A New VISTA in Intracellular Checkpoint Signaling in Triple-Negative Breast Cancer

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Immune checkpoint inhibitors have transformed treatment paradigms in multiple tumor types including triple-negative breast cancer. However, clinical benefit in metastatic disease is restricted to patients with PD-L1–positive tumors, and primary and acquired resistance remains common among treated patients. These challenges have prompted interest in novel immune checkpoints such as V-domain immunoglobulin suppressor of T-cell activation (VISTA), a B7 family protein with complex immunoregulatory functions. Although VISTA is expressed in multiple tumor types and increasingly explored as a therapeutic target, its exact signaling mechanisms remain unclear. In this issue of *Cancer Research*, Zhao and colleagues demonstrated that VISTA suppresses tumor growth in a subset of VISTA-expressing triple-negative breast cancers via a cell-intrinsic, immune-independent mechanism. They discovered a conserved four–amino acid motif (NPGF) in VISTA's intracellular domain that mediates its antiproliferative effects by recruiting and sequestering the adapter protein NUMB at endosomes, thereby impairing trafficking of multiple growth factor receptors. VISTA-expressing tumors exhibit marked sensitivity to VISTA-blocking antibodies, a response that depends on the presence of the NPGF motif. Together, these findings redefine VISTA as a cell-intrinsic regulator of tumor growth and introduce new potential therapeutic avenues that extend beyond receptor–ligand disruption to interfere with checkpoint-mediated intracellular trafficking and signaling.

See related article by Zhao et al., p. 3399

Immune checkpoint inhibitor (ICI) therapy has transformed cancer treatment, including in triple-negative breast cancer (TNBC), in which pembrolizumab plus chemotherapy improves outcomes in both neoadjuvant and first-line metastatic settings, as demonstrated in the KEYNOTE-522 and KEYNOTE-355 trials. However, in metastatic TNBC, clinical benefit is restricted to patients with PD-L1–positive tumors, representing less than half of those with metastatic TNBC. Among those treated, many experience primary or acquired resistance, limiting long-term efficacy. CTLA4 blockade with ipilimumab has been tested in combination with anti–PD-1, but this did not result in superior responses and came with added toxicities (1, 2). In metastatic melanoma, the anti-LAG3 antibody relatlimab is approved in combination with the anti–PD-1 antibody nivolumab as first-line therapy (3). These challenges and successes in other cancer types underscore the need to identify novel immune regulatory pathways beyond PD-1/PD-L1 to enable combination strategies or next-line therapies following ICI resistance.

V-domain immunoglobulin suppressor of T-cell activation (VISTA) is an emerging immune checkpoint of the B7 family, sharing ~25% sequence identity with PD-1. It is broadly expressed on normal hematopoietic cells, including myeloid antigen-presenting cells (APC) and T cells, and binds to several known ligands including PSGL-1. VISTA plays a multifaceted role in immune regulation. An early study by Noelle and colleagues in 2011 identified that VISTA expression on APCs inhibits T-cell proliferation and cytokine production. Accordingly, VISTA blockade exacerbated T-cell–mediated autoimmune disease, and VISTA overexpression on tumor cells impaired antitumor immunity in mice. A growing body of evidence has implicated VISTA in regulating T-cell receptor signaling, maintaining peripheral tolerance, modulating innate immunity, and enforcing T-cell quiescence as an early immune checkpoint.

Given VISTA's complex role in immune regulation, its relevance in tumor biology is increasingly recognized. VISTA is highly expressed on tumor-infiltrating immune cells, including T cells, myeloid-derived suppressor cells, and tumor-associated macrophages, and is also found on tumor cells in cancers such as melanoma and gastrointestinal, ovarian, and non–small cell lung cancers (4). Its prognostic significance is context-dependent, with studies showing associations ranging from poor to favorable outcomes and variable patterns of coexpression or exclusivity with PD-1/PD-L1. VISTA expression can increase after ICI treatment, suggesting that it plays a role in acquired resistance and could be a potential target post-ICI progression (5). Mechanistically, VISTA functions both as a receptor, interacting with distinct binding partners, and as a ligand on APCs, delivering inhibitory signals to T cells. A deeper understanding of these context-dependent mechanisms is critical for guiding therapeutic strategies.

In this issue of *Cancer Research*, Zhao and colleagues (6) redefine VISTA as a cell-intrinsic regulator of tumor growth in a subset of TNBC. Through transcriptomic and IHC profiling of TNBC samples, the authors identified a population of TNBCs with high VISTA expression in tumor cells themselves—distinct from immune infiltrates—and show that this expression is largely mutually exclusive with PD-L1. VISTA-high tumors were associated with lower proliferation rates, evidenced by decreased Ki-67 levels, and maintained high expression even in matched metastatic lesions, suggesting a stable phenotype. Functional modeling using engineered TNBC cell lines and murine orthotopic tumors demonstrated that VISTA expression suppressed proliferation and tumor growth independent of an intact immune system, pointing to a noncanonical, immune-independent mechanism of tumor suppression.

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Investigating the mechanism of this putative nonimmune function, the authors identified a conserved four–amino acid motif (NPGF) in VISTA's intracellular domain as critical for its antiproliferative effects. Using proximity-labeling proteomics and targeted mutagenesis, they showed that this motif recruits NUMB, an adapter protein known for regulating endocytosis and receptor trafficking (7). VISTA sequestered NUMB on $EEA1^+$ early endosomes, disrupting NUMB's interaction with clathrin adapter complexes and thereby impairing trafficking of receptor tyrosine kinases like EGFR and the insulin receptor. In $VISTA^+$ cells, EGFR phosphorylation was paradoxically increased at the membrane and early endosomes, but downstream Akt signaling was diminished, suggesting receptor misrouting rather than simple inactivation. Mutation or deletion of the NPGF motif reversed these phenotypes: NUMB mislocalization was corrected, EGFR trafficking normalized, and tumor growth inhibition abolished. Importantly, these effects were unique to malignant epithelial cells and were not observed in nontransformed mammary cells, indicating tumor-selective vulnerability.

The presence of the NPGF motif not only governed VISTA's tumor-suppressive functions but also determined therapeutic response to anti-VISTA antibodies. In multiple murine models, $VISTA^+$ tumors showed marked sensitivity to VISTA-blocking antibodies, with significantly reduced tumor growth and enhanced intratumoral $CD8^+$ T-cell infiltration. These responses were abolished in tumors expressing NPGF-mutant VISTA, indicating that antibody efficacy depends on disrupting this specific intracellular signaling and trafficking axis. Interestingly, $VISTA^+$ tumors also had fewer myeloid-derived suppressor cells and tumor-infiltrating lymphocytes at baseline, suggesting that they may evade immune surveillance through nonclassical mechanisms and could benefit from VISTA-directed therapies not captured by PD-1/PD-L1 biomarkers. In total, this study enhances our understanding of immune checkpoint biology by uncovering a previously unrecognized, tumor-intrinsic signaling role for VISTA, mediated through a conserved intracellular NPGF motif that governs receptor trafficking and suppresses tumor growth.

Although current VISTA-targeting strategies in clinical trials focus on disrupting its extracellular interactions with ligands such as PSGL-1 and VSIG-3 (8–10), this study reveals a novel therapeutic opportunity, targeting VISTA's intracellular signaling machinery, specifically the conserved NPGF motif and its binding partners, such as NUMB. This motif not only provides mechanistic insights but also introduces new avenues for drug development, including small molecules or engineered peptides that selectively disrupt VISTA–NUMB interactions.

Notably, the authors identified a distinct biomarker-defined subset of TNBCs characterized by high VISTA and low PD-L1 expression, supporting VISTA blockade as a rational approach for immunotherapy-refractory and/or PD-L1–negative tumors. Although some clinical trials are testing anti-VISTA/anti–PD-1 combinations based on coexpression patterns (9, 10), this work suggests that VISTA may also function as a nonredundant checkpoint in tumors lacking canonical immune activation. Furthermore, because VISTA modulates trafficking of multiple growth factor receptors—including EGFR and the insulin receptor—these findings may extend to malignancies driven by dysregulated receptor signaling. More broadly, they raise the possibility that other immune checkpoints, particularly within the B7 family, may exert critical intracellular cancer cell–autonomous functions that have been overlooked. Therapeutic strategies that extend beyond receptor–ligand disruption to interfere with checkpoint-mediated intracellular trafficking and signaling could redefine the future landscape of immunotherapy across TNBC and other solid tumors.

Authors' Disclosures

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References

1. Nederlof I, Isaeva OI, de Graaf M, Gielen RCAM, Bakker NAM, Rolfes AL, et al. Neoadjuvant nivolumab or nivolumab plus ipilimumab in early-stage triple-negative breast cancer: a phase 2 adaptive trial. Nat Med 2024;30:3223–35.
2. Trédan O, Loirat D, Chabaud S, Petit T, Viret F, Levy C, et al. Abstract RF3-03: Nivolumab + Ipilimumab (NIVO + IPI) compared to capecitabine for triple-negative breast cancer patients with residual disease after neoadjuvant chemotherapy – final results of BreastImmune-03, a multicenter randomized open-label phase II trial. Clin Cancer Res 2024;31:RF3–03.
3. Tawbi HA, Schadendorf D, Lipson EJ, Ascierto PA, Matamala L, Castillo Gutiérrez E, et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. N Engl J Med 2022;386:24–34.
4. Nishizaki D, Kurzrock R, Miyashita H, Adashek JJ, Lee S, Nikanjam M, et al. Viewing the immune checkpoint VISTA: landscape and outcomes across cancers. ESMO Open 2024;9:102942.
5. Gao J, Ward JF, Pettaway CA, Shi LZ, Subudhi SK, Vence LM, et al. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. Nat Med 2017;23:551–5.
6. Zhao Y, Andoh T, Charles F, Reddy P, Paul K, Goar H, et al. VISTA-induced tumor suppression by a four amino acid intracellular motif. Cancer Res 2025;85:3399–415.
7. Ortega-Campos SM, García-Heredia JM. The multitasker protein: a look at the multiple capabilities of NUMB. Cells 2023;12:333.
8. Noelle R, Johnson M, Rodon J, Zauderer M, Lewis L, Severgnini M, et al. Pharmacokinetic and pharmacodynamic data from a phase 1 study of CI-8993 Anti-VISTA antibody in patients with advanced solid tumors. J ImmunoTherapy of Cancer 2022;10:A793.
9. Rodon Ahnert J, Gruber JJ, Telli ML, Mita MM, Mita AC, Kim JW, et al. A phase 1 first-in-human clinical trial of HMBD-002, an IgG4 monoclonal antibody targeting VISTA, in advanced solid tumors. J Clin Oncol 2023;41:TPS2664.
10. Iadonato S, Ovechkina Y, Lustig K, Cross J, Eyde N, Frazier E, et al. A highly potent anti-VISTA antibody KVA12123 - a new immune checkpoint inhibitor and a promising therapy against poorly immunogenic tumors. Front Immunol 2023;14:1311658.

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