Clinical Cancer Research

Interferon Gamma Messenger RNA Signature in Tumor Biopsies Predicts Outcomes in Patients with Non–Small Cell Lung Carcinoma or Urothelial Cancer Treated with Durvalumab

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Abstract

Purpose: To identify a predictive biomarker for durvalumab, an anti–programmed death ligand 1 (PD-L1) mAb.

Experimental Design: RNA sequencing of 97 advanced-stage non–small cell lung carcinoma (NSCLC) biopsies from a nonrandomized phase Ib/II clinical trial (1108/NCT01693562) were profiled to identify a predictive signature; 62 locally advanced or metastatic urothelial cancer tumors from the same study were profiled to confirm predictive utility of the signature. Thirty NSCLC patients provided pre- and posttreatment tumors for messenger RNA (mRNA) analysis. NSCLC with ≥25% tumor cells and urothelial cancer with ≥25% tumor or immune cells stained for PD-L1 at any intensity were scored PD-L1 positive (PD-L1$^+$). Kaplan–Meier and Cox proportional hazards analyses were used to adjust for gender, age, prior therapies, histology, ECOG status, liver metastasis, and smoking. Tumor mutation burden (TMB) was calculated using data from The Cancer Genome Atlas (TCGA).

Results: In the NSCLC discovery set, a four-gene IFNγ-positive (IFNγ$^+$) signature comprising *IFNγ*, *CD274*, *LAG3*, and *CXCL9* was associated with higher overall response rates, longer median progression-free survival, and overall survival compared with signature-low patients. IFNγ$^+$-signature NSCLC patients had improved survival regardless of IHC PD-L1 status. These associations were replicated in a urothelial cancer cohort. The IFNγ$^+$ signature was induced 2-fold ($P = 0.003$) by durvalumab after 8 weeks of therapy in patients with NSCLC, and baseline signature was associated with TMB but not survival in TCGA data.

Conclusions: The IFNγ$^+$ mRNA signature may assist in identifying patients with improved outcomes with durvalumab, independent of PD-L1 assessed by IHC. *Clin Cancer Res; 24(16); 3857–66. ©2018 AACR.*

Introduction

Tumors can negatively regulate the immune response by modulating inhibitory cell surface receptors to instill T-cell exhaustion. Sustained expression of these receptors imparts a dysfunctional T-cell state, which reduces effector and subsequent antitumor function (1). Clinical trials evaluating immune checkpoint inhibitors (ICI), such as antibodies to anti-programmed cell death protein 1 (PD-1) and its ligand (PD-L1), have demonstrated that blocking such inhibitory receptors and ligands provides benefit to patients with cancers such as non–small cell lung cancer (NSCLC), urothelial cancer, renal cell carcinoma, melanoma, and ovarian cancer (2–11). Furthermore, patients in whom the immune checkpoint protein PD-L1 is overexpressed on tumor or immune cells (12–16) show even greater clinical benefit from these therapies.

Durvalumab is a human monoclonal immunoglobulin G1 antibody that inhibits PD-L1 binding to PD-1 and CD80, thereby restoring the T-cell dysfunctional state and driving antitumor immunity. Durvalumab in a dosage of 10 mg/kg every 2 weeks has demonstrated an acceptable safety profile and was recently approved by the FDA for patients with locally advanced or metastatic urothelial cancer. This PD-L1–specific mAb is currently under investigation in multiple solid tumors, including NSCLC. Results from the recently published PACIFIC trial demonstrated that consolidation therapy with durvalumab after platinum-based chemoradiotherapy elicited significantly longer median progression-free survival (PFS), overall response rate (ORR), and time to death or distant metastases compared with placebo in patients with stage III NSCLC (17). Similar to other anti-PD-1 and PD-L1 therapies, durvalumab has shown improved clinical outcomes in patients in whom PD-L1 is overexpressed before treatment, although a proportion of patients still do not benefit.

In light of the demonstrated success of PD-1 and PD-L1 ICIs to significantly increase response rates and PFS in patients with certain cancers, recent research has focused on identifying biomarkers that may assist in predicting clinical outcomes before ICI treatment. These biomarkers include PD-L1 (*CD274*)

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

An unmet need in cancer treatment is identification of biomarkers of response to PD-1 or PD-L1 immune checkpoint inhibitors that predict therapeutic outcomes in patients with non–small cell lung carcinoma (NSCLC) and urothelial cancer. Expression of PD-L1 measured by IHC has limited capacity for predicting outcomes of immune checkpoint therapy because expression of this biomarker does not correlate highly with treatment response. In addition, PD-L1 as measured on tumor cells, which has been approved by the FDA for both companion and complementary diagnostic utility, does not necessarily reflect antitumor activity of tumor-infiltrating lymphocytes. Results presented in this report demonstrate that gene expression levels of four IFNγ-inducible mRNAs correlate with improved clinical outcomes in patients with NSCLC or urothelial cancer treated with the PD-L1 inhibitor durvalumab. This IFNγ gene signature may augment the predictive value of PD-L1 and other biomarkers in identifying cancer patients most likely to respond to therapy with durvalumab or other immune checkpoint inhibitors.

protein identified by IHC staining of tumor cells and/or tumor-infiltrating immune cells within the tumor microenvironment, and immune cell–derived, IFNγ–inducible gene transcripts or factors involved in T-cell regulation or activation, immune cell recruitment, and signal transduction, such as *CXCL9*, *CXCL10*, *IFNG*, *STAT1*, *STAT4*, *HLA-DRA*, *CD4*, and *CD8A*, among others (18).

Results from numerous studies evaluating the predictive utility of PD-L1 IHC for response to ICIs have yielded mixed results (19), as response to treatment does not strictly correlate with PD-L1 tumor expression in all patients (3). The equivocal utility of PD-L1 as a predictive biomarker may result from (i) heterogeneity of PD-L1 expression within the tumor microenvironment, (ii) different performance characteristics of IHC assay methods and reagents, (iii) lack of definition of critical cutoffs for PD-L1 staining associated with clinical outcomes, (iv) limited number of binding sites on the hydrophobic PD-L1 molecule, and (v) possible denaturing effects of formalin fixation on archived or freshly obtained tumor biopsies (20). For these reasons, identification of a collective set of gene expression biomarkers strongly associated with and predictive of response to ICIs such as durvalumab may assist in the selection of patients who are most likely to derive clinical benefit with these agents.

A high tumor mutation burden (TMB) has also been proposed to serve as a positive predictive biomarker, based on increased expression of tumor neoantigens that may be recognized by immune cells (2, 21). However, data from the CheckMate 026 study of nivolumab versus placebo in patients with stage IV or recurrent NSCLC showed that TMB at any of three quantitative mutational boundaries did not correlate with either increased OS or tumor PD-L1 expression, despite higher PFS with nivolumab versus placebo in patients with high TMB (21). Therefore, the predictive utility of TMB for response to ICIs remains to be determined, as it may be influenced by factors such as neoantigen heterogeneity as well as neoantigen loss through clonal selection pressures (22).

In addition to PD-L1 tissue expression and TMB, IFNγ messenger RNA (mRNA) expression and the downstream genes induced by this cytokine may serve as positive predictive biomarkers for improving ICI treatment across multiple tumor types. IFNγ produced by T cells and natural killer (NK) cells can promote cancer cell cytotoxicity through recruitment of tumor-infiltrating macrophages, induction of nitric oxide, and increases in cytotoxic T-cell proliferation and activity. Hence, in addition to PD-1 and PD-L1, certain composite signatures of IFNγ-inducible genes within the tumor microenvironment may represent combined biomarker signatures that are associated with increased likelihood of clinical benefit achievable with ICI therapy. Examples of IFNγ-inducible biomarkers that may collectively be associated with increased antitumor activity include differentiation and MHC antigens on tumor-infiltrating lymphocytes, chemokines, and markers of cellular metabolism, signal transduction, and DNA repair (18, 23, 24).

Together, these studies suggest that the IFNγ pathway is an important component within the tumor microenvironment for launching cytotoxic responses after blockade of PD-1 or PD-L1.

To further assess the association between an IFNγ mRNA signature and clinical outcomes, we conducted an exploratory analysis from a phase Ib/II trial evaluating durvalumab in patients with NSCLC or urothelial cancer who were pretreated primarily with chemotherapy (CP1108/NCT01693562; ref. 25).

Materials and Methods

All participants provided written informed consent before undergoing study procedures. This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The clinical protocol for this study was approved by appropriate Institutional review boards and ethics committees.

Study patients

As of October 24, 2016, 407 patients were eligible for efficacy analysis. Of these patients, 304 had been previously treated for squamous or nonsquamous NSCLC, and 103 had stage IIIB/IV urothelial cancer. All patients had received durvalumab at 10 mg/kg every 2 weeks (≥13 weeks before July 24, 2016) in the 1-year follow-up, dose expansion phase of the study. At that time, the median duration of follow-up was 19.4 months (NSCLC) and 8.4 months (urothelial cancer), respectively. Within the NSCLC cohort, 97 patients had fresh tumor biopsies with measurable mRNA for gene expression profile analysis, and 285 patients had fresh or archival tumor biopsies with measurable PD-L1 by IHC. Within the urothelial cancer cohort, 62 patients had fresh tumor count with measurable mRNA for gene expression profile analysis and 100 patients had fresh or archival tumor count with measurable PD-L1 by IHC (Supplementary Fig. S1).

Analyses

IHC. IHC staining for PD-L1 on fresh or archival tissue biopsies of sufficient quality were available for 285 NSCLC patients and 100 urothelial cancer patients. Tumor tissue samples were collected, processed, and analyzed for cellular expression of PD-L1 as described previously, using the analytically validated Ventana SP263 assay and automated BenchMark ULTRA platform

(Ventana Medical Systems; ref. 5). Tissue biopsies were scored PD-L1 positive by IHC analysis, using a minimum threshold of PD-L1 expression on ≥25% of tumor cells in NSCLC samples or ≥25% of tumor or immune cells in urothelial cancer samples at any intensity (5).

mRNA sequencing. RNA sequencing was conducted on frozen biopsies from 97 NSCLC and 62 urothelial cancer tumors of sufficient quality, using the Illumina NextSeq instrument (Atlantic Lab Equipment) and sequencing protocols as described previously (23, 26). Technicians were blind to clinical data. To summarize, RNA was extracted utilizing the Zymo Quick-RNA MicroPrep according to the manufacturer's protocol and checked for quantity and quality via spectrophotometry and Agilent bioanalyzer. Sufficient quality RNA was converted to libraries utilizing TruSeq RNA chemistry, and libraries were sequenced on the Illumina HiSeq instrument. For all sequencing data, reads were quality checked for read counts, quality values, kmer usage, GC content, and all other relevant parameters with FastQC (v0.10.1) and custom scripts. A minimum read count of 80 million (100 base pairs, paired end) was required, and reads were mapped to human reference genome (UCSC hg19; February 2009 release; Genome Reference Consortium GRCh37; gtf annotation file GRCh37.68), using STAR (v2.5.2) with at least 70% mapping rates and were quantified as transcripts per million using RSEM (v1.2.30; refs. 27, 28). These RNA-sequencing data have been deposited into the Gene Expression Omnibus (GEO) repository (ID number GSE110390).

A total of 21 genes were preidentified for relevance to immune activation based on the literature and internal *in vitro* experiments and then were individually associated with ORR (responders versus nonresponders) and OS after durvalumab treatment in tumor biopsies from 97 NSCLC patients (Supplementary Table S1). Expression of each gene was partitioned into low or high groups using receiver operator characteristic calculations with AUC; time-to-event analysis such as Kaplan–Meier and Cox proportional hazards (PH) models were then used. A gene signature was then developed as the mean level of the four genes correlating with ORR and OS. Patients with signature scores above the upper tertile were scored as IFNγ sig^+. This analysis was first performed on tumor biopsies from NSCLC patients, and then the same IFNγ signature was applied to tumor biopsies from 62 urothelial cancer patients (Supplementary Table S2). The effect of durvalumab therapy on individual IFNγ-induced genes in the signature was evaluated in matched pre- and post-treatment (6 weeks) tumor biopsies from a subgroup of 30 NSCLC study patients.

Tumor mutation burden in TCGA whole-exome data

The Cancer Genome Atlas (TCGA) variant calls from whole-exome tumor data was used for indications of NSCLC adenocarcinoma, squamous NSCLC, and bladder adenocarcinoma. TCGA-Biolinks was used to extract nonsilent mutation burden (nonsynonymous, nonsense, or frameshift mutations) with Mutect2 calls per subject.

Response criteria and statistical analyses

Investigator-confirmed (NSCLC) and independently confirmed (urothelial cancer) antitumor activity was assessed by RECIST v1.1 criteria for response to treatment (ORR). Kaplan–Meier analyses and Cox PH regression models were used for survival analyses (OS and PFS). HRs for OS and PFS were adjusted for baseline ECOG status, smoking status, histology (NSCLC), tumor stage (NSCLC), race, gender, age, number of previous lines of therapy, and presence of liver metastasis. Exact binomial calculation was used for 95% confidence intervals of ORR, and intervals based on log(−log (survival)) were used for 95% confidence intervals of median OS and median PFS. Kaplan–Meier analysis was also conducted with RNA-sequencing data (TPM values) from TCGA for tumors from patients with NSCLC adenocarcinoma, squamous NSCLC, or bladder adenocarcinoma. Concordance analyses between IFNγ gene signature, PD-L1 status, and nonsilent TMB were performed with a Fisher exact test.

Results

Identification of the IFNγ gene signature

Gene expression analysis of 21 preidentified immune-related genes in NSCLC tumors showed that pretreatment levels of *IFNγ*, *LAG3*, *CXCL9*, and *PD-L1* mRNAs individually correlated best with ORR, both without and with adjustment for covariates described in the Methods and evident in the receiver operating characteristics for AUC as well as negative predictive value for treatment response (Supplementary Table S1). A signature was formed from the mean expression level of the four genes, and the upper 33% of mean expression indicated IFNγ sig^+ status. The individual genes selected for the signature in NSCLC also correlated well with ORR in tumor biopsies from urothelial cancer patients, although *CD274* ranked lower in AUC than the other three genes in this tumor type (Supplementary Table S2).

Baseline demographic and disease characteristics

Baseline characteristics of both NSCLC and urothelial cancer patients were similar across IFNγ and PD-L1 status, including current/former smoking and Eastern Cooperative Oncology Group (ECOG) status, gene mutations (*KRAS*, *ALK*, and *EGFR*), number of lines of therapy, tumor stage, and liver metastases (Tables 1 and 2). More than 80% of NSCLC patients had stage III or stage IV disease, and the majority of both NSCLC and urothelial cancer patients had received two or more previous cancer therapies. Higher percentages (38.1%–46.3%) of urothelial cancer patients had liver metastases at baseline compared with NSCLC patients (21.9%–35.4%; Table 2). The majority of patients were former smokers.

Clinical outcomes by baseline IFNγ gene signature and PD-L1 status

Across the Response Evaluation Criteria in Solid Tumors (RECIST) criteria for treatment response, both PD-L1 positivity by IHC and IFNγ sig^+ at baseline, were statistically associated with response to durvalumab therapy. The ORR in NSCLC patients was approximately 6- or 4-fold higher in patients with IFNγ sig^+ or PD-L1–positive (PD-$L1^+$) tumors, respectively, compared with those who were negative for these two biomarkers (Table 3). Similarly, the ORR in urothelial cancer patients was approximately 3.5- or 7.5-fold higher in patients with IFNγ sig^+ or PD-$L1^+$ tumors, respectively, compared with those who were negative for these two biomarkers (Table 4). Summaries of full Cox PH model statistical correlations of patients' general baseline demographic and disease characteristics, including PD-L1 expression, with OS and PFS in NSCLC and urothelial

Table 1. NSCLC baseline demographic and disease characteristics by IFNγ mRNA signature and PD-L1 status

Characteristic	IFNγ sig$^+$ ($n = 32$)	IFNγ sig$^-$ ($n = 65$)	Total ($N = 97$)	PD-L1$^+$ ($n = 165$)	PD-L1$^-$ ($n = 120$)	Total ($N = 285$)
Mean age, years (range)	61.9 (26–87)	64.5 (31–83)	63.7 (26–87)	63.8 (26–85)	63.7 (35–87)	63.7 (26–87)
Male gender, *n* (%)	21 (65.6)	33 (50.8)	54 (55.7)	100 (60.6)	60 (50)	160 (56.1)
Race, *n* (%)						
White	23 (71.9)	45 (69.2)	68 (70.1)	115 (69.7)	97 (80.8)	212 (74.4)
Asian	7 (21.9)	11 (16.9)	18 (18.6)	41 (24.8)	15 (12.5)	56 (19.6)
Baseline ECOG 1, *n* (%)	23 (71.9)	48 (75)	71 (74)	123 (74.5)	91 (76.5)	214 (75.4)
Mutant *EGFR*, *n* (%)	0 (0)	7 (10.8)	7 (7.2)	8 (4.8)	16 (13.3)	24 (8.4)
Mutant *ALK*, *n* (%)	0 (0)	3 (4.6)	3 (3.1)	2 (1.2)	1 (0.8)	3 (1.1)
Mutant *KRAS*, *n* (%)	5 (15.6)	7 (10.8)	12 (12.4)	13 (7.9)	14 (11.7)	27 (9.5)
Smoking history, *n* (%)						
Current	1 (3.1)	5 (7.7)	6 (6.2)	21 (12.7)	14 (11.7)	35 (12.3)
Former	27 (84.4)	46 (70.8)	73 (75.3)	122 (73.9)	84 (70)	206 (72.3)
Squamous, *n* (%)	16 (50)	28 (43.1)	44 (45.4)	100 (60.6)	52 (43.3)	152 (53.3)
Line of therapy, *n* (%)						
1L	7 (21.9)	8 (12.3)	15 (15.5)	49 (29.7)	9 (7.5)	58 (20.4)
2L	13 (40.6)	14 (21.5)	27 (27.8)	54 (32.7)	27 (22.5)	81 (28.4)
3L$^+$	12 (37.5)	43 (66.2)	55 (56.7)	62 (37.6)	84 (70)	146 (51.2)
Tumor stage, *n* (%)						
I	0 (0)	4 (6.2)	4 (4.1)	9 (5.5)	3 (2.5)	12 (4.2)
II	3 (9.4)	5 (7.7)	8 (8.2)	12 (7.3)	9 (7.5)	21 (7.4)
III	9 (28.1)	20 (30.8)	29 (29.9)	42 (25.5)	33 (27.5)	75 (26.3)
IV	19 (59.4)	33 (50.8)	52 (53.6)	98 (59.4)	70 (58.3)	168 (58.9)
Liver metastasis,[a] *n* (%)	7 (21.9)	23 (35.4)	30 (30.9)	46 (27.9)	35 (29.2)	81 (28.4)

[a]Baseline liver metastases were derived from the baseline disease assessment by investigator and independent confirmation.

cancer patients are listed in Supplementary Tables S4 and S5, respectively.

Kaplan–Meier analysis for patients treated with durvalumab therapy demonstrated longer median OS in patients with IFNγ sig$^+$ or PD-L1$^+$ tumors (18.1–22.7 months) than in patients with biomarker-negative tumors (6.5–7.7 months), and this association was statistically significant between IFNγ sig$^+$ and IFNγ sig$^-$ status only (Fig. 1A and B). The IFNγ sig$^+$ gene signature also performed better than PD-L1$^+$ status as a biomarker for longer PFS in NSCLC patients as compared with biomarker-negative patients (Fig. 1C and D).

Differences in survival in urothelial cancer patients between high and low IFNγ or PD-L1 expression were generally similar to those observed in NSCLC patients. Both PD-L1$^+$ and IFNγ sig$^+$ tumor status were associated with statistically longer median OS (approximately 6-fold) compared with biomarker-negative status, although final interpretation of OS by IFNγ sig$^+$ awaits more mature data (i.e., longer patient follow-up time; Fig. 1E and F). Both PD-L1$^+$ and IFNγ sig$^+$ tumor status was also associated with significantly longer PFS compared with biomarker-negative status, although IFNγ sig$^+$ tumor status was associated with 3.5-fold longer median PFS (9.3 months) compared with PD-L1$^+$ status (2.6 months; Fig. 1G and H).

Next, we evaluated the association between IFNγ signature threshold values and ORR to determine whether increased expression of the gene signature improved ORR at the cost of a smaller patient population. In baseline NSCLC and urothelial cancer tumors, higher IFNγ signature thresholds (67% and 75%, respectively) were associated with higher ORR, such that ORR increased from approximately 20% at the 25% and 33% thresholds to approximately 35%–40% at the 67% and 75%

Table 2. Urothelial cancer baseline demographic and disease characteristics by IFNγ mRNA signature and PD-L1 status

Characteristic	IFNγ sig$^+$ ($n = 21$)	IFNγ sig$^-$ ($n = 41$)	Total ($N = 62$)	PD-L1$^+$ ($n = 61$)	PD-L1$^-$ ($n = 39$)	Total ($N = 100$)
Mean age, years (range)	66.8 (53–79)	64.9 (48–82)	65.5 (48–82)	66.4 (34–88)	65.1 (48–82)	65.9 (34–88)
Male gender, *n* (%)						
Male	15 (71.4)	26 (63.4)	41 (66.1)	43 (70.5)	26 (66.7)	69 (69)
Race, *n* (%)						
White	14 (66.7)	27 (65.9)	41 (66.1)	41 (67.2)	23 (59)	64 (64)
Asian	5 (23.8)	8 (19.5)	13 (21)	9 (14.8)	8 (20.5)	17 (17)
Baseline ECOG 1, *n* (%)	13 (61.9)	31 (75.6)	44 (71)	42 (68.9)	31 (79.5)	73 (73)
Smoking history, *n* (%)						
Current	2 (9.5)	3 (7.3)	5 (8.1)	5 (8.2)	3 (7.7)	8 (8)
Former	13 (61.9)	14 (34.1)	27 (43.5)	35 (57.4)	18 (46.2)	53 (53)
Line of therapy, *n* (%)						
1L	0 (0)	2 (4.9)	2 (3.2)	3 (4.9)	6 (15.4)	9 (9)
2L	12 (57.1)	20 (48.8)	32 (51.6)	35 (57.4)	18 (46.2)	53 (53)
3L	5 (23.8)	15 (36.6)	20 (32.3)	15 (24.6)	8 (20.5)	23 (23)
4L	2 (9.5)	2 (4.9)	4 (6.5)	4 (6.6)	5 (12.8)	9 (9)
5L$^+$	2 (9.5)	2 (4.9)	4 (6.5)	4 (6.6)	2 (5.1)	6 (6)
Liver metastasis,[a] *n* (%)	8 (38.1)	19 (46.3)	27 (43.5)	30 (49.2)	20 (51.3)	50 (50)

[a]Baseline liver metastases were derived from the baseline disease assessment by investigator and independent confirmation.

Table 3. ORR, OS, and PFS by IFNγ mRNA signature or PD-L1 status in NSCLC patients

Status	Patients, *n* [events, *n* (OS; PFS)]	ORR, % (95% CI)[a]	Median OS, months (95% CI)[b]	OS adjusted HR[c] (*P*)	Median PFS, months (95% CI)[b]	PFS adjusted HR[c] (*P*)
IFNγ sig$^+$	32 (19; 23)	37.5 (21.7–56.3)	22.7 (9.5–NR)	0.41 (0.0061)	7.5 (2.7–14.6)	0.29 (0.0001)
IFNγ sig$^-$	65 (43; 52)	6.2 (2.0–15.8)	6.5 (4.3–14.2)		1.4 (1.3–2.4)	
PD-L1$^+$	165 (77; 126)	24.8 (18.5–32.2)	18.1 (13.6–22.7)	0.62 (0.0078)	3.0 (2.6–4.8)	0.64 (0.0039)
PD-L1$^-$	120 (83; 101)	5.8 (2.4–11.6)	7.7 (5.7–10.1)		1.5 (1.4–2.6)	

Abbreviations: CI, confidence interval; NR, not reached.
[a]Exact binomial calculation.
[b]Intervals based on log (−log (survival)).
[c]Adjusted for baseline ECOG, smoking status, histology, tumor stage, race, gender, age, previous lines of therapy, and liver metastasis.

thresholds (Supplementary Fig. S2). All IFNγ sig$^+$ threshold cutoffs were associated with higher ORR compared with IFNγ sig$^-$. Higher thresholds (50%–75%) for tumor IFNγ sig$^+$ were also associated with longer median OS (approximately 21–22 months) and median PFS (approximately 5.5–7.5 months) in NSCLC patients, as well as median PFS (approximately 9 months) in urothelial cancer patients (Supplementary Fig. S3). All IFNγ sig$^+$ threshold cutoffs, except the 75% cutoff, were associated with longer OS (median not reached) compared with IFNγ sig$^-$ in urothelial cancer patients (Supplementary Fig. S3).

Evaluation of IFNγ signature correlation with OS in TCGA

To assess whether the correlations observed between the IFNγ gene signature and clinical outcomes in NSCLC or urothelial cancer patients could be prognostic (i.e., whether the IFNγ signature could predict treatment response regardless of durvalumab), we conducted a Kaplan–Meier analysis of the IFNγ signature in TCGA database of tumors from patients with NSCLC adenocarcinoma, squamous NSCLC, and bladder adenocarcinoma. Tumors were partitioned into high or low groups at the upper tertile of IFNγ signature expression, similar to the threshold used in study 1108, and correlated with OS. In contrast to tumors from patients treated with durvalumab, OS in high-IFNγ signature tumors did not significantly differ from low signature tumors (Supplementary Fig. S4), which is consistent with the idea that IFNγ signature is predictive and not prognostic.

Durvalumab induction of IFNγ-inducible gene expression in NSCLC tumors

To determine the effect of durvalumab on intratumoral immune cell gene expression, we examined paired tumor biopsies at baseline and 6 weeks after treatment in a subset of 30 NSCLC patients. Durvalumab treatment elicited statistical increases in all component mRNAs of the total IFNγ signature except *CD274* (PD-L1; Fig. 2). Durvalumab treatment significantly increased intratumoral gene expression of the T-cell chemotactic chemokine *CXCL9*, the checkpoint molecule *LAG3*, and *IFNγ*, but not *CD274* (PD-L1), during 6 weeks of treatment. Individually, the *CXCL9* mRNA appeared to present the most consistent increase during durvalumab treatment across all patients in the subgroup, followed in decreasing statistical order by *LAG3* mRNA, IFNγ signature, and *IFNγ* mRNA. Among the group of gene expression profiles, tumor levels of IFNγ were the most widely variable quantitatively from pretreatment to 6 weeks of durvalumab treatment (Fig. 2).

Predicted utility of IFNγ signature compared with PD-L1 IHC status in NSCLC

Because *CD274* (PD-L1) mRNA is one of the four genes within the IFNγ signature, it is not surprising that the signature and PD-L1 IHC are significantly associated in NSCLC and urothelial cancer tumors (Table 5). However, in NSCLC, 20% of PD-L1$^-$ tumors (11 of 54) were IFNγ sig$^+$, whereas 37% of IFNγ sig$^+$ tumors (11 of 30) were PD-L1$^-$ (OR = 4.1). It is noteworthy that, among IFNγ sig$^+$ tumors, regardless of PD-L1 status, the median OS was similar. Within urothelial cancer, the majority of IFNγ sig$^+$ tumors (14 of 15) were also PD-L1$^+$ by IHC, precluding analysis of IFNγ sig$^+$, PD-L1$^-$ subjects.

Association of IFNγ$^+$ signature and tumor mutational burden in NSCLC and urothelial cancer

The genetic biomarker TMB, typically defined as the count of nonsilent somatic mutations detected in the tumor of a patient, has been shown to be correlated with characteristics such as disease subtype, microsatellite instability, CD8$^+$ T-cell presence, activation of oncogenic pathways, and clinical outcomes after treatment with ICIs (2, 23, 29–32). One hypothesis

Table 4. ORR, OS, and PFS by IFNγ mRNA signature or PD-L1 status in urothelial cancer patients

Status	Patients, *n* [events, *n* (OS; PFS)]	ORR, % (95% CI)[a]	Median OS, months (95% CI)[b]	OS adjusted HR[c] (*P*)	Median PFS, months (95% CI)[b]	PFS adjusted HR[c] (*P*)
IFNγ sig$^+$	21 (4; 11)	52.4 (29.8–74.3)	NR (7.8–NR)	0.21 (0.03)	9.3 (2.6–NR)	0.22 (0.0007)
IFNγ sig$^-$	41 (15; 34)	14.6 (5.6–29.2)	8.2 (3.4–NR)		1.4 (1.4–1.9)	
PD-L1$^+$	61 (23; 44)	37.7 (25.6–51.0)	18.4 (7.8–NR)	0.39 (0.0046)	2.6 (1.4–3.9)	0.53 (0.016)
PD-L1$^-$	39 (22; 33)	5.1 (0.6–17.3)	3.4 (2.4–14.3)		1.5 (1.4–2.4)	

Abbreviations: CI, confidence interval; NR, not reached.
[a]Exact binomial calculation.
[b]Intervals based on log (−log (survival)).
[c]Adjusted for baseline ECOG, smoking status, race, gender, age, previous lines of therapy, and liver metastasis.

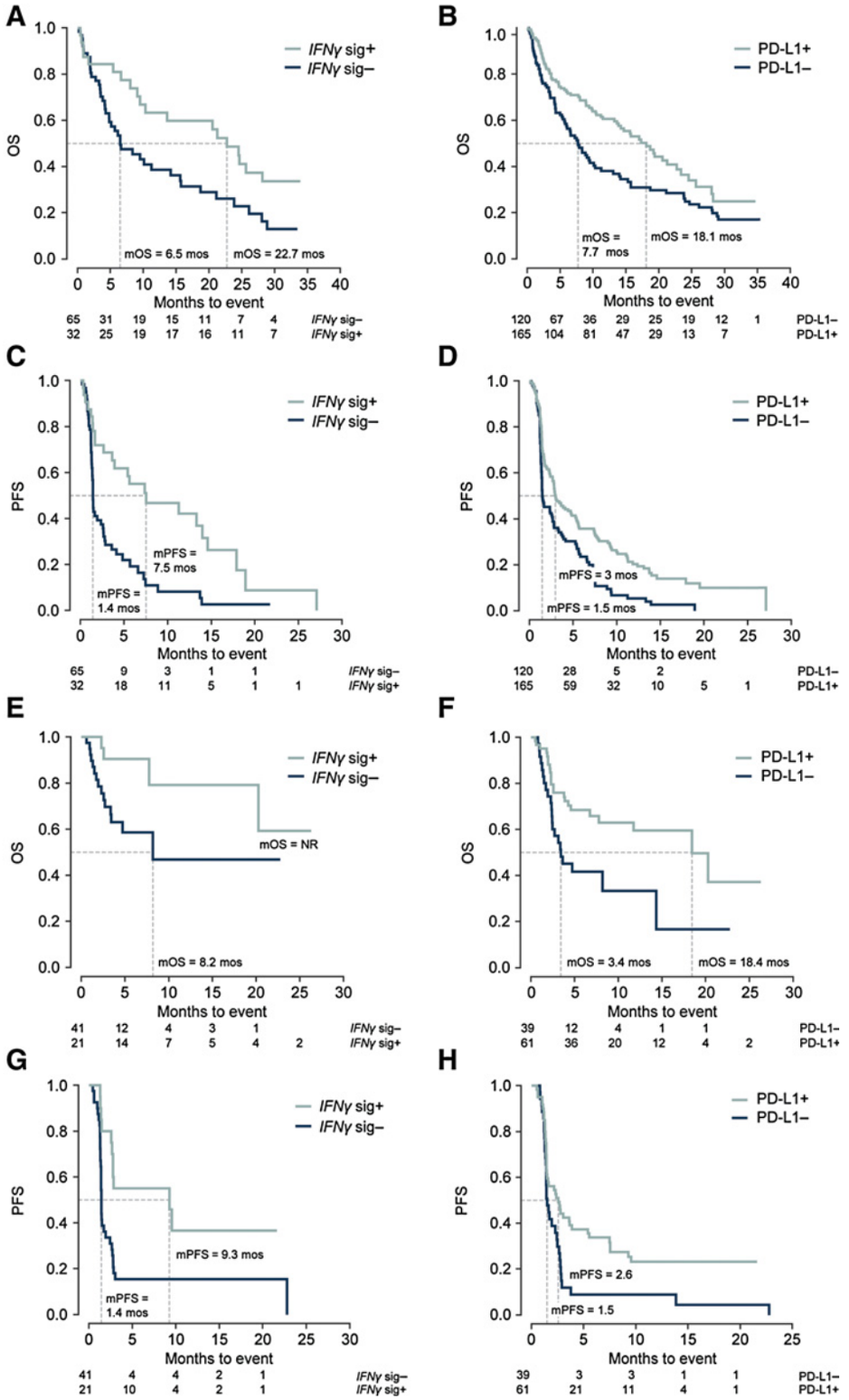

Figure 1.
Kaplan–Meier analysis of OS and PFS in NSCLC patients by IFNγ mRNA signature (**A** and **C**) and PD-L1 status (**B** and **D**) and in urothelial cancer patients by IFNγ mRNA signature (**E** and **G**) and PD-L1 status (**F** and **H**). Adjusted analysis; NR, not reached.

to explain the association between outcomes and TMB is the creation of neoantigens that elicit an immune response. Thus, a consequence of TMB could be increased IFNγ signature in the tumor microenvironment. For this reason, we used TCGA to evaluate the association between TMB and both the IFNγ signature and *CD274* mRNA, with the latter serving as surrogate

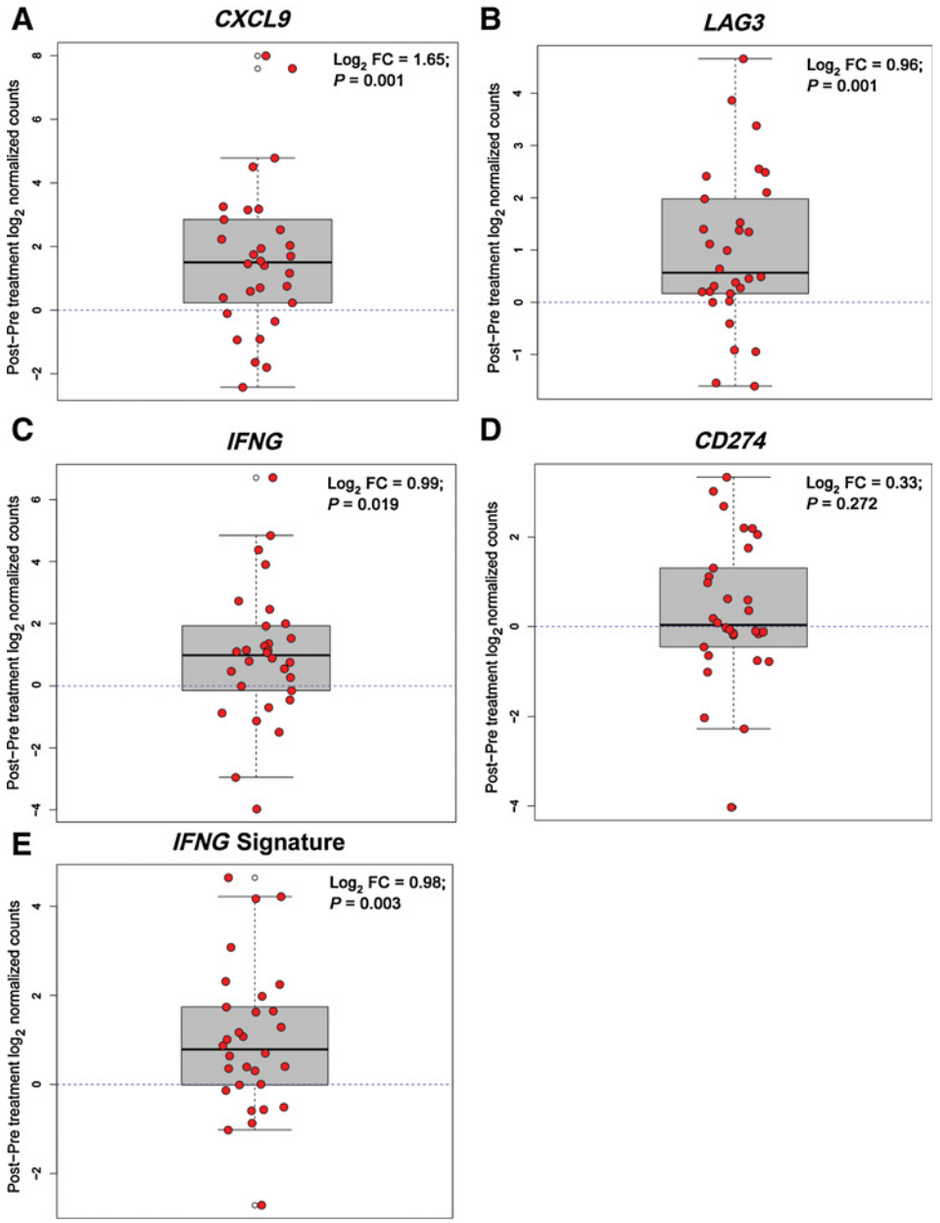

Figure 2.

Multiple components of the IFNγ gene signature induced by durvalumab in NSCLC tumors (*n*/30 with greater than twofold increase from baseline). (**A**) 16/30; (**B**) 13/30; (**C**) 15/30; (**D**) 9/30; (**E**) 15/30. *P* = Student paired *t* test; *n* = 30 patient paired samples.

for PD-L1 cellular expression in the TCGA database that lacks IHC data for this biomarker. TMB was partitioned into high or low at the median value for NSCLC and urothelial cancer separately. In NSCLC, *CD274* expression was partitioned into high or low at the upper tertile to mimic the approximate prevalence of PD-L1 IHC positivity (tumor cell expression) identified in an unselected patient population in this tumor type (30%–35%). In urothelial cancer tumor samples, *CD274* was partitioned at the lower tertile for consistency with the prevalence of tumor or immune-cell PD-L1 expression as measured by IHC (60%–70%). The IFNγ signature and TMB were associated in NSCLC and urothelial cancer, whereas *CD274* mRNA was not associated with TMB in NSCLC or urothelial cancer (Supplementary Table S3), consistent with a previous report in NSCLC (21).

Discussion

The IFNγ signature described here likely identifies a subset of NSCLC and urothelial cancer with a preexisting immune response that is blocked by the checkpoint PD-L1 and relieved by treatment with durvalumab. We selected a four-gene IFNγ signature for study of its predictive utility for durvalumab treatment response based on the screened expression levels of 21 genes associated with robust immune activation in a cohort of NSCLC tumor biopsies. We then tested this signature on a cohort of urothelial cancer tumor biopsies and demonstrated consistent predictive performance. In keeping with this strategy, the unique baseline four-gene IFNγ signature utilized in this study identifies several key biological pathways required for a robust antitumor response. IFNγ is a key immunoregulatory

Table 5. Concordance between tumor IFNγ gene signature and PD-L1 status in NSCLC and urothelial cancer patients

NSCLC	IFNγ sig$^+$ mOS (*n*)	IFNγ sig$^-$ mOS (*n*)
PD-L1$^+$, mOS (*n*)	23 mo (19)	6 mo (18)
PD-L1$^-$, mOS (*n*)	25 mo (11)	7 mo (43)

Fisher's exact test for count data; $P = 0.003$; OR = 4.1.

UC[a]	IFNγ sig$^+$ mPFS (*n*)	IFNγ sig$^-$ mPFS (*n*)
PD-L1$^+$, mPFS (*n*)	NR (14)	NR (11)
PD-L1$^-$, mPFS (*n*)	NR (1)	NR (16)

NOTE: Fisher's exact test for count data; $P = 0.001$; OR = 19.0.
Abbreviations: mPFS, median progression-free survival; NR, median not reached; UC, urothelial carcinoma.
[a]UC OS data are not fully mature for subgroup analysis.

cytokine that is produced by activated T, NK, and NK/T cells and orchestrates the immune response to tumors (33). IFNγ also induces expression of the chemokine CXCL9, which is part of this gene signature. CXCL9 is a T-cell chemoattractant produced by macrophages and tumor cells and drives necessary infiltrates to provide cytotoxic activity. LAG3 is an inhibitory receptor primarily expressed on chronically stimulated or exhausted T cells and thus is a hallmark of an immune response in the tumor microenvironment. CD274 (PD-L1) is the target of durvalumab and the primary tumor cell gene expressed among those in the IFNγ signature, although it is also expressed on lymphocytes and other immune cells. Taken together, these observations provide a strong biological rationale for genes that make up the IFNγ signature.

Our data showed that the four-gene IFNγ signature in baseline tumor biopsies was a robust predictive biomarker for longer OS and PFS after durvalumab treatment. This IFNγ sig$^+$ tumor status may identify tumors that are scored as PD-L1$^-$, but may otherwise remain sensitive to the therapeutic effects of ICIs, thus providing an opportunity to expand the benefit of checkpoint blockers. These results are consistent with those from the POPLAR study of atezolizumab treatment of NSCLC, in which OS was significantly longer in patients with tumors testing positive for a Teff and IFNγ gene signature, but differed in a statistical association observed between IFNγ and PD-L1 status in tumor cells (4).

Our study results are consistent with other recent study reports demonstrating that expanded immune gene signatures of different sizes have been associated with longer response rates and survival in patients with squamous or nonsquamous NSCLC, HNSCC, gastric cancer, or other tumor types treated with ICIs (34). In an open-label, phase II, randomized controlled trial evaluating atezolizumab (an anti–PD-L1 antibody) versus docetaxel in 287 treatment-experienced patients with either squamous or nonsquamous NSCLC (POPLAR study), atezolizumab treatment showed significant improvement in OS in patients with tumors that had high expression of a Teff/IFNγ gene signature measured with a commercial eight-gene mRNA (*CD8A*, *GZMA*, *GZMB*, *IFNγ*, *EOMES*, *CXCL9*, *CXCL10*, and *TBX21*) platform (4). In a cohort of patients with either gastric cancer ($n = 33$) or head and neck squamous cell carcinoma (HNSCC; $n = 43$) from the KEYNOTE-012 phase Ib clinical trial, an IFNγ-associated six-gene (*CXCL9*, *CXCL10*, *IDO1*, *IFNγ*, *HLA-DRA*, and *STAT1)* signature showed positive predictive values of 45% and 40%, respectively, for response rates to pembrolizumab in patients with high gene signature above the Youden index cut-off values (61% and 50% prevalence for gastric cancer and HNSCC tumors, respectively; refs. 34, 35). An analysis of 65 patients with multiple cancer types who were treated with either pembrolizumab or nivolumab showed that 11- to 14-gene signatures of immunologic-constant-of-rejection biomarkers (including IFNγ signaling and chemokine/chemokine receptor transcripts) were significantly associated with PFS and nonprogressive disease, respectively (18). Karachaliou and colleagues reported a statistically significant association between baseline high IFNγ gene expression and PFS after pembrolizumab or nivolumab treatment in small cohorts of patients with melanoma or NSCLC tumors, as well as trends between high IFNγ gene expression and OS in NSCLC tumors (36). In that study, the IFNγ signaling inducer molecule I kappa B kinase epsilon (IKBKE) was found to be positively, but not statistically, correlated with both IFNγ and PD-L1 baseline tumor expression, whereas expression of the other genes assessed (*STAT1*, *STAT3*, and *CD274*) and PD-L1 protein were not. Of interest, the increased survival benefits with durvalumab that are associated with high expression of the four-gene IFNγ signature in our study is similar to the increased OS and PFS associated with high expression of 6-, 8-, or 18-gene IFNγ signatures in NSCLC and urothelial cancer patients treated with other ICIs (Supplementary Table S6).

In this study, an association between the IFNγ gene signature and TMB was also demonstrated, although there was a sizable population of patients that were discordant between these two biomarkers (37%–43% of IFNγ sig$^+$ had low TMB and 44%–47% IFNγ sig$^-$ had high TMB). Thus, it would be interesting to determine whether a combination of both biomarkers may be more predictive than either alone or whether the benefit of increased immune infiltration caused by TMB is fully captured by the IFNγ signature.

This study has some important limitations. First, the initial number of study patients in the IFNγ signature cohort of NSCLC patients and both biomarker cohorts of urothelial cancer patients was low ($N < 100$), trailing off to very low patient numbers within 20 months of durvalumab treatment. This makes statistical extrapolations to larger patient populations difficult, despite available statistical comparisons. Second, the median OS in the total urothelial cancer patient cohort had not been reached by the data cutoff for this study, which hinders final determination of the differences in response to durvalumab treatment between biomarker cohorts and concordance between biomarkers in urothelial cancer patients. Third, the TMB analysis was based on TCGA data, as opposed to the patient population in study 1108, where differences such as disease stage and exposure to therapy are apparent. Furthermore, *CD274* mRNA was used as a surrogate for PD-L1 IHC, where inherent analyte and technological differences are known. As a result, the interpretations of the TMB associations with PD-L1 in NSCLC and urothelial cancer should be made with these caveats in mind. Finally, the true predictive effect of this gene signature should be evaluated in a randomized control trial. It should also be noted that patients registered in TCGA database had resectable cancer, whereas those enrolled in the current study had unresectable, advanced disease. Thus, conclusions around the predictive utility of the four-gene IFNγ signature described herein should be considered cautiously in patients who do not have advanced NSCLC or urothelial cancer or who

have different disease characteristics than those of the study participants.

Despite these limitations, the study data presented here contribute to the body of scientific evidence for the value of IFNγ gene expression as a predictive biomarker for response to ICI treatment in NSCLC and urothelial cancer patients. Additional studies focusing on the correlative predictive capacity of an IFNγ signature with other immunoregulatory molecules may provide insight into the mechanisms contributing to the antitumor effects of durvalumab treatment.

Disclosure of Potential Conflicts of Interest

B.W. Higgs, C.A. Morehouse, K. Streicher, P.Z. Brohawn, F. Pilataxi, and A. Gupta are employees of MedImmune/AstraZeneca. A. Gupta holds ownership interest (including patents) in AstraZeneca and Bristol–Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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