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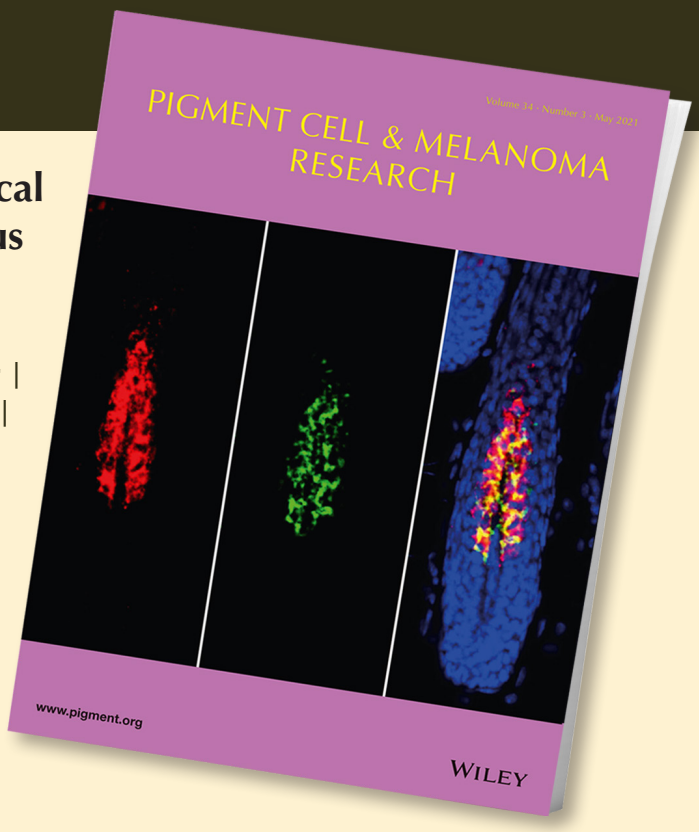


## The impact of BRAF mutation status on clinical outcomes with anti-PD-1 monotherapy versus combination ipilimumab/nivolumab in treatment-naïve advanced stage melanoma

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# The impact of BRAF mutation status on clinical outcomes with anti-PD-1 monotherapy versus combination ipilimumab/nivolumab in treatment-naïve advanced stage melanoma

Vincent T. Ma[1] | Stephanie Daignault-Newton[2] | Jessica J. Waninger[3,4] | Sara Journey[4] | Zoey Chopra[4] | Alangoya Tezel[4] | Bruce G. Redman[1] | Leslie A. Fecher[1] | Michael D. Green[5] | Ajjai S. Alva[1] | Christopher D. Lao[1]

[1]Division of Hematology and Oncology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

[2]Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA

[3]Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, MI, USA

[4]Department of Medical Education, University of Michigan, Ann Arbor, MI, USA

[5]Department of Radiation Oncology, University of Michigan, Ann Arbor, MI, USA

**Correspondence**
Vincent T. Ma, Division of Hematology and Oncology, Department of Internal Medicine, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-5848, USA.
Emails: vma@med.umich.edu

## Abstract

Nearly half of all metastatic melanoma patients possess the BRAF V600 mutation. Several therapies are approved for advanced stage melanoma, but it is unclear if there is a differential outcome to various immunotherapy regimens based on BRAF mutation status. We retrospectively analyzed a cohort of metastatic or unresectable melanoma patients who were treated with combination ipilimumab/nivolumab (ipi/nivo) or anti-PD-1 monotherapy, nivolumab, or pembrolizumab, as first-line treatment. 235 previously untreated patients were identified in our study. Our univariate analysis showed no statistical difference in progression-free survival (PFS) or overall survival (OS) with ipi/nivo versus anti-PD-1 monotherapy in the BRAF V600 mutant cohort, but there was improved PFS [HR: 0.48, 95% CI, 0.28–0.80] and OS [HR: 0.50, 95% CI, 0.26–0.96] with ipi/nivo compared to anti-PD-1 monotherapy in the BRAF WT group. After adjusting for known prognostic variables in our multivariable analysis, the BRAF WT cohort continued to show PFS and OS benefit with ipi/nivo compared to anti-PD-1 monotherapy. Our single-institution analysis suggests ipi/nivo should be considered over anti-PD-1 monotherapy as the initial immunotherapy regimen for metastatic melanoma patients regardless of BRAF mutation status, but possibly with greater benefit in BRAF WT.

**KEYWORDS**

anti-CTLA-4 inhibitor, anti-PD-1 inhibitor, BRAF mutation, immune checkpoint inhibitor, melanoma

## 1 | INTRODUCTION

Several immune checkpoint inhibitors (ICIs) are approved for unresectable or metastatic melanoma including anti-programmed cell death 1 (PD-1) inhibitors (pembrolizumab and nivolumab) and anti-cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) inhibitor (ipilimumab) (Ma et al., 2020). Published data from the CheckMate 067 trial evaluating metastatic melanoma patients demonstrated that first-line treatment with combination ipilimumab/nivolumab (ipi/nivo) or single-agent nivolumab fared significantly better in terms of response rate, progression-free survival (PFS), and overall survival (OS) than ipilimumab alone (Larkin et al., 2019). In a similar cohort, pembrolizumab was superior to ipilimumab in terms of response rate and survival (Schachter et al., 2017). As

the CheckMate 067 trial was not powered to directly compare survival outcomes between ipi/nivo and single-agent nivolumab, it is unknown if combination therapy or anti-PD-1 monotherapy is superior.

Although observational data suggest better survival outcomes with ipi/nivo, results show that combination therapy compared to single-agent ICIs leads to higher rates and severity of treatment-related adverse events (Larkin et al., 2019). This consequently leads to more emergency department visits, hospitalizations, use of systemic immunosuppressants, and greater financial burden on the patient and healthcare system (Oh et al., 2017). Further studies are being explored to identify subgroups of patients that warrant combination therapy over anti-PD-1 monotherapy. A descriptive subgroup analysis from CheckMate 067 showed a statistical PFS favorability with ipi/nivo over nivolumab in patients with tumor PD-L1 expression of less than 5% or less than 1%, elevated lactate dehydrogenase (LDH) levels, and BRAF V600 mutation (Wolchok et al., 2017).

Nearly half of all metastatic melanoma patients possess a BRAF V600 mutation (Kim et al., 2015). In this group, targeted therapy with BRAF and MEK inhibitors are alternative treatment options in the front-line setting. Many oncologists favor first-line ICI therapy regardless of BRAF mutational status. However, the optimal first-line ICI therapy in BRAF-mutant and BRAF wild-type (WT) patients has yet to be determined (Pavlick et al., 2019). Additionally, there is uncertainty about whether BRAF mutation status can predict survival outcomes in patients treated with ICIs.

The purpose of this retrospective study is to evaluate the survival outcomes of metastatic melanoma patients comparing first-line treatment with anti-PD-1 monotherapy (nivolumab and pembrolizumab) versus combined ipi/nivo stratified by BRAF mutation status.

**Significance**

Single-agent PD-1 inhibitors, nivolumab and pembrolizumab, and combination ipilimumab/nivolumab (ipi/nivo), are frequently used first-line immunotherapy options for advanced stage melanoma. As combination therapy carries a higher risk of toxicity, an improved understanding of which patients benefit most from combination ipi/nivo can guide clinical management. In our study, we investigate whether BRAF mutation status has any impact on survival following either regimen. Our findings showed a trend toward better survival in patients with BRAF wild-type status when treated with ipi/nivo compared to anti-PD-1 monotherapy. Our observations should be validated in randomized prospective trials.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

We identified 327 patients diagnosed with advanced, metastatic, or unresectable melanoma between February 2012 and October 2019 from the University of Michigan. Uveal melanoma patients were excluded from the study. After excluding patients who received any prior systemic therapy, a retrospective analysis was performed on a cohort of 235 patients. These patients were treated with standard ipi/nivo, single-agent nivolumab, or single-agent pembrolizumab. Patients with incomplete clinical data or insufficient follow-up (less than 30 days) from initiation of the designated therapy were excluded. Patients were selected based on having histologically proven unresectable stage III or IV melanoma following American Joint Committee on Cancer (AJCC) 8th edition criteria (Gershenwald et al., 2017). Patients and data were collected via electronic medical record system and a pharmacy database hosted by the University of Michigan.

### 2.2 | Study design

We characterized baseline patient demographics including age, gender, and BRAF mutation status. To characterize prognostic factors, Eastern Cooperative Oncology Group (ECOG) performance status, serum LDH levels, and absence or presence of brain and liver metastases were documented before initiation of examined therapy options. Efficacy endpoints of each treatment included progression-free survival (PFS) and overall survival (OS). We assessed best response by utilizing the revised RECIST guideline (version 1.1) as measured by complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) (Eisenhauer et al., 2009).

The anti-PD-1 monotherapy group included patients who were treated with: nivolumab at 240 mg IV every 2 weeks, 3 mg/kg IV every 2 weeks, or 480 mg IV every 4 weeks; or pembrolizumab at 200 mg IV every 3 weeks or 2 mg/kg IV every 3 weeks. For the combination ipi/nivo cohort, these were patients who received ipilimumab (3 mg/kg) in combination with nivolumab (1 mg/kg). The combination scheduling was typically administered for up to 4 infusions every 3 weeks followed by nivolumab therapy at 3 mg/kg every 2 weeks or 240 mg every 2 weeks or 480 mg every 4 weeks.

### 2.3 | Statistical methods

OS was determined based on electronic health record documentation. PFS was defined as time from date of therapy initiation to clinical progression on physical examination or on imaging by RECIST v1.1 with the noted exception of identifying the largest target lesions in retrospect; or date of death, whichever occurred first. In cases of pseudoprogression, as defined by iRECIST (Seymour et al., 2017), these were not characterized as progressive disease. Patients who

**TABLE 1** Patient characteristics

| Subgroup | Number of Patients | | | *p*-value[a] |
|---|---|---|---|---|
| | BRAF Mutant | BRAF WT | Unknown | |
| All Patients | 81 (100%) | 152 (100%) | 2 (100%) | |
| BRAF Mutation | | | | |
| V600E | 53 (65%) | N/A | N/A | |
| V600K | 26 (32%) | N/A | N/A | |
| V600 Unspecified | 2 (3%) | N/A | N/A | |
| Treatment | | | | .05 |
| Ipi/Nivo | 45 (56%) | 63 (41%) | 2 (100%) | |
| Nivolumab | 6 (7%) | 27 (18%) | 0 | |
| Pembrolizumab | 30 (37%) | 62 (41%) | 0 | |
| Age | | | | <.0001* |
| <65 | 59 (73%) | 68 (45%) | 1 (50%) | |
| ≥65 | 22 (27%) | 84 (55%) | 1 (50%) | |
| Gender | | | | 1.00 |
| Male | 54 (67%) | 101 (66%) | 2 (100%) | |
| Female | 27 (33%) | 51 (34%) | 0 | |
| ECOG | | | | .90 |
| 0–1 | 79 (98%) | 148 (97%) | 2 (100%) | |
| 2 | 2 (2%) | 3 (2%) | 0 | |
| 3 | 0 | 1 (1%) | 0 | |
| LDH[b] | | | | .41 |
| ≤ULN | 50 (62%) | 109 (72%) | 1 (50%) | |
| >ULN | 28 (35%) | 38 (25%) | 0 | |
| >2xULN | 7 (9%) | 7 (5%) | 0 | |
| Unknown | 3 (4%) | 5 (3%) | 1 (50%) | |
| Brain Metastases | | | | .11 |
| No | 61 (75%) | 113 (74%) | 0 | |
| Yes | 20 (25%) | 39 (26%) | 2 (100%) | |
| Liver Metastases | | | | .30 |
| No | 65 (80%) | 116 (76%) | 2 (100%) | |
| Yes | 16 (20%) | 36 (24%) | 0 | |

Abbreviations: ECOG, Eastern Cooperative Oncology Group (Performance Status); Ipi/Nivo, ipilimumab/nivolumab; LDH, lactate dehydrogenase; ULN, upper limit of normal.

[a]Chi-square test.

[b]LDH upper limit of normal is 240 IU/L

* indicates statistical significance of $p < .05$.

were alive at the time of the analysis were censored at last known follow-up.

The Jonckheere–Terpstra test was used to assess the trend of best response rate by BRAF mutation status. PFS and OS between the combination ipi/nivo treatment group and the anti-PD-1 monotherapy group were compared using Kaplan–Meier methods and log-rank tests. PFS and OS were compared between the two treatment groups with these markers in stratified Cox models. Hazard ratios, 95% confidence intervals, and log-rank *p*-values are reported in forest plots. Multivariable Cox regression of PFS and OS was performed to compare the effects of the two treatment groups on survival from the initiation of therapy adjusted by age, gender, pretreatment lactate dehydrogenase (LDH) measured in IU/L obtained within 30 days of starting therapy, and presence of brain and liver metastases at time of starting therapy with hazard ratios, 95% confidence intervals, and Wald chi-square *p*-values reported. The analysis was completed with 2-sided significance testing assuming a type I error of 0.05 using SAS 9.4 (SAS Institute Inc.). Statistical difference between two comparative groups is defined as log-rank test *p*-value of < .05 and/or a hazard ratio with its 95% confidence interval range excluding the value of 1.00.

## 3 | RESULTS

Two hundred thirty-five patients were analyzed in our study. 81 patients had BRAF-mutant (V600E, V600K, or V600 unspecified) melanoma, 152 patients had BRAF WT melanoma, and 2 patients had unknown BRAF mutation status. As first-line treatment, 110 patients received combination ipi/nivo and 125 patients received anti-PD-1 monotherapy (33 with nivolumab and 92 with pembrolizumab).

For the patients included in our analysis, the median patient age was 61 with an ECOG performance status of 1 and a normal LDH level (≤240 IU/L). BRAF WT patients had a statistically significant higher median age (66 years vs. 59 years) at time of treatment (Table 1). Between BRAF-mutant versus BRAF WT patients, there was no significant difference in distribution of the designated therapy, gender, ECOG performance status, pretreatment LDH, presence or absence of brain metastases, and presence or absence of liver metastases (Table 1).

Median follow-up was 14.9 months (Min-Max: 0.1–55 months). Median PFS for all patients treated with ipi/nivo was not reached, and for patients treated with anti-PD-1 monotherapy was 19.7 months (Figure 1). PFS statistically favored ipi/nivo compared to anti-PD-1 monotherapy [HR: 0.52, 95% CI, 0.35–0.77, $p = .0010$]. The median OS for patients treated with ipi/nivo was not reached and for patients treated with anti-PD-1 monotherapy was 44.4 months (Figure 1). OS statistically favored ipi/nivo compared to anti-PD-1 monotherapy [HR: 0.53, 95% CI, 0.31–0.90, $p = .0170$] (Figure 1). Rates of therapy discontinuation due to adverse events from ICI were 53% (58/110) with ipi/nivo and 16% (20/125) with anti-PD-1 monotherapy (Table S1).

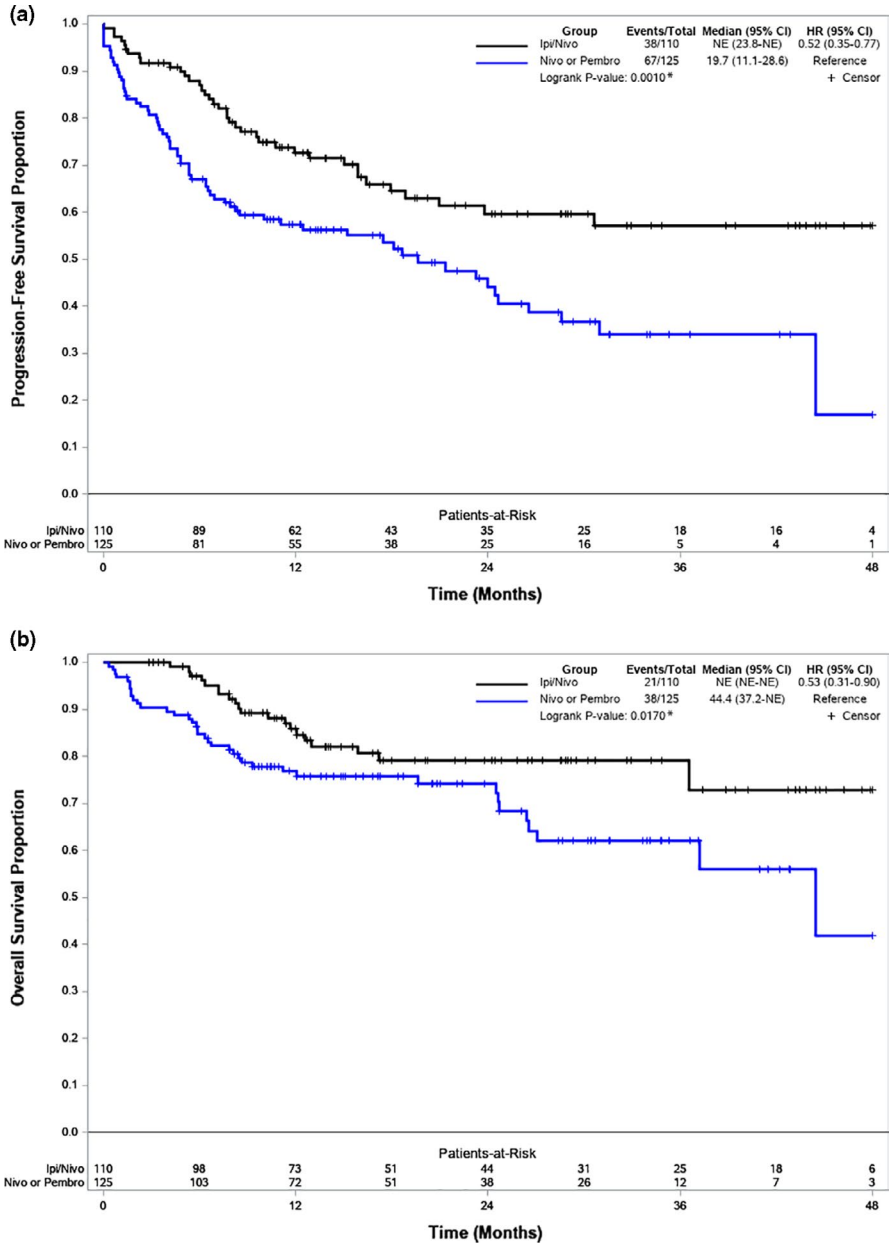


**FIGURE 1** Kaplan–Meier curves comparing all advanced stage melanoma patients treated with Ipi/Nivo versus anti-PD-1 monotherapy (Nivo or Pembro) by (a) progression-free survival and (b) overall survival. Ipi, ipilimumab; Nivo, nivolumab; Pembro, pembrolizumab

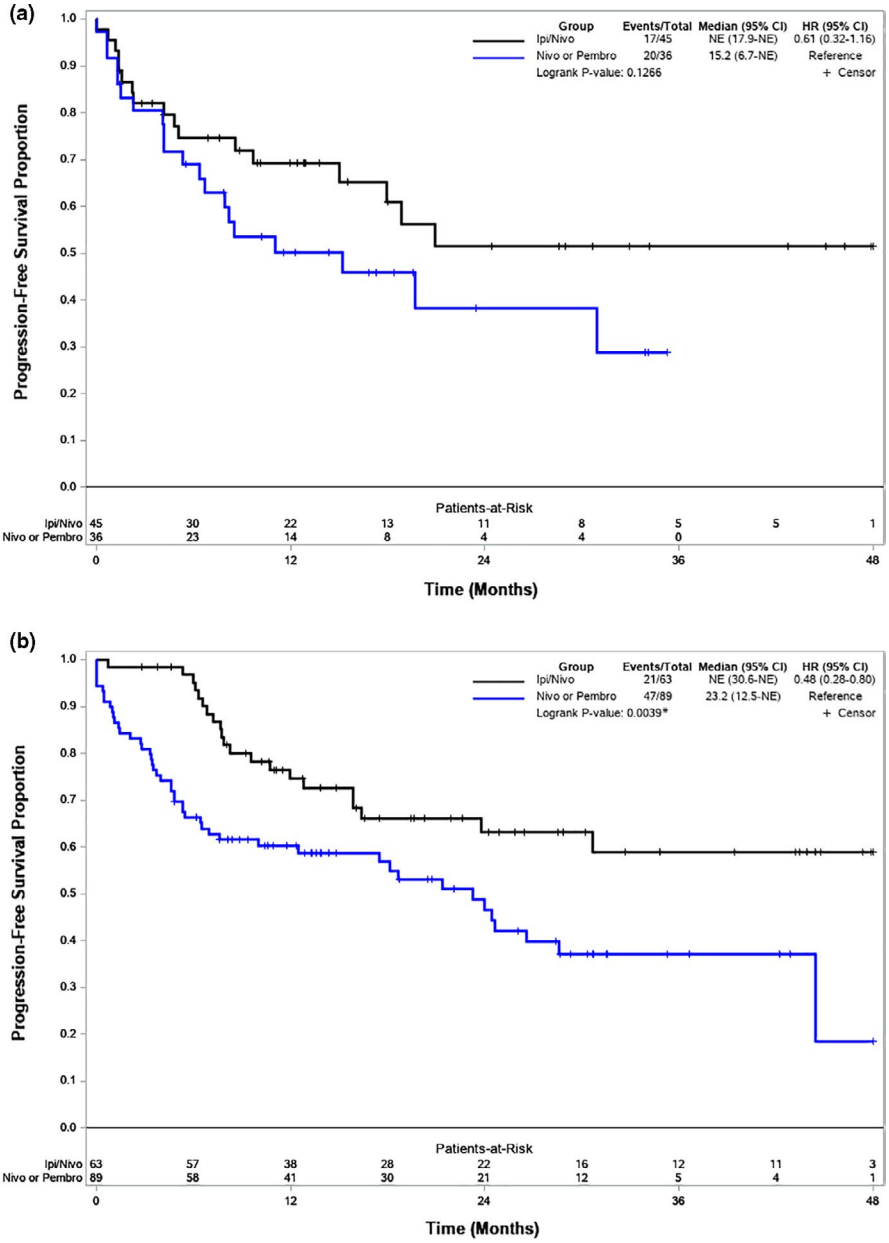


**FIGURE 2** Kaplan–Meier curves of progression-free survival comparing advanced stage melanoma patients treated with Ipi/Nivo versus anti-PD-1 monotherapy (Nivo or Pembro) in the (a) BRAF-mutant cohort and (b) BRAF WT cohort. Ipi, ipilimumab; Nivo, nivolumab; Pembro, pembrolizumab

## 3.1 | Univariate analysis

Best response rate by treatment type was assessed based on BRAF mutation status (Table S1). Rates of objective response (PR + CR), regardless of BRAF mutation status, were higher in patients treated with ipi/nivo compared to anti-PD-1 monotherapy. Respective rates of PR and CR in patients treated with anti-PD-1 monotherapy were 28% and 25% in the BRAF-mutant group; and 25% and 30% in the BRAF WT group (Table S1). Respective rates of PR and CR in patients treated with ipi/nivo were 36% and 38% in the BRAF-mutant group; and 29% and 46% in the BRAF WT group (Table S1).

The effect of BRAF mutation status on OS and PFS stratified by individual treatment type was assessed using a univariate model. There was no statistical difference in PFS among the two groups when treated with anti-PD-1 monotherapy [BRAF mutant as reference, HR: 0.84, 95% CI, 0.50–1.43, $p = .526$] or with ipi/nivo [BRAF mutant as reference, HR: 0.69, 95% CI, 0.36–1.31, $p = .252$] (Figure S1). Similarly, there was no statistical difference in OS among the two groups when treated with anti-PD-1 monotherapy [BRAF mutant as reference, HR: 1.35, 95% CI, 0.62–2.98, $p = .4483$] or with ipi/nivo [BRAF mutant as reference, HR: 0.95, 95% CI, 0.39–2.30, $p = .9151$] (Figure S2).

A univariate model for OS and PFS was then performed to assess the effect of different treatment types by BRAF mutation status. The median PFS for BRAF V600-mutant patients treated with ipi/nivo and anti-PD-1 monotherapy was not reached and 15.2 months, respectively, with no statistically significant difference in treatment favorability [HR: 0.61, 95% CI, 0.32–1.16, $p = .1266$] (Figure 2). The median PFS for BRAF WT patients treated with ipi/nivo and anti-PD-1 monotherapy was not reached and 23.2 months, respectively, with statistical favorability in the combination therapy arm, [HR: 0.48, 95% CI, 0.28–0.80, $p = .0039$]

(Figure 2). In the OS analysis, the BRAF V600-mutant patients had no statistically significant survival favorability [HR: 0.71, 95% CI, 0.26–1.93, $p = .5002$] with ipi/nivo (median time not reached) compared to anti-PD-1 monotherapy (median 37.2 months) (Figure 3). Whereas combination therapy was statistically favored [HR: 0.50, 95% CI, 0.26–0.96, $p = .0345$] among the BRAF WT cohort with median OS not reached in the ipi/nivo arm and 44.4 months in the anti-PD-1 monotherapy arm (Figure 3).

Higher rates of PFS with combination therapy over anti-PD-1 monotherapy were seen in all clinically relevant subgroups, but with statistical difference notably in patients with BRAF WT status ($p = .005$), males ($p = .005$), age < 65 ($p = .001$), normal LDH ($p = .016$), LDH greater than 2 times the upper limit of normal (ULN) ($p = .008$), absence of brain metastases ($p = .001$), and absence ($p = .017$) or presence ($p = .016$) of liver metastases (Figure 4). A similar pattern of superior OS with combination therapy over anti-PD-1 monotherapy was observed, but with statistical favorability in the following patient subsets: BRAF WT status ($p = .038$), males ($p = .012$), age < 65 ($p = .011$), and presence of liver metastases ($p = .009$) (Figure 5).

## 3.2 | Multivariable analysis

In the multivariable Cox regression analysis, we adjusted for treatment type stratified by BRAF mutation status, age, gender, pretreatment LDH level, and presence or absence of brain and liver metastases. The analysis confirmed that treatment with ipi/nivo over single-agent PD-1 inhibitor in BRAF WT patients [HR: 0.477, 95% CI, 0.222–0.916, $p = .025$] and LDH level greater than 2 times the ULN compared to a normal LDH level [HR: 0.249, 95% CI,

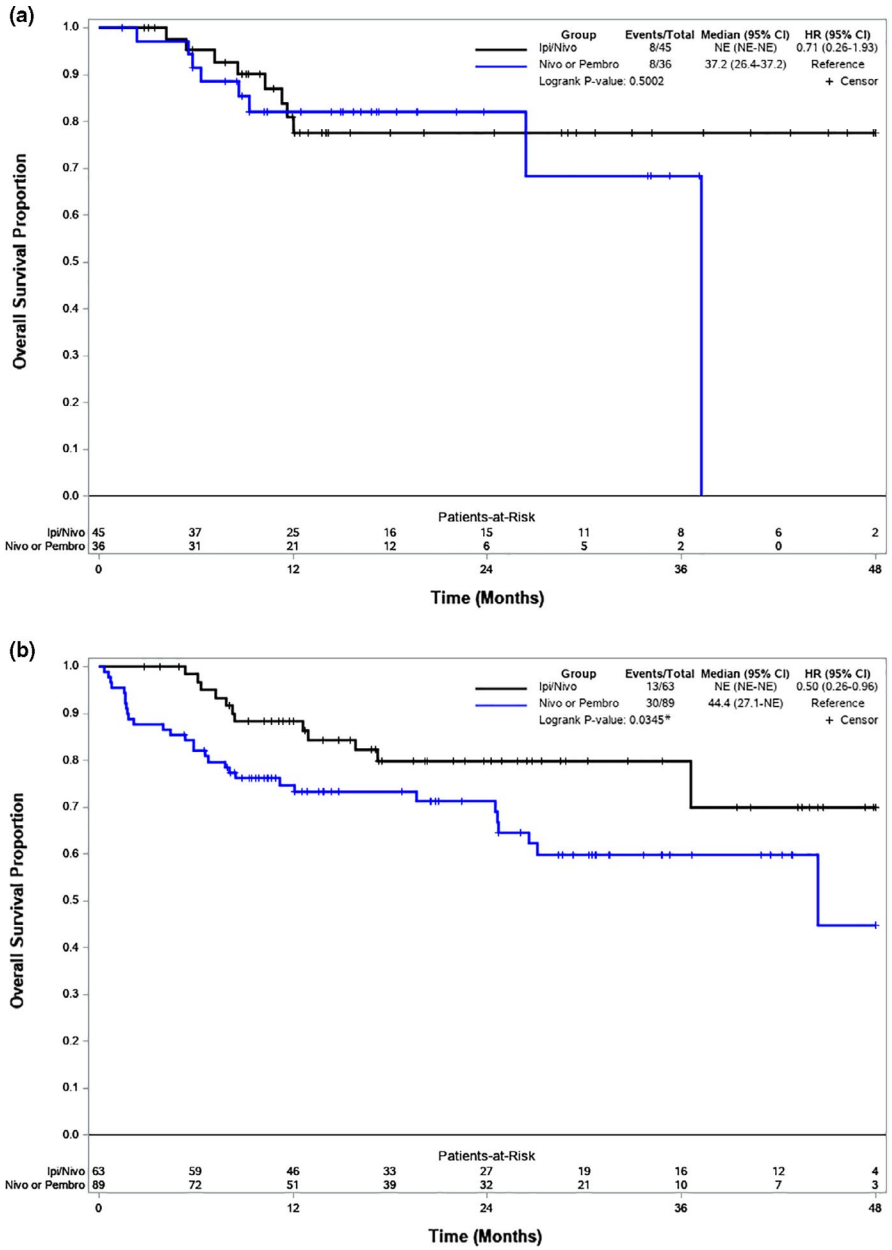


**FIGURE 3** Kaplan–Meier curves of overall survival comparing advanced stage melanoma patients treated with Ipi/Nivo versus anti-PD-1 monotherapy (Nivo or Pembro) in the (a) BRAF-mutant cohort and (b) BRAF WT cohort. Ipi, ipilimumab; Nivo, nivolumab; Pembro, pembrolizumab

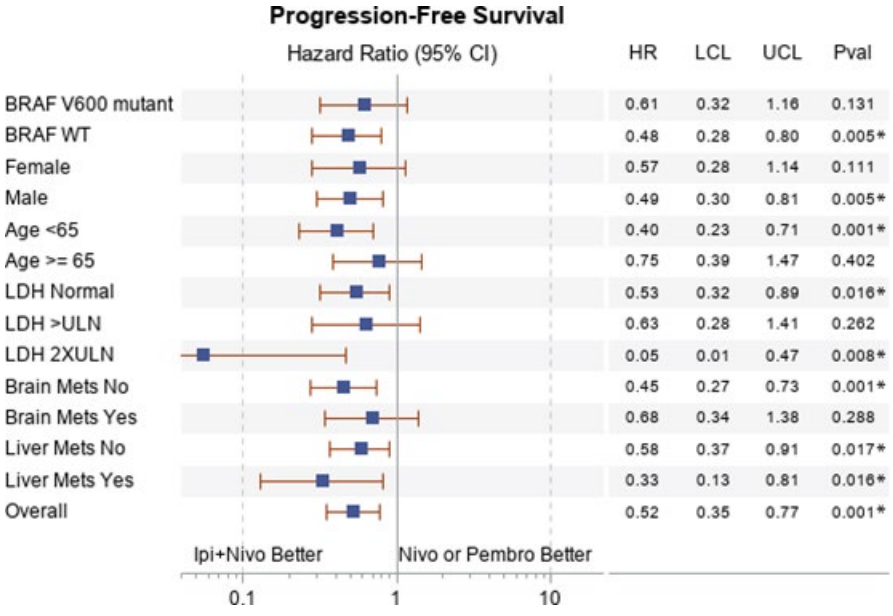


**FIGURE 4** Forest plot for progression-free survival using univariate Cox models by stratification groups. 2XULN, two times the upper limit of normal; Ipi, ipilimumab; LDH, lactate dehydrogenase; Nivo, nivolumab; Pembro, pembrolizumab; ULN, upper limit of normal

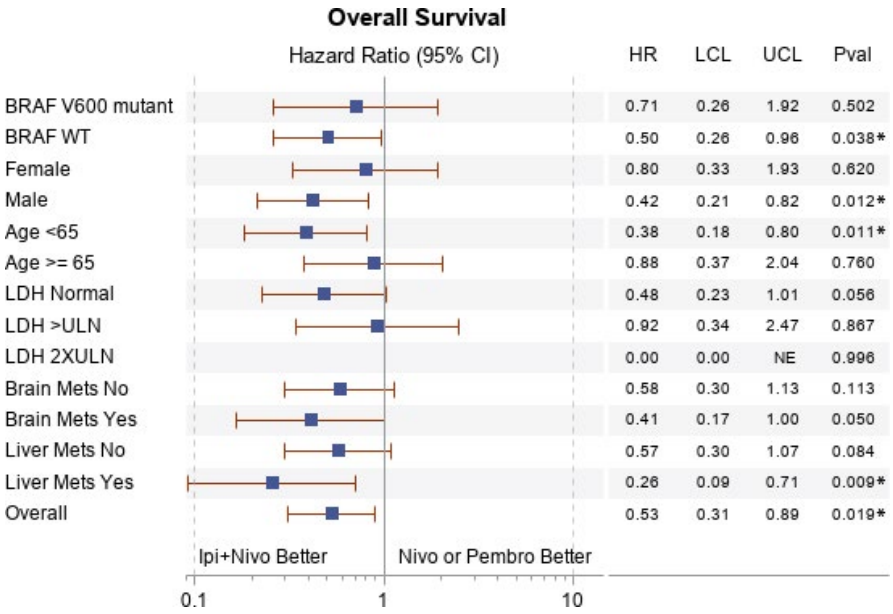


**FIGURE 5** Forest plot for overall survival using Cox models using univariate Cox models by stratification groups. 2XULN, two times the upper limit of normal; Ipi, ipilimumab; LDH, lactate dehydrogenase; Nivo, nivolumab; Pembro, pembrolizumab; ULN, upper limit of normal

0.114–0.543, $p = .001$] were statistically significant markers for PFS (Table 2). While not statistically significant, there was a trend toward improved PFS with ipi/nivo over anti-PD-1 monotherapy in BRAF-mutant patients [HR: 0.500, 95% CI, 0.273–1.126, $p = .060$] (Table 2).

After adjusting for similar pertinent prognostic variables, there was statistical favorability in OS with ipi/nivo over anti-PD-1 monotherapy in patients with BRAF WT [HR: 0.306, 95% CI, 0.095–0.905, $p = .027$] and BRAF mutant [HR: 0.417, 95% CI, 0.192–0.989, $p = .048$] (Table 3). The multivariable Cox regression analysis also demonstrated that LDH level greater than 2 times the ULN compared to a normal LDH level [HR: 0.086, 95% CI, 0.035–0.212, $p = <.0001$] was a statistically significant marker for OS (Table 3).

## 4 | DISCUSSION

In our retrospective analysis, patients with metastatic or unresectable melanoma with BRAF WT status treated with combination ipi/nivo had a statistically increased likelihood of PFS and OS compared to patients treated with anti-PD-1 monotherapy agents, nivolumab, or pembrolizumab. These findings persisted after adjusting for several prognostic variables including age, pretreatment LDH levels, prior treatment status, and presence or absence of brain and liver metastases. Among BRAF V600 mutant patients, the survival benefit also favors ipi/nivo over anti-PD-1 monotherapy, but this trend was not as statistically significant. Based on our findings, ipi/nivo, instead of anti-PD-1 monotherapy, should be considered as initial ICI therapy for metastatic melanoma

**TABLE 2** Multivariate Cox regression of treatment and prognostic variables and effect on progression-free survival with hazard ratios

| Variable | Description | Hazard Ratio | 95% Wald Confidence Limits | | p-value[a] |
|---|---|---|---|---|---|
| Therapy by BRAF Status | BRAF WT: Ipi/Nivo versus Nivo or Pembro | 0.477 | 0.222 | 0.916 | .025* |
| | BRAF V600 mutant: Ipi/Nivo versus Nivo or Pembro | 0.500 | 0.273 | 1.126 | .060 |
| Brain Metastases | No Brain Mets versus Brain Mets | 0.696 | 0.449 | 1.078 | .104 |
| Liver Metastases | No Liver Mets versus Liver Mets | 1.037 | 0.600 | 1.793 | .895 |
| LDH[b] | Normal LDH versus > ULN LDH | 0.778 | 0.475 | 1.272 | .317 |
| | Normal LDH versus > 2xULN LDH | 0.249 | 0.114 | 0.543 | .001* |
| Age | Age < 65 versus Age ≥ 65 | 0.966 | 0.591 | 1.578 | .890 |
| Gender | Male versus Female | 0.921 | 0.607 | 1.398 | .700 |

Abbreviations: 2x, two times; Ipi, ipilimumab; LDH, pretreatment lactate dehydrogenase; Mets. metastases; Nivo, nivolumab; Pembro, pembrolizumab; Tx, Treatment; ULN, upper limit of normal; WT, wild type.

[a]Wald chi-square test.

[b]LDH upper limit of normal is 240 IU/L

* indicates statistical significance of $p < .05$.

**TABLE 3** Multivariate Cox regression of treatment and prognostic variables and effect on overall survival with hazard ratios

| Variable | Description | Hazard Ratio | 95% Wald Confidence Limits | | p-value[a] |
|---|---|---|---|---|---|
| Therapy by BRAF Status | BRAF WT: Ipi/Nivo versus Nivo or Pembro | 0.306 | 0.095 | 0.905 | .027* |
| | BRAF V600 mutant: Ipi/Nivo versus Nivo or Pembro | 0.417 | 0.192 | 0.989 | .048* |
| Brain Metastases | No Brain Mets versus Brain Mets | 0.586 | 0.334 | 1.027 | .062 |
| Liver Metastases | No Liver Mets versus Liver Mets | 0.566 | 0.292 | 1.094 | .091 |
| LDH[b] | Normal LDH versus > ULN LDH | 0.577 | 0.305 | 1.093 | .092 |
| | Normal LDH versus > 2xULN LDH | 0.086 | 0.035 | 0.212 | <.0001* |
| Age | Age < 65 versus Age ≥ 65 | 1.148 | 0.609 | 2.165 | .669 |
| Gender | Male versus Female | 0.966 | 0.552 | 1.690 | .904 |

Abbreviations: 2x, two times; Ipi, ipilimumab; LDH, pretreatment lactate dehydrogenase; Mets, metastases; Nivo, nivolumab; Pembro, pembrolizumab; Tx, Treatment; ULN, upper limit of normal; WT, wild type.

[a]Wald chi-square test.

[b]LDH upper limit of normal is 240 IU/L.

* indicates statistical significance of $p < .05$.

regardless of BRAF mutation status, but possibly with greater survival benefit in BRAF WT patients.

Single-agent PD-1 inhibitors, nivolumab and pembrolizumab, are approved first-line immunotherapy agents for metastatic melanoma (Schachter et al., 2017; Topalian et al., 2012). Although direct comparisons between anti-PD-1 monotherapy are lacking, we combined patients receiving either nivolumab or pembrolizumab as a single cohort since they share a similar biological target and historic data suggests similar efficacy (Moser et al., 2019). Currently, optimal selection of anti-PD-1 monotherapy versus combination

anti-CTLA-4 and anti-PD-1 inhibitor in the front-line treatment setting hinges on the treating physician's thorough assessment of the disease status and patient characterization. Our study noted higher rates of objective response and trend toward better survival with ipi/nivo compared to anti-PD-1 monotherapy overall. Similar to historic data (Hodi et al., 2018), we observed higher rates of therapy discontinuation due to toxicities with combination ICI (Table S1). To date, there are no validated biomarker tests for ICI therapy in melanoma that identifies which patients are likely to benefit or to experience immune-related toxicities. Extensive data have demonstrated that PD-L1 expression weakly correlates with clinical response to anti-PD-1 therapy (Daud et al., 2016; Topalian et al., 2012). Studies have evaluated the possible role of interleukin (IL)-6 levels (Valpione et al., 2018) and somatic copy number alterations (Davoli et al., 2017) in predicting survival with anti-CTLA-4 therapy, but their clinical use is still in its infancy. Biomarkers of efficacy and resistance with ipi/nivo in melanoma are under further investigation. Identifying better predictive and prognostic biomarkers is becoming essential to better optimize precision immunotherapy.

Based on limited studies, the effectiveness of PD-1 inhibition is thought to be independent of the BRAF mutation status (Schachter et al., 2017; Tumeh et al., 2014). This generalization is somewhat limited, particularly with nivolumab. In CheckMate 066, the authors evaluated nivolumab in the first-line setting for metastatic melanoma, but excluded BRAF V600 mutant patients (Ascierto et al., 2019). The CheckMate 037 trial, which evaluated nivolumab as subsequent therapy, noted statistically better objective response rates in the BRAF WT group with nivolumab compared to chemotherapy, but no apparent statistical difference in the BRAF V600 mutant cohort (Weber et al., 2015). Congruent with historic findings (Kim et al., 2015), our BRAF-mutant cohort was about 40%–60% of the metastatic melanoma patients and was generally younger in age on initial diagnosis/treatment compared to the BRAF WT group. Our study found that the efficacy of individual anti-PD-1 inhibitor-based regimens is independent of the BRAF mutation status (Table S1, Figures S1 and S2). In our univariate analysis, markers statistically favoring ipi/nivo over anti-PD-1 monotherapy that were concordant in PFS and OS included BRAF WT, age < 65, male, and presence of liver metastases. Differential outcomes to ICI therapy based on gender (Conforti et al., 2018), age (Kugel et al., 2018), and liver metastases (Tumeh et al., 2017) have been reported in the literature. After controlling for these variables, our multivariable analysis was able to demonstrate BRAF WT as a statistically significant factor impacting differential response to therapy.

Our findings were surprisingly discordant with the observational trend seen in the landmark CheckMate 067 trial (Long et al., 2019). In their study, patients with BRAF V600 mutations had a descriptively better 5-year PFS and OS with combination ipi/nivo, 38% and 60% respectively, compared to single-agent nivolumab, 22% and 46%, respectively. In contrast, the patients with BRAF WT had a smaller absolute difference in PFS and OS with combination ipi/nivo, 35% and 48%, respectively, at 5 years, compared to nivolumab monotherapy, 32% and 43%, respectively, at 5 years. Unlike their study, which permitted accrual of patients who had received prior adjuvant or neoadjuvant treatment for melanoma (Hodi et al., 2018), we analyzed a cohort of patients who never received any prior systemic anti-neoplastic therapy. Our retrospective study was also inclusive of patients with brain metastases in order to reflect a real-world heterogeneous group of patients in clinical practice. Furthermore, our multivariable analysis accounts for multiple known prognostic variables in metastatic melanoma that may impact therapy outcomes. Mindful of the limitations associated with our study including the retrospective nature, single-institution site, and median duration follow-up of less than 2 years; the contrasting results still warrant future prospective analysis of different cohorts to clarify these dissimilar findings.

Our findings may be explained by the distinct biomolecular features between BRAF WT and BRAF-mutant melanoma. Several studies have found the oncogenic signaling associated with the BRAF V600 mutation can drive the transcription of multiple genes that promote immune suppression. These mechanisms include upregulating immunomodulatory chemokines that promote recruitment of suppressive immune cell subsets into the tumor microenvironment (Khalili et al., 2012); internalization of MHC class I molecules which leads to reduced CD8 + T-cell recognition and function (Bradley et al., 2015); and downregulating expression of melanoma differentiation antigens that can be recognized by cytotoxic T lymphocytes (Boni et al., 2010). All of these may have counteractive anti-tumor effect with ICI therapy, but their role in differential outcomes with various ICI regimens remains unknown. BRAF V600E melanoma cells express higher levels of cytokines, including IL-6, than their BRAF WT counterpart (Bjoern et al., 2016; Whipple & Brinckerhoff, 2014). Low levels of IL-6 are associated with increased OS in melanoma patients treated with anti-CLTA-4 inhibitors (Valpione et al., 2018), suggesting the addition of anti-CTLA-4 with anti-PD-1 inhibitor may be driving the comparative differences. We also postulate that tumor mutational burden (TMB) may be a contributing factor in ICI therapy outcomes. Molecular studies have shown a higher average TMB rate in BRAF WT than BRAF-mutant tumors (Mar et al., 2013; Park et al., 2019). Several studies have shown a positive association with tumor mutational load and response to immunotherapy (Snyder et al., 2014; Danilova et al., 2016). In other studies, combination ICIs may have greater clinical efficacy in tumors with higher TMBs (Forschner et al., 2019; Hellmann et al., 2019). Confirmation of these clinical findings still requires validation in larger cohorts.

While our data demonstrated a statistically favorable PFS with ipi/nivo over anti-PD-1 monotherapy in BRAF WT only, the statistical benefit in OS for both BRAF WT and BRAF mutant is likely to reflect the availability of BRAF/MEK inhibitors as salvage therapy for BRAF-mutant patients. BRAF-targeted therapies are an alternative treatment option for BRAF V600-mutant patients, but there is a paucity of robust data on the outcomes of patients treated with ICIs following BRAF-targeted therapy or vice versa (Saab et al., 2019). Several studies suggest that resistance to BRAF inhibition may attenuate the subsequent benefit of ICI therapy (Ascierto et al., 2014; Hugo et al., 2017). In one retrospective study, BRAF V600-mutant

patients who received prior BRAF-targeted therapies had inferior outcomes after starting anti-PD-1 therapy than if they had received anti-PD-1 therapy initially (Johnson et al., 2017). For this reason, we preferentially analyzed treatment-naïve patients in our study. This helped limit a guarantee-time bias where patients with more indolent diseases would survive long enough to receive salvage anti-PD-1 inhibitor and it excluded the confounding BRAF-mutant patients that might preferentially be treated with up-front BRAF-targeted therapy due to aggressive disease requiring rapid response.

A notable finding in our study is the significantly higher median PFS, for both anti-PD-1 monotherapy and ipi/nivo, compared to historical data in clinical trials. We suspect the exaggerated PFS may be largely attributed to our preferred assessment of response to account for pseudoprogression, a phenomenon occasionally seen with immunotherapy where initial increase in tumor size is followed by reduction in tumor burden (Seymour et al., 2017). Other plausible explanations include the available resources at our tertiary medical center for therapy monitoring and toxicity management; the permitted use of adjunct radiation therapy prior to and during initiation of ICI therapy; and the flexible adherence to the treatment regimen without the clinical trial restraints.

There are several limitations associated with our analysis. Our cohort included melanoma patients who elected to be treated at a tertiary referral medical center. We are unable to account for certain differences among patients that could have driven the selection of ipi/nivo or anti-PD-1 monotherapy. Although we attempted to control for a potential selection bias by using a multivariable Cox regression, we could not account for other pertinent variables including patient co-morbidities, TMB, disease burden, or other sites of metastases. We did not account for ECOG performance status in our multivariable analysis as the vast majority in the cohort had a value of 0 to 1. While our cohort of BRAF-mutant patients appear to trend toward better PFS with ipi/nivo compared to anti-PD-1 monotherapy, the lack of statistical difference may be attributed to its relatively small sample size. The findings of our study are interesting and if validated, may have implications in clinical practice decisions when selecting initial line of immunotherapy. Further long-term clinical outcomes of melanoma patients treated in clinical trials comparing ipi/nivo versus anti-PD-1 monotherapy are eagerly awaited and the argument for selection of front-line ICIs will continue to broaden as other therapy combinations are developed.

## CONFLICT OF INTEREST

Dr. Ajjai Alva serves as a consultant for Merck, AstraZeneca, Bristol-Myers Squibb, and Pfizer. Dr. Ajjai Alva receives research funding through the University of Michigan from Merck, Genentech, Prometheus Laboratories, Mirati Therapeutics, Roche, Bayer, Progenics, Astellas Pharma, Arcus Biosciences, AstraZeneca, Bristol-Myers Squibb, and Clovis Oncology. Stephanie Daignault-Newton serves as a consultant for Boston Scientific and receives research funding from Pfizer and Bayer. Dr. Leslie Fecher serves as a consultant for ViaOncology/Elsevier, Hoosier Cancer Research Network, Elsevier. Dr. Leslie Fecher receives research funding through the University of Michigan from Merck, Incyte, Bristol-Myers Squibb, Pfizer/EMD Serono, Array BioPharma, and Kartos Therapeutics. Dr. Leslie Fecher has uncompensated relationships with NCCN. Dr. Christopher Lao serves as a consultant for Immunocore. Dr. Christopher Lao receives travel, accommodations, and expenses from Bristol-Myers Squibb and Immunocore. Dr. Christopher Lao receives research funding from Bristol-Myers Squibb, Merck, Novartis, and Dynavax. Dr. Vincent Ma, Dr. Michael D. Green, Dr. Bruce Redman, Jessica J. Waninger, Sara Journey, Zoey Chopra, and Alangoya Tezel have no conflict of interest to declare.

## ETHICAL APPROVAL

The study protocol is approved by the University of Michigan institutional ethical guidelines and complies with the guidelines of the responsible governmental agency.

## ORCID

*Vincent T. Ma* https://orcid.org/0000-0002-8417-2787

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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