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ORIGINAL REPORT

Antitumor Activity of Nivolumab in Recurrent and Metastatic Nasopharyngeal Carcinoma: An International, Multicenter Study of the Mayo Clinic Phase 2 Consortium (NCI-9742)

Brigette B.Y. Ma, Wan-Teck Lim, Boon-Cher Goh, Edwin P. Hui, Kwok-Wai Lo, Adam Pettinger, Nathan R. Foster, Jonathan W. Riess, Mark Agulnik, Alex Y.C. Chang, Akhil Chopra, Julie A. Kish, Christine H. Chung, Douglas R. Adkins, Kevin J. Cullen, Barbara J. Gitlitz, Dean W. Lim, Ka-Fai To, K.C. Allen Chan, Y.M. Dennis Lo, Ann D. King, Charles Erlichman, Jun Yin, Brian A. Costello, and Anthony T.C. Chan

Author affiliations and support information (if applicable) appear at the end of this article.

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B.A.C. and A.T.C.C. contributed equally to this work.

J.Y., B.A.C., and A.T.C.C. are joint senior authors.

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Corresponding author: Brigette B.Y. Ma, MD, Department of Clinical Oncology, Prince of Wales Hospital, Basement, LKS Specialist Clinic, Ngan Shing Street, Shatin, New Territories, Hong Kong, Special Administrative Region, People's Republic of China; e-mail: brigette@clo.cuhk.edu.hk.

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ABSTRACT

Purpose

This multinational study evaluated the antitumor activity of nivolumab in nasopharyngeal carcinoma (NPC). Tumor and plasma-based biomarkers were investigated in an exploratory analysis.

Patients and Methods

Patients with multiply pretreated recurrent or metastatic NPC were treated with nivolumab until disease progression. The primary end point was objective response rate (ORR) and secondary end points included survival and toxicity. The expression of programmed death-ligand 1 (PD-L1) and human leukocyte antigens A and B in archived tumors and plasma clearance of Epstein-Barr virus DNA were correlated with ORR and survival.

Results

A total of 44 patients were evaluated and the overall ORR was 20.5% (complete response, n = 1; partial response, n = 8). Nine patients received nivolumab for > 12 months (20%). The 1-year overall survival rate was 59% (95% CI, 44.3% to 78.5%) and 1-year progression-free survival (PFS) rate was 19.3% (95% CI, 10.1% to 37.2%). There was no statistical correlation between ORR and the biomarkers; however, a descriptive analysis showed that the proportion of patients who responded was higher among those with PD-L1 positive tumors (> 1% expression) than those with PD-L1-negative tumors. The loss of expression of one or both human leukocyte antigen class 1 proteins was associated with better PFS than when both proteins were expressed (1-year PFS, 30.9% *v* 5.6%; log-rank $P = .01$). There was no association between survival and PD-L1 expression or plasma Epstein-Barr virus DNA clearance. There was no unexpected toxicity to nivolumab.

Conclusion

Nivolumab has promising activity in NPC and the 1-year overall survival rate compares favorably with historic data in similar populations. Additional evaluation in a randomized setting is warranted. The biomarker results were hypothesis generating and validation in larger cohorts is needed.

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INTRODUCTION

Nasopharyngeal carcinoma (NPC) is endemic to parts of Asia and North Africa, and is etiologically associated with the Epstein-Barr virus (EBV). Circulating fragments of EBV-derived DNA can be detected in > 95% of patients with advanced NPC and have been shown to closely reflect tumor burden.[1] This virus-associated cancer represents the archetypal "inflamed tumor," which often exhibits a dense lymphocytic infiltrate and increased programmed death-ligand 1 (PD-L1) expression.[2] In a recent study on the whole-exome sequencing (WES) and whole-genome sequencing (WGS) of microdissected NPC primary tumors, researchers found that the mutational load of NPC may be higher than once reported.[3,4] A third of primary NPC tumors harbor major histocompatibility complex (MHC) class I gene aberrations, with inactivating mutations and rearrangements in the human leukocyte antigen (HLA) -A and *HLA-B* genes being the most common, which invariably results in the loss of HLA-A and HLA-B protein expression.[4]

Given these unique biologic characteristics of NPC, this is, to our knowledge, the first completed

ASSOCIATED CONTENT

Appendix
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Data Supplement
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report on the activity of the immune-checkpoint inhibitor nivolumab in patients with recurrent or metastatic NPC. To date, there is a lack of prospective data on the biomarkers of response to checkpoint inhibitors in NPC. Therefore, this study also investigated the clinical significance of PD-L1, HLA-A, and HLA-B expression in NPC tumors and plasma EBV DNA.

This study was a multinational trial sponsored by the National Cancer Institute. The protocol was approved by the Central Institutional Review Board of the National Cancer Institute and the institutional ethics committees in Hong Kong and Singapore.

PATIENTS AND METHODS

Patient Selection and Treatment

Eligible patients had histologically or cytologically confirmed NPC that had recurred at locoregional and/or distant sites and were not amenable to curative treatment. The target lesions had to be measurable by the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, criteria. All patients had to receive at least one prior line of platinum-based chemotherapy for recurrent disease and have adequate organ function. They underwent a baseline contrast-enhanced computed tomography of the chest, abdomen, and pelvis, and magnetic resonance imaging or computed tomography scan for locoregional disease. Radiologic assessments were performed every 8 weeks for 6 months and then every 12 weeks thereafter. Archived tumor samples were retrieved and plasma samples were obtained at baseline, then weekly for the first 4 weeks of treatment. Eligible patients were treated with nivolumab at a dosage of 3 mg/kg intravenously every 2 weeks on a 4-week cycle until they experienced disease progression. Patients were allowed to continue treatment beyond RECIST progression occurring during the initial 12 weeks, as long as they satisfied all the criteria set out in the protocol.

Biomarker Study

In a preplanned biomarker study, archived, paraffin-embedded NPC tumors were retrieved for the immunohistochemical analysis of PD-L1 (anti-human PD-L1 antibody, clone 22C3, PD-L1 IHC 22C3; pharmDx assay; Agilent Technologies, Santa Clara, CA) and HLA-B (anti-HLA-B antibody, HLA-*N*-20; Santa Cruz Biotechnology, Dallas, TX) protein expression. PD-L1 expression in tumor cells and immune cells was scored as the percentage of tumor cells and immune cells with membranous straining, respectively.[5] PD-L1 expression of $<$ 1% was regarded as negative and expression of $\geq$ 1% was regarded as positive. HLA-A and HLA–B expression was scored as the percentage of tumor cells with membranous straining. This is based partly on Garrido et al,[6] who used a threshold of $\geq$ 25% as positive expression and defined HLA positivity as PD-L1 expression in $\geq$ 20% of the cells and HLA negativity as PD-L1 expression in $<$ 20% of the cells. The results were independently scored by two pathologists who were blinded to the clinical status of the study participants. Plasma EBV DNA level was determined using real-time quantitative polymerase chain reaction and the clearance (half-life) during the first cycle of nivolumab, as previously described.[1]

Statistics and Sample Size Calculation

The primary end point of this study was objective response by the RECIST criteria (version 1.1), and the secondary end points were overall survival (OS), progression-free survival (PFS), duration of response and toxicity (Common Terminology Criteria for Adverse Events, version 4.0). Time-to-event variables were estimated using the Kaplan-Meier method and survival rates were compared using the log-rank test. Two-sided P values $<$ .05 were considered statistically significant. In an exploratory analysis, Fisher exact test was used to correlate binary clinical data with biomarker data.

The sample size of this study was estimated on the assumption that response rates (RRs) to nivolumab should be around 20%, based on a report that was available at the time this study was planned.[7] Furthermore, the RR to noncytotoxic, experimental agents such as pazopanib and cetuximab in similarly pretreated patient cohorts was approximately 5% to 10%.[8,9] This study's design was based on the modified Simon two-stage optimal design (power, 90%; $\alpha = 0.09$; $P_0 = .05$; $P_1 = .20$; $n_1 = 20$; $n = 35$ with an additional 10 patients to allow for ineligibility, major protocol violations, or other reasons). Because four responses were observed during the first stage, enrollment was continued until a total of 45 patients was reached.

RESULTS

Efficacy and Tolerability of Nivolumab

A total of 45 patients were enrolled, of whom one patient was ineligible. This study accrued patients across 11 sites; most patients were Asian (83%; Table 1). The enrollment period spanned from October 28, 2015, until June 1, 2016, and the data were frozen on July 10, 2017. The median follow-up was 12.5 months (range, 2.2 to 22.0 months) for the 28 patients who were still alive.

Of the 44 eligible patients, one patient had complete response (CR) lasting $>$ 12 months (2.3%); eight had a partial response (PR; 18.2%; median duration of response, 9.3 months [95% CI, 3.6 to 13.1 months]); 15 had stable disease (SD; 34.1%; three patients had SD $>$ 12 months); 18 had disease progression (40.9%); and two patients (4.5%) were not assessed for response. The overall RR was 20.5% (95% CI, 9.8 to 35.3), and the disease control rate was 54.5% (Fig 1). There was no relationship with the number of prior lines of chemotherapy and the response pattern to the last line of chemotherapy before enrollment (Table 1). The median OS was 17.1 months (95% CI, 10.9 months to not reached) and the 1-year OS rate was 59% (95% CI, 44.3% to 78.5%; Appendix Fig A1, online only). The median progression-free survival (PFS) was 2.8 months (95% CI, 1.8 to 7.4 months) and the 1-year PFS rate was 19.3% (95% CI, 10.1% to 37.2%; Appendix Fig A2, online only). As illustrated in the swimmer plot in Figure 2, most responders achieved a PR at the first radiologic assessment; two patients had a delayed response at the second assessment. All 18 patients whose best response was progressive disease were detected at the first radiologic assessment. Thirteen patients (29.5%) were still receiving treatment 6 months after registration, and nine patients (20.5%) received treatment for $>$ 12 months.

Compliance with treatment was good, with a median of three cycles of nivolumab administered (range, 1 to 19 cycles). The main reason for permanent discontinuation of the study was disease progression (69.2% of cases), with a minority of cases (10.3%) due to adverse events. Of the 45 patients who were evaluable for toxicities, 10 (22.2%) experienced grade 3 or higher adverse events that were possibly related to nivolumab (Appendix Table A1). Grade 3 or higher toxicities occurred in 22% of patients and included colitis, diarrhea, fatigue, increase in aspartate transaminase or alanine aminotransferase levels, neutropenia, hyponatremia, and lymphopenia. One patient died of pulmonary tuberculosis during treatment.

Correlative Studies With Biomarkers

The archived tumors of 42 patients were retrieved and the plasma samples of 43 patients were prospectively collected for

Table 1. Patient Characteristics

Characteristic	Data (%)
No. of patients	45
Median age, years (range)	57.0 (37.0-76.0)
Sex	
Male	35 (77.8)
Female	10 (22.7)
Race	
Asian	37 (82.2)
African American	1 (2.2)
Native Hawaiian/other Pacific Islander	1 (2.2)
White	4 (8.9)
Unknown/not reported	2 (4.4)
ECOG PS	
0	17 (37.8)
1	27 (60)
2	1 (2.2)
Histology (WHO)	
Undifferentiated NPC	25 (55.6)
Poorly differentiated NPC	12 (26.6)
NPC (not otherwise specified)	8 (17.8)
Most common sites of recurrent disease	
Lung	42 (95.5)
Liver	42 (95.5)
Bone	16 (36.4)
Lymph nodes	35 (79.5)
Nasopharynx	13 (29.5)
No. of prior lines of chemotherapy, median (range)	
Median (range)	3 (1-9)
1-2	17 (38.6)
3-4	16 (36.4)
> 5	11 (25)
Response to the last line of chemotherapy before study enrollment	
Responders to nivolumab (n = 9):	
CR or PR to last chemotherapy	3 (33.3)
SD or PD to last chemotherapy	6 (66.7)
Nonresponders to nivolumab (n = 35)	
CR or PR to last chemotherapy	6 (17.1)
SD or PD to last chemotherapy	29 (82.9)
Prior radical radiotherapy*	
Yes	37 (82.2)
No	8 (17.8)
No. of nivolumab cycles, median (range)	3 (1-24)
Follow-up for 44 evaluable patients	
Progression	35 (79.5)
No progression	9 (20.5)
Alive	28 (63.6)
Dead	16 (36.4)
Reasons for withdrawal from study for the 44 evaluable patients	
Still receiving treatment	5 (11.1)
Adverse events/ toxicity	4 (10.3)
Intercurrent illness	1 (2.6)
Death	1 (2.6)
Disease progression	27 (69.2)
Patient refusal	3 (7.7)
Reasons not specified	3 (7.7)

Abbreviations: CR, complete response; ECOG PS, Eastern Cooperative Group performance status; NPC, nasopharyngeal carcinoma; PR, partial response; SD, stable disease.
*Data presented as No. (%) unless otherwise indicated. Data cut off at July 10, 2017.

biomarker analysis. The results of plasma EBV DNA clearance and expression of PD-L1, HLA-A, and HLA-B are listed in Table 2, and the prognostic value of these markers was investigated. There was no statistical difference between patients with PD-L1–negative versus PD-Ll–positive tumors (expressed in tumor or immune cells) in terms of OS or PFS (Fig 1). In terms of correlation with response, six of 18 patients (33%) with PD-L1–positive tumors responded to nivolumab, whereas only three of 23 patients (13%) with PD-L1–negative tumors responded, but this did not reach statistical significance. The swimmer plot in Figure 2 illustrates the PD-L1 status and duration of response in patients who received nivolumab. In a descriptive summary of objective RRs and different levels of PD-L1 expression (Appendix Fig A3 and Appendix Table A2), a higher proportion of patients with higher levels of PD-L1–expressing tumors responded to nivolumab than those with PD-L1–negative tumors.

Of the 41 samples that were of sufficient quality for determining HLA expression, a statistical difference in PFS was observed between patients with tumors showing loss of expression of HLA-A and/or HLA-B (1-year PFS, 30.9% [95% CI, 16.2% to 59.1%]; median, 4.8 months [95% CI, 2.7 to 14 months]; log-rank $P = .01$), and patients with tumors expressing both HLA-A and HLA-B (1-year PFS, 5.6% [95% CI, 0.8% to 37.9%]; median PFS, 1.8 months [95% CI, 1.7 to 7.4 months]; Fig 3). A similar difference in OS was observed between patients with tumors exhibiting loss of expression of HLA-A and/or HLA-B expression (1-year OS, 75.7% [95% CI, 59.2% to 96.8%]; median OS was not reached) and patients with tumors expressing both HLA proteins (1-year OS, 33.8%; 95% CI, 15.5% to 73.7% median OS was 10.9 [9.7-NE]), though the difference was not statistically significant (log-rank $P = .08$). There was no association between HLA expression and RR (HLA-A and HLA-B expressed, RR, 22.22%; HLA-A and/or HLA-B loss, RR, 19.23%; Fisher $P = 1.0$).

Plasma EBV DNA was detectable in 97.8% of patients at baseline (Table 2), which is consistent with the literature of a detection rate > 96%.[1] Patients with plasma EBV DNA clearance above or below the median half-life (14.1 days) did not differ statistically in terms of RR and survival. There was no statistical difference in RR and survival between patients with a rising versus falling trend of EBV DNA during the first month of nivolumab.

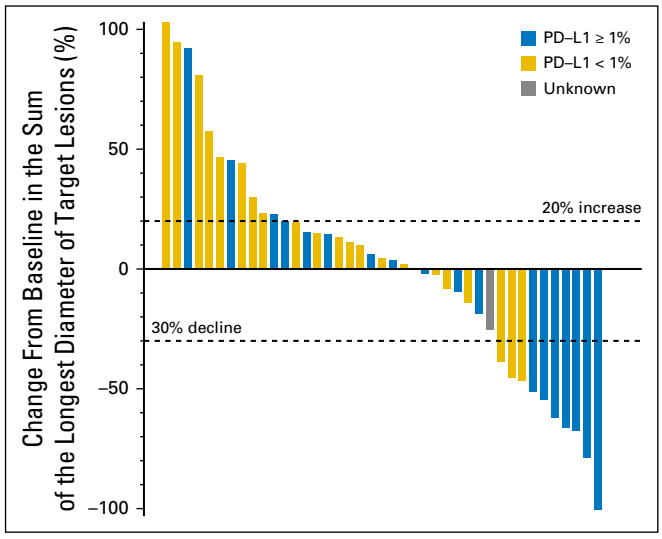

Fig 1. Waterfall plot. Changes in sum of unidimensional tumor dimension from baseline and Response Evaluation Criteria in Solid Tumors response in individual patients. Partial response was defined as a ≥ 30% decline in tumor dimension. Progressive disease was defined as a > 20% increase in tumor dimensions.

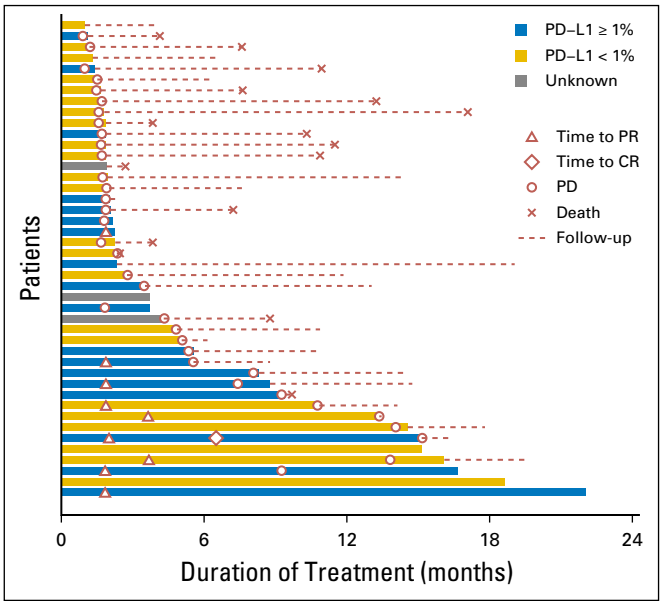

Fig 2. Swimmer plot. Duration of response and time to response in patients receiving nivolumab. CR, complete response; PD, progressive disease; PR, partial response.

DISCUSSION

To our knowledge, this is the first completed report on the clinical activity and biomarker of response to nivolumab in patients with recurrent and metastatic NPC. There was no statistical association between PD-L1 expression in tumor cells or immune cells with survival and response to nivolumab; however, there was a higher proportion of patients with PD-L1–positive tumors who responded to nivolumab numerically in a descriptive analysis. Intriguingly, loss of HLA-A and HLA-B was associated with better survival than in patients with HLA-A– and HLA-B–intact tumors.

The pattern of response observed in this study was consistent with that reported with PD-1 inhibitors in other cancers (Fig 4).[10] In the swimmer plot (Fig 2), some delayed responses (including a CR) are seen beyond the first 4 months of treatment. Disease in nearly all primary nonresponders progressed within the first 2 months of therapy, with a minority displaying a sharp increase in tumor dimensions. The latter may well represent hyperprogression, a newly reported pattern of response occurring in 9% of patients undergoing treatment with immune-checkpoint inhibitors, but this cannot be confirmed without comparison with tumor growth rate from prior therapy.[11]

Insights from studies of immune-checkpoint inhibitors have now shown that survival milestones (eg, 1-year survival rates) can better reflect the unique patterns of activity of these agents over other end points such as median survival, because clinical trials of PD-1 inhibitors often exhibit a late separation of survival curves and durable SD without tumor shrinkage.[10] The 1-year OS rate of nivolumab (59%; 95% CI, 44.3% to 78.5%) compares favorably with phase II studies of similar populations. In these studies, the 1-year OS rates were consistently reported at approximately 45% to cytotoxic and noncytotoxic drugs.[8,12-14] In a recently published phase Ib study of 27 patients with a mixed background of treatment-naïve or pretreated squamous and nonsquamous NPC, treatment with pembrolizumab resulted in an RR of 25.9% and 1-year OS of 63%.[15] Compared with those in that phase Ib study,[15] the patients in our study were all treatment refractory, nearly all had detectable plasma EBV DNA at baseline, and > 80% had nonkeratinizing NPC that was more reflective of endemic NPC.

There is significant variability in the literature on the prevalence and prognostic significance of PD-L1 expression in NPC, probably because of the differences in the assays and scoring methods used across studies.[2,16-18] The predictive utility of PD-L1 expression could also depend on the differential expression in immune cells versus tumor cells.[19] Studies that had made such a distinction found that PD-L1 was expressed in 24% to 33% of tumor cells and 40% to 75% of immune cells.[17,18] This study was powered based on objective response to nivolumab but not biomarker end points; thus, the lack of statistical correlation with PD-L1 expression could be due to the limited sample size. Interestingly, as shown in Appendix Fig A3, a higher proportion of objective responses was observed in patients with PD-L1–positive tumors (≥ 1% expression) than those with PD-L1–negative tumors. In Checkmate 141 (Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck), a numerically higher (but nonstatistical) benefit in survival was reported in patients with PD-L1–positive tumors who received nivolumab than in those who received chemotherapy.[20] In the Pembrolizumab for patients with PD-L1–Positive Advanced Gastric Cancer (Keynote 012) study, patients with PD-L1–positive tumors had higher RR to pembrolizumab than those with PD-L1–negative tumors.[21] Notably, the complete responder in this study also possessed the highest level of PD-L1 expression in the immune cells (Appendix Table A2). It is also possible that the clinical significance of PD-L1 expression in NPC

Table 2. Biomarker Characteristics: Summary

Biomarker	No. (%)
Plasma EBV DNA (n = 45)	
Trend in cycle 1	
Not detectable	1 (2.2)
Increasing	19 (42.2)
Decreasing	25 (55.6)
Plasma EBV DNA, median (range)	
Baseline, copies/mL	6,438 (0-1.18 × 10^6)
Half-life, days	14.1 (4.0-994.3)
PD-L1 expression in tumor cells (n = 45)	
Expressed < 1%	24 (53.3)
Expressed ≥ 1%	18 (40)
Unknown	3 (6.7)
PD-L1 expression in immune cells (n = 45)	
Expressed < 1%	31 (68.9)
Expressed ≥ 1%	10 (22.2)
Unknown	4 (8.9)
HLA-A expression (n = 45)	
Expressed	26 (57.8)
Loss	15 (33.3)
Unknown	4 (8.9)
HLA-B expression (n = 45)	
Expressed	21 (46.7)
Loss	20 (44.4)
Unknown	4 (8.9)

Abbreviations: EBV, Epstein-Barr virus; HLA, human leukocyte antigen; PD-L1, programmed death-ligand 1.

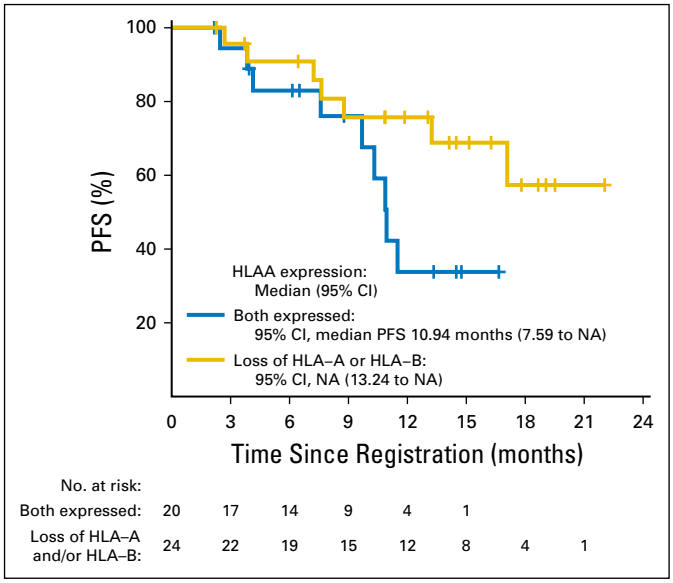

Fig 3. Progression-free survival curves of patients with tumors expressing both HLA-A and HLA-B (blue line), versus loss of HLA-A and/or HLA-B expression (gold line). HLA, human leukocyte antigen; NA, not achieved; PFS, progression-free survival.

may be contingent on the disease stage or other factors in the tumor microenvironment that are yet to be identified, such as the intratumor level of CD3+ cells.[18] We used archived (as opposed to fresh) tumor samples for the determination of PD-L1 expression, and tissue fixation and storage may potentially undermine the detection of PD-L1 protein, as reported in renal cell cancers.[22] However, archived tumors have been used effectively to determine the eligibility of enrollment in some studies of PD-1 inhibitors in lung cancer.[23]

Genetic alterations in MHC class 1 genes are found in 30% of NPC primary tumors and have been linked to inferior OS and disease-free survival.[4] Approximately 50% of these alterations were gene rearrangements and inactivating mutations in HLA-A and HLA-B genes, and this invariably would result in varying loss of the respective protein expression in NPC tumors.[4] In this study, HLA-A and HLA-B expression did not predict response to nivolumab; however, there was a statistical association between loss of expression of HLA-A and/or HLA-B and PFS. This finding is intriguing in light of the result of our WGS/WES study, in which patients with somatic alterations of MHC class I genes had poorer outcomes.[4] This difference could be partly explained by the differences in the patient's clinical stage and origin of the tumor specimens. The WGS/WES study involved mainly the primary tumors of patients with nonmetastatic disease, whereas our study focused on recurrent and/or metastatic NPC and using a mixture of archived primary and metastatic tumors. The heterogeneity of HLA class 1 expression in solid tumors was highlighted by Garrido et al,[6] who described a progressive transition of tumor cells from an MHC class 1–positive "permissive" phase during early carcinogenesis, to a "nonpermissive" phase in a later state, as typified by growing populations of MHC class 1–negative cancer cells that have escaped T-cell killing. Thus, the frequency of MHC class 1 downregulation could be higher in metastatic than in primary tumors.[24]

In the literature, loss of HLA class 1 expression in solid tumors has mostly been associated with poorer prognosis and a more immune-suppressive microenvironment.[6,25] However, the literature is lacking on the clinical significance of MHC class 1 downregulation in the tumors of patients undergoing anti-PD-1/PD-L1 therapy.[26,27] In melanoma, a nonstatistical association between response to PD-L1 inhibitor and intact MHC-1 gene expression has been found,[28,29] whereas another study found an association with increased HLA-A mRNA expression.[26] Although the presence of an intact MHC antigen–presenting machinery may be a prerequisite for successful therapy with PD-1 inhibitors in most cancers, some tumors, such as Hodgkin lymphoma, often exhibit total loss of MHC class 1 expression in $>$ 50% of patients and are responsive to PD-1 inhibitors.[30] In our study, the favorable prognostic significance of loss of HLA-A and/or HLA-B may raise speculation about whether there could be alternative

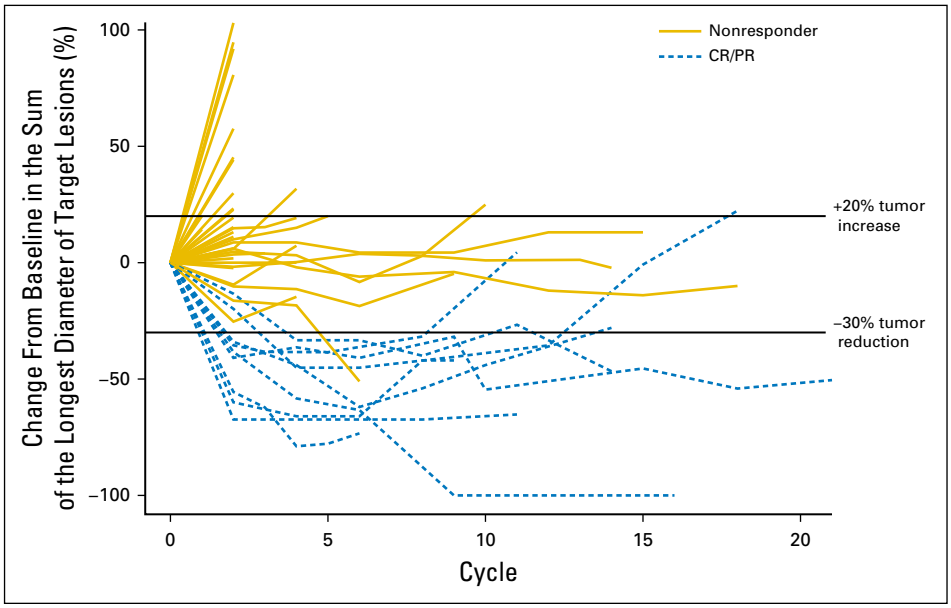

Fig 4. Spider plot of changes in the sum of unidimensional tumor measurements over time. The dotted blue line represents responders (according to Response Evaluation Criteria in Solid Tumors); solid gold line represents nonresponders. CR, complete response; PR, partial response.

mechanisms of action of PD-1 inhibitors in heavily pretreated NPC besides reversing T-cell exhaustion, such as via natural killer (NK) cells.[31] For instance, preclinical studies have found that PD-1/PD-L1 blockade may affect the functional exhaustion of NK cells in ovarian cancer and Kaposi sarcoma cells by potentiating NK-cell cytotoxicity.[32,33] Additional studies are needed to elucidate the mechanism of action of PD-1 blockade in NPC.

Cell-free plasma EBV DNA exists as DNA fragments and its release into the patient's circulation may arise from apoptosis of cancer cells after cytotoxic therapy.[34] Although the data were not shown, seven of the eight responders with detectable baseline plasma EBV DNA had a decreasing trend for this marker during the first month of therapy. It is possible that the sample size of this study was inadequate to demonstrate a statistical significance.

In conclusion, nivolumab has promising clinical activity in heavily pretreated recurrent and/or metastatic NPC and should be investigated in other clinical settings. This clinical study was primarily designed to investigate the activity of nivolumab in NPC and the biomarker component of this study was intended to be hypothesis generating. The clinical utility of PD-L1 expression on response to nivolumab and the prognostic significance of HLA-A and HLA-B expression in patients treated with nivolumab should be validated in independent cohorts. The possibility of non–T-cell dependent mechanisms of nivolumab in NPC should be explored.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Brigette B.Y. Ma, Wan-Teck Lim, Boon-Cher Goh, Nathan R. Foster, Kevin J. Cullen, Ann D. King, Charles Erlichman, Jun Yin, Brian A. Costello, Anthony T.C. Chan
Provision of study materials or patients: Brigette B.Y. Ma, Jonathan W. Riess, Mark Agulnik, Barbara J. Gitlitz
Collection and assembly of data: Brigette B.Y. Ma, Wan-Teck Lim, Boon-Cher Goh, Edwin P. Hui, Kwok-Wai Lo, Adam Pettinger, Nathan R. Foster, Jonathan W. Riess, Mark Agulnik, Alex Y.C. Chang, Akhil Chopra, Julie A. Kish, Christine H. Chung, Douglas R. Adkins, Kevin J. Cullen, Barbara J. Gitlitz, Dean W. Lim, Ka Fai To, K.C. Allen Chan, Ann D. King
Data analysis and interpretation: Brigette B.Y. Ma, Wan-Teck Lim, Boon-Cher Goh, Edwin P. Hui, Kwok-Wai Lo, Adam Pettinger, Nathan R. Foster, Jonathan W. Riess, K.C. Allen Chan, Y.M. Dennis Lo, Ann D. King, Charles Erlichman, Jun Yin, Brian A. Costello, and Anthony T.C. Chan
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Affiliations

Brigette B.Y. Ma, **Edwin P. Hui**, **Kwok-Wai Lo**, **Ka-Fai To**, **K.C. Allen Chan**, Y.**M. Dennis Lo**, **Ann D. King**, and **Anthony T.C. Chan**, The Chinese University of Hong Kong, Shatin, Hong Kong, Special Administrative Region, People's Republic of China; **Wan-Teck Lim**, National Cancer Centre; **Boon-Cher Goh**, National University Cancer Institute of Singapore; **Alex Y.C. Chang**, Johns Hopkins University School of Medicine; **Akhil Chopra**, OncoCare Cancer Centre, Singapore; **Adam Pettinger**, **Nathan R. Foster**, **Charles Erlichman, Jun Yin**, and **Brian A. Costello**, Mayo Clinic, Rochester, MN; **Jonathan W. Riess**, University of California Davis Comprehensive Cancer Center, Sacramento; **Barbara J. Gitlitz**, University of Southern California Keck School of Medicine, Los Angeles; **Dean W. Lim**, City of Hope Comprehensive Cancer Center, Duarte, CA; **Mark Agulnik**, Northwestern University, Evanston, IL; **Julie A. Kish** and **Christine H. Chung**, Moffitt Cancer Center, University of South Florida, Tampa, FL; **Douglas R. Adkins**, Washington University School of Medicine, St Louis, MO; **Kevin J. Cullen**, University of Maryland, Baltimore, MD.

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Brigette B.Y. Ma
Honoraria: Novartis, Merck Sharp & Dohme, Merck Serono, Roche
Consulting or Advisory Role: Novartis, Merck Serono, Merck Sharp & Dohme
Research Funding: Novartis
Travel, Accommodations, Expenses: Amgen

Wan-Teck Lim
Research Funding: Bristol-Myers Squibb

Boon-Cher Goh
No relationship to disclose

Edwin P. Hui
Honoraria: Merck Serono, Merck Sharp & Dohme
Consulting or Advisory Role: Bristol-Myers Squibb

Kwok-Wai Lo
No relationship to disclose

Adam Pettinger
No relationship to disclose

Nathan R. Foster
No relationship to disclose

Jonathan W. Riess
Consulting or Advisory Role: Celgene, ARIAD, Clovis Oncology, Medtronic, Abbvie, Biodesix
Research Funding: Millennium (Inst), Novartis (Inst), Merck (Inst), AstraZeneca/MedImmune (Inst)
Travel, Accommodations, Expenses: Roche

Mark Agulnik
Consulting or Advisory Role: Novartis, Janssen Oncology, Eisai, Eli Lilly
Speakers' Bureau: Bristol-Myers Squibb, Eli Lilly, Janssen Oncology

Alex Y.C. Chang
Consulting or Advisory Role: Bristol-Myers Squibb, Celgene, Pfizer

Akhil Chopra
Consulting or Advisory Role: Bristol-Myers Squibb, Roche India, Bayer, Merck, AstraZeneca, Astellas Pharma, Eli Lilly, MSD Oncology
Travel, Accommodations, Expenses: Astellas Pharma, Bristol-Myers Squibb

Julie A. Kish
No relationship to disclose

Christine H. Chung
Consulting or Advisory Role: AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Vigilant Biosciences, Celgene
Research Funding: AstraZeneca, Bristol-Myers Squibb Eli Lilly, Merck, Regeneron, Ignyta, IRX Therapeutics, Pfizer, Iovance Biotechnologies
Travel, Accommodations, Expenses: AstraZeneca, Bristol-Myers Squibb, Eli Lilly

Douglas R. Adkins
No relationship to disclose

Kevin J. Cullen
No relationship to disclose

Barbara J. Gitlitz
Employment: Roche
Honoraria: Roche, Eli Lilly
Speakers' Bureau: Roche, Eli Lilly
Travel, Accommodations, Expenses: Roche

Dean W. Lim
No relationship to disclose

Ka Fai To
No relationship to disclose

K.C. Allen Chan
Stock or Other Ownership: Grail
Consulting or Advisory Role: Grail
Speakers' Bureau: BioRad
Patents, Royalties, Other Intellectual Property: Patent portfolios on molecular diagnostics, Grail, Illumina, Sequenom

Y.M. Dennis Lo
Stock or Other Ownership: Grail, Sequenom, Xcelom, Cirina
Consulting or Advisory Role: Grail, Decheng Capital, Sequenom
Research Funding: Grail/Cirina (Inst)
Patents, Royalties, Other Intellectual Property: Patent portfolio in molecular diagnostics using circulating nucleic acids

Ann D. King
No relationship to disclose

Charles Erlichman
Research Funding: National Cancer Institute

Jun Yin
No relationship to disclose

Brian A. Costello
Honoraria: GU CONNECT
Research Funding: GlaxoSmithKline/Novartis (Inst)

Anthony T.C. Chan
Research Funding: Bristol-Myers Squibb, Pfizer, MSD Oncology

Appendix

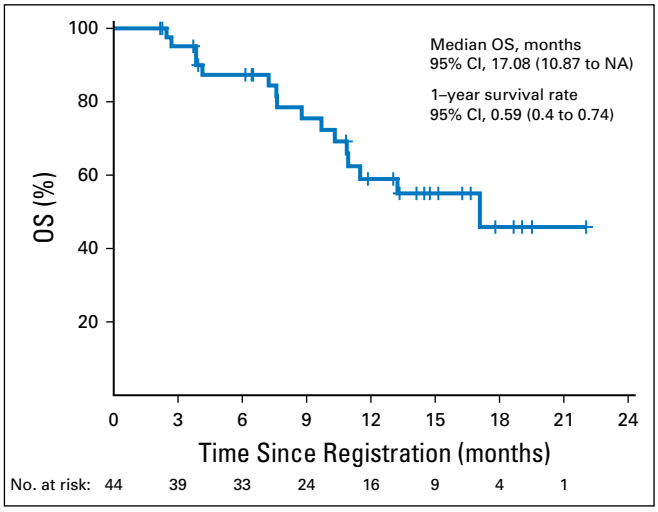

Fig A1. Overall survival (OS) of 44 evaluable patients.

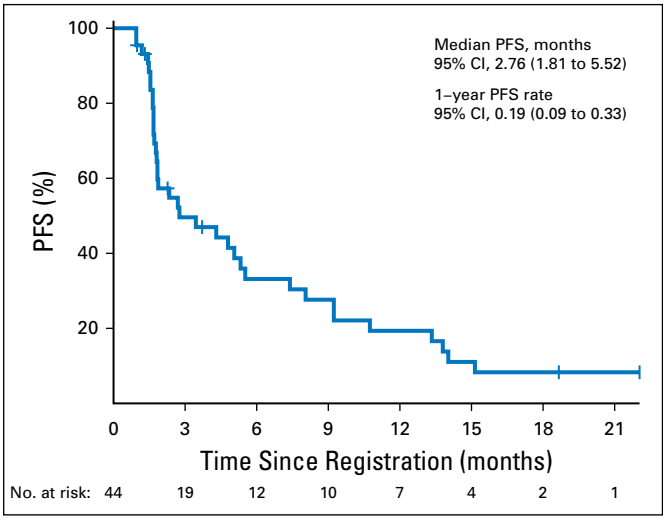

Fig A2. Profession-free survival (PFS) of 44 evaluable patients.

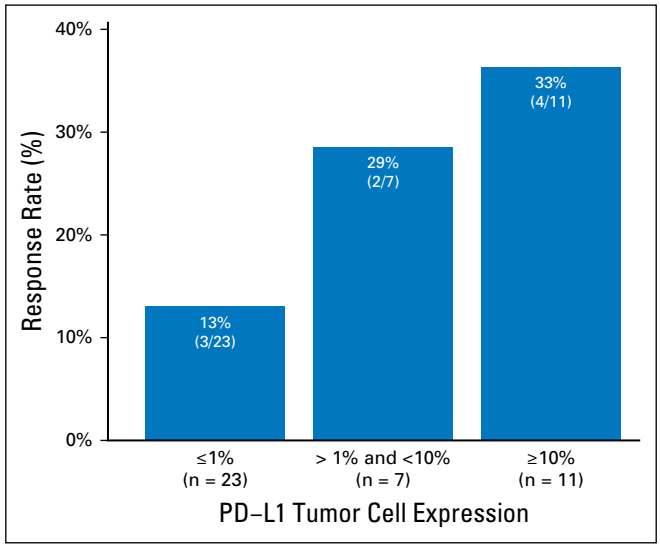

Fig A3. Descriptive summary on the proportion of patient with response (defined by Response Evaluation Criteria in Solid Tumors) to nivolumab according to the level of programmed death-ligand 1 (PD-L1) expression in tumor cells.

Table A1. Selected Adverse Events Possibly Related to Nivolumab

Toxicity	Grade, No.				
	1 and 2	2	3	4	5
Hypothyroidism	3	3	0	0	0
Colitis	0	0	1	0	0
Diarrhea	3	1	1	0	0
Nausea	2	2	0	0	0
Fatigue	9	5	1	0	0
Sepsis	0	0	0	0	1
Lung infection	0	1	0	1	0
ALT level increased	2	0	2	0	0
AST level increased	2	3	1	0	0
Lymphocyte count decreased	1	1	1	0	0
Neutrophil count decreased	0	0	1	0	0
Serum amylase increased	0	3	0	0	0
Weight loss	1	2	0	0	0
WBC count decreased	0	0	1	0	0
Hyponatremia	0	0	1	0	0
Myalgia	2	2	0	0	0
Rash, acneiform	1	0	0	0	0
Rash, maculopapular	1	0	0	0	0
Skin hypopigmentation	1	0	0	0	0
Rash pustular	0	1	0	0	0

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table A2. Biomarker Characteristics in Responders

Patient No.	PD-L1 Expression, Tumor Cells, %	PD-L1 Expression, Immune Cells, %	HLA-A	HLA-B	RECIST Response	Site of Metastases
015	10	35	Expressed	Expressed	PR	Lung
022	70	< 1	Expressed	Expressed	PR	Lung
001	< 1	< 1	Loss	Loss	PR	Liver, nodal
005	40	< 1	Expressed	Loss	PR	Liver, lung
043	< 1	< 1	Loss	Loss	PR	Liver, nodal
016	< 1	< 1	Expressed	Expressed	PR	Liver, lung, bone
044	5	< 1	Expressed	Expressed	PR	Distant nodes
040	90	< 5	Expressed	Loss	PR	Bone, locoregional
027	5	50	Loss	Loss	CR	Soft tissue/bone

Abbreviations: CR, complete response; HLA, human leukocyte antigen; PD-L1, programmed death-ligand 1, PR, partial response; RECIST, Response Evaluation Criteria In Solid Tumors.