Editorial

VISTA/PD-1H: a potential target for non-small cell lung cancer immunotherapy

Juan-Manuel Hernandez-Martinez[1,2], Edgar Vergara[1], Zyanya Lucia Zatarain-Barrón[1], Feliciano Barrón-Barrón[1], Oscar Arrieta[1]

[1]Thoracic Oncology Unit and Experimental Oncology Laboratory, Instituto Nacional de Cancerología de México (INCan), Mexico City, Mexico; [2]CONACYT-Instituto Nacional de Cancerología, Mexico City, Mexico

Correspondence to: Dr. Oscar Arrieta. Thoracic Oncology Unit, Instituto Nacional de Cancerología, San Fernando 22 Sección XVI, Tlalpan, Mexico City 14080, Mexico. Email: ogar@unam.mx.

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The development of immunotherapeutic agents for oncology is based on the insight that tumor development and progression is highly dependent on the ability of tumor cells to avoid immune recognition and destruction. Numerous immunomodulatory alterations, enabling tumor cells to evade the immune system, have been described. However, on the basis of their mechanism of action, they can be broadly classified into three categories: (I) downregulation of molecules involved with appropriate antigen processing and presentation; (II) downregulation of immune activation pathways; and (III) upregulation of immunosuppressive pathways (1). Not surprisingly, many of the mechanisms that enable tumor cells to evade the immune system have also been implicated in the resistance to immunotherapies.

To date, the most successful approach for activating therapeutic anti-tumor immunity has been the use of immune checkpoint inhibitors (ICIs), which have achieved unprecedented clinical responses. ICIs restore T-cell activation and antitumor responses by targeting co-inhibitory molecules such as cytotoxic T lymphocyte-associated antigen 4 (CTLA4), programmed cell death-1 (PD-1) and its ligands (PD-L1, PD-L2).

Anti-PD-1 ICIs, like nivolumab (Bristol-Myers Squibb) and pembrolizumab (Merck), have become a standard of care treatment for patients with metastatic non-small cell lung cancer (NSCLC) after progression following first-line platinum-based doublet chemotherapy (2-4). Pembrolizumab is the first anti-PD-1 approved in the first-line setting both as a single agent therapy for metastatic NSCLC in patients with tumor PD-L1 expression ≥50% and no *EGFR* or *ALK* genomic aberrations (5), or in combination with pemetrexed and platinum chemotherapy in patients with nonsquamous NSCLC and no *EGFR* or *ALK* genomic tumor aberrations, regardless of PD-L1 status (6,7).

The assessment of tumor PD-L1 expression by immunohistochemistry (IHC) has been useful to identify patients that are more likely to respond to anti-PD-1/PD-L1 therapies. However, it is far from an ideal biomarker and considerable debate continues regarding the predictive value of tumor PD-L1 expression. For instance, the response rates of NSCLC patients to anti-PD-L1/PD-1 antibodies range from approximately 20% to 50% depending on the clinical setting, underscoring that a significant number of patients exhibit primary resistance. Notably, it has been reported that a significant number of NSCLC patients with PD-L1 negative tumors respond to PD-1/PD-L1 blockade. Furthermore, the majority of patients who initially respond to PD-1/PD-L1 blockade, eventually develop adaptive or acquired resistance leading to disease progression. Consequently, a continuing priority within the field of clinical oncology is to identify the factors underlying the responsiveness to checkpoint blockade in order to develop better predictive biomarkers and novel ICIs that could potentially improve the efficacy

of immunotherapies.

Several mechanisms of primary, adaptive and acquired resistance to anti-PD-1/PD-L1 have been described (8). In NSCLC, resistance to anti-PD-1 therapy has been associated with the overexpression of multiple co-inhibitory molecules like CTLA4, T cell immunoglobulin mucin receptor 3 (TIM3), lymphocyte activation gene 3 (LAG3), B and T lymphocyte attenuation (BTLA) (9,10). These findings suggest that the expression of other co-inhibitory molecules, that negatively regulate T cell function can have a profound effect on anti-tumor immunity and on the survival outcomes of cancer patients (11).

V-domain Immunoglobulin suppressor of T cell activation (VISTA), an immune-checkpoint protein whose extracellular domain bears homology to PD-L1, has been found to be highly expressed on monocytic myeloid-derived suppressor cells (M-MDSCs) and regulatory T cells (Tregs). V-domain Ig suppressor of T cell activation (VISTA) modulates a broad spectrum of innate and adaptive immune responses (12), by mechanisms that do not overlap with that of other immune checkpoints, like PD-1 (13). Thus, VISTA is a particularly attractive candidate for the development of specific inhibitors against it.

Therefore, the research paper published in the journal of "*Clinical Cancer Research*" by Villarroel-Espindola *et al*. (Spatially Resolved and Quantitative Analysis of VISTA/PD-1H as a Novel Immunotherapy Target in Human Non–Small Cell Lung Cancer), is an important contribution towards characterizing the immune contexture of NSCLC (14). In this original study, the authors demonstrate that VISTA is frequently expressed in human NSCLC where it is also commonly associated with increased tumor-infiltrating lymphocytes. Furthermore, compelling evidence is presented which support the immunomodulatory role of VISTA in human NSCLC and suggest its potential as a therapeutic target.

The results of this study revealed that VISTA is expressed in the majority of human NSCLC samples, predominately in stromal cells with a cytoplasmic and membranous pattern. High VISTA levels were correlated with PD-1 axis markers, tumor associated macrophages and effector T-cells. Moreover, VISTA mRNA levels were associated with low tumor mutational burden (TMB) in lung adenocarcinomas. The overall results on this study suggest that VISTA plays an immunomodulatory role in human NSCLC.

For this study multiplexed quantitative immunofluorescence (QIF), a technique that the authors have thoroughly standardized and validated in previous reports, was used to evaluate the expression of VISTA and other markers related to the PD-1-PD-L1 axis in three cohorts of human NSCLC. Unlike traditional IHC methods, in which the use of a semiquantitative scale to evaluate staining intensity leads to considerable interobserver variability, multiplexed immunofluorescent staining with automated quantitative analysis (AQUA) allows for the spatial identification of immune cells (within and outside the tumor boundaries as well as at the epithelial-stromal interface) while also providing highly accurate and precise values of protein expression. Nevertheless, the number of markers that can be simultaneously analyzed is still very limited, which poses some uncertainty as to the identity of the immune cells described. For instance, although CD68 is widely used as a marker for cells of myeloid lineage (particularly staining macrophages and monocytes), its expression has also been reported in other non-myeloid cell types such as fibroblasts, activated endothelial cells, B lymphocytes and T lymphocytes (15). CD68 staining has also been reported in the stroma of carcinomas and melanomas, perhaps indicating phagocytic activity (16) or macrophage-tumor cell fusions. Therefore, future studies with a more thoroughly detailed immunophenotypification (and that incorporate morphological and molecular data) are necessary to confirm the identity of the immune cells found.

We were particularly interested in the negative association between VISTA expression and tumor *EGFR* mutations, particularly in view of the ongoing debate about the relationship between *EGFR* activating mutations and PD-L1 overexpression. A recent study in NSCLC cell lines and tumors showed that *EGFR* mutations and *ALK* rearrangements induce the upregulation of PD-L1 by activating PI3K-AKT and MEK-ERK (17). Another study found that tumor PD-L1 expression increased after gefitinib treatment in a subset of NSCLC, this group of patients showed a tendency towards improved overall survival (OS) (18). Whereas some studies in NSCLC patients have found no association between PD-L1 expression and *EGFR* mutations, others have found that: (I) high PD-L1 expression is associated with tumor *EGFR* mutations (19); (II) PD-L1 expression is more commonly found among patients with no tumor *EGFR* mutations (20). The results from a recent meta-analysis of forty-seven studies (N=11,444) indicate that high PD-L1 expression is associated with *EGFR* wild-type status (OR =0.61, 95% CI: 0.42–0.90, P=0.01) in NSCLC (21).

The results by Villarroel-Espindola *et al*., seem to confirm that the expression of proteins of the B7 family of immune regulatory molecules is more commonly found in *EGFR*^{wt} tumors.

It was also interesting to note that tumor mutation burden (TMB) was not linearly associated with VISTA mRNA expression in squamous lung cancer (LC) (R^2 =0.025) or in adenocarcinomas (R^2 =0.010), these results are consistent with those of other studies showing that TMB does not correlate with PD-L1 expression (22).

We believe that the study by Villarroel-Espindola *et al*. will form the basis for future studies evaluating potential mechanisms immunotherapy resistance in NSCLC, and as such, it is important to take this opportunity to draw attention to some points. First, the reader should keep in mind that the results regarding protein expression come from the retrospective analysis of archival samples from three different cohort, thus these results need to be verified in prospective studies

Additionally, it has been shown that sample age affect staining results and the archival samples used in this study were of a wide range of different ages (collected from 1988–2003). Indeed, based on the prevalence of PD-L1, it has been recommended that specimen age for IHC testing should be fewer than 3 years. Similarly, because of its retrospective nature, it is uncertain if tumor staging and classification were updated to with current standards.

We were interested in the clinicopathological characteristics of patients analyzed, which were reported to have been previously described in detail (23-25). We noticed that the cohort sizes do not match between said studies, probably due to the number of viable samples that were ultimately used, it is thus difficult to assess to what extent the clinicopathological characteristics previously reported are representative of this study population.

Of note, the majority of patients had early stage NSCLC, were treatment-naïve before tumor resection and did not receive anti-PD-1/PD-L1 therapy. Furthermore, there is no information regarding subsequent lines of treatment, which are likely to have had an impact on the survival outcomes reported. Therefore, an important future avenue of research will be to prospectively assess these parameters in NSCLC patients treated with immunotherapies.

It is puzzling, thus that the authors of the current study found that elevated expression of VISTA (measured exclusively in the tumor area) was significantly associated with longer 5-year OS, considering that recent studies have demonstrated the potential therapeutic applicability of VISTA inhibitors. For instance, in a melanoma model, the use of an anti-VISTA antibody increased the infiltration, proliferation and effector function of tumor-reactive T lymphocytes while simultaneously reducing the number and activity of M-MDSCs and Tregs, which led to reduced tumor growth (26). Furthermore, macrophage PD-L1 and VISTA expression was shown to increase following ipilimumab (anti-CTLA-4) therapy in patients with localized prostate cancer, indicating that VISTA is a compensatory inhibitory pathway (27).

Another recent study in metastatic melanoma patients evaluated the expression of VISTA and PD-L1 in biopsies collected prior to treatment (anti-PD-1 alone or in combination with ipilimumab) and after disease progression. This study found that, compared to pretreatment biopsies, the majority of biopsies collected at the time of progression showed a significant increase in the density of intratumoral lymphocytes positive for VISTA, PD-L1 and FOXP3 as well as a loss of tumor PTEN and downregulation of tumor HLA-A (28).

Immune-based therapy offers a novel strategy to eliminate tumor cells by activating the immune system (29). The available information suggest that VISTA might be a potential immunotherapeutic target, targeting VISTA with monoclonal antibodies may result beneficial not only as monotherapy, but in combination with other treatments.

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Footnote

Conflicts of Interest: Oscar Arrieta has received honoraria as advisor, participated in speakers' bureau and given expert opinions to Pfizer, AstraZeneca, Boehringer-Ingelheim, Roche, Lilly, and Bristol-Myers Squibb. The other authors have no conflicts of interest to declare

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