Combined Transcriptome and Circulating Tumor DNA Longitudinal Biomarker Analysis Associates With Clinical Outcomes in Advanced Solid Tumors Treated With Pembrolizumab

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ABSTRACT

PURPOSE Immune gene expression signatures are emerging as potential biomarkers for immunotherapy (IO). VIGex is a 12-gene expression classifier developed in both nCounter (Nanostring) and RNA sequencing (RNA-seq) assays and analytically validated across laboratories. VIGex classifies tumor samples into hot, intermediate-cold (I-Cold), and cold subgroups. VIGex-Hot has been associated with better IO treatment outcomes. Here, we investigated the performance of VIGex and other IO biomarkers in an independent data set of patients treated with pembrolizumab in the INSPIRE phase II clinical trial (ClinicalTrials.gov identifier: NCT02644369).

MATERIALS AND METHODS Patients with advanced solid tumors were treated with pembrolizumab 200 mg IV once every 3 weeks. Tumor RNA-seq data from baseline tumor samples were classified by the VIGex algorithm. Circulating tumor DNA (ctDNA) was measured at baseline and start of cycle 3 using the bespoke Signatera assay. VIGex-Hot was compared with VIGex I-Cold + Cold and four groups were defined on the basis of the combination of VIGex subgroups and the change in ctDNA at cycle 3 from baseline (ΔctDNA).

RESULTS Seventy-six patients were enrolled, including 16 ovarian, 12 breast, 12 head and neck cancers, 10 melanoma, and 26 other tumor types. Objective response rate was 24% in VIGex-Hot and 10% in I-Cold/Cold. VIGex-Hot subgroup was associated with higher overall survival (OS) and progression-free survival (PFS) when included in a multivariable model adjusted for tumor type, tumor mutation burden, and PD-L1 immunohistochemistry. The addition of ΔctDNA improved the predictive performance of the baseline VIGex classification for both OS and PFS.

CONCLUSION Our data indicate that the addition of ΔctDNA to baseline VIGex may refine prediction for IO.

ACCOMPANYING CONTENT

Appendix

Data Sharing Statement

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INTRODUCTION

Improved understanding of the biological drivers of immune responses has enabled the identification of tumor types beyond the classic immunoresponsive tumor histologies that may respond to immunotherapy (IO).[1-3] However, only a small fraction of biomarker-selected patients will benefit from IO treatment.[1,4] Approved IO biomarkers, such as tumor mutation burden (TMB) or PD-L1 immunohistochemistry (IHC), have limited positive predictive value to detect responders and their performance is variable across tumor histologies.[5] Recently, RNA-based gene expression signatures have been identified that are predictive of IO benefit.[6] Gene expression signatures capture tumor intrinsic features as well as different components of the tumor microenvironment (TME).[7] Despite multiple studies supporting the role of the tumor transcriptome as a potential predictive IO biomarker, none of these have been implemented in the

CONTEXT

Key Objective

Different predictive biomarkers for immune checkpoint treatment have been investigated in the past few years. Yet, their limited predictive performance across tumor types limit routine clinical implementation. VIGex is a 12-gene immune response signature. The predictive performance of the immune signature VIGex and tumor-informed circulating tumor DNA (ctDNA) longitudinal analysis was investigated in a cohort of patients with advanced solid tumors treated with pembrolizumab in the INSPIRE phase II clinical trial.

Knowledge Generated

Leveraging on the INSPIRE phase II clinical trial (ClinicalTrials.gov identifier: NCT02644369) data set, we investigated the combined performance of ctDNA changes and VIGex immune gene expression signature categories. The additive predictive performance of these two biomarkers was observed supporting its combined use to refine immunotherapy predictions.

Relevance

The findings of our study support the use of predictive biomarker combinations for patient selection or treatment de-escalation strategies.

clinical arena because of lack of validation across laboratories or high expenses associated with signatures developed in RNA sequencing (RNA-seq). VIGex is a pan-cancer transcriptomic signature encompassing genes involved in immune response and validated in patients treated with early-phase IO agents and publicly available data sets. VIGex is reported as a continuous score and three categories (hot, intermediate-cold [I-Cold], and cold) depending on the inflammatory status of the TME. VIGex-Hot was associated with increased $CD8^+$ tumor-infiltrating lymphocytes (TILs) by IHC compared with VIGex I-Cold/Cold.[8] VIGex was associated with IO benefit in a pan-cancer cohort of patients treated with early-phase IO clinical trials and in a meta-analysis across publicly available data sets but its performance varied across tumor types, suggesting that combinations of biomarkers may be required to improve predictions. Emerging evidence suggests that dynamic changes in circulating tumor DNA (ctDNA) correlate with tumor burden evolution.[9] However, there are still unanswered questions about preferred testing assays and its clinical utility as a predictive or prognostic biomarker across tumor types.[9]

INSPIRE was a phase II clinical trial (ClinicalTrials.gov identifier: NCT02644369) that evaluated the PD-1 immune checkpoint inhibitor pembrolizumab in IO-naïve patients with advanced or metastatic solid tumors across five cohorts: head and neck squamous cell carcinoma (HNSCC), high-grade serous ovarian carcinoma, triple-negative breast cancer (TNBC), metastatic melanoma (MM), and a fifth cohort of mixed solid tumors (MST). Patients enrolled in INSPIRE had serial plasma samples for ctDNA analysis.[10] Previous INSPIRE reports showed that early reductions in ctDNA assayed by a bespoke panel identify patients who are more likely to respond to IO treatment.[10] Moreover, complete clearance of ctDNA during treatment with pembrolizumab correlates with long-term disease control.[10] Whether adding ctDNA dynamics to existent baseline biomarkers such as gene expression signatures can refine IO predictions has not been investigated.

Here, we aimed to externally validate the predictive performance of VIGex in the INSPIRE independent data set of patients with advanced solid tumors treated with pembrolizumab and longitudinal ctDNA evaluation. Moreover, we evaluated the additive predictive performance of multiple IO response biomarkers, including VIGex, ctDNA dynamics, PD-L1, and TMB.

MATERIALS AND METHODS

Study Design

This study received institutional and research ethical approval at both Vall d'Hebron Institute of Oncology (VHIO) and Princess Margaret Cancer Centre (CAPCR ID: 20-6266). RNA data from baseline samples from patients enrolled in INSPIRE phase II clinical trial were sent to VHIO for VIGex analysis blinded to clinical data. Statistical and integrated biomarker analysis was performed at Princess Margaret Cancer Centre.

INSPIRE Clinical Trial Patients and Treatment

A total of 106 patients were enrolled from March 2016 to May 2018 in the INSPIRE Phase II clinical trial (ClinicalTrials.gov identifier: NCT02644369). The primary objective of INSPIRE was to evaluate pharmacodynamic changes in patients with advanced or metastatic solids tumors receiving pembrolizumab. Patients were prospectively enrolled into one of five cohorts: recurrent unresectable or metastatic HNSCC, TNBC, high grade serous ovarian cancer (HGSOC), MM, and

MST. Participants received pembrolizumab 200 mg intravenously once every 3 weeks until disease progression, intolerable toxicity, or for a maximum of 35 cycles. Radiologic assessments were performed every 9 weeks and response was evaluated by RECIST v1.1. Eligible patients were required to have disease amenable to serial tumor biopsies. Biopsies were required during the screening period and after two to three cycles of pembrolizumab.

Bulk Tumor Tissue RNA-Seq

RNA libraries were prepared from 200 ng of total RNA using the TruSeq Stranded Total RNA Library Prep Gold following the PM/OICR Genomics Platform. Libraries were sequenced with pair-end 100 cycles using Illumina NovaSeq6000 to a target depth of 100 million reads per sample. FASTQs were aligned to human genome transcript reference GENCODE version 31 (Ensembl 97) using STAR 2.7.2b aligner with default settings.[11] Data quality was assessed using RNA-seQC (v.1.1.8).[12] Expression levels of all transcripts were quantified using RSEM 1.3.0.[13] Differential gene expression analysis between groups was conducted using DESeq2 R package (v1.34.0).[14]

VIGex Score

VIGex score for each sample was computed as previously reported by using the 12 gene sum of the log2 of individual gene expression levels minus the average of the log2 of the individual specific gene expression level in the training data set divided by 12. This was based on the VIGex 12 selected genes: *CTLA-4*, *CD274*, *PDCD1*, *IL7R*, *FOXP3*, *CXCL9*, *CXCL10*, *CXCL11*, *IFNG*, *PRF1*, *GZMA*, and *GZMB*.[8] VIGex score follows a normal distribution, and the cutoffs for defining Hot (VIGex score >0.75), I-Cold (VIGex score between −0.75 and 0.75), and Cold groups (VIGex score <[−0.75]) were selected on the basis of the concordance with the groups obtained by two different clustering methods: hierarchical with three groups and partitioning around medoids clustering as previously reported.[8]

Whole-Exome Sequencing and TMB Analysis

DNA from tumor and pretherapy peripheral blood mononuclear cells was extracted from frozen cell pellets stored at −80°C (Qiagen DNA/RNA coisolation kit). Agilent SureSelect V5 + UTR probes were used for hybrid selection to enrich for exonic sequences. Prepared libraries were sequenced with paired-end 125 bp reads on the Illumina HiSeq2000 or 2500 platform per manufacturer's protocols at the Princess Margaret Genomic Centre or Translational Genomics Laboratory in Toronto, ON. Tumor samples were sequenced to a median depth of 250X and normals to a median depth of 50X. Sequence alignment to human reference genome version hg38 using the Burrows-Wheeler Alignment tool (v.0.7.12), cocleaning, and duplicate removal was performed following previously reported methodology.[15] TMB was defined as the number of nonsynonymous mutations per megabase.

Circulating Tumor DNA

Whole-exome sequencing–generated patient-specific tumor somatic mutation profiles were used to design bespoke ctDNA assays by Natera Inc (San Carlos, CA) using their proprietary Signatera assay as previously described.[10] Illumina sequencing platform was used to perform amplicon deep sequencing of products obtained from targeted PCR. For each baseline and on-treatment time point plasma sample, ctDNA was quantified in units of mean tumor molecules per mL of plasma. This takes into account mean allele frequencies across all mutations, cell-free DNA extracted, and plasma volume. The early change in ctDNA was calculated as the percentage difference in absolute ctDNA levels between third treatment cycle and baseline time points. An increase was defined greater than zero, while decrease was <0.

PD-L1 Analysis

IHC staining for PD-L1 using the mouse monoclonal anti–PD-L1 antibody (clone 22C3 at 2 μg/mL, Merck, Palo Alto, CA) was performed by Qualtek Molecular Laboratories (Newtown, PA). The PD-L1 IHC assay has been previously validated and being used in the Merck Investigator Studies Program.[16] The level of PD-L1 staining is reported by Qualtek as a modified proportion score (range, 0-100), indicating the percentage of PD-L1–expressing tumor cells and mononuclear inflammatory cells within the tumor nest. PD-L1 IHC reactivity interpretation was conducted as previously reported and evaluated by a board-certified pathologist at Qualtek.[10,15]

CIBERSORT Immune Cell Infiltration Estimation

Infiltration level of 22 types of immune cell was estimated for each sample with CIBERSORT in absolute mode,[17] using gene expression data (transcript per million) from RNA-seq. Twenty two types of immune cells include: B-cell naïve, B-cell memory, B-cell plasma, T-cell naïve, T-cell $CD4^+$ naïve, T-cell $CD4^+$ memory resting, T-cell $CD4^+$ memory activated, T-cell $CD8^+$, T-cell follicular helper, T-cell regulatory, T-cell gamma delta, macrophage M0, macrophage M1, macrophage M2, mast cell resting, mast cell activated, NK cell resting, NK cell activated, eosinophils, neutrophils, myeloid dendritic cell resting, and myeloid dendritic cell activated. CIBERSORT was ran using the web portal[18] with LM22 signature matrix.

Statistical Analysis

Kaplan-Meier curves were used to illustrate overall survival (OS) and progression-free survival (PFS) by VIGex categories. Objective response rate (ORR) was defined by RECIST (version 1.1). Cox proportional hazards regression models were fitted to assess the association of VIGex categories and ctDNA dynamics on OS and PFS while adjusting for TMB, PD-L1 status, and tumor type. Spearman's rank-order correlation was used to examine the association between

continuous markers, and chi-squared test was used for association between categorical markers. All statistical analyses were performed in the R Statistical Computing Environment v3.3.1 (R Foundation for Statistical Computing, Vienna, Austria[19]).

Ethics Approval and Consent for Publication

This research project and the consent to participate were submitted and approved by the institutional review board of Princess Margaret Cancer Centre. All patients enrolled in the INSPIRE clinical trial signed an informed consent to participate.

Consent for Publication

The investigators of the INSPIRE clinical trial (ClinicalTrials.gov identifier: NCT02644369) consent the publication of these data as collected in the protocol.

RESULTS

Vigex Categories Are Associated With Different Immune Cell Populations

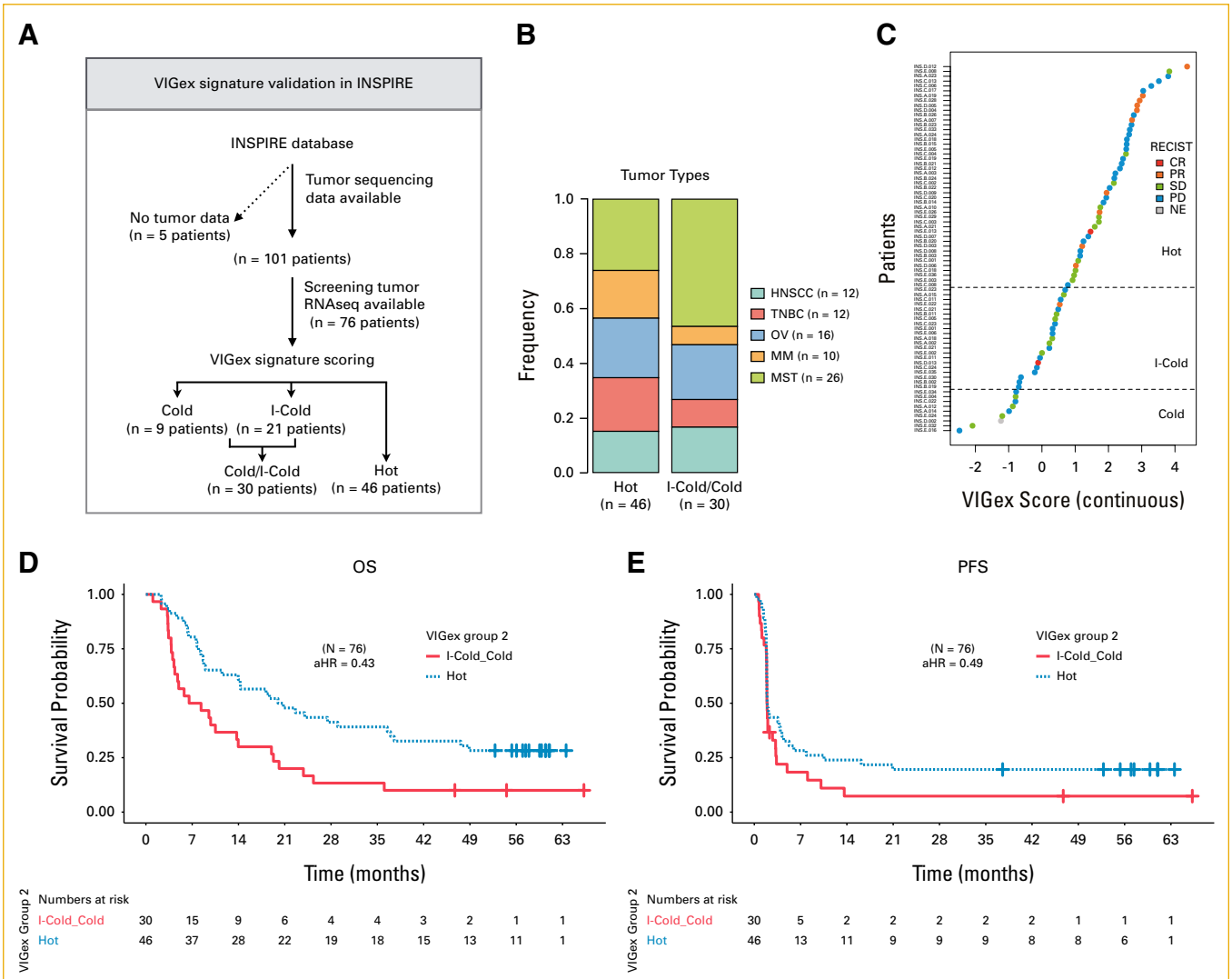
We first aimed to evaluate if VIGex signature categories were associated with different immune compositions assessed by CIBERSORT. Increased $CD8^+$ TILs were observed for VIGex-Hot patients compared with I-Cold ($P < .001$) or Cold ($P < .001$; Data Supplement, Fig S1). Other fundamental immune cells in antitumor immune responses such as NK cells activated or $CD4^+$ T cells were also increased in VIGex-Hot compared with the I-Cold or Cold VIGex categories ($P = .002$, $P = .012$; and $P = .015$, $P = .018$, respectively). However, no differences were observed in these immune subsets between I-Cold and Cold VIGex categories ($P = .78$ and $P = .24$, respectively). Overall, higher CIBERSORT absolute immune scores were observed in VIGex-Hot ($P < .001$) with no differences in the I-Cold and Cold VIGex categories ($P = .24$) suggesting not identical but similar immune cell composition.

VIGex-Hot Is Associated With Benefit to Immune Checkpoint Inhibitors

Of the 106 patients enrolled in INSPIRE, 76 patients had screening tumor RNA-seq available (Fig 1A). A total of 46 patients were categorized as Hot, 21 patients as I-Cold, and nine patients as Cold. Considering the similar CIBERSORT absolute immune scores observed in both VIGex I-Cold and Cold categories, patients were grouped together for further biomarker analyses. The distribution of tumor types across VIGex categories is described in Figure 1B. Although MST and HNSCC tumor cohorts were more frequently represented in the I-Cold/Cold VIGex category, TNBC, MM, or HGSOC had more representation in the VIGex-Hot category. Responses were observed in 11 (24%) patients categorized as Hot and two (10%) patients categorized as I-Cold. No patients categorized as Cold by VIGex achieved a partial or complete response. ORR was 24% in the Hot category and 10% in the I-Cold/Cold VIGex category (Fig 1C). We next evaluated the association of VIGex category with OS and PFS. Median OS for VIGex-Hot was 20.4 (95% CI, 14 to 37.6) months and 7.14 (95% CI, 4.4 to 19) months for VIGex I-Cold/Cold category. The 2-year and 5-year OS rates were 46% and 28% for VIGex-Hot and 17% and 10%, respectively, in the I-Cold/Cold VIGex category. Importantly, VIGex categories were significantly associated with OS when assessed in a multivariable model adjusted for tumor type, PD-L1, and TMB ($P = .009$; Figs 1D-1F). Median PFS was 2 months (95% CI, 1.9 to 4.4) for VIGex-Hot and 1.9 (95% CI, 1.9 to 4) months for VIGex I-Cold/Cold category. The 1-year and 2-year PFS rates were 24% and 20% for VIGex-Hot and 11% and 7%, respectively, in the I-Cold/Cold VIGex category. VIGex categories were significantly associated with PFS when included in a multivariable analysis adjusted for TMB, PD-L1 status, and tumor type ($P = .036$; Figs 1E-1G). Overall, these results support the external validity of VIGex as a pan-cancer IO predictive biomarker independent of TMB and PD-L1.

The Addition of ΔctDNA Further Improves the Performance of VIGex

We aimed to explore the combined predictive performance of VIGex categories and ctDNA dynamics. A total of 59 patients had both baseline RNA-seq tumor samples and serial ctDNA assessments (baseline and cycle 3). Four groups were defined on the basis of the combination of VIGex categories and the change in ctDNA at cycle 3 from baseline (ΔctDNA; Fig 2A). Previous reports described that complete clearance of ctDNA is associated with better IO outcomes.[10] A total of 10 patients had clearance of ctDNA as defined by changes from detectable on baseline to undetectable during treatment course. Notably, among the 10 patients in our cohort who had ctDNA clearance, nine patients (90%) were categorized as VIGex-Hot in baseline tumor samples (Fig 2B). An ORR of 53% was observed in the group defined by VIGex-Hot + decreased ΔctDNA. Moderate responses were observed for patients categorized as VIGex-Hot + increased ΔctDNA or VIGex-I-Cold/Cold + decreased ΔctDNA. No responses were observed in the group categorized as VIGex-I-Cold/Cold + increased ctDNA (Fig 2C). The categories defined by the combination of VIGex and ΔctDNA biomarkers were associated with OS ($P = .001$ log-rank). Median OS for VIGex-Hot + decreased ΔctDNA was 46 months (95% CI, 28 to not applicable [NA]), VIGex-I-Cold/Cold + decreased ΔctDNA 22.4 months (95% CI, 17.6 to NA), VIGex-Hot + increased ΔctDNA 13 months (95% CI, 6.6 to NA), and VIGex-I-Cold/Cold + increased ΔctDNA 4.8 months (95% CI, 3 to NA). Better OS was observed for VIGex-Hot + decreased ΔctDNA or VIGex-Hot + increased ΔctDNA compared with I-Cold/Cold + increased ΔctDNA ($P = .0004$ and $P = .015$, respectively) when adjusted by tumor cohorts. In terms of PFS, the groups defined by the combination of VIGex and ΔctDNA



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Overall Survival, Multivariable Analysis			
Covariate	**HR**	**95% CI**	***P***
VIGex Category			
I-cold/cold	(1.00)	—	—
Hot	0.43	0.23 to 0.81	**.009**
TMB	0.54	0.21 to 1.38	.197
PD-L1 (IHC)	0.99	0.98 to 1.0	.109
Tumor cohort			
HNSCC	(1.00)	—	—
TNBC	1.25	0.47 to 3.35	.655
OV	0.39	0.15 to 1.02	.054
MM	0.17	0.04 to 0.76	**.021**
MST	0.235	0.09 to 0.64	**.005**

G

Progression-Free Survival, Multivariable Analysis			
Covariate	**HR**	**95% CI**	***P***
VIGex Category			
I-cold/cold	(1.00)	—	—
Hot	0.49	0.25 to 0.96	**.036**
TMB	0.54	0.24 to 1.22	.139
PD-L1 (IHC)	0.99	0.98 to 1.00	.076
Tumor cohort			
HNSCC	(1.00)	—	—
TNBC	3.36	1.18 to 9.57	**.024**
OV	1.20	0.47 to 3.04	.7
MM	0.25	0.07 to 0.95	**.042**
MST	0.37	0.14 to 0.93	**.034**

FIG 1. Association of VIGex-Hot with immunotherapy outcomes in INSPIRE. (A) Consort diagram. (B) Distribution of tumor types across VIGex categories. (C) Distribution of responses across VIGex categories. (D) Kaplan-Meier curves of OS stratified by VIGex categories. (E) Kaplan-Meier curves of PFS stratified by VIGex categories. (F) Table showing multivariable analysis for OS. (G) Table showing multivariable analysis for PFS. aHR, adjusted hazard ratio; CR, complete response; HNSCC, head and neck squamous cell carcinoma; I-Cold, intermediate-cold; MM, metastatic melanoma; MST, mixed solid tumors; NE, not evaluable; OS, overall survival; OV, ovarian cancer; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; TNBC, triple-negative breast cancer.

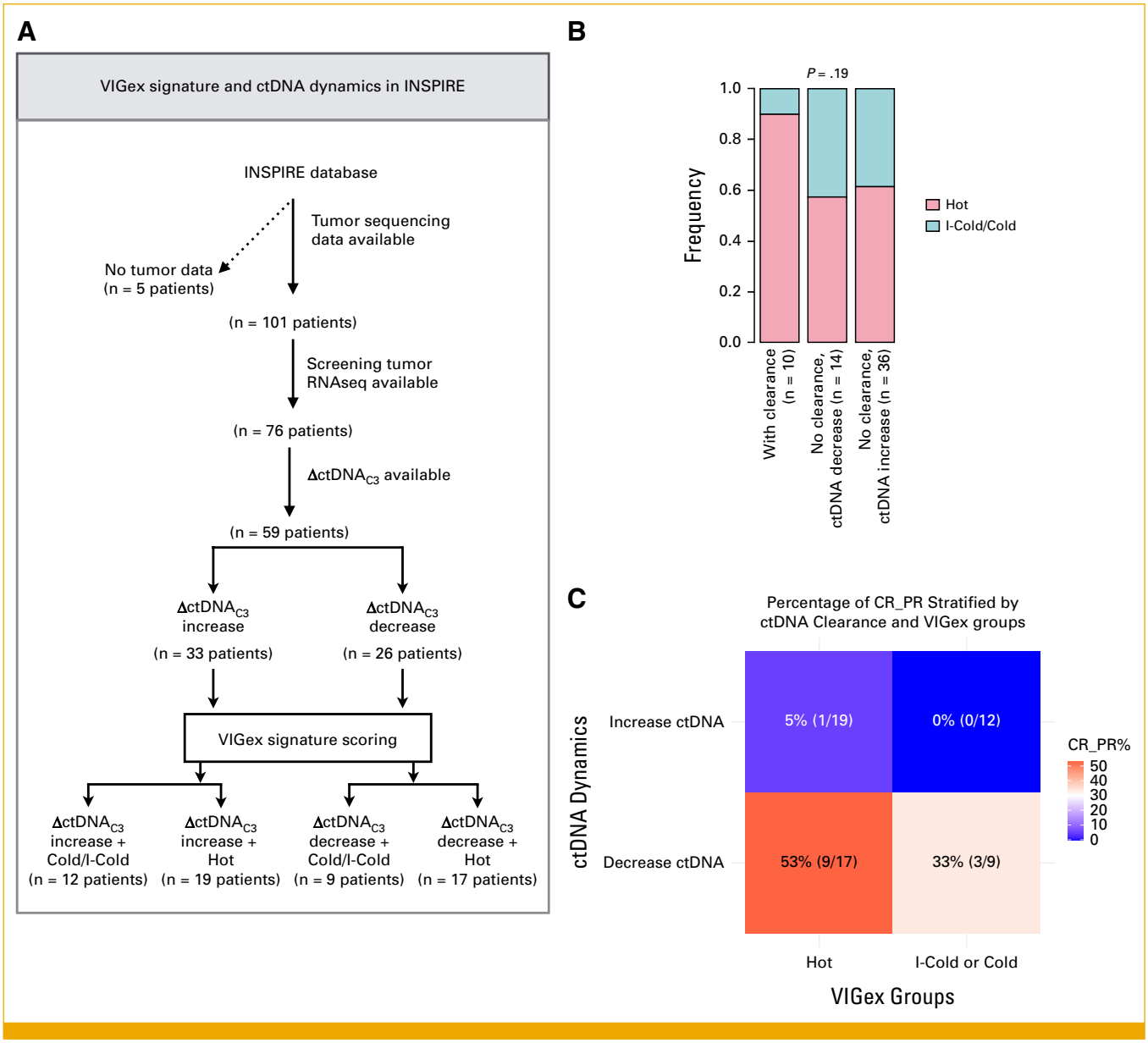

FIG 2. Association of VIGex categories and △ctDNA with immunotherapy outcomes in INSPIRE. (A) Consort diagram. (B) Distribution of VIGex categories across subgroups defined by ctDNA clearance. (C) Plot showing objective response rate in each of the groups defined by VIGex and △ctDNA categories. (D) Kaplan-Meier curve of OS stratified by VIGex and △ctDNA categories. Survival analyses were performed from cycle 3. (E) Kaplan-Meier curve of PFS stratified by VIGex and △ctDNA categories. Survival analyses were performed from cycle 3. (F) Forest plot for Cox proportional hazards model for OS, reporting HR and the 95% confidence intervals for each covariate in the model. (G) Forest plot for Cox proportional hazards model for PFS, reporting HR and the 95% CIs for each covariate in the model. CR, complete response; ctDNA, circulating tumor DNA; HR, hazard ratio; I-Cold, intermediate-cold; OS, overall survival; PFS, progression-free survival; PR, partial response. (continued on following page)

were associated with PFS ($P < .001$ log-rank). Significantly longer PFS was observed for VIGex-Hot + decreased ctDNA compared with VIGex-I-Cold/Cold + increased ctDNA ($P = .004$; Figs 2D-2G). Importantly, the addition of ΔctDNA to VIGex categories significantly improved the performance over baseline VIGex for OS ($P = .002$) and PFS ($P = .026$). Altogether, these results support the combined use of baseline and dynamic biomarkers to improve the performance over single baseline analytes.

VIGex Association With Other Predictive Biomarkers

Next, we investigated whether VIGex continuous scores would be also independent of TMB and other predictive biomarkers of immune checkpoint inhibitors efficacy. No correlation was observed between VIGex and TMB. A significant but slight correlation was observed with PD-L1 expression (Figs 3A-3D). Overall, these findings support the combined use of VIGex with other approved baseline

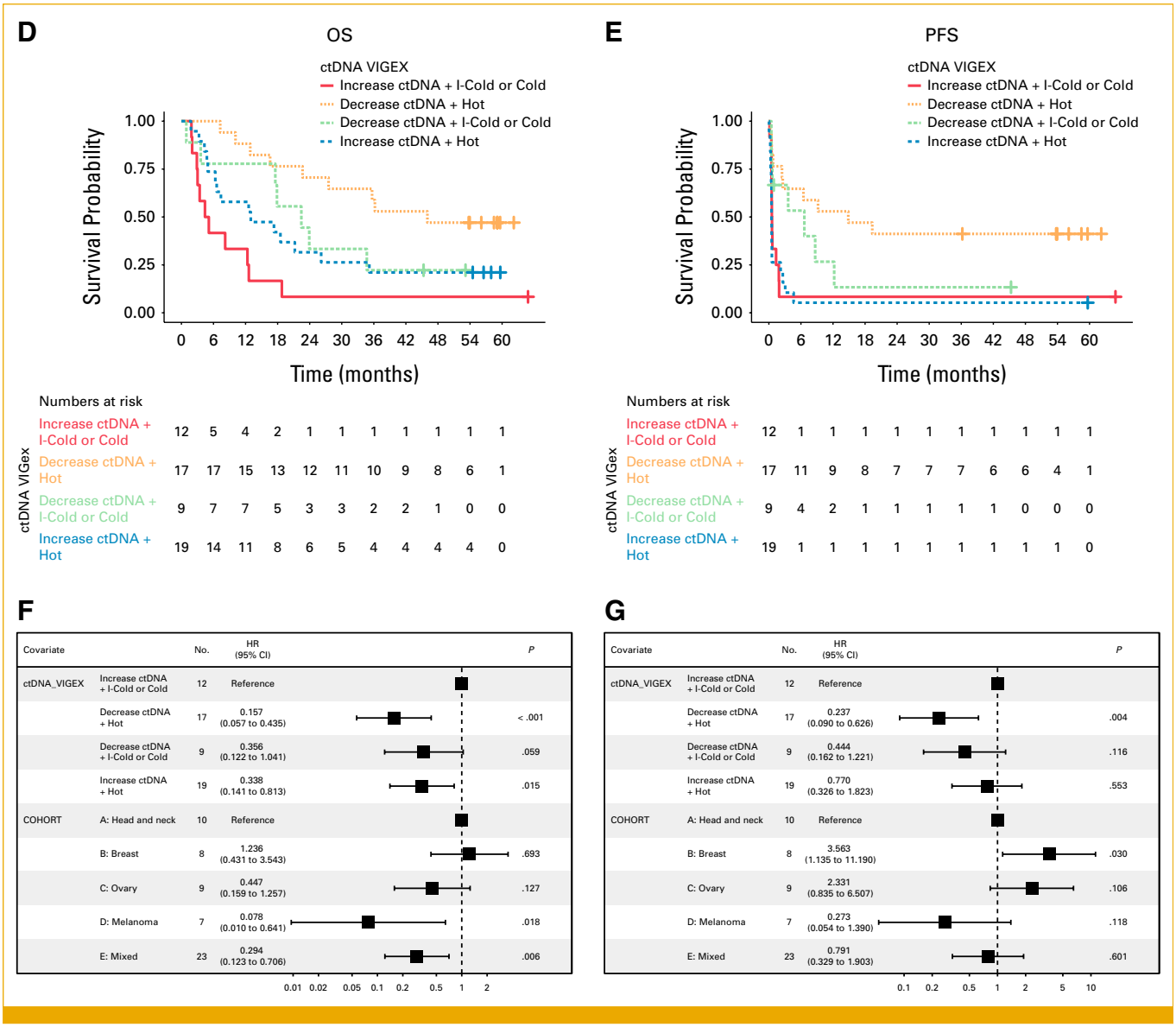

FIG 2. (Continued).

biomarkers. Emerging evidence suggests that chromosome 9p21 loss may be associated with negative outcomes in patients treated with IO.[20] These findings have been further validated in an independent cohort,[21] supporting its role as a negative predictive biomarker for IO. Hence, we aimed to evaluate if VIGex-I-Cold/Cold was enriched in this genomic alteration. No significant differences were observed in 9p21 loss distribution across VIGex categories (P = .351; Fig 3E).

DISCUSSION

There are a variety of gene expression signatures reflecting an immunogenic TME and defining patients more likely to benefit from IO treatment. However, most signatures were developed in either Nanostring or RNA-seq assays and trained in clinical data sets with a limited number of tumor histologies,[22] limiting their generalizability to nonimmunogenic cancers. VIGex was initially developed from real-world formalin-fixed paraffin-embedded tumor samples from 45 different tumor histologies with nCounter platform (Nanostring) and RNA-seq.[8] Its predictive accuracy was initially validated in patients treated across early-phase IO trials at VHIO and through an in silico meta-analysis of publicly available data sets.[8] In this study, we confirmed the predictive performance of VIGex assessed by RNA-seq in an independent multihistology cohort of patients with advanced solid tumors treated with pembrolizumab at Princess Margaret Cancer Centre. We demonstrated that VIGex-Hot was associated with improved PFS and OS when adjusted by tumor type, TMB, and PD-L1. Increased $CD8^+$ TILs by CIBERSORT were observed in VIGex-Hot samples. It is noteworthy that VIGex was not correlated with TMB and only moderately correlated with PD-L1 expression by IHC. Our findings provide support for the idea that multimodal

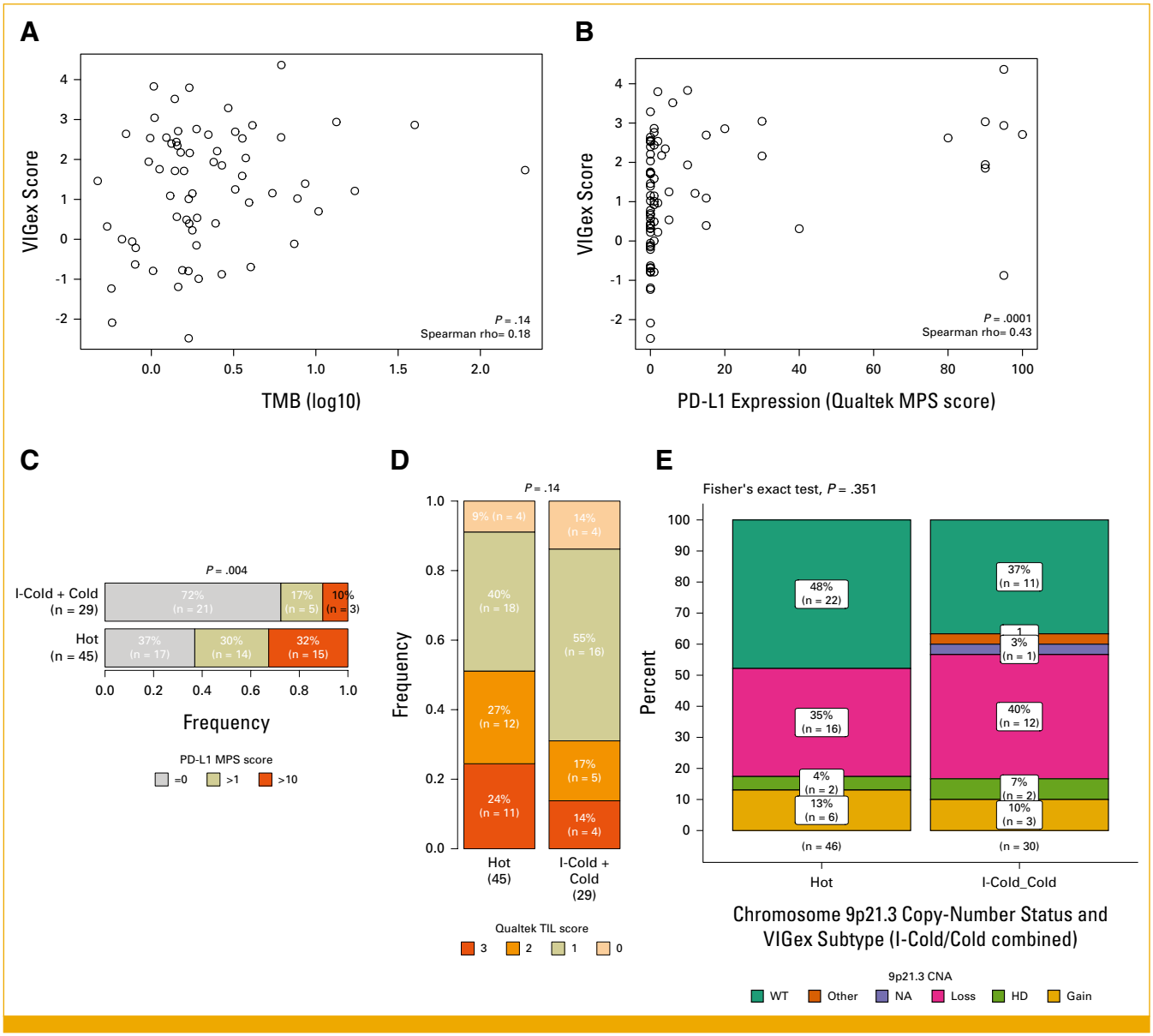

FIG 3. Association of VIGex categories with other approved predictive biomarkers for immunotherapy. (A) Scatter plot showing the association of VIGex continuous score and tumor mutational burden. (B) Scatter plot showing the association of VIGex continuous score and PD-L1 by Qualtek MPS score. (C) Bar plot showing the association of VIGex and PD-L1 score. (D) Bar plot showing the association of VIGex and QualTek TIL score. (E) Bar plot showing the association of 9p21 loss and VIGex categories. HD, homozygous deletion; I-Cold, intermediate-cold; MPS, modified proportion score; NA, not applicable; TIL, tumor-infiltrating lymphocyte; TMB, tumor mutation burden; WT, wild type.

predictive biomarkers ascertained from baseline tumor samples are needed to identify patients most likely to benefit from IO therapy. Cristescu et al[6] described a potential additive effect with the combined used of an 18-gene T-cell–inflamed gene expression profile (GEP) with TMB in a pooled analysis from pembrolizumab clinical trials. Importantly, numerically higher ORR was observed in the group defined by the intersection of TMB high and an immune-inflamed GEP.[6]

In our study, the addition of the assessment of ctDNA dynamics from baseline to before cycle 3 of pembrolizumab to the VIGex classifier improved outcome prediction. The highest ORR was observed in patients with VIGex-Hot tumors who achieved a decreased ΔctDNA from baseline to before cycle 3. Moreover, none of the patients with VIGex I-Cold/Cold tumors who had an increased ΔctDNA responded to treatment. These findings could be used in future clinical trials to identify patients appropriate for intensification or deintensification of IO therapy. For example, patients with a nonimmunogenic TME as defined by baseline VIGex classification that show an early increase in ctDNA levels after initiating IO treatment might benefit from interruption of IO therapy and switching to an alternative treatment.

Conversely, patients with an immunogenic TME as defined by baseline VIGex that show an early decrease in ctDNA levels after initiating IO treatment might be candidates for early cessation of therapy followed by observation. This patient stratification approach could reduce the financial toxicities associated with IO therapy and minimize the risk of long-term immune-related adverse events.

Our study has several limitations. First, this was an unplanned retrospective analysis using tumor samples and ctDNA data from a single-institution, multihistology clinical trial. Future prospective studies are needed to establish the pan-cancer clinical utility of baseline immune gene signatures and ctDNA kinetics monitoring for patient selection and IO treatment decisions. Second, our cohort is small and only a subset was evaluable for baseline VIGex classification and ctDNA assessment. Third, the time point selected to evaluate ctDNA dynamics before cycle 3 of pembrolizumab administrated was based on results from previous INSPIRE publications.[10,15] However, the optimal timing of ctDNA response assessment after initiation of IO is uncertain.[9] Finally, pretreatment tumor tissue availability in routine practice outside of an investigator-initiated clinical trial that included mandatory baseline tumor biopsies is also an important limitation. Gene expression signatures that include a small number of genes, low RNA input thresholds, and that can be performed on less expensive testing platforms such as Nanostring could improve scalability. Although our study included a variety of tumor types, the number of patients within each individual tumor type was small. It is well established that there are differences in levels of ctDNA shedding across cancer types, which may affect the generalizability of combined biomarker approaches that include gene expression signatures and ctDNA dynamics.[9] Our study used a bespoke, tumor-informed ctDNA assay (Signatera) that is highly sensitive for ctDNA detection. Whether our findings are reproducible with other ctDNA detection methods, such as tumor-naïve targeted ctDNA panels, methylation, or shallow whole-genome sequencing, requires further investigation.

In conclusion, we demonstrated that VIGex is associated with IO outcomes independent of other predictive biomarkers. Our findings provide proof of principle for the combined use of tissue-based gene expression signatures and ctDNA dynamics to improve IO response prediction that may be used to inform the development of future clinical trials testing individualized IO treatment approaches.

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EQUAL CONTRIBUTION

A.V. and P.L.B. contributed equally as last authors.

PRIOR PRESENTATION

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DATA SHARING STATEMENT

Anonymized patient normal, tumor exome, and RNA-seq bam files containing alignments of the original raw sequencing reads used in this study have been deposited in the European Genome-phenome Archive repository under accession code EGAS00001003280. The processed variant calls are available at EGAD00001006569. The data sets are available under restricted access in compliance with patient consent for data sharing, and access can be obtained by approval from the University Health Network data access committee. Custom code to reproduce key figures and results reported in the manuscript are available at https://github.com/pughlab/inspire-genomics.

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REFERENCES

1. André T, Shiu KK, Kim TW, et al: Pembrolizumab in microsatellite-instability–high advanced colorectal cancer. N Engl J Med 383:2207-2218, 2020
2. Yarchoan M, Hopkins A, Jaffee EM: Tumor mutational burden and response rate to PD-1 inhibition. N Engl J Med 377:2500-2501, 2017
3. Samstein RM, Lee CH, Shoushtari AN, et al: Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet 51:202-206, 2019
4. Marabelle A, Fakih M, Lopez J, et al: Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: Prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol 21:1353-1365, 2020
5. McGrail DJ, Pilié PG, Rashid NU, et al: High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. Ann Oncol 32:661-672, 2021
6. Cristescu R, Mogg R, Ayers M, et al: Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science 2018:362, 1979
7. Lau D, Bobe AM, Khan AA: RNA sequencing of the tumor microenvironment in precision cancer immunotherapy. Trends Cancer 5:149-156, 2019
8. Hernando-Calvo A, Vila-Casadesús M, Bareche Y, et al: A pan-cancer clinical platform to predict immunotherapy outcomes and prioritize immuno-oncology combinations in early-phase trials. Med 4:710-727.e5, 2023
9. Cescon DW, Bratman SV, Chan SM, et al: Circulating tumor DNA and liquid biopsy in oncology. Nat Cancer 1:276-290, 2020
10. Bratman SV, Yang SYC, Iafolla MAJ, et al: Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nat Cancer 1:873-881, 2020
11. Dobin A, Davis CA, Schlesinger F, et al: STAR: Ultrafast universal RNA-seq aligner. Bioinformatics 29:15-21, 2013
12. DeLuca DS, Levin JZ, Sivachenko A, et al: RNA-SeQC: RNA-seq metrics for quality control and process optimization. Bioinformatics 28:1530-1532, 2012
13. Li B, Dewey CN: RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323, 2011
14. Love MI, Huber W, Anders S: Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550, 2014
15. Cindy Yang SY, Lien SC, Wang BX, et al: Pan-cancer analysis of longitudinal metastatic tumors reveals genomic alterations and immune landscape dynamics associated with pembrolizumab sensitivity. Nat Commun 12:5137, 2021
16. Dolled-Filhart M, Locke D, Murphy T, et al: Development of a prototype immunohistochemistry assay to measure programmed death ligand-1 expression in tumor tissue. Arch Pathol Lab Med 140: 1259-1266, 2016
17. Chen B, Khodadoust MS, Liu CL, et al: Profiling tumor infiltrating immune cells with CIBERSORT. Methods Mol Biol 1711:243-259, 2018
18. Newman AM, Liu CL, Green MR, et al: Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 12:453-457, 2015
19. R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2021. https://www.R-project.org/
20. Han G, Yang G, Hao D, et al: 9p21 loss confers a cold tumor immune microenvironment and primary resistance to immune checkpoint therapy. Nat Commun 12:5606, 2021
21. Spiliopoulou P, Yang SYC, Bruce JP, et al: All is not lost: Learning from 9p21 loss in cancer. Trends Immunol 43:379-390, 2022
22. Bareche Y, Kelly D, Abbas-Aghababazadeh F, et al: Leveraging big data of immune checkpoint blockade response identifies novel potential targets. Ann Oncol 33:1304-1317, 2022

APPENDIX

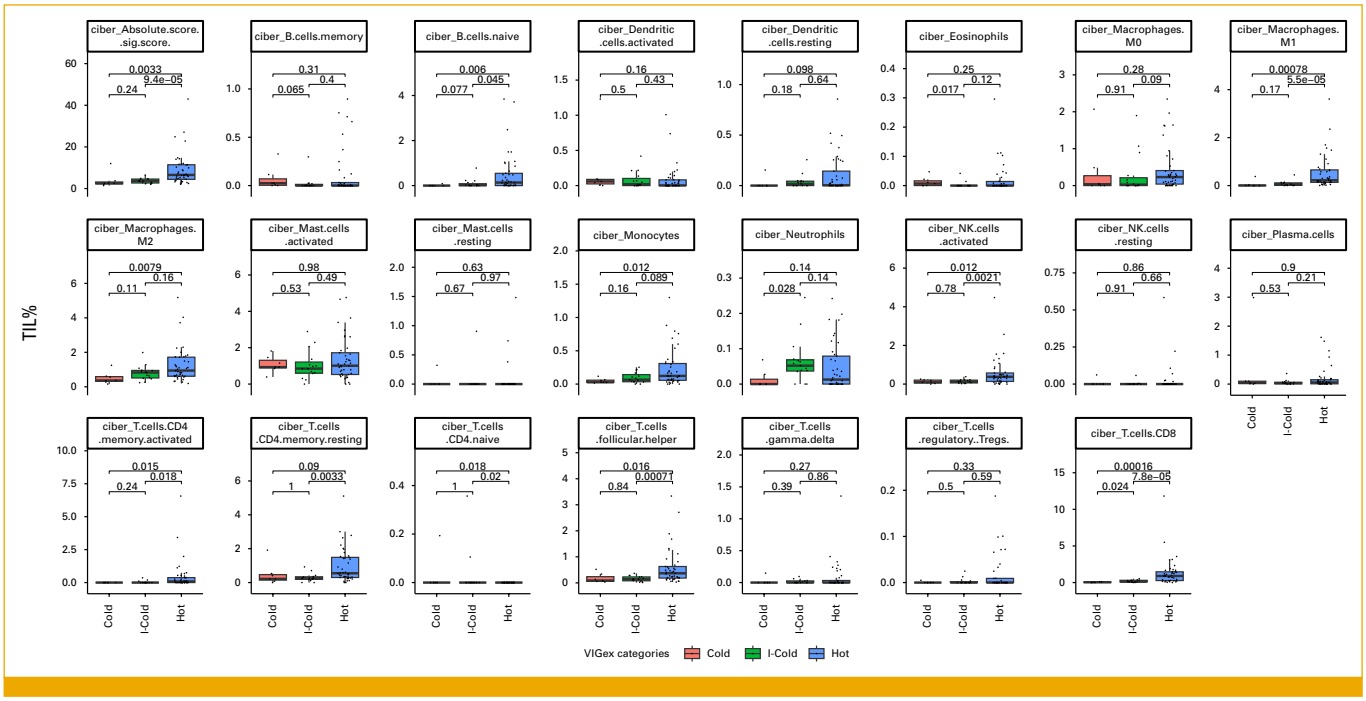

FIG A1. Association of VIGex categories with immune cell infiltration assessed by CIBERSORT. I-Cold, intermediate-cold; TIL, tumor-infiltrating lymphocyte.