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REVIEWS

Predictive factors of neoadjuvant immune checkpoint blockade in melanoma

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ABSTRACT

This review describes the current body of literature and ongoing clinical trials examining neoadjuvant immune checkpoint inhibitors (ICI) for patients with resectable stage III and IV melanoma. Based on prior success in treating metastatic melanoma and as adjuvant therapy, ICIs are being explored in the neoadjuvant setting. There have been initial trials and there are many ongoing trials examining neoadjuvant ICI. Herein, we will review the clinical feasibility and efficacy of various neoadjuvant ICI regimens, explore pathologic and cellular responses, and present factors associated with predictive tumor response.

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Introduction

The introduction of immune checkpoint inhibition (ICI) has revolutionized the care of patients with metastatic melanoma. In the last 11 years, the anti-CTLA-4 drug ipilimumab (ipi), multiple anti-PD-1 agents, and the combination of anti-PD-1 therapy with ipi have been US Food and Drug Administration (FDA) approved as regimens for patients with metastatic melanoma. The anti-PD-1/PD-L1 drugs now FDA approved in melanoma include nivolumab (nivo), pembrolizumab,[1–3] and atezolizumab (anti-PD-L1) when used in combination with cobimetinib and vemurafenib.[4] These approvals were based on clinical trials demonstrating these therapies improved recurrence-free survival compared to historic therapies.[1–3,5,6] For example, in patients with previously untreated metastatic melanoma, the overall rate of survival at 1 year after treatment with nivolumab was 72.9% (95% confidence interval [CI], 65.5 to 78.9) as compared with 42.1% (95% CI, 33.0 to 50.9) in the dacarbazine group (hazard ratio for death, 0.42; 99.79% CI, 0.25 to 0.73; $P < .001$).[7] In addition to clinically evident metastatic disease, ICI has also been approved for adjuvant therapy after complete resection of stage III or IV disease to decrease chance of recurrence. Adjuvant therapy is defined as treatment that is given in addition to the primary (initial) treatment to decrease the risk of cancer recurrence. For example, adjuvant pembrolizumab after resection of high risk stage III melanoma, administered every 3 weeks for up to 1 year resulted in significantly longer recurrence-free survival than placebo.[8]

Given their success in patients with metastatic disease and as adjuvant treatment, exploration of neoadjuvant ICI in patients with resectable American Joint Committee on Cancer (AJCC) stage III and IV disease has rapidly emerged.[9] Neoadjuvant therapy is the administration of therapeutic agents before definitive therapy. For example, the administration of systemic therapy before surgical excision of a tumor. Similar to the use of neoadjuvant strategies used in other solid tumors, the preoperative utilization of ICI has many potential advantages. These include treating clinically occult metastatic disease, the ability to assess tumor treatment effects, and the potential to limit the extent of surgery and therefore morbidity from surgery.[10]

The use of ICI in the neoadjuvant setting in melanoma is further supported by the hypothesis that having abundant tumor antigen may augment anti-tumor responses to immune-directed therapies.[11] However, the disadvantages to the neoadjuvant approach include the potential for progression to unresectable disease while on neoadjuvant therapy and missed opportunity for surgery. Additionally, some patients may be cured with surgery alone, and thus may be unnecessarily exposed to the risks of ICI therapy.

Background

In addressing the advantages and disadvantages to the neoadjuvant approach, it is important to understand the distinct risks of both surgery and ICI. Clinically significant morbidity from complete lymph node dissection (CLND) is reported in up to 50% of patients. This includes both short-term (wound breakdown or infection, seroma, skin flap necrosis) and long-term (neuropathy, functional impairment, swelling, lymphedema) complications.[12] Inguinal lymphadenectomy as compared to axillary or cervical may have even higher rates of complications.[13,14] The potential advantages of forgoing CLND were highlighted in a recent large randomized trial: in patients who did not undergo CLND after a positive sentinel lymph node, the rate of lymphedema was only 6% compared to 24% of patients who underwent CLND ($p < 0001$).[15] These findings exemplify the potential benefits of strategies to minimize extensive surgical resection in patients with macroscopic nodal disease, where the current standard is CLND. For example, instead of the need for complete lymph node dissection, resection of clinically involved lymph nodes only may be an option, thereby reducing morbidity in patients treated with

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neoadjuvant therapy. Moreover, neoadjuvant therapy may also allow for surgical excision of oligometastatic disease after a mixed response to immune therapy, instead of the need for resection of multiple sites. For example, a patient with three sites of disease and complete radiographic response in two sites may undergo resection of the persistent site only.[16] One study demonstrated significantly longer mean overall survival in mixed responders who underwent surgery of isolated remaining disease compared to those who did not.[17]

On the contrary, some patients may be cured with surgery alone, and the application of either adjuvant or neoadjuvant ICI therapy may expose patients to unnecessary risk of ICI therapy. Because ICI can have distinct immunologic toxicities from non-tumor-specific activation of T cells (e.g., colitis), common terminology for toxicities was developed for immunotherapy. These toxicities are reported as immune related adverse events (irAEs, using the Common Terminology Criteria for Adverse Events (CTCAE) grading system to define grade 3 as severe and grade 4 as life-threatening).[18] Other examples of irAEs, which are graded by severity, include colitis, diarrhea, hepatitis, maculopapular rash, endocrine dysfunction, and hematologic abnormalities, among others. In a large trial of ICI in patients with metastatic melanoma, treatment-related adverse events of grade 3 or 4 occurred in 16.3% of the patients in the nivolumab group, 55.0% of those in the nivolumab-plus-ipilimumab group, and 27.3% of those in the ipilimumab group.[19]

As additional background on the advantages and disadvantages to treatment, it's important to understand disease assessment. For a patient with metastatic melanoma on ICI therapy, treatment response is typically determined with whole body imaging at defined intervals. However, response to ICI therapy can be complex and determination of benefit or disease progression not precise. Due to the complexity, criteria have been developed to define resistance to ICI therapy which includes having confirmatory imaging no sooner than 4 weeks to determine disease progression or therapy failure.[20] In short, determining benefit of ICI therapy from images can be challenging. With neoadjuvant therapy, the ability to precisely determine treatment effects is possible by examining the pathologic response to ICI at the time of surgical resection. After immunotherapy treatment, the tumor can be examined microscopically for treatment effects, including viability of cells, otherwise known as the pathological response. In fact, specialized pathologic criteria have been developed to standardize the assessment of tumor after ICI therapy.[21] In this review of neoadjuvant ICI therapy, the term pathological complete response (pCR) indicates no residual cancer cells on histology, whereas microscopic residual disease (MRD) indicates no clinically evident disease but the presence of microscopic tumor on final histology. The pathologic response after surgery may more clearly define which patients will benefit from ICI therapy and versus which patients should receive alternative treatments. For example, patients with pCR after surgery appear to have a survival advantage compared to those without pCR across many neoadjuvant ICI studies.[22]

Ultimately, the risks and benefits must be balanced as we refine which patients are appropriate for the neoadjuvant approach. In this review, we will investigate current clinical results from neoadjuvant immune therapy trials in melanoma. We focus on factors that may predict appropriate patient selection for the neoadjuvant approach and examine the predictive information that neoadjuvant therapy can provide.

Review of clinical data

For patients with unresectable melanoma, response to ipilimumab is around 15%, response to anti-PD-1 therapy is around 40%, and the response to anti-PD-1 plus ipi is around 60%.[23,24] Anti-PD-1 based therapy is now considered as one of the a standard first line therapies for patients with metastatic melanoma.[25,26] While serious irAE rates are just less than 20% with anti-PD-1 monotherapy, grade 3 or 4 irAEs are reported in more than 50% of patients receiving dual checkpoint inhibitor therapy.[19] Given the clinical implications in a potentially curative setting, neoadjuvant regimens have subsequently been designed to maximize benefit while minimizing the potential for severe irAEs. A summary of neoadjuvant trials is shown in Table 1.

Tarhini and colleagues investigated the use of neoadjuvant ipi and high-dose interferon alfa-2b (HDI) in patients with locally regionally advanced melanoma.[27] Thirty patients were randomized to neoadjuvant ipi 3 mg/kg q3 weeks for two doses before surgery followed by ipi 3 mg/kg q3 weeks x 2 doses then 12qweeks for four doses after surgery or ipi 10 mg/kg q3 weeks for two doses before surgery followed by ipi 10 mg/kg for q3 weeks x 2 doses then 12 qweeks for four doses after surgery.[27] Neoadjuvant HDI was administered to both groups concurrently at 20 MU/m^2/day intravenous (IV), 5 days/week then by a subcutaneous regimen (SC) at 10 MU/m^2/day before surgery.[27] Following surgery, the SC regimen was resumed as adjuvant therapy for 46 weeks in both groups.[27] Of 30 patients, 15 completed the entire course including HDI.[27] Grade 3–4 irAEs were significantly higher in patients who received ipi 10 mg/kg compared to those who received ipi 3 mg/kg (p - = .042).[27] For 28 evaluable patients, pathological complete response (pCR) was 32% (95% CI, 18–51) with no significant differences between the ipilimumab doses (pCR was 36% for ipi 3 mg/kg and 29% for ipi 10 mg/kg).[27] Additionally, two patients had only microscopic residual disease (MRD) at the time surgery.[27] Patients with a pCR appeared to have durable responses; at a median follow up of 32 months, 10/11 patients with a pCR/MRD and 8/10 patients with a radiological response were found to be disease free.[27] Overall, the pCR rates correlated well to clinical prognosis, supporting pCR as a potential predictor of outcome.[27] However, use of neoadjuvant ipi alone was not further explored given that anti-PD-1 regimens were proving to be less toxic and more efficacious in the metastatic setting and further neoadjuvant trials appropriately began to explore anti-PD-1 and combination therapies as neoadjuvant regimens.

Huang and colleagues explored the immunologic response and clinical outcomes for patients with stage III and oligometastatic stage IV melanoma treated with neoadjuvant pembrolizumab monotherapy.[28] Twenty-nine patients with resectable melanoma were given one dose of pembrolizumab (200 mg) prior to surgery.[28] Patients then underwent surgery at a median

Table 1. Neoadjuvant studies.

Study	Population	Neoadjuvant regimen	N: Total/ underwent surgery	Outcomes pCR* (%)/No. Recur after pCR	Total Grade ¾ irAEs (%)**	Median RFS^{+} (mo)	Median FU (mo)
NCT01608594 Tarhini 2018[27]	Locally/ Regionally advanced	Ipi 3 mg/kg x → surgery → Ipi 3 mg/kg OR Ipi 10 mg/kg → surgery → Ipi 10 mg/kg PLUS HDINF (both groups)	15/14 15/14	36% (5/14) 1 of 5 29% (4/14) 0 of 5	8 events 17 events	NR	32
NCT02434354 Huang 2019[28]	Stage III, Stage IV	Pembrolizumab x 1 → surgery → pembrolizumab x1 year	29/27 (path assessed)	30% (8/27) 0 of 8	7%	NR	25
NCT02437279 Blank 2018 OpACIN[29]	Stage III	I3N1 q3w x 2 → surgery → I3N1 x 2	10/ 10	78% (7/9) 0 of 7	90%	3 year 80%[30]	36[30]
NCT02977052 Rozeman2019OpACIN-neo[31]	Stage III	**A**: I3N1 q3w x 2 → surgery **B**: I1N3 q3w x 2 → surgery **C**: I3 q3w x 2 → N3 q2w x 2 → surgery	A: 30/30 B: 30/30 C: 26/25	A: 80% (24/30) 0 of 19 B: 77% (23/30) 0 of 17 C: 65% (17/26) 0 of 17	A:40% B: 20% C: 50%	NR	24[30]
NCT02519322 Amaria 2019[32]	Stage III, IV	A: nivo 3 mg/kg x 4 → surgery → nivo 3 mg/kg q2w x 13 B: I3N1 x 3 → surgery → nivo 3 mg/kg q2w x 13	A: 12/ 10 B: 11/ 11	A: 25% (3/12***) 0 of 3 B: 45% (5/11) 0 of 5	A: 8% B: 73%	NR NR	A: 15.0 B: 15.6

*pCR – pathologic complete response.
**irAEs – iimmune-related adverse events.
*** study reports 2 of 12 patients did not undergo surgery but still reports 3/12 patients had pCR after surgery, although seem like 3/10, 30% should be correct.
$^{+}$RFS – recurrence-free survival.
$^{-}$FU – follow up.

of 3 weeks (median 21 days, range 17–42) after pembrolizumab; all patients then initiated adjuvant pembrolizumab after surgery.[28] The rate of grade 3 or higher adverse events not attributed to pembrolizumab or to surgery alone was not higher than 30%, the prespecified safety endpoint (observed rate was 0%, $p = .0002$, z test); only 7% of all adverse events were grade 3.[28] On histologic assessment, 8 of 27 patients (29.6%, 95% CI 13.8–50.2%) had a complete (no residual tumor identified; n = 5) or major (10% or less viable tumor cells; n = 3) pathologic response.[28] At median follow up of 25 months, all patients with pathologic complete response remained disease free.[28] In contrast, patients without robust pathological responses at surgery had a poor prognosis with greater than 50% risk of recurrence despite adjuvant therapy.[28] This trial also demonstrated that pathological response after a single neoadjuvant dose can be used to predict clinical outcome, however limitations of this data include only having a 2 year follow-up period.[28]

The OpACIN (Study to Identify the Optimal Adjuvant Combination Scheme of Ipilimumab and Nivolumab in Melanoma Patients) trial investigated the feasibility of neoadjuvant ipi (3 mg/kg) + nivolumab (1 mg/kg).[29] Twenty patients with palpable stage III melanoma were randomized to receive either four courses of ipi 3 mg/kg + nivolumab 1 mg/kg every 3 weeks starting at week 6 post-complete regional lymph node dissection (CLND) (adjuvant arm), or to receive two courses of ipi 3 mg kg + nivolumab 1 mg kg every 3 weeks pre-surgery, followed by CLND at week 6 and another two courses ipi + nivolumab starting at week 12 (thus 6 weeks post-CLND; neoadjuvant arm).[29] All patients (n = 10) in the neoadjuvant arm underwent surgery and only 1/10 patients within each arm received all four courses of ipilimumab + nivolumab.[29] Toxicity was higher than expected with grade 3 or 4 adverse events occurring in 9/10 patients in each arm.[29] In the neoadjuvant arm, 7/9 (78%) of patients were reported to achieve profound pathological responses, with three pathological complete responses (pCRs), three near pCR (≤ 10% viable tumor cells), and 1 patient achieving a pathological partial response (pPR ≤ 50% viable tumor cells); of note, radiographic response did not necessarily correlate and tended to underestimate those with complete pathologic responses.[29] Again, the potential to gain important prognostic information early in a patients' treatment course was seen: none of the patients who achieved a pathological response within the neoadjuvant arm have relapsed thus far (median follow-up of 21.6 months after surgery).[29] Recently reported updated results revealed that after a median follow up of 36 months, the estimated 3-year recurrence free survival for the neoadjuvant arm was 80% (95% CI: 59%-100%) compared to 60% (95% CI: 36–100%) for the adjuvant arm.[30] This is the first evidence supporting that neoadjuvant IO therapy may be more beneficial than adjuvant, although given the low number of patients (10 in each arm), further study is required. Moreover, the toxicity in this trial was high, prompting need to explore potentially safer regimens that preserve efficacy.

The OpACIN-neo trial aimed to identify a dosing schedule of ipilimumab plus nivolumab that was less toxic than the OpACIN regimen but equally effective.[31] Rozeman and colleagues tested three different neoadjuvant dosing schedules of ipi + nivo without adjuvant therapy in 86 patients with resectable stage III melanoma.[31] Patients were randomized to one of three neoadjuvant therapy schedules.[31] The grouping cohort was

structured as follows: group A received two cycles of ipi 3 mg/kg plus nivo 1 mg/kg once every 3 weeks (n = 30), group B received two cycles of ipi 1 mg/kg plus nivo 3 mg/kg once every 3 weeks (n = 30), and group C received two cycles of ipi 3 mg/kg once every 3 weeks followed by two cycles of nivo 3 mg/kg once every 2 weeks (n = 26).[31] Group C was discontinued early due to significant toxicity and 1 patient in Group A died as a result of toxicity likely related to study drug.[31] Pathologic response appeared similar in all groups and occurred in 24 (80% [61–92]) patients in group A, 23 (77% [58–90]) in group B, and 17 (65% [44–83]) in group C.[31] Updated results revealed that after a median follow-up of 24 months, there were no differences in estimated RFS between the 3 groups; 90% for arm A (95% CI: 80–100%), 78% for arm B (95% CI 63–96%), and 83% for arm C (95% CI: 70–100%), although median recurrence free survival (RFS) was not reached in any of the arms.[30] Therefore, group B neoadjuvant dosing schedule (2 cycles of ipi 1 mg/kg plus NIVO 3 mg/kg) appeared to best tolerated with comparable rates of pathologic response.[31] Updated results combining patients from both OpACIN (median follow up 36 months) and OpACIN-neo (median follow up 24 months) trials reported that only 1/71 (1.4%) of patients who achieved a pathological response with neoadjuvant therapy relapsed, compared to 16/23 (69.6%) who relapsed without a pathological response.[30]

Amaria et al. aimed to establish the optimal neoadjuvant regimen in a randomized phase II trial of 23 patients with clinical stage III or oligometastatic stage IV melanoma by comparing neoadjuvant nivolumab to neoadjuvant nivolumab plus ipi.[32] Twelve patients were randomized to monotherapy of neoadjuvant nivo 3 mg/kg every 14 days for up to 4 doses and 11 patients were randomized to combined therapy of ipi 3 mg/kg plus nivo 1 mg/kg every 21 days for up to 3 doses, both groups received by adjuvant nivo 3 mg/kg every two weeks for 13 doses.[32] While radiologic outcomes were significantly improved in patients who received combination therapy vs monotherapy alone (p = .039), the higher pCR rates in the combination group was not significant (45% vs 25%, p = .40).[32] Substantial toxicity (73% grade 3 treatment-related adverse events [trAEs]), was seen in the combination group compare to treatment with nivolumab monotherapy (8% grade 3 trAESs).[32] The trial was stopped early because of progression while on neoadjuvant nivolumab that prevented surgical resection in 2 of 12 patients and because of the high rates of grade 3 trAEs during neoadjuvant combination treatment (73%; 8 out of 11 patients).[32] Improved recurrence-free survival, distant metastasis-free survival, and overall survival (OS) were observed in patients who achieved a pCR following neoadjuvant therapy versus those who did not.[32]

Clinical data summary

Overall, each of the aforementioned trials demonstrates that pathological responses from neoadjuvant ICI correlate to improved patient survival.[27–32] The OpACIN trial was the only to provide comparative data between neoadjuvant and adjuvant ICI therapy, with a 3 year RFS of 80% and 60%, respectively; however, no differences in irAEs were observed in neoadjuvant vs adjuvant arm.[29] Each study used different dosing strategies including dose and timing, such that the optimal dosing and timing remains to be defined. In general, greater toxicity was seen with higher doses or combination of ICIs,[27,31,32] although combination therapy did yield higher pCR rates and improved radiologic outcomes.[32] The greater toxicity and higher response rates seen with neoadjuvant combination ICI therapy are a similar pattern to what has been observed in patients with metastatic melanoma. Specifically, the regimen of nivo 3 mg/kg+ ipi 1 mg/kg has been associated with significantly less grade 3 to 5 adverse events compared to nivo 1 mg/kg+ ipi 3 mg/kg in patients with metastatic melanoma.[33] In the neoadjuvant studies discussed, one study arm and one study using the ipi 3 mg/kg+ nivo 1 mg/kg dosing regimen were discontinued to high rates of toxicity using these doses. Thus, it appears if using a combination ICI neoadjuvant strategy, the regimen of ipi 1 mg/kg+ nivo 3 mg/kg may be favored for the lower toxicity profile. For monotherapy, rates of grade 3 or higher irAEs with a single dose of 200 mg neoadjuvant pembrolizumab were the lowest (7%) compared to all other aforementioned neoadjuvant ICI regimens.[28] This may be due to cumulative toxicities with additional cycles, but no direct comparisons between a single dose of neoadjuvant therapy and multiple cycles has been performed.

The rates of adverse events with neoadjuvant ICI therapy can also be compared to those of adjuvant ICI therapy. The OpACIN trial had a rate of 90% irAEs in both the neoadjuvant + adjuvant arm and in the adjuvant arm (both ipi 3 mg/kg plus nivo 1 mg/kg).[29] In the Huang et al study of a single dose of anti-PD-1, the rate of grade 3 toxicity was 7%. Comparatively, a study with adjuvant pembrolizumab therapy (200 mg every 3 weeks for 18 doses) in resected stage III melanoma demonstrated grades 3 to 5 adverse events at a rate of 14.7%.[8] It's possible that the additional cycles in the adjuvant setting could lead to increases in toxicity, although this remains unknown.

Several other neoadjuvant regimens are currently being evaluated for their efficacy in treating advanced melanoma. Anti-PD-1 agents such as atezolizumab (NCT04020809) and pembrolizumab (NCT03757689) are being further investigated as neoadjuvant monotherapy. Many studies are also examining combinations of PD-1 blockade plus alternative drugs (NCT04303169, NCT02519322, NCT04330430, NCT04207086) that may act as equally effective but safer alternatives for melanoma treatment, relative to the toxicities associated with ipi. Lastly, given the established clinical benefit of adjuvant immunotherapy and the promising investigations of neoadjuvant immunotherapy, a current phase II trial with 500 patients is underway to compare the efficacy and safety of pembrolizumab given after surgery versus administration both before and after surgery in patients with stage III/IV melanoma (NCT03698019). This type of trial may further define the role of neoadjuvant therapy compared to adjuvant therapy. Other neoadjuvant options include talimogene laherparepvec (T-VEC) and radiation therapy or combination of either with ICI therapy. Ongoing trials have demonstrated synergism between radiotherapy and ICIs, revealing another possible avenue for neoadjuvant ICI use.[34]

Tumor and patient specific factors predictive of response to ICI

Factors predictive of response to checkpoint inhibitor therapy have been studied in the metastatic setting as well as the neoadjuvant setting. Since the majority of patients do not respond to anti-PD1 therapy and treatment comes with risk of adverse events, developing predictors could maximize patient benefit while minimizing toxicity. One advantage of the neoadjuvant approach is that the tumor can be more readily studied for effects of therapy given tissue availability at the time of surgery. Examining the tumor microenvironment (TME) is critical to the evaluation of immunotherapy.[35] T cell recruitment, DC activation, and IFN-γ activity within the TME are essential for tumor response to PD-1 antagonist therapy.[36–38] Furthermore, tumor mutational burden and tumor PD-L1 expression have been explored to predict response to ICI in the metastatic and neoadjuvant setting.[28,32,39–41] Although many tumor features have been reported to predict response to ICI in the literature to date, there are currently no predictive biomarkers informing standard of care use of ICI in melanoma. The confirmed pathologic diagnosis of Stage IIII or IV melanoma is currently the only necessary information needed to make treatment decisions. However, there are many prediction tools being explored.

PD-L1 expression on tumor is the most well studied in terms of ability to predict response to anti PD-1 therapy. However, in melanoma, PD-L1 tumor expression does not reliably predict response to therapy. In review of 451 patients, PD-L1 expression in pretreatment tumor biopsy samples was correlated with response rate, progression free survival, and overall survival; however, a subset of patients with PD-L1–negative tumors also achieved durable responses.[39] Thus while PD-L1 expression can be predictive of benefit in some patients, no standard exists in melanoma for defining PD-L1 positivity. Furthermore, given the potential for responses in PD-L1 negative patients, PD-L1 is is currently not used to guide treatment decisions. Importantly, PD-L1 expression was not required for entry in OpACIN, nor other neoadjuvant immune therapy trials in melanoma.

In the neoadjuvant setting, assessment of surgical specimens post therapy has yielded preliminary predictive markers. Pre-treatment biopsies and post treatment surgical specimens were studied in detail to identify distinguishing factors of patients with pathologic response and to dissect underlying mechanisms of checkpoint blockade by Huang et al.[28] They observed a distinct inflammatory gene signature of the TME prior to therapy that correlated with response to neoadjuvant anti-PD1 therapy. Contrarily, the authors observed the proliferation of regulatory T cells in response to anti-PD-1 monotherapy in the post-treatment surgical specimen was associated with recurrence.[28] This type of information could identify patients who should receive combination checkpoint as adjuvant therapy or perhaps switch to another class of therapy. Blank et al. (OpACIN) also studied pre- and post-treatment tumor and blood in patients undergoing neoadjuvant ICI.[29] Peripheral blood analysis was compared between patients who underwent neoadjuvant ipilimumab plus nivolumab compared to adjuvant ipilimumab plus nivolumab. Neoadjuvant ipi + nivolumab was associated with expansion of more tumor-resident T cell clones in the peripheral blood compared to adjuvant ipi + nivolumab.[29] Reduced T cell infiltrates and lower productive T cell clonality were found in pretreatment tumor biopsies from patients who were more likely to develop recurrence after ipilimumab plus nivolumab.[29] Regardless of adjuvant or neoadjuvant therapy, baseline tumor biopsies revealed that low CD3, β 2 microglobulin, and PD-L1 expression were associated with recurrence.[29] Since T cells are the primary targets of ICI therapy, the ability to evaluate the TME and peripheral blood including the T cell compartment is important and may be especially relevant and feasible in the neoadjuvant setting.

In addition to T cells, B cells and tertiary lymphoid structures are increasingly being recognized as predictive of response to ICI. In Amaria et al. study of neoadjuvant ipilimumab plus nivolumab versus nivolumab, B cell markers were noted to be higher in baseline and on-treatment biopsies in patients who responded to therapy.[32] This was followed by additional work showing that the formation of tertiary lymphoid structures in the TME was a predictor of response in patients receiving ICI.[42,43] Switched memory B cells and unique functional states of B cells were observed in responders to therapy compared to non-responders.[42] Ultimately, pretreatment tumor or blood analysis that could precisely predict response would significantly reduce unnecessary toxicities in patients not expected to have a response. On treatment and post treatment tumor biopsies could also delineate mechanisms of response and expected recurrence free survival which could tailor future therapy in the individual patient. While much can be learned from study of the tumor microenvironment, notable limitations include the intra-tumor heterogeneity or the difference in biological, morphological, phenotypic and genotypic profiles within the same tumor from one patient that may not be represented well in a single biopsy.[44] Tumor heterogeneity makes it challenging to reproduce findings. Other limitations to studying or relying on pre and post tumor samples include no tumor available for patients who achieve clinical CR and do not undergo surgery or patients with disease progression that prohibits surgery and limits sample analysis.

The utility of pathologic response

In other solid tumor malignancies, pathologic response after neoadjuvant therapy serves as an early readout of patient outcomes, correlates with subsequent recurrence free survival, is a marker of patient-specific therapy response, and potentially informing the choice of future therapies.[45–47] Data from clinical trials of neoadjuvant therapy in melanoma using both immune checkpoint and targeted therapy (BRAFi/MEKi) have also demonstrated pathologic response as an early read out patient outcomes, specifically recurrence free survival.[32,48] In a recent pooled analysis from the international neoadjuvant melanoma consortium of six trials and 149 patients with surgically resectable clinical stage III melanoma, the pathologic

CR after anti-PD-1 plus or minus ipi ranged from 19–57%.[49] This pooled analysis included the aforementioned studies (see Clinical Data section), which all reported that pCR predicted prognosis. In the pooled analysis, for those achieving a pathologic CR, the 24 month RFS was 100% for those who had received an immune therapy regimen compared to 78% in the targeted regimen. For those with non-path CR, the 24 RFS was 72% in the immune therapy regimen compared to 8% in the targeted therapy group. Notably, in the pooled analysis, seven patients (4%) progressed before surgery and thus did not have pathologic assessment. In summary, pathologic response after neoadjuvant therapy in melanoma appears to correlate with recurrence free survival.

In addition to prognostic information, pathologic response may be used to reduce surgical morbidity. In the Magnetic Seed Localization for Melanoma (MeMaLoc) study, which included 12 patients from the OpACIN-neo trial, a magnetic bead was placed in the largest pathologic lymph node prior to treatment. At the time of surgery, which was a complete lymphadenectomy, this "index node" with bead was analyzed separately. The pathologic response was 100% congruent with response in the remaining nodes.[50] Based on these results, the PRADO (Personalized response-driven adjuvant therapy after combination ipilimumab and nivolumab in high-risk resectable stage III melanoma) study aimed to determine if complete lymphadenectomy could be avoided in patients who had complete pathologic response in the index node.[51] PRADO is an extension cohort of the multi-center phase 2 OpACIN-neo study; patients received 2 cycles of ipi 1 mg/kg+ nivo 3 mg/kg followed by surgical removal of the index lymph node.[51] Patients that achieved major pathologic response in the lymph node did not undergo complete lymphadenectomy. Early results presented at ASCO 2020 showed that complete lymphadenectomy was omitted in 58 (97%) of the patients with major pathologic response, which reduced surgical mordibity.[51] Long-term results to determine disease outcomes are still needed, but the concept of using pathologic response to reduce surgical morbidity may benefit patients.

Pathologic response can also be important in guiding treatment decisions especially for patients with activating BRAF mutations. If no pathologic response to neoadjuvant checkpoint is observed, patients with activating mutations can be changed to BRAFi/MEKi therapy. Moving forward, detailed pathologic response may continue to provide prognostic information and help guide treatment decisions including need for adjuvant therapy. In order to standardize pathologic reporting after neoadjuvant therapy in melanoma, guidelines have been developed which include these three elements: percent viable tumor, percent tumor melanosis/necrosis, and percent fibrosis/fibroinflammatory stroma.[21] This standardization may help further refine prognostic factors particularly in regards to need for additional adjuvant therapy after surgery or the extent of surgery. This concept is being studied in NCT04013854, where patients' adjuvant therapy regimen will be determined by pathologic response after neoadjuvant therapy. We have observed in a small number of patients that pathologic response when reported as 100% fibrosis/fibroinflammatory stroma is associated excellent prognosis and in the future these types of patients may forego adjuvant therapy (unpublished). Patients undergoing neoadjuvant therapy should have pathology appropriately evaluated which will also assist in combing data from all studies.

Future directions

For melanoma treatment overall, the optimal timing of therapy in patients with surgically resectable advanced disease remains under active investigation. Currently, adjuvant therapy after surgical resection of patients with stage III or IV melanoma with ICI is recommended and approved. Early data suggest potential benefits of neoadjuvant ICI therapy including the benefit of having abundant tumor antigen at the time of treatment which may result in more robust anti-tumor T cell responses. In addition, neoadjuvant ICI can provide important prognostic information discussed above. Importantly, data on surgical resectability, delays in surgical interventions, and toxicities that alter surgical planning have not been well studied. Whether or not ICI in the neoadjuvant setting is an advantage compared to adjuvant therapy in terms of adverse events is understudied. There is also a challenge in defining the proper follow up time and sample size needed to ascertain if neoadjuvant therapy has a significant over survival advantage over adjuvant therapy.

Additional ongoing issues related to the application of neoadjuvant ICI include the selection of therapeutic agents, as well as duration, dose, and need for adjuvant therapy after surgical resection. Specifically, there remains a question whether neoadjuvant therapy should be given until a specified response or for a set number of treatment courses. Defining surgical resectability can also be very subjective in some cases and applying strict anatomic criteria can be challenging. Additionally, consideration of disease-free interval or patient fitness may also be important in determining which patients may benefit or not benefit from a neoadjuvant approach.

Further, differentiating radiographic pseudoprogression from true disease progression and how this impacts the decision to proceed with surgical resection must be defined.[20] The International Neoadjuvant Melanoma Consortium with experts in medical oncology, surgical oncology, pathology, radiation oncology, radiology, and translational research has developed recommendations for investigating neoadjuvant therapy in melanoma to address many of these issues.[52] Their task will be to determine if larger trials are needed to compare dosing strategies and make recommendations on optimal combinations and appropriate patient selection. They will need to discuss the need and priority for randomized controlled trials comparing neoadjuvant, neoadjuvant+adjuvant, and adjuvant therapy treatment strategies.

At our institution, patients receive neoadjuvant therapy as part of clinical trials or with careful assessment and agreement between medical oncology and surgical oncology. Because neoadjuvant ICI therapy can be associated with toxicity and the possibility of foregoing surgery, we consider this approach for patients with increased chance for morbidity with local surgical resection or a known higher risk for development of distant metastases. Some examples would be patients with bulky lymphadenopathy compressing

vasculature structures, two sites of disease (e.g. dermal metastases plus lymph node disease), or a short disease free interval (i.e. lymph node recurrence less than 3 months from primary diagnosis). The choice of anti-PD-1 monotherapy or anti-PD -1 plus ipilimumab is based on patient fitness and disease burden given the known increase in adverse events with ipilimumab but also increased pathologic response rates. Given concerns about loss of window for resectability, patients on neoadjuvant regimens are followed closely by both surgical and medical oncology with serial imaging after 3 or 4 doses or earlier if physical exam is concerning for progression. . If toxicity limits further therapy before 3 or 4 cycles, we perform surgery when patient is deemed recovered to tolerate surgery. At present, additional management as to the timing of surgery is tailored to the individual patient depending on toxicity, physical exam and radiographic response, and assessment of surgical morbidity.

Conclusion

Systemic treatment options for patients with advanced melanoma have expanded dramatically over the past decade. The development of novel immunotherapies has made the possibility of durable tumor control a reality for this cohort of patients who historically had limited or no possibility of long-term survival. While surgery remains a cornerstone of therapy for patients with regionally advanced, but resectable disease, adjuvant systemic therapies have now consistently shown a recurrence free survival benefit in this patient population. With this proven existence of effective agents, there has been a shift toward utilizing systemic treatments earlier in the course of the disease. Studies exploring neoadjuvant approaches have shown early promise, both in terms of clinical outcomes and the potential for the development of novel biomarkers. However, risks of systemic therapies, particularly in the curative setting, need to be carefully considered and this should be central to future trial designs and dosing regimens. The management of patients with regionally advanced melanoma benefits from a multidisciplinary approach, with a goal of improving clinical outcomes.

Disclosure of potential conflicts of interest

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