Genomic Landscape of Late-Stage Gastric Cancer: Analysis From KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 Studies

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ABSTRACT

PURPOSE The Cancer Genome Atlas (TCGA) classifies gastric cancer (GC) into four molecular subtypes: Epstein-Barr virus–positive, microsatellite instability–high (MSI-H), genomically stable (GS), and chromosomal instability (CIN). This exploratory analysis compared the genomic landscape of late-stage GC from KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 studies with early-stage GC from TCGA and evaluated the genomic characteristics of late-stage GC in patients of Western and Asian origin.

MATERIALS AND METHODS Using pretreatment tumor samples, bulk DNA was analyzed via whole-exome sequencing (WES; KEYNOTE-059/KEYNOTE-061) and FoundationOneCDx (KEYNOTE-062) to determine TCGA-defined molecular subtypes (only MSI-H is determinable from FoundationOneCDx), genomic alterations, homologous recombination deficiency (HRD), and tumor mutational burden (TMB); gene expression signatures were analyzed using RNA sequencing.

RESULTS When comparing KEYNOTE-059/061/062 combined WES and FoundationOneCDx data with data from TCGA, the MSI-H subtype prevalence was numerically lower in patients of Western (5% *v* 22%) and Asian origin (5% *v* 19%). When comparing KEYNOTE-059/061 WES data with the TCGA data set, the GS subtype prevalence was numerically higher (36% *v* 21%) in patients of Western or Asian origin. Among subtypes in KEYNOTE-059/061, HRD scores and TMB trended highest in CIN and MSI-H subtypes, respectively. *TP53* mutation was the most prevalent genomic characteristic per KEYNOTE-059/061/062 combined analysis in patients of Western or Asian origin. Gene expression signature distributions were generally similar between patients of Western and Asian origin.

CONCLUSION Numerical differences in the prevalence of MSI-H and GS subtypes were observed between early-stage and late-stage GC. Genomic characteristics of late-stage GC were generally similar between patients of Western and Asian origin.

ACCOMPANYING CONTENT

Data Sharing Statement

Data Supplement

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INTRODUCTION

Gastric cancer (GC) is a heterogeneous disease with both histologic and molecular diversity. Traditionally, GC classification was based on morphology[1]; however, recognition of the molecular heterogeneity and its implications as the driver for tumorigenesis has led to the development of a molecular-based classification. The Cancer Genome Atlas (TCGA) proposed a molecular classification system on the basis of data from 295 patients with early-stage GC not previously treated with chemotherapy or radiotherapy.[2] The tumor tissue samples were then categorized into four subtypes on the basis of molecular analyses: Epstein-Barr virus (EBV)–positive tumors, microsatellite instability–high (MSI-H) tumors, genomically stable (GS) tumors, and chromosomal instability (CIN) tumors. EBV-positive tumors were identified in 9% of GC samples and displayed recurrent *PIK3CA* mutations, extreme DNA hypermethylation, and amplification of *JAK2*, *PD-L1*, and *PDCD1LG2*. Tumors with MSI-H comprised 22% of the GC samples and displayed elevated mutation rates, including mutations of genes encoding targetable oncogenic signaling proteins. A similar proportion of tumors was identified as GS (20%) and was enriched for the diffuse histologic variant and mutations of

CONTEXT

Key Objective

What is the genomic landscape of late-stage gastric cancer (GC) and how does it compare with early-stage disease? Do characteristics differ between patients of Western and Asian origin?

Knowledge Generated

Classification into one of the four The Cancer Genome Atlas (TCGA) subtypes revealed a numerically lower prevalence of the microsatellite instability–high subtype, a numerically higher prevalence of the genomically stable subtype, and subtle numerical differences in the prevalence of Epstein-Barr virus–positive and chromosomal instability subtypes in late-stage disease versus early-stage disease from the previously reported TCGA data set. No evidence of major molecular differences between patients of Western and Asian origin was observed.

Relevance

Our analyses examining the association between the molecular characteristics of late-stage GC and clinical outcomes following treatment with pembrolizumab among patients of Western and Asian origin add to the existing body of scientific evidence.

RHOA or fusions involving RHO-family GTPase-activating proteins. Tumors with CIN comprised 50% of the GC samples and were characterized by marked aneuploidy and focal amplification of receptor tyrosine kinases. No differences in the distribution of the four GC molecular subtypes were observed between patients of Western or East Asian origin.[2]

Gene expression analyses of GC tumors revealed differences in immunity signatures related to T-cell function between patients of Asian and non-Asian origin.[3] Compared with GC tissue samples collected from patients of Asian origin, samples from patients of non-Asian origin were enriched for signatures related to T-cell biology, including CD28 and cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) signaling.[3] In a phase II clinical trial of pembrolizumab in patients with metastatic GC from Asia (ClinicalTrials.gov identifier: NCT02589496), extremely high objective response rates (ORRs) were reported in patients with MSI-H (100%) and EBV-positive (100%) tumors; in patients with GS and CIN tumors, ORRs were 12% and 5%, respectively.[4] In contrast, an exploratory analysis of the KEYNOTE-061 trial that examined response to pembrolizumab by TCGA subtypes in patients predominantly (60%) from Western countries (Europe, Israel, North America, and Australia) showed that the ORR in patients with MSI-H and EBV-positive tumors was 43% and 13%, respectively; the ORR in patients with GS and CIN tumors was 9% and 11%, respectively.[5]

In this exploratory analysis, the genomic landscape of late-stage GC was evaluated using data from pretreatment tumor tissue samples from patients with GC or gastroesophageal junction (GEJ) cancer enrolled in the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 studies examining pembrolizumab with or without chemotherapy, and the results were compared with published data for early-stage GC from TCGA. Additionally, the genomic and tumor microenvironment (TME) features of advanced GC were characterized in patients of Western and Asian origin enrolled in these studies.

MATERIALS AND METHODS

Published molecular data from patients with early-stage GC from TCGA database were used as a reference data set.[2] The current analysis used pretreatment tumor samples collected from patients with primary GC or GEJ in KEYNOTE-059 (ClinicalTrials.gov identifier: NCT02335411), KEYNOTE-061 (ClinicalTrials.gov identifier: NCT02370498), and KEYNOTE-062 (ClinicalTrials.gov identifier: NCT02494583; Data Supplement).[6-8] The studies were conducted in accordance with principles of Good Clinical Practice and were approved by the appropriate institutional review boards and regulatory agencies. Written informed consent was provided by all study participants before enrollment.

Details regarding the data sets used and DNA and RNA analyses are provided in the Data Supplement. Whole-exome sequencing (WES), FoundationOneCDx, and/or RNA-sequencing data obtained from the KEYNOTE-059/061 combined analysis and the KEYNOTE-062 analysis were compared with TCGA.[2] The prevalence of TCGA subtypes and molecular genomic features was analyzed in the KEYNOTE studies' populations. Correlations between genomic features and molecular subtypes or other clinical/molecular features were assessed. DNA alterations and demographic features were stratified by patient origin (Western *v* Asian), TCGA subtype, and tumor histology (diffuse *v* intestinal).

Heat maps of T-cell–inflamed gene expression profile ($Tcell_{inf}GEP$), PD-L1, and gene expression signatures were generated to determine correlations between these

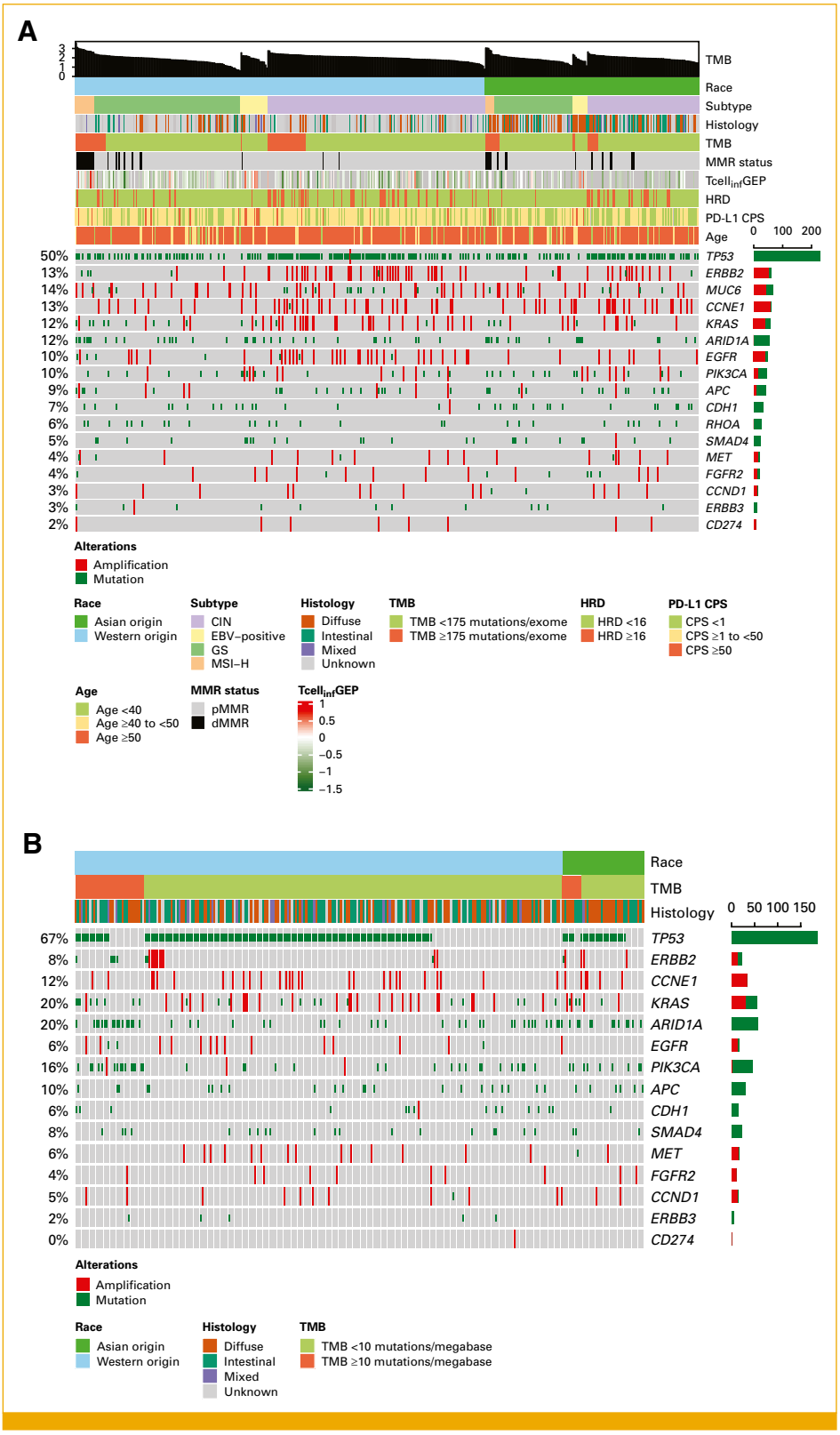

FIG 1. OncoPrint for gastric cancer data from (A) the KEYNOTE-059/061 combined analysis using WES (n = 464) and (B) the KEYNOTE-062 data analyzed using FoundationOneCDx (n = 279). The OncoPrints were generated using the ComplexHeatmap (version 2.6.2) package of R (version 4.0.5). CIN, chromosome instability; CPS, combined positive score; dMMR, mismatch repair deficient; EBV, Epstein-Barr virus; GS, genomically stable; HRD, homologous recombination deficiency; MMR, mismatch repair; MSI-H, microsatellite instability–high; pMMR, mismatch repair proficient; $Tcell_{inf}GEP$, T-cell–inflamed gene expression profile; TMB, tumor mutational burden; WES, whole-exome sequencing.

signatures and genetic alterations. RNA-sequencing data were also categorized by race and TCGA subtype. Descriptive statistics were used to provide nominal numerical comparisons between the KEYNOTE studies' and TCGA data sets.[2] Area under the receiver operating characteristic curve (AUROC) and chi-square statistics were performed for descriptive analyses.

RESULTS

Overall, 743 samples were included in this analysis (Western, n = 544; Asian, n = 199) comprising 79 samples from KEYNOTE-059 (Western, n = 62; Asian, n = 17), 385 samples from KEYNOTE-061 (Western, n = 243; Asian, n = 142), and 279 samples from KEYNOTE-062 (Western, n = 239; Asian, n = 40). The numbers of patient tumor samples in KEYNOTE-059 and KEYNOTE-061 and TCGA data sets with both RNA-sequencing and WES data, by origin and TCGA subtype, are presented in the Data Supplement (Tables S1 and S2).

Molecular Subtype Classification

In the KEYNOTE-059/061 combined analysis (n = 464), most patients had tumors with CIN, tumor mutational burden (TMB) <175 mutations/exome, and proficient mismatch repair (Fig 1A). In KEYNOTE-062 (n = 279), most patients had tumors with TMB <10 mutations/megabase (Fig 1B).

In patients of Western origin, the prevalence of the MSI-H subtype was numerically lower in the KEYNOTE-059/061/062 combined analysis versus TCGA data set (5% *v* 22%; Table 1). The prevalence of the GS subtype was numerically higher in the KEYNOTE-059/061 analysis versus TCGA data set (36% *v* 21%); subtle numerical differences in the prevalence of the EBV-positive subtype (7% *v* 10%) and CIN subtype (53% *v* 48%) were also observed (Table 1). In patients of Asian origin, the prevalence of the MSI-H subtype was numerically lower in the KEYNOTE-059/061/062 combined analysis versus TCGA data set (5% *v* 19%; Table 1). The prevalence of the GS subtype was numerically higher in the KEYNOTE-059/061 analysis versus TCGA data set (36% *v* 21%); numerical differences in the prevalence of the EBV-positive subtype (7% *v* 10%) and CIN subtype (52% *v* 49%) were observed (Table 1).

Assessment of homologous recombination deficiency (HRD) and TMB by TCGA subtypes indicated that the distribution of HRD scores was highest in the CIN subtype (Fig 2A); the distribution of TMB (continuous scale) was highest in the MSI-H subtype (Fig 2B). Gene expression profiles were similar regardless of the TMB status (not shown).

Genomic Alterations

There were no major differences in the prevalence of select genomic features of late-stage GC between patients of Western and Asian origin in the KEYNOTE-059/061 combined analysis or in KEYNOTE-062, except for a numerically higher prevalence of *EGFR* gene amplification in patients of Western origin versus patients of Asian origin in the KEYNOTE-059/061 combined analysis (10% *v* 5%; Fig 3A) and in KEYNOTE-062 (6% *v* 0%; Fig 3B). *TP53* mutation was the most prevalent genomic characteristic in patients of Western and Asian origin in the KEYNOTE-059/061 combined analysis (Figs 1A and 3A) and in KEYNOTE-062 (Figs 1B and 3B).

In the KEYNOTE-059/061/062 combined analysis, *TP53* mutation remained the most prevalent genomic characteristic in both patients of Western and Asian origin (Western, 58%; Asian, 52%; Data Supplement, Table S3), followed by *ARID1A* mutation (Western, 15%; Asian, 16%; Data Supplement, Table S3). In the KEYNOTE-059/061/062 combined analysis, the gene with the most copy number variation (CNV) was *CCNE1* (Western, 12%; Asian, 16%), followed by *ERBB2* (Western, 9%; Asian, 10%; Data Supplement, Table S4). The prevalence of most genes with mutations and/or CNVs was similar between patients of Western and Asian origin, except for a slightly higher prevalence of *EGFR* with CNVs in patients of Western origin (8% *v* 4%, respectively [chi-square, 4.25]) and *CCNE1* with CNVs in patients of Asian origin (12% *v* 16%, respectively

TABLE 1. Prevalence of TCGA Subtypes

	KEYNOTE-059/061 Combined Analysis		KEYNOTE-059/061/062 Combined Analysis[a]		TCGA	
Subtype	Western Origin (n = 305), No. (%)	Asian Origin (n = 159), No. (%)	Western Origin (n = 658), No. (%)	Asian Origin (n = 210), No. (%)	Western Origin (n = 172), No. (%)	Asian Origin (n = 77), No. (%)
CIN	162 (53)	83 (52)	NA	NA	82 (48)	38 (49)
EBV-positive	20 (7)	11 (7)	NA	NA	17 (10)	8 (10)
GS	109 (36)	58 (36)	NA	NA	36 (21)	16 (21)
MSI-H	14 (5)	7 (4)	36 (5)	11 (5)	37 (22)	15 (19)

NOTE. Percentages might not total 100 because of rounding.
Abbreviations: CIN, chromosome instability; EBV, Epstein-Barr virus; GS, genomically stable; MSI-H, microsatellite instability–high; NA, not applicable; TCGA, The Cancer Genome Atlas; WES, whole-exome sequencing.
[a]Combined analysis of WES and FoundationOneCDx data. FoundationOneCDx does not enable the determination of CIN, EBV-positive, and GS subtypes.

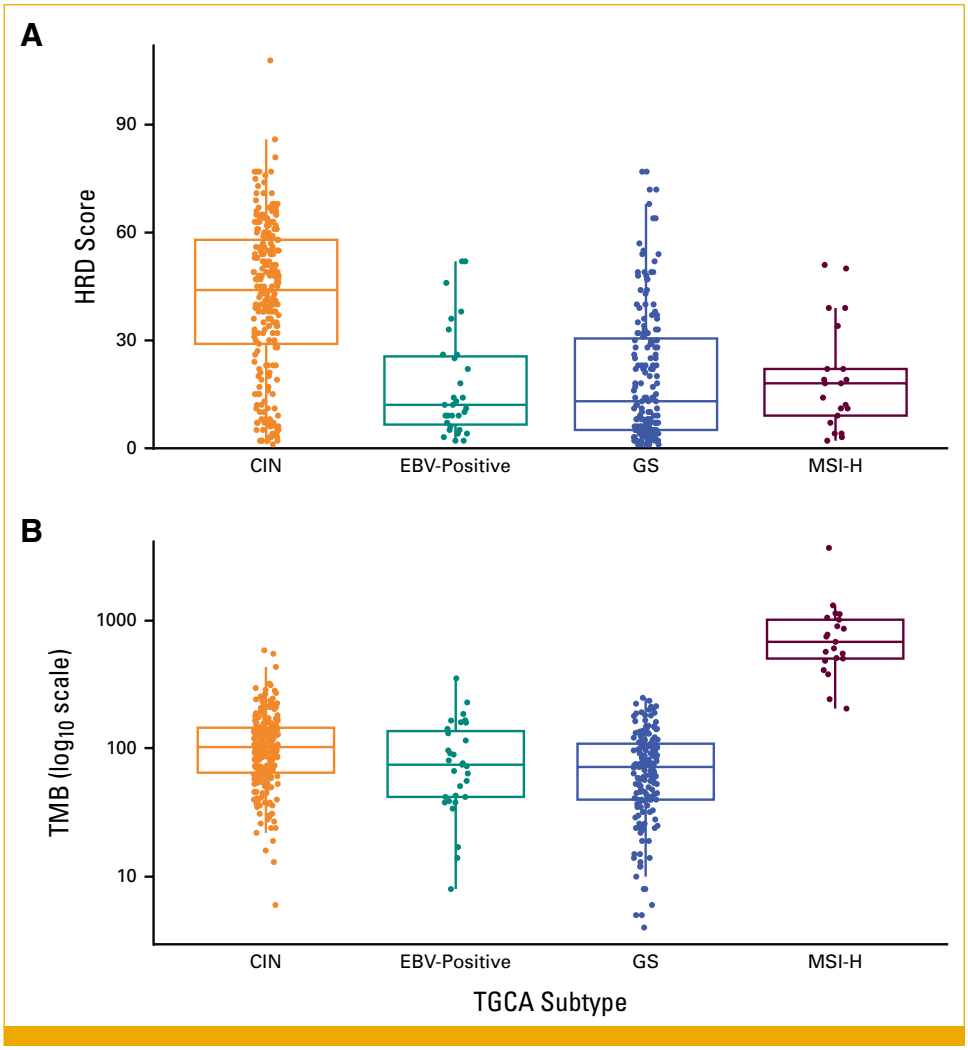

FIG 2. Genomic scores by TCGA subtype in the KEYNOTE-059/061 combined analysis: (A) HRD score and (B) TMB score. CIN, chromosome instability; EBV, Epstein-Barr virus; GS, genomically stable; HRD, homologous recombination deficiency; MSI-H, microsatellite instability–high; TCGA, The Cancer Genome Atlas; TMB, tumor mutational burden.

[chi-square, 2.89]; Data Supplement, Tables S3 and S4). *CLDN18* fusion prevalence in the overall populations of the KEYNOTE-059/061 combined analysis and the published TCGA data set was 3% (13/374 samples) and 5% (11/225), respectively[2]; in patients of Asian origin, *CLDN18* fusion prevalence was low for both data sets (KEYNOTE-059/061, 0% [0/374 samples]; TCGA, <1% [1/225 samples]).

Gene Expression Analysis

Expression of *EGFR*, *ERBB2*, *VEGFA*, and *CCNE1* increased with CNVs in the KEYNOTE-059/061 combined analysis (Data Supplement, Fig S1). In the KEYNOTE-059/061 combined analysis, the expression of *MLH1* had a lower distribution in the MSI-H subtype relative to the rest of TCGA subtypes (Data Supplement, Fig S2).

In the TCGA data set, *CLDN18* gene expression appeared to be elevated in the EBV-positive subtype relative to the rest of TCGA subtypes (Fig 4A). *CLDN18* gene expression scores were higher in the presence of the *CLDN18* fusion gene versus the *CLDN18* wild-type gene in both the KEYNOTE-059/061 combined analysis and TCGA data set, although the AUROC for *CLDN18* gene expression scores in discriminating between the *CLDN18* fusion gene and the *CLDN18* wild-type gene in TCGA was modest (AUROC, 0.56 [95% CI, 0.44 to 0.67]; Fig 4B).

Integrated Pathway Analysis

Similar expression patterns of the $Tcell_{inf}GEP$ and 10 non–$Tcell_{inf}GEP$ signatures were observed between the KEYNOTE-059/061 combined analysis and TCGA data set, with no differences by origin (Data Supplement, Fig S3).

RNA gene expression signatures by race indicated that the expression of the hypoxia signature was numerically higher in patients of Western origin versus patients of

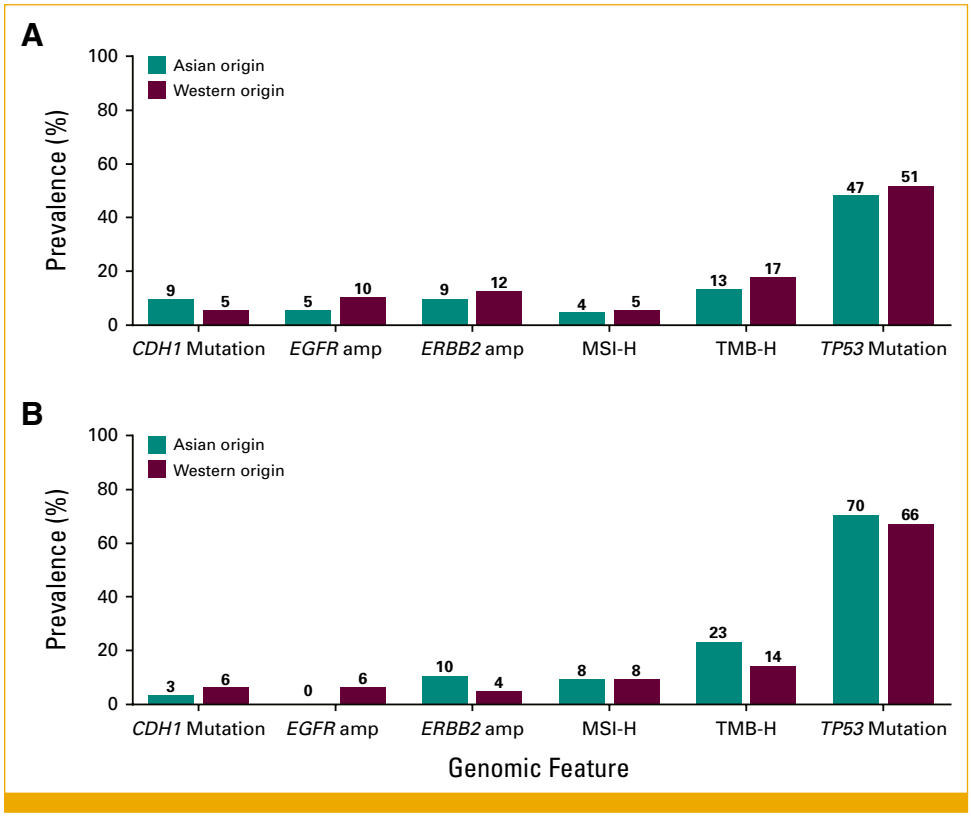

FIG 3. Prevalence of select genomic features: (A) KEYNOTE-059/061 combined analysis and (B) KEYNOTE-062 analysis. amp, amplification; MSI-H, microsatellite instability–high; TMB-H, high tumor mutational burden.

Asian origin for both the KEYNOTE-059/061/062 combined analysis and TCGA data sets (Fig 5). The expression of glycolysis, granulocytic myeloid-derived suppressor cells (gMDSC), and angiogenesis signatures was nominally higher in patients of Western origin versus Asian origin for the KEYNOTE-059/061/062 combined analysis (Fig 5). Analyses of gene expression signatures in patients of Western versus Asian origin by TCGA subtype revealed trends toward higher gMDSC and angiogenesis signature expressions in the MSI-H subtype and higher gMDSC signature expression in the EBV-positive subtype for patients of Western versus Asian origin in both the KEYNOTE-059/061 combined analysis and TCGA data sets (Data Supplement, Fig S4). Gene expression signature distribution from TCGA and the KEYNOTE-059/061 combined analysis by race and TCGA subtype is shown in the Data Supplement (Table S5). Observations for the angiogenesis and gMDSC signatures were consistent with those of similar analyses of individual gene expression signature distribution by both race and TCGA subtype in the KEYNOTE-059/061 combined analysis (Data Supplement, Figs S5B and S5C). In the KEYNOTE-059/061 combined analysis, $Tcell_{inf}GEP$ trended toward higher distribution in the EBV-positive subtype, with no difference between patients of Western and Asian origin (Data Supplement, Fig S5A). The glycolysis signature trended toward a higher expression distribution in MSI-H and EBV-positive subtypes for patients of Western origin versus Asian origin in the KEYNOTE-059/061 combined analysis but not in TCGA data set (Data Supplement, Fig S5D). Additionally, MYC signature expression trended toward higher distribution in the MSI-H subtype for patients of Asian origin (Data Supplement, Fig S5G). The expression of the proliferation signature trended toward lower distribution in the GS subtype, with no differences between patients by origin (Data Supplement, Fig S5H). No clear trends by race or TCGA subtype were observed for the expression of RAS or WNT signatures (Data Supplement, Figs S5I and S5K).

Analyses of the correlation between gene expression signatures and gene mutations showed a moderate positive correlation between PD-L1 combined positive score and $Tcell_{inf}GEP$, and a negative correlation between PD-L1 CPS and *WNT* mutations (Data Supplement, Fig S6A); similar results were obtained for gene expression signatures and genes with CNVs (Data Supplement, Fig S6B).

DISCUSSION

This exploratory genomic analysis of data from the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 clinical trials represents a comprehensive evaluation of the clinical and molecular characteristics of late-stage GC using TCGA network classification of GC molecular subtypes.[2] Compared

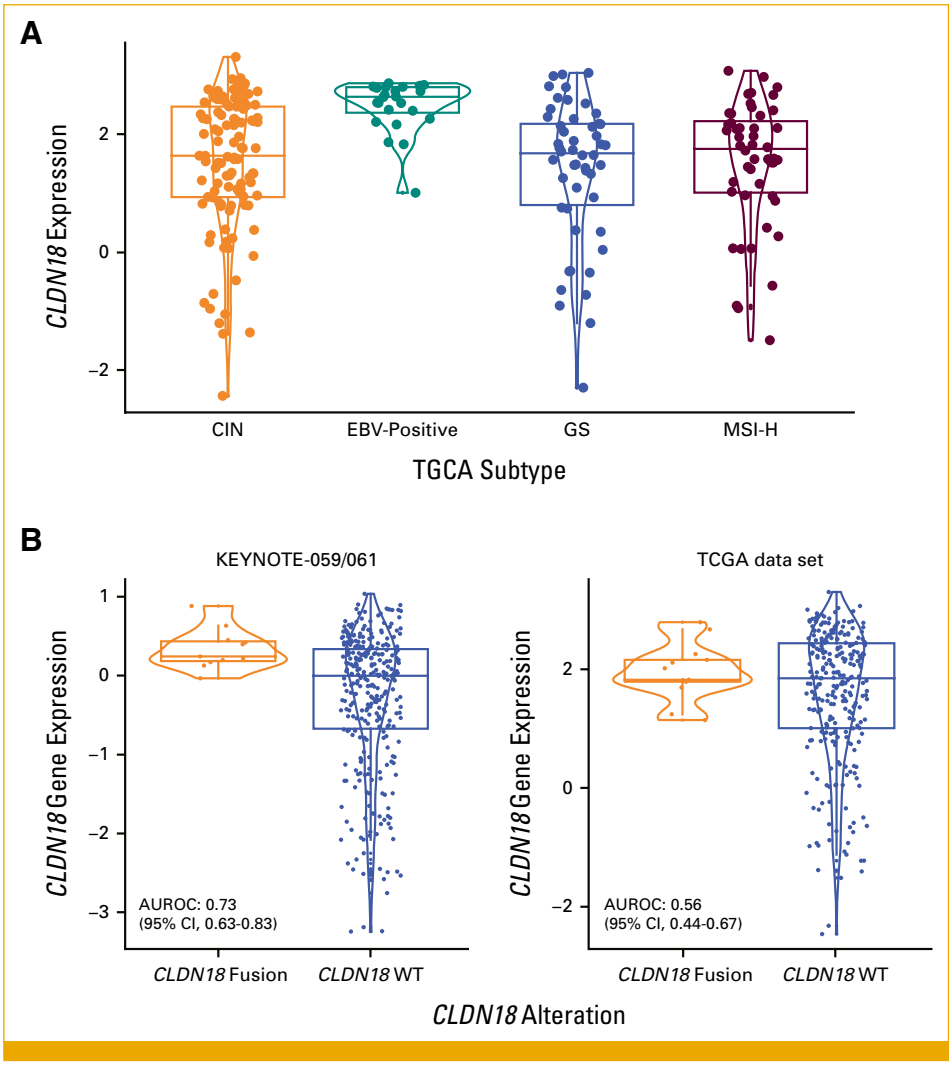

FIG 4. *CLDN18* gene expression. (A) Expression by subtypes for TCGA data set (n = 232). (B) Expression in the presence of *CLDN18* fusion and wild-type genes in the overall KEYNOTE-059/061 combined analysis (n = 333) and TCGA data set (n = 284). The AUROC analysis was performed using pROC package in R and plots were visualized using the ggplot2 package. AUROC, area under the receiver operating characteristics curve; CIN, chromosome instability; EBV, Epstein-Barr virus; GS, genomically stable; MSI-H, microsatellite instability–high; TCGA, The Cancer Genome Atlas; WT, wild-type.

with the TCGA data set, the KEYNOTE-059/061/062 or KEYNOTE-059/061 combined analyses had a lower prevalence of MSI-H, a higher prevalence of GS, and subtle numerical differences in the prevalence of EBV-positive and CIN subtypes; results were similar between patients of Asian and Western origin. The prevalence of MSI-H was consistent with expectations on the basis of stage.

A post hoc analysis of patients enrolled in South Korea with stage II/III GC in the phase III CLASSIC trial also reported low rates of MSI-H GC (6.8%).[9] Furthermore, a phase II clinical trial in patients from Korea with metastatic GC reported a high proportion of patients with the GS subtype (45%), along with low rates of the EBV-positive (7%) and MSI-H (11%) subtypes.[4] In a large multicenter study, the prevalence of the EBV-positive subtype in patients with GC from the United Kingdom and Japan was 5% (United Kingdom, 4%; Japan, 6%)[10]; other studies conducted in Europe have reported similarly low rates of the EBV-positive subtype (4%-6%) in GC.[11,12] Taken together, data from the literature support the findings of the current analysis.

In the KEYNOTE-059/061 combined analysis, trends for HRD and TMB, which are associated with or contribute to the modulation of the TME,[13,14] varied across TCGA subtypes, with a higher HRD distribution score in the CIN subtype and a higher TMB in the MSI-H subtype versus the other TCGA subtypes. In an analysis of patients with advanced GC, a higher median TMB (21.93 mutations/megabase *v* 3.42 mutations/megabase) was reported in the

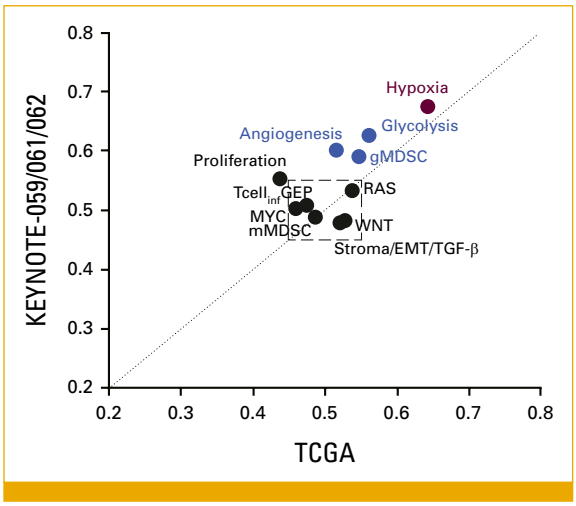

FIG 5. Gene expression signatures in gastric cancers from TCGA (n = 169) and KEYNOTE-059/061/062 combined analysis (n = 472) by race. This scatter plot presents the AUROC of patients of Western origin versus patients of Asian origin in TCGA (*x*-axis) and combined KEYNOTE (*y*-axis) data. Analysis of the TCGA data set was restricted to stage 3 and 4 tumors. AUROC >0.5 means the signature has higher expression in patients of Western origin versus patients of Asian origin. Statistically significant nominal *P* values were observed for the expression of the hypoxia signature (red) in both the combined KEYNOTE (AUROC, 0.67; $P < .001$) and TCGA (AUROC, 0.64; $P = .008$) data sets. Statistically significant nominal *P* values were also observed in the KEYNOTE-059/061/062 combined analysis data set for the expression of the glycolysis (blue; AUROC, 0.63; $P < .001$), angiogenesis (AUROC, 0.60; $P < .001$), and gMDSC (blue; AUROC, 0.59; $P = .003$) signatures (blue). The dotted box around AUROC = 0.5 separates variables that did not show a significant nominal *P* value (shown in black) versus those that showed a significant nominal *P* value (shown in red and blue). AUROC, area under the receiver operating characteristics curve; EMT, epithelial-mesenchymal transition; gMDSC, granulocytic myeloid-derived suppressor cell; mMDSC, monocyte myeloid-derived suppressor cell; Tcell$_{inf}$GEP, T-cell–inflamed gene expression profile; TCGA, The Cancer Genome Atlas; TGF-β, transforming growth factor-beta.

subgroup of patients with MSI-H GC versus those with non–MSI-H GC.[15] Higher TMB in patients with GC has been associated with HRD, with a median TMB of 8.28 mutations/megabase versus 3.07 mutations/megabase in HRD versus non–HRD GC.[16] The current study confirmed these findings, as median TMB was higher for patients with HRD ≥16 in all analyzed subtypes.

Combined analysis of data from all three KEYNOTE studies included in the current analysis identified the tumor suppressor genes *TP53* (59%) and *ARID1A* (16%) as the most frequently mutated genes in advanced-stage GC. Similar findings were reported in another study of patients with late-stage GC.[17] In an analysis of samples from tumor biopsy and matched peripheral blood collected from previously untreated patients with advanced GC, targeted next-generation sequencing of genomic DNA on the basis of a 118-cancer gene panel identified 92 mutated genes; the most commonly mutated gene was *TP53* (35%).[17] In another study, comprehensive genomic profiling of 116 primarily late-stage GC samples using FoundationOneCDx identified 501 cancer-related genomic alterations; the most common alterations were in *TP53* (58/116 [50%]) and *ARID1A* (28/116 [24%]).[18] We observed a modest numerically higher prevalence of *EGFR* gene amplification in patients of Western origin versus patients of Asian origin in both the KEYNOTE-059/061 combined analysis and KEYNOTE-062; however, additional studies are needed to confirm this observation. The observation of *CCNE1* and *ERBB2* as the most prevalent genes with CNVs in the KEYNOTE-059/061/062 combined analysis is similar to results of another study of 89 predominantly late-stage GC samples that identified *ERBB2* and *CCNE1* as the most prevalent genes with CNVs.[19]

The high prevalence of GC in Asian countries has been well documented.[20,21] Previous reports have demonstrated immunologic differences between patients of Asian and non-Asian origin. Analyses of gene expression profiles of >1,600 tumor tissue samples from patients of Asian and non-Asian origin with GC revealed differences in the expression of gene signatures that regulate T-cell pathways between the two populations.[3] Molecular differences in signaling markers were also observed between the two populations, with tumor tissue samples from patients of non-Asian origin exhibiting increased CD28 and CTLA-4 signaling and lower levels of the regulatory T-cell marker, FOXP3.[3] In our analysis, there were no appreciable differences in the molecular characteristics between patients of Asian and Western origin. The lower distribution of *MLH1* expression observed in the MSI-H subtype relative to the other TCGA subtypes is consistent with the critical role of loss of function in this gene for the MSI-H genotype. Despite the low prevalence of genetic drivers, including *CLDN18* fusions (KEYNOTE-059/061 combined analysis, 3%; TCGA data set, 5%[2]), trends toward higher *CLDN18* gene expression scores were observed in the presence of the *CLDN18* fusion gene versus the *CLDN18* wild-type gene for both the KEYNOTE-059/061 combined analysis and TCGA data set, suggesting the existence of other drivers of overexpression that are specific for cancers of the GI tract. Stratification of gene expression signatures by race and TCGA subtype was limited by the small sample sizes of the subgroups. However, analysis showed differences in the expression of selected individual TME-related pathways, including hypoxia, which showed the strongest consistent pattern of differentiation between patients of Western and Asian origin for both the KEYNOTE-059/061/062 combined analysis and TCGA data sets. This consistent pattern of differentiation suggests further study is needed to determine the potential for therapeutic strategies targeting hypoxia in GC. Furthermore, trends indicating differences in the distribution of the expression of immune-suppressive pathways in the TME, such as gMDSC and stroma/epithelial-mesenchymal transition (EMT)/transforming growth factor-beta (TGF-β) in the MSI-H subtype and angiogenesis in the EBV-positive subtypes, were observed for

patients of Western origin. Similarly, a trend toward higher expression of monocytic MDSCs (mMDSC) was observed in patients of Western origin with MSI-H tumors or CIN tumors versus patients of Asian origin. Three of these gene expression signatures (angiogenesis, mMDSC, and stroma/EMT/TGF-β) were recently shown to be negatively associated with response to pembrolizumab monotherapy in an analysis of nine clinical trials across seven tumor types, including GC.[22] These findings may contribute to our understanding of the biology of immune checkpoint pathways in different GC subtypes, which may lead to the development of tailored therapeutic strategies comprising treatments that modulate these key elements of the TME.

The current study is limited by its retrospective and exploratory design, and all analyses were descriptive in nature. Additionally, anticancer therapy exposures at the time the tumor samples were acquired varied between studies; patients enrolled in KEYNOTE-061 and cohort 1 of KEYNOTE-059 had received previous therapy, whereas patients enrolled in KEYNOTE-062 were previously untreated. These differences should be considered when interpreting the results. Furthermore, enrollment on the basis of PD-L1 selection in KEYNOTE-061 and KEYNOTE-062 may have affected the molecular characteristics of this GC population.[6-8] For example, an analysis of patients with lung adenocarcinoma who underwent PD-L1 testing demonstrated associations between PD-L1 expression and distinct molecular features, including TMB, individual gene mutations (ie, *KRAS*, *TP53*, and *MET*), and signaling pathway alterations (ie, WNT and PI3K pathways).[23] Therefore, PD-L1 selection of patients in the three KEYNOTE studies may result in a GC population with distinct molecular features, which limits the generalizability of the present findings. Furthermore, KEYNOTE-062 excluded patients with HER-positive disease, which may have contributed to the relatively low prevalence of HER2 amplification in the combined cohort.

In conclusion, this exploratory analysis described the genomic landscape of late-stage GC using data from the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 clinical trials. Classification into one of the four TCGA subtypes revealed a numerically lower prevalence of the MSI-H subtype, a numerically higher prevalence of the GS subtype, and subtle numerical differences in the prevalence of the EBV-positive and CIN subtypes in late-stage disease versus early-stage disease from the previously reported TCGA data set. Furthermore, no evidence of major molecular differences between patients of Western and Asian origin was observed. On the basis of these findings, additional analyses examining the association between the molecular characteristics of late-stage GC and clinical outcomes following treatment with pembrolizumab among patients of Western and Asian origin add to the body of evidence.

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DATA SHARING STATEMENT

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so that the requestor can perform the proposed analyses. All authors had full access to all data and take responsibility for the integrity of the data and the accuracy of the data analysis.

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