patients with advanced melanoma who were treated with ipilimumab, data were pooled for 1861 patients from ten prospective and two retrospective studies, including two Phase III trials. Median OS was 11.4 months, including 254 patients with at least 3 years of survival follow-up. The 3-year survival rate was 22%, and the survival curve began to demonstrate a plateau at year 3. These data suggest that in patients who respond to immunotherapy, responses tend to be durable. However, while the duration of response with ipilimumab and with tremelimumab is unprecedented, the fact remains that most patients do not respond to treatment with these agents [3,33]. Combined modality approaches to overcome multiple immune checkpoints at one time, or integrating targeted antitumor therapy with immunomodulator therapy, or to induce tumor inflammatory infiltrates that appears to be critical to achieving benefit from these agents may allow us to induce responses in higher numbers of patients. This strategy has been successful in the metastatic setting; in a Phase I study, ipilimumab and nivolumab given concurrently yielded an objective response rate of over 40% [34]. This has been followed by Phase II and III trials that have suggested significant improvements in the overall response rate and CR rate that may be anticipated to improve overall survival. In a randomized, double-blind Phase II study of previously untreated patients with metastatic melanoma, ipilimumab plus nivolumab was compared with ipilimumab plus placebo [35]. The primary end point was the objective response rate. The rate of confirmed objective response was 61% in the combination group versus 11% in the ipilimumab monotherapy group ($p < 0.001$). A total of 22% obtained a CR in the combination group, with no CRs in the monotherapy group. Median PFS was not reached with the combination therapy group, and was 4.4 months with ipilimumab monotherapy (HR: 0.40; 95% CI: 0.23–0.68; $p < 0.001$). Interestingly, similar results for RR and PFS were observed

Table 2. Ongoing trials of neoadjuvant immunotherapy and targeted therapy in potentially resectable melanoma.

Study/NCT ID	Design	Randomized?	Primary outcome	Treatment used	Main findings/status
NCT02306850	Phase IIB	N/A	Resectability rate at 24 weeks	Pembrolizumab	Recruiting
NCT01608594	Phase II	Yes	Safety	10 mg/kg ipilimumab + HDI- α -2b or 3 mg/kg ipilimumab + HDI- α -2b	Recruiting
NCT02339324	Phase II	N/A	Safety	Pembrolizumab $\pm$ HDI- α -2b	Recruiting
NCT02519322	Phase II	Yes	Pathologic response	Neoadjuvant/adjuvant nivolumab vs neoadjuvant ipilimumab and nivolumab and adjuvant nivolumab	Not yet open
Targeted therapy					
NCT02303951	Phase II	N/A	Percentage of patients who become resectable at 18 weeks	Vemurafenib and cobimetinib	Recruiting
NCT02036086	Phase II	N/A	Feasibility	Vemurafenib and cobimetinib	Recruiting
NCT01972347	Phase II	N/A	Proportion of viable tissue after 12 weeks of treatment	Dabrafenib + trametinib	Recruiting
NCT02231775	Phase II	Yes	1-year recurrence-free survival	Surgery vs dabrafenib + trametinib followed by surgery	Recruiting
T-VEC					
NCT02211131	Phase II	Yes	Recurrence-free survival	Surgery vs T-VEC followed by surgery	Recruiting

HDI: High-dose IFN- α -2b; T-VEC: Talimogene laherparepvec.

in 33 patients with *BRAF* mutation-positive tumors [35]. In a randomized, double-blind, Phase III study of patients with previously untreated metastatic melanoma, nivolumab, nivolumab plus ipilimumab or ipilimumab alone were compared in patients with metastatic melanoma. PFS and OS were co-primary end points. Combined PD-1 and CTLA-4 blockade was more efficacious than either agent alone. Median PFS with ipilimumab and nivolumab was 11.5 months (95% CI: 8.9–16.7), as compared with 2.9 months (95% CI: 2.8–3.4) with ipilimumab (HR: 0.42). PFS with nivolumab was 6.9 months (95% CI: 4.3–9.5; HR for the comparison with ipilimumab: 0.57) [36]. Based on these results, the FDA approved the combination of anti-CTLA-4 and anti-PD1 in September 2015.

As combinatorial strategies have demonstrated efficacy in the metastatic setting, it stands to reason that they may be even more efficacious in less advanced disease, when tumor burden is lower and the tumor has inhibited the host immune system to a lesser extent. Several studies of neoadjuvant combination immunotherapy are currently under way (Table 2). NCT01608594 is a neoadjuvant ipilimumab and HDI trial in a randomized, open label Phase I study, in which patients with resectable disease will receive ipilimumab 10 mg/kg or 3 mg/kg plus HDI. Patients will undergo baseline biopsy and receive

treatment with ipilimumab and HDI concurrently; ipilimumab will be given every 3 weeks for two doses, and HDI will be given for 5 days out of 7 for 4 weeks, followed by thrice weekly injections for 2 weeks, followed by definitive surgery. HDI will be resumed after recovery from surgery with thrice weekly injections for 46 additional weeks. The primary outcome is safety, and secondary outcome measures include pathologic response rate, radiologic response rate, PFS and OS.

NCT02339324 is a trial of neoadjuvant pembrolizumab and HDI. It is a single arm study in which patients with resectable disease will receive pembrolizumab every 3–4 weeks for two doses, given concurrently with HDI on 5 consecutive days out of 7 for 4 weeks, followed by maintenance HDI for 2 weeks, followed by definitive surgery. Following definitive surgery, patients will receive maintenance therapy with pembrolizumab every 3 weeks given concurrently with HDI thrice weekly injections for 46 weeks. Safety is the primary outcome, and secondary outcome measures include immunologic biomarker assessment in the blood and tumor, radiologic and pathologic response rate, PFS and OS.

NCT02519322 is a Phase II randomized open-label trial of neoadjuvant checkpoint blockade in patients with clinical stage III or oligometastatic stage IV melanoma. Patients will receive

nivolumab followed by surgery and adjuvant nivolumab, versus adjuvant nivolumab alone, or neoadjuvant ipilimumab and nivolumab followed by surgery and adjuvant nivolumab. The primary outcome and secondary outcomes are pathologic response and immunologic response, respectively.

Molecularly targeted agents

BRAF inhibitors have yielded unprecedented antitumor results in the patient population with *BRAF* mutant (V600 E or K) tumors. *BRAF* mutations that lead to activation of the MAPK pathway occur in approximately 50% of cutaneous melanomas. The development of *BRAF* inhibitors (*BRAF*i) has represented a significant breakthrough in the treatment of advanced *BRAF*-mutant (V600E/K) melanoma, as they induce antitumor responses in >50% of patients bearing the V600E or V600K *BRAF*-activating mutations [37,38]. The ORR to *BRAF*i is 48.4%, but most responders only have partial responses; the median duration of response is still relatively short, in the range of approximately 6–7 months, with virtually all patients developing secondary resistance [39–41]. In the neoadjuvant setting, ongoing trials include neoadjuvant *BRAF*/MEK inhibitors (NCT02303951, NCT 02036086, NCT 01972347, NCT02231775) (Table 2) [42].

This is particularly interesting when one considers recent data showing that *BRAF*i drive T cells into the tumor microenvironment and increase melanoma antigen recognition, without impeding their function [43,44]. Future trials will likely include combination therapy with *BRAF*i and immunotherapy, in order to potentiate the T cell effect, or *BRAF*/MEKi and immunotherapy.

Intratumoral immunotherapy

Several intralesional immunotherapeutic agents have been evaluated, with the goal of inducing local tumor regression in the injected tumor, as well as inducing systemic immune responses that may impact distant tumors [42]. Intralesional Bacillus Calmette–Guérin was initially reported to be promising, with local and distant disease control, but these early findings were not reproduced in later studies [45–47]. Intralesional granulocyte–macrophage colony-stimulating factor (GM-CSF), and IFN- α have also been tested, with modest results reported [42,48,49]. The most successful intral-
esional therapy to date has been talimogene

laherparepvec (T-VEC). T-VEC is a herpes simplex type I-derived oncolytic immunotherapy that is injected directly into melanoma lesions. It is designed to selectively replicate in tumors and produce GM-CSF to enhance systemic antitumor immune responses. In a Phase III trial, T-VEC was compared with subcutaneous GM-CSF in stage IIIB/IV melanoma. Patients were randomized 2:1 to T-VEC or SC GM-CSF. The primary end point was durable response rate, defined as partial or complete months for >6 months, starting within 12 months. In an analysis that included 436 patients, ORR with T-VEC was 26%, with 11% CR, versus 6% with GM-CSF, with 1% CR. A 4.4-month improvement in OS was noted in the T-VEC arm, and this approached statistical significance ($p = 0.051$; HR: 0.787; CI: 0.62–1.00) [35,50]. In October 2015, T-VEC was approved by the FDA for use in unresectable melanoma of the skin or lymph nodes. Neoadjuvant combination trials of T-VEC with anti-CTLA-4 and anti-PD-1 antibodies are already underway in the metastatic setting. A Phase II, randomized clinical trial is evaluating single agent T-VEC in the neoadjuvant setting (Table 2, NCT02211131). Patients will receive neoadjuvant T-VEC followed by surgery versus surgery alone in resectable advanced stage melanoma. The primary outcome is efficacy of T-VEC plus surgery versus surgery alone on 2-year RFS, and secondary outcomes include efficacy of each regimen on 3-year and 5-year RFS, respectively, and pathologic response rate.

Conclusion

The current paradigm for systemic therapy of advanced melanoma is to treat patients who are *BRAF* mutant with *BRAF*i + MEKi, while the remainder of patients are treated with immunotherapy. There has been considerable debate whether, for *BRAF* mutant melanoma the proper order of therapeutic intervention ought to begin with *BRAF*/MEKi therapy, or with immunotherapy. This question is now under Phase III evaluation in the ECOG-ACRIN led intergroup trial EA6134, activated recently across the US cooperative groups. In future, additional factors such as ulceration, PD-1/PD-L1 expression and the extent of T-cell infiltration are likely to add new determinants that may permit the identification of patients who may benefit most from the anti-PD-1 and PD-L1-directed therapies. Moreover, immunophenotyping patients' tumor samples

may allow us to rationally determine which combination of therapies is most likely to work best, and to predict responses to treatment. A recent study aimed to investigate the roles of tumor-specific neoantigens and tumor microenvironment alterations in the response to ipilimumab. Whole exomes were analyzed from pretreatment melanoma tumor biopsies and matching germline tissue samples from 110 patients, and in a subset of 40 patients, transcriptome data were also analyzed. Overall mutational load, neoantigen load and expression of cytolytic markers in the immune microenvironment were significantly associated with clinical benefit, though no recurrent neoantigen peptide sequences predicted responder patient populations [51]. Other studies have also evaluated gene expression signatures, exome sequencing and IHC analysis of the tumor microenvironment [22,42,52].

In addition to potentially improving survival outcomes, tumor resectability and local control, neoadjuvant therapy has the major advantage of allowing us to witness the clinical and pathologic response of treatment in real time; studying the immune infiltrate in tumor tissue and peripheral blood will likely allow us to more rapidly and rationally develop combination strategies, and to define the basis for optimal selection of patients for the benefits of new therapies. Capturing data on pivotal cell populations such as myeloid derived suppressor cells, Tregs and tumor-associated macrophages, in addition to cytokines and chemokines in the peripheral blood and the tumor parenchyma across time-points while patients are receiving neoadjuvant treatment will allow us to understand who responds, and what the immune mechanisms of response are.

While HDI and ipilimumab are the only agents approved in the adjuvant setting, and thus far no therapy has been approved for treatment of melanoma in the neoadjuvant setting, an unprecedented number of immunotherapy and targeted antitumor therapy agents have been approved for use in the metastatic setting. Treatment with immune therapy and particularly immune checkpoint inhibitors has revolutionized the way we treat metastatic melanoma in a way that few could have imagined a mere 10 years ago; these agents need to be further studied in the neoadjuvant setting, where they may have an even higher likelihood of achieving meaningful survival benefits.

Future perspective

The ipilimumab experience has taught us that in patients who respond to immunotherapy, responses tend to be durable. Immune checkpoint inhibitors have revolutionized the treatment of metastatic melanoma, and most importantly, have elevated the prospects for overall survival in this patient population. However, the fact remains that most patients do not respond to treatment with these agents [3,33]. There is therefore an urgent need to optimize neoadjuvant therapy in patients with locally advanced disease. Neoadjuvant therapy has several potential advantages over adjuvant therapy, and has been shown to improve outcomes in several malignancies [8–10]. Neoadjuvant treatment may shrink tumors and render them resectable, and immunotherapy may harness the immune system and minimize the risk of local recurrence and distant metastasis by eradicating micrometastatic disease. A key factor will be to obtain tumor samples before and after treatment in order to assess tumor response, capture the immune infiltrate and develop a robust understanding of the immunologic mechanisms of tumor response and modulation of the tumor microenvironment. Immune checkpoint inhibitors such as ipilimumab, pembrolizumab and nivolumab, and molecularly targeted antitumor agents such as *BRAF* and MEK inhibitors may even more efficacious in less advanced disease, when tumor mediated immune suppression is likely less pervasive. Promising data relating to the combination of ipilimumab and HDI in the neoadjuvant setting have paved the way to move forward; current neoadjuvant combination trials include the evaluation of ipilimumab and HDI, pembrolizumab and HDI, ipilimumab and nivolumab, and combinations of *BRAF*/MEK inhibitors. The combination of *BRAF*/MEK inhibitors and other molecularly targeted antitumor agents with immunotherapy will be the next frontier for neoadjuvant studies in melanoma.

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