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ORIGINAL ARTICLE

Association between *PD1* mRNA and response to anti-PD1 monotherapy across multiple cancer types

L. Paré[1,2†], T. Pascual[1,2†], E. Seguí[1,2†], C. Teixidó[1,3], M. Gonzalez-Cao[4], P. Galván[1,2], A. Rodríguez[1,2], B. González[3], M. Cuatrecasas[3], E. Pineda[1,2], A. Torné[5], G. Crespo[6], S. Martin-Algarra[7], E. Pérez-Ruiz[8], Ò. Reig[1,2], M. Viladot[1,2], C. Font[1,2], B. Adamo[1,2], M. Vidal[1,2], L. Gaba[1,2], M. Muñoz[1,2], I. Victoria[1,2], G. Ruiz[1,2], N. Viñolas[1,2], B. Mellado[1,2], J. Maurel[1,2], J. Garcia-Corbacho[1,2], M. Á. Molina-Vila[9], M. Juan[10], J. M. Llovet[11–13], N. Reguart[1,2], A. Arance[1,2] & A. Prat[1,2*]

[1]Translational Genomic and Targeted Therapeutics in Solid Tumors, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona; [2]Department of Medical Oncology; [3]Pathology Service, Hospital Clínic of Barcelona, Barcelona; [4]Quironsalud Group, Dr. Rosell Oncology Institute (IOR), Dexeus University Hospital, Barcelona; [5]Gynecology Service, Hospital Clínic of Barcelona, Barcelona; [6]Department of Medical Oncology, Hospital Universitario de Burgos, Burgos; [7]Department of Medical Oncology, Clínica Universitaria de Navarra, Pamplona; [8]Department of Medical Oncology, Hospital Costa del Sol REDISSEC, Marbella; [9]Pangaea Oncology, Laboratory of Molecular Biology, Quirón-Dexeus University Institute, Barcelona; [10]Immunology Department, Hospital Clinic of Barcelona, Barcelona; [11]BCLC Group, Translational Research Lab in Hepatic Oncology, IDIBAPS, Hospital Clínic, CIBERehd, Barcelona, Universitat de Barcelona; Barcelona, Spain; [12]Mount Sinai Liver Cancer Program, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, USA; [13]Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

Correspondence to: Dr Aleix Prat, Department of Medical Oncology, Translational Genomics and Targeted Therapeutics in Solid Tumors, IDIBAPS, Hospital Clínic of Barcelona, Villarroel 170, Barcelona 08036, Spain. Tel: +34-93-227-54-00; Fax: +33-1-40-03-24-50; E-mail: alprat@clinic.cat
[†]These authors contributed equally.

Background: We hypothesized that the abundance of *PD1* mRNA in tumor samples might explain the differences in overall response rates (ORR) observed following anti-PD1 monotherapy across cancer types.

Patients and methods: RNASeqv2 data from 10 078 tumor samples representing 34 different cancer types was analyzed from TCGA. Eighteen immune-related gene signatures and 547 immune-related genes, including *PD1*, were explored. Correlations between each gene/signature and ORRs reported in the literature following anti-PD1 monotherapy were calculated. To translate the in silico findings to the clinical setting, we analyzed the expression of *PD1* mRNA using the nCounter platform in 773 formalin-fixed paraffin embedded (FFPE) tumor samples across 17 cancer types. To test the direct relationship between *PD1* mRNA, PDL1 immunohistochemistry (IHC), stromal tumor-infiltrating lymphocytes (sTILs) and ORR, we evaluated an independent FFPE-based dataset of 117 patients with advanced disease treated with anti-PD1 monotherapy.

Results: In pan-cancer TCGA, *PD1* mRNA expression was found strongly correlated ($r > 0.80$) with CD8 T-cell genes and signatures and the proportion of *PD1* mRNA-high tumors (80th percentile) within a given cancer type was variable (0%–84%). Strikingly, the *PD1*-high proportions across cancer types were found strongly correlated ($r = 0.91$) with the ORR following anti-PD1 monotherapy reported in the literature. Lower correlations were found with other immune-related genes/signatures, including *PDL1*. Using the same population-based cutoff (80th percentile), similar proportions of *PD1*-high disease in a given cancer type were identified in our in-house 773 tumor dataset as compared with TCGA. Finally, the pre-established *PD1* mRNA FFPE-based cutoff was found significantly associated with anti-PD1 response in 117 patients with advanced disease (*PD1*-high 51.5%, *PD1*-intermediate 26.6% and *PD1*-low 15.0%; odds ratio between *PD1*-high and *PD1*-intermediate/low = 8.31; $P < 0.001$). In this same dataset, PDL1 tumor expression by IHC or percentage of sTILs was not found associated with response.

Conclusions: Our study provides a clinically applicable assay that links *PD1* mRNA abundance, activated CD8 T-cells and anti-PD1 efficacy.

Key words: *PD1*, gene expression, anti-PD1, immunotherapy, solid tumors

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Introduction

Cancer is characterized by the accumulation of different genetic alterations which lead to the expression of different neoantigens, which are displayed on the surface of cancer cells [1]. Simultaneously, cancer cells have developed sophisticated ways of escaping from the immune system, which has been a major limitation of cancer immunotherapy [1]. To date, many immune escape mechanisms have been identified, including expression of endogenous 'immune checkpoints' that normally terminate immune responses after antigen activation [1]. These observations have resulted in the development of immune checkpoint-pathway inhibitors such as anti-PD1 or anti-CTLA4.

Anti-PD1 drugs such as nivolumab or pembrolizumab have demonstrated unprecedented clinical efficacy in more than 15 cancer types [2]. The overall response rates (ORR) achieved by these drugs across 22 cancer types varies and ranges from 0% to 50% [3]. Patients responding to these therapies usually gain a large survival benefit, leading to impressive outcomes. Thus, pre-selecting patients most likely to respond to anti-PD1 is necessary.

Recent studies support the role of PDL1 expression by immunohistochemistry (IHC) as a potential biomarker for pembrolizumab in non-small-cell lung cancer (NSCLC) [4]. However, its predictive value in other cancer types, or with other anti-PD1 drugs, is controversial. Moreover, IHC-based detection of PDL1 has important limitations, such as different sensitivities of the antibodies used and its subjectivity in scoring and cut-off determination [4].

Therefore, identification of reproducible biomarkers that can be applied to predict benefit of anti-PD1 monotherapy might be of clinical value. Previously, we reported that the expression of immune-related genes using the nCounter platform, including *PD1*, is reproducible and is associated with anti-PD1 monotherapy efficacy in 65 patients with advanced cancer [5]. Here, we hypothesized that the abundance of *PD1* mRNA in tumor samples might explain the ORR differences observed across different cancer types following anti-PD1 monotherapy.

Methods

The cancer genome atlas (TCGA) dataset

Data from a total of 10 462 tumor samples representing 36 cancer types with available RNASeqv2 data at the TCGA portal was downloaded. Gene expression values were represented as RNA-seq by Expectancy-Maximization data and normalized within-sample to the upper quartile of total reads. Information regarding the tumor mutational burden (TMB) in each TCGA tumor sample was obtained from Hoadley et al. [6].

Immune-related genes and signatures

In the TCGA pan-cancer dataset, 547 immune-related genes, including *PD1*, were explored (supplementary material, available at *Annals of Oncology* online). In addition, 18 immune-related gene expression signatures (GES) were evaluated. Among them, 15 tracked various immune cell types and the gene lists were obtained from previously published literature [7]. 'Tumor inflammation signature' (TIS) reported by Ayers et al. [8] was also evaluated. Finally, two additional immune GES were newly defined from the unsupervised clustering. Gene lists for all 18 GES are included in supplementary material, available at *Annals of Oncology* online. To obtain a single score for each signature and sample, the mean expression of the genes composing the signature was calculated, except for the TIS whose algorithm is protected and scores were directly provided to us by Nanostring.

Anti-PD1 efficacy in the literature

To recapitulate all the data presented to date from clinical trials published assessing anti-PD1 monotherapy in patients with advanced solid tumors, we searched for articles published until June 2018 in PubMed or in abstracts from the American Society of Clinical Oncology annual meeting, using the search terms 'Nivolumab' and 'Pembrolizumab'. We included studies that enrolled at least 15 patients without biomarker pre-selection.

PD1 mRNA from FFPE tumor tissues

Methods for RNA extraction, quality assessment and gene expression analysis can be found in supplementary data, available at *Annals of Oncology* online.

Independent validation cohort

We selected consecutive patients with advanced cancer mainly treated at Hospital Clínic in Barcelona (HCB) with anti-PD1 monotherapy as standard practice or in a clinical trial from January 2013 to April 2018. Patients received either pembrolizumab or nivolumab until progression or unacceptable toxicity. From a total of 155 patients, tumor samples were available for 117 patients. Extraction of RNA and gene expression were carried out successfully in all patients.

Stromal tumor-infiltrating lymphocytes (sTILs) and PDL1 IHC

Percentages of sTILs were evaluated as described previously [9]. IHC was carried out on FFPE tissue sections using the anti-PDL1 mouse monoclonal antibody (22C3, Dako) on a Dako Autostainer following manufacturer's recommendations. PDL1 IHC expression was evaluated in tumor cells.

Statistical analysis

All gene expression cluster analyses were displayed using Java Treeview v1.1.3. Average linkage hierarchical clustering was carried out using Cluster v2.12. Biologic analysis of microarray data was carried out with the DAVID annotation tool. Univariate and multivariable logistic regression analyses were done to investigate the association of *PD1* with response. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. Univariate and multivariable cox model analyses were done to investigate the association of *PD1* with progression-free survival (PFS) and overall survival (OS). The significance level was set to a two-sided α of 0.05. We used R v3.4.2 for all the statistical analyses.

Results

PD1 and immune-related gene expression in TCGA

To start approaching our hypothesis, we combined RNA-seq data from TCGA dataset for a total of 10 078 samples representing 34 different cancer types (Figure 1A and supplementary Table S1, available at *Annals of Oncology* online). A total of 547 immune-related genes, including *PD1*, were selected from 15 557 genes (Figure 1A and supplementary Figure S1, available at

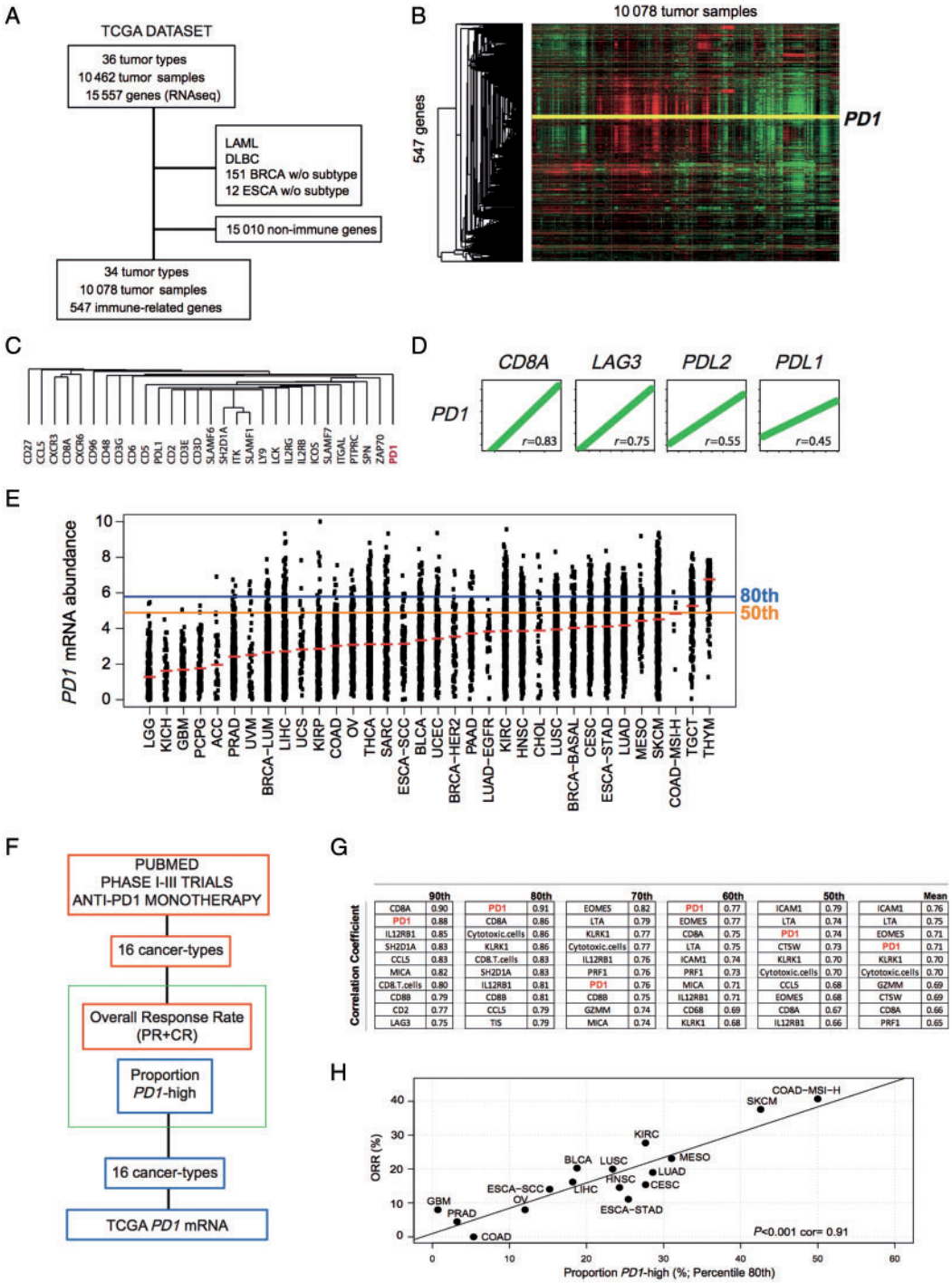


Figure 1. Expression of *PD1* mRNA and other immune-related genes across cancer types in The Cancer Genome Atlas (TCGA) dataset. (A) Consort diagram reflecting the number of tumor samples and genes evaluated in the study. (B) Unsupervised hierarchical clustering using the 547 immune genes (rows) and 10 078 tumor samples (columns). Each colored square on the heatmap represents the relative median signature score for each sample with highest expression being red, lowest expression being green and average expression being black. The yellow horizontal line indicates the location of *PD1*. (C) Gene cluster obtained from the unsupervised clustering that contains *PD1* and the rest of immune genes with an overall intraclass correlation of 0.85. (D) Correlation between *PD1* and four other immune-related genes (*CD8A*, *LAG3*, *PDL1* and *PDL2*) in the combined TCGA dataset. Numbers within the plot indicate the Pearson correlation coefficient. (E) Expression of *PD1* mRNA across 34 cancer types. The red line within each cancer type denotes the median expression. Samples have been ordered according to the median expression of *PD1*. Horizontal lines indicate the percentile 50th and 80th of *PD1* expression in the entire cohort. (F) Plot depicting the analysis carried out by comparing the proportions of *PD1*-high disease within each cancer type and the reported overall response rates (ORRs) following anti-PD1 monotherapy. (G) Top 10 Pearson correlation coefficients obtained for a given gene based on different percentile cutoffs obtained from the entire cohort. (H) Correlation between the % of *PD1*-high described in our study (defined as percentile 80th) and the reported ORR in previous paper of different solid tumors following anti-PD1 monotherapy.

Annals of Oncology online). Eighteen GES tracking different immune cell types were identified, including TIS, which has previously been shown to enrich for a population of patients who respond to pembrolizumab [8]. In addition, we carried out an unsupervised analysis using all immune-related genes and all samples, and hand-picked two gene clusters that met the following criteria: >20 genes and a correlation coefficient (r) among the genes >0.8 (Figure 1B and supplementary Figure S1, available at *Annals of Oncology* online).

Next, we explored the association of *PD1* expression with other immune-related genes in the combined matrix (Figure 1C). As expected, *PD1* strongly correlated ($r > 0.80$) with a group of 30 genes, including *CD3* and *CD8A*, which were found significantly enriched in biological processes such as CD8 T-cell activation ($P < 0.001$, DAVID tool) (Figure 1C). Concordant with this single-gene analysis, strong correlation ($r > 0.80$) was found between *PD1* and GES tracking T-cell activation. Lower correlations were found between *PD1* expression and other immune checkpoint inhibitors such as *LAG3* ($r = 0.75$), *PDL2* ($r = 0.55$) and *PDL1* ($r = 0.45$) (Figure 1D).

Immune-related gene expression and reported anti-PD1 efficacy

When *PD1* expression was evaluated across 34 cancer types, large expression variability across and within each cancer type was observed (Figure 1E). For example, the proportion of *PD1*-high tumors within each cancer type ranged from 0% to 84% when percentile 80th in the combined matrix was used as the cutoff to define *PD1*-high versus *PD1*-low (Figure 1E and supplementary material, available at *Annals of Oncology* online).

Among the cancer types with the highest mean expression of *PD1*, the ones with the highest reported ORR following anti-PD1 monotherapy (e.g. melanoma) were identified. Conversely, cancer types showing the lowest *PD1* mean expression were those with the lowest reported ORR following anti-PD1 monotherapy (e.g. pancreatic adenocarcinoma).

These previous data suggested that the abundance of *PD1* expression within and across cancer types might be associated with anti-PD1 efficacy. To test this hypothesis indirectly, we correlated, for each signature and gene, the percentage of high-expressing tumors with the ORR reported from clinical trials that evaluated anti-PD1 monotherapy with either nivolumab or pembrolizumab (supplementary Table S2, available at *Annals of Oncology* online and Figure 1F). To define the proportion of biomarker-high tumors within each cancer type, six different cutoffs (i.e. percentiles 50th to 90th and the mean) were evaluated in the combined matrix. The results revealed that among the 565 biomarkers evaluated (i.e. 547 individual genes and 18 GES), *PD1* was found highly correlated ($r = 0.74$–0.91) with ORR and consistently within the top three biomarkers across the six cutoffs evaluated (Figure 1G and supplementary material, available at *Annals of Oncology* online). *PDL1* was found moderate correlated ($r = 0.06$–0.53) with ORR across the six cutoffs evaluated (supplementary Figure S2, available at *Annals of Oncology* online). Overall, the highest correlation coefficient was obtained with *PD1* when percentile 80th was used as the cutoff ($r = 0.91$, $P < 0.001$) (Figure 1G and H). The correlation coefficient of 0.91 suggests that 82.8% of the differences in the ORR across cancer types may be explained by the proportion of *PD1*-high expressers within each cancer type.

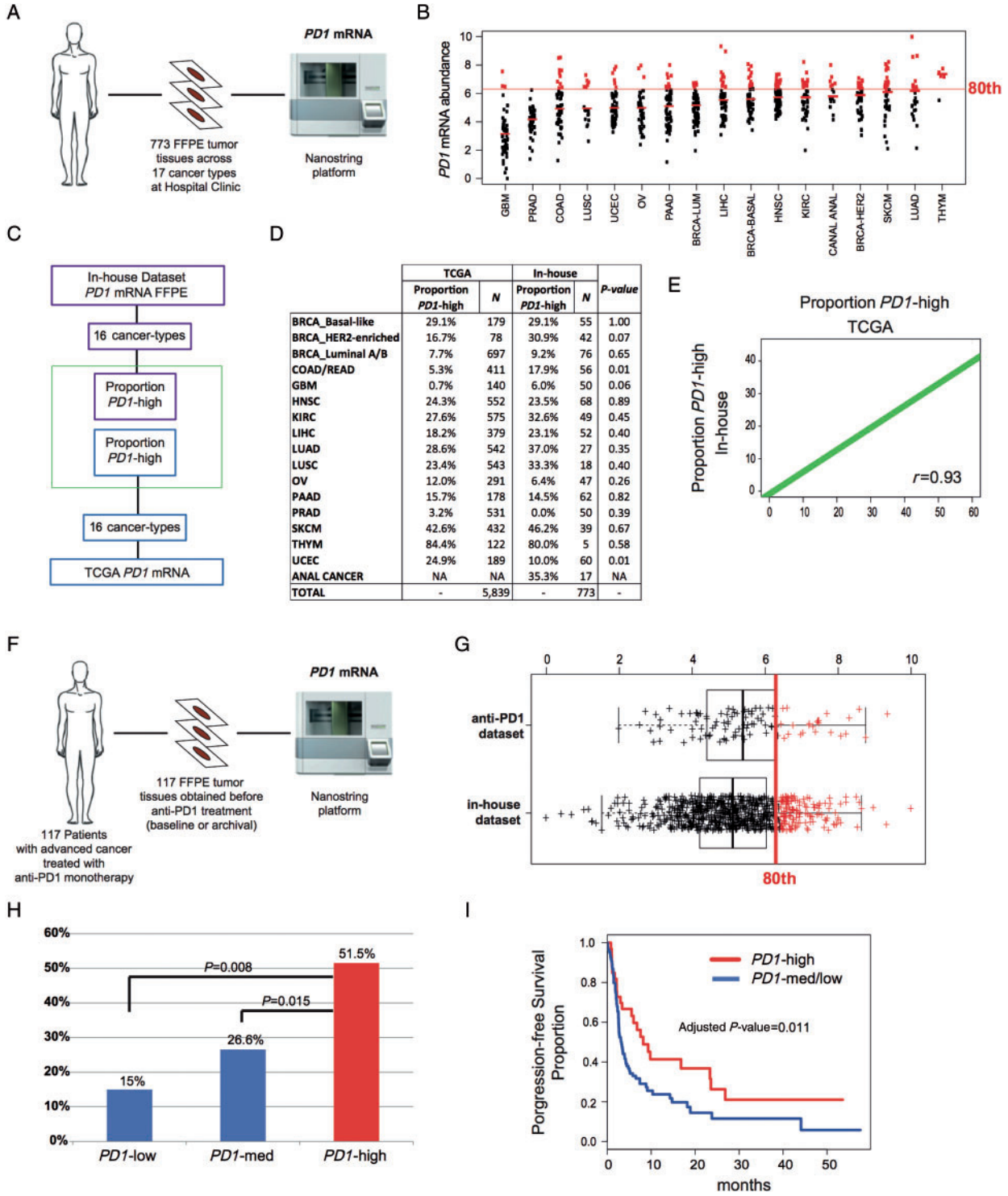
The previous *in silico* and indirect analysis suggested that (i) there is a strong association between *PD1* mRNA absolute levels and reported ORR following anti-PD1 monotherapy and (ii) percentile 80th in the combined matrix of 34 cancer types might be an appropriate *PD1* expression cutoff to identify responding tumors following anti-PD1 monotherapy. Thus, we aimed to estimate the expected ORR following anti-PD1 monotherapy for any *PD1*-high tumor. Hence, we obtained a regression line that best fits the relationship between the proportion of high *PD1*-expressing tumors within each cancer type (percentile 80th) versus the reported ORR. The formula for the regression line was ORR = Proportion of *PD1*-high tumors*0.75 + 0.96. Thus, the *in silico* analysis estimated that a given *PD1*-high tumor would have an expected ORR of 76.0% (95% CI 54.8% to 94.8%) if treated with anti-PD1 monotherapy.

PD1 mRNA, TMB and reported anti-PD1 efficacy

Recent *in silico* studies have linked the median number of somatic mutations (i.e. TMB) within each cancer type with reported ORR following anti-PD1/PDL1 monotherapy across cancer types [3]. To explore the association between *PD1* mRNA and TMB, we used TCGA data from 8 792 tumor samples and 31 cancer types with both types of information. In this combined dataset, no correlation between *PD1* mRNA and TMB was found ($r = 0.09$). However, the mean TMB in *PD1*-high tumors (9.44) was significantly larger than in *PD1*-low tumors (5.61; $P < 0.001$). Finally, the correlation between TMB and reported ORR was 0.61 ($P = 0.011$), whereas the correlation between *PD1* mRNA (percentile 80th as cutoff) with reported ORR was 0.91 ($P < 0.001$) (supplementary Figure S3, available at *Annals of Oncology* online). These *in silico* results suggest that *PD1* mRNA might be a better predictor of anti-PD1 treatment than TMB.

Implementing *PD1* expression in the clinical setting

To translate the previous *in silico* findings in clinical samples, we analyzed the expression of *PD1* in 773 FFPE tumor samples representing 17 cancer types from a retrospective in-house dataset at HCB using nCounter (Figure 2A). In this combined dataset, we plotted the expression of *PD1* across cancer types and identified the expression value at percentile 80th which defines *PD1*-high versus *PD1*-low disease (*PD1* score = 6.3) (Figure 2B). Next, we compared the proportion of *PD1*-high disease within each cancer type between our in-house dataset and TCGA dataset (Figure 2C). Anal cancer could not be compared since it is not represented in TCGA. Strikingly, 15 of the 16 cancer types did not show a significant difference in the proportion of *PD1*-high tumors between both datasets (Figure 2D). Concordant with this finding, the correlation between the proportion of *PD1*-high tumors across the 16 cancer types in TCGA dataset and our in-house dataset was strong ($r = 0.93$, $P < 0.001$) (Figure 2E). The only cancer type with a significant difference in the proportion of *PD1*-high tumors between both datasets was endometrial carcinoma (24.9% versus 10.0%). This difference might be due to sample selection since our in-house dataset of endometrial



	TCGA		In-house		P-value
	Proportion PD1-high	N	Proportion PD1-high	N	
BRCA_Basal-like	29.1%	179	29.1%	55	1.00
BRCA_HER2-enriched	16.7%	78	30.9%	42	0.07
BRCA_Luminal A/B	7.7%	697	9.2%	76	0.65
COAD/READ	5.3%	411	17.9%	56	0.01
GBM	0.7%	140	6.0%	50	0.06
HNSC	24.3%	552	23.5%	68	0.89
KIRC	27.6%	575	32.6%	49	0.45
LIHC	18.2%	379	23.1%	52	0.40
LUAD	28.6%	542	37.0%	27	0.35
LUSC	23.4%	543	33.3%	18	0.40
OV	12.0%	291	6.4%	47	0.26
PAAD	15.7%	178	14.5%	62	0.82
PRAD	3.2%	531	0.0%	50	0.39
SKCM	42.6%	432	46.2%	39	0.67
THYM	84.4%	122	80.0%	5	0.58
UCEC	24.9%	189	10.0%	60	0.01
ANAL CANCER	NA	NA	35.3%	17	NA
TOTAL	-	5,839	-	773	-

Figure 2. Implementing *PD1* mRNA in the clinical setting. (A) Creation of an in-house dataset of 773 formalin-fixed paraffin embedded (FFPE) tumor samples representing 17 cancer types. *PD1* mRNA was measured using the nCounter platform. (B) Expression of *PD1* mRNA across 17 cancer types. The red line within each cancer type denotes the median expression. Samples have been ordered according to the median expression of *PD1*. Horizontal line indicate percentile 80th obtained from the entire cohort. (C) Plot depicting the analysis carried out by comparing the proportions of *PD1*-high disease within each cancer type between the in-house dataset and the TCGA dataset. (D) Table with the actual proportions of *PD1*-high disease in both datasets. (E) Correlation of the proportions of *PD1*-high disease of each cancer type between the in-house dataset and the TCGA dataset. The number within the plot indicate the Pearson correlation coefficient. (F) Creation of a validation dataset of 117 FFPE tumor samples from patients with advanced solid tumors treated with anti-PD1 monotherapy. *PD1* mRNA was measured using the nCounter platform. (G) *PD1* mRNA expression in tumor samples from both cohorts (in-house and validation). The vertical red line indicates percentile 80th obtained from the in-house dataset. Red samples identify those with *PD1*-high disease. (H) Overall response rates based on *PD1* mRNA expression. (I) Progression-free survival based on *PD1* mRNA expression. *P*-value was obtained from the log-rank rest.

carcinomas did not include high-grade carcinomas whereas the TCGA had 59.9% high-grade carcinomas. Overall, these data suggested that the proportion of *PD1*-high tumors within each cancer type is a common phenomenon across different datasets (i.e. in-house and TCGA), tissues (i.e. fresh-frozen and FFPE) and genomics platforms (i.e. RNAseq and nCounter).

Direct association between *PD1* expression and anti-PD1 efficacy

To evaluate the direct association of *PD1* FFPE-based mRNA expression with ORR following anti-PD1 monotherapy, we evaluated the *PD1* mRNA pre-established cutoff (i.e. 6.3) in a retrospective and independent cohort of 117 patients with advanced melanoma ($n = 59$), NSCLC ($n = 32$), renal cell cancer ($n = 14$) and others ($n = 12$) treated with anti-PD1 monotherapy [nivolumab ($n = 55$) or pembrolizumab ($n = 62$)] at HCB (Figure 2F). Most patients were male ($n = 83$), mean age was 61 years and median follow-up was 15.4 months (supplementary Tables S3 and S4, available at *Annals of Oncology* online). In the entire population, the ORR was 31.6% and median PFS was 3.93 months (95% CI 2.7–6.5).

In this validation cohort, 28.2% of the samples were identified as *PD1*-high according to our pre-established cutoff (Figure 2G and supplementary Table S5, available at *Annals of Oncology* online). In terms of efficacy, the ORR in *PD1*-high tumors was 51.5% (95% CI 33.9% to 68.8%) compared with 23.8% (95% CI 15.5% to 34.6%) in the rest of tumors (adjusted OR = 8.31; 2.7–30.5; $P < 0.001$). A gradient of response was observed if the rest of tumors were subdivided into two equal groups based on *PD1* expression. The ORR in the *PD1*-intermediate and *PD1*-low groups was 26.6% (95% CI 16.7% to 39.3%) and 15.0% (95% CI 4.0% to 38.9%), respectively (Figure 2H). The median PFS of *PD1*-high versus others was 8.17 versus 3.18 months [adjusted hazard ratio (HR) = 2.04; 95% CI 1.2–3.5; $P = 0.011$) (Figure 2I). No clinical–pathological variable was found associated with ORR or PFS. The median OS for *PD1*-high versus others was 23.4 versus 14.9 months (HR 1.31; 95% CI 0.8–2.3; $P = 0.330$).

Correlation of *PD1* mRNA with other biomarkers

First, we investigated the correlation of *PD1* mRNA with sTILs in 84 evaluable samples (72%) from the validation dataset. The correlation between the two biomarkers was moderate ($r = 0.53$) (supplementary Figure S4A, available at *Annals of Oncology* online). In terms of association with anti-PD1 efficacy, sTILs as a continuous variable was not found associated with response (OR = 1.01; 95% CI 1–1.1; $P = 0.197$), whereas *PD1* mRNA as a continuous variable was found significantly associated with response (OR = 1.32; 95% CI 1.0–1.73; $P = 0.034$).

Second, we investigated the correlation of *PD1* mRNA with PDL1 IHC in 74 evaluable samples (63%) from the validation dataset. No correlation between the two biomarkers was found ($r = -0.04$) (supplementary Figure S4B, available at *Annals of Oncology* online). PDL1 IHC as a continuous variable was not found associated with response (OR = 0.99; 95% CI 0.97–1.0; $P = 0.28$), whereas *PD1* mRNA as a continuous variable was found significantly associated with response (OR = 1.43; 95% CI 1.1–1.9; $P = 0.01$).

Discussion

In recent years, anti-PD1 drugs have demonstrated significant efficacy across various tumors becoming a new game-changer in oncology [2]. However, not all patients benefit from this treatment strategy; in addition, no clear predictive biomarker exist that can be used across cancer types. Thus, there is a need to better understand the biology associated with the activity of these drugs and to identify predictive biomarkers of response. At present time, PDL1 expression by IHC remains the only approved companion diagnostic for pembrolizumab in NSCLC, but important technical limitations exist with this biomarker [4], together with the fact that PDL1 was neither found predictive in other cancer types nor with other anti-PD1 drugs such as nivolumab. Therefore, clinical implementation of robust and reproducible genomic assays using platforms such as the nCounter is needed. Thereby, high reproducibility of *PD1* expression measurement has been reported using the nCounter platform starting from either already purified RNA or from tissue [5].

Two recent studies evaluating the association of immune-related gene expression in patients with various solid tumors treated with anti-PD1/PDL1 have been reported. In the first one, Ayers et al. [10] developed a predictive 'tumor inflammation' signature using the nCounter platform in 19 patients with advanced melanoma treated with pembrolizumab. Interestingly, *PD1* was not part of the gene list of this signature. Furthermore, they tested the predictive ability of TIS and three additional GES, in patients treated with pembrolizumab in the KEYNOTE-001/012 trials. Overall, they observed that patients with tumors that had low scores of the GES did not respond to anti-PD1 therapy [10]. We observed that TIS in TCGA showed a lower correlation coefficient with reported ORR than with *PD1* alone (0.79 versus 0.91). In the second study, Fehrenbacher et al. [11] evaluated 224 NSCLC samples from a trial where patients were randomized to docetaxel or atezolizumab. The authors observed that patients with high T-effector-interferon-γ-associated gene expression, measured using the Nimblegen platform, had improved OS with atezolizumab.

These prior studies support our findings that immune gene expression tracking T-cell activation is associated with anti-PD1 sensitivity across multiple cancer types. On the one hand, however, *PD1* by itself is more strongly associated with anti-PD1 sensitivity than any other immune marker evaluated. Our hypothesis is that *PD1* is tracking activated CD8 T cells, and the higher amount of activated CD8 T cells, the more likely a tumor is to respond to anti-PD1. Indeed, Gros et al. [12] reported that intratumoral expression of *PD1* in melanoma can guide the identification of the patient-specific repertoire of tumor-reactive CD8+ lymphocytes that reside in the tumor. Further supporting this hypothesis, a recent study using flow cytometry on metastatic melanoma samples reveals that increasing fractions of *PD1*-high/*CTLA4*-high cells within the tumor-infiltrating CD8+ T-cell subset strongly correlates with response and PFS following anti-PD1 monotherapy [13]. Finally, Thommen et al. have shown that sTILs with high *PD1* expression strongly predicted both response and survival in a small cohort of NSCLC patients treated with anti-PD1 blockade [14].

An interesting observation is the similar proportions of *PD1*-high disease observed between our in-house samples and the

TCGA samples. TCGA pan-cancer dataset is largely (>90%) composed of early disease and gene expression was carried out in surgically removed fresh-frozen tissues and the Illumina RNAseq platform. On the contrary, our in-house pan-cancer dataset came from a variety of cohorts, many samples were obtained from core-needle biopsies, and gene expression was carried out in FFPE tumor tissues and the nCounter platform. This result suggests that the amount of T-cell activation within and across cancer types is a consistent and stable population-based phenomenon.

Beyond gene expression, other genomic biomarkers of anti-PD1 sensitivity are emerging. One of the most promising is the number of mutations (TMB) [15]. Indeed, cancer types with the highest ORR following anti-PD1 therapy are the ones with the highest TMB. By performing a similar in silico analysis as carried out in our study between a large pan-cancer dataset and the reported ORR in the literature following anti-PD1/PDL1 therapy, Yarchoan et al. [3] observed a significant correlation between TMB and ORR. The correlation coefficient was 0.74, suggesting that 55% of the differences in the ORR across cancer types may be explained by the TMB. Moreover, Goodman et al. [16] analyzed TMB in 102 patients with advanced melanoma or NSCLC treated with anti-PD1 monotherapy and observed a direct association with efficacy. However, whether TMB directly predicts anti-PD1 efficacy in other cancer types needs to be demonstrated. In addition, further studies are needed to establish the analytical validity and clinical utility of TMB.

There are several caveats to our study. First, the direct association of *PD1* expression and anti-PD1 efficacy was evaluated in a limited and heterogeneous dataset of patients with advanced cancer (mostly melanoma and NSCLC) treated outside of a clinical trial. Thus, biases inherent to the 'real-world setting' are likely affecting the results, including the fact that archival biopsies were used. Nonetheless, we were able to demonstrate a gradient of anti-PD1 response based on *PD1* expression. Second, further studies are needed to elucidate if *PD1* is associated with anti-PD1 response in cancer types less represented or not represented at all in our validation cohort. Third, although the current study establishes the analytical and clinical validity of the *PD1* mRNA biomarker, further prospective and randomized studies are needed to establish its clinical utility and optimal cut-off. Fourth, we do not know if *PD1* might be a good biomarker of anti-PDL1 sensitivity. In-silico analyses comparing TCGA data with reported ORR as carried out in our study and others [3] might provide a first answer.

In summary, our results are consistent with the hypothesis that identification of a pre-existing and stable adaptive immune response using *PD1* mRNA expression predicts outcome across cancer types following anti-PD1 monotherapy. Further clinical validation of *PD1* seems warranted.

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Disclosure

Advisory role of AP for Nanostring Technologies. All remaining authors have declared no conflicts of interest.

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