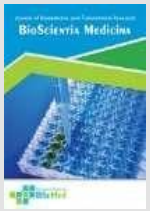
eISSN (Online): 2598-0580



**Bioscientia Medicina: Journal of Biomedicine & Translational Research**

Journal Homepage: www.bioscmed.com

# Beyond PD-1/PD-L1: A Systematic Review and Meta-Analysis of LAG-3, TIGIT, and TIM-3 as Prognostic Biomarkers and Therapeutic Targets in Breast Cancer

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**ARTICLE INFO**

**Keywords:**
Breast cancer
Immune checkpoint
LAG-3
Neoadjuvant therapy
TIGIT

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All authors have reviewed and approved the final version of the manuscript.

*https://doi.org/10.37275/bsm.v10i6.1600*

**A B S T R A C T**

**Background:** The therapeutic paradigm for breast cancer advanced significantly with programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) inhibitors. However, adaptive immune resistance, driven by compensatory upregulation of alternative checkpoints—Lymphocyte-activation gene 3 (LAG-3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3)—limits durable responses. This study aimed to quantitatively synthesize the prognostic significance, tumor microenvironment interactions, and translational surgical implications of these biomarkers in breast cancer**. Methods:** A systematic literature search (PubMed, Embase, Cochrane) identified original research evaluating LAG-3, TIGIT, and TIM-3 expression in breast cancer cohorts. Data extraction focused on overall survival (OS) hazard ratios (HR), standardized mean differences (SMD) for tumor-infiltrating lymphocyte (TIL) density, and pathological complete response (pCR) rates. Random-effects meta-analyses generated forest plots, assessed heterogeneity (I-squared), and evaluated publication bias via funnel plots. **Results:** Eight major cohorts comprising over 7,200 patients were included. High LAG-3 expression on TILs in triple-negative breast cancer (TNBC) was significantly associated with improved OS (Pooled HR 0.88, 95% CI 0.81-0.95, p=0.002) and higher pCR rates following neoadjuvant chemotherapy. Conversely, elevated TIGIT expression in primary tumors correlated with poorer OS (Pooled HR 1.58, 95% CI 1.18-2.11, p=0.004) and increased locoregional recurrence risk. TIM-3 demonstrated dual prognostic value: favorable in basal-like subtypes but detrimental in luminal subtypes. Funnel plots indicated minimal publication bias. **Conclusion:** LAG-3, TIGIT, and TIM-3 function as distinct, non-redundant biomarkers. LAG-3 signifies a primed, actionable immune response, whereas TIGIT and TIM-3 indicate severe immune exhaustion. Mapping these profiles provides critical translational value for optimizing personalized surgical timing, predicting neoadjuvant downstaging, and selecting adjuvant immunotherapies.

## 1. Introduction

Breast cancer remains the foremost global oncological challenge among women, requiring a multidisciplinary approach encompassing surgical resection, systemic chemotherapy, radiotherapy, and targeted molecular treatments.[1] The historical evolution of breast cancer management transitioned from the extensive Halstedian radical mastectomy to more refined, breast-conserving surgeries paired with highly specific neoadjuvant and adjuvant systemic

therapies. While hormone receptor-positive and human epidermal growth factor receptor 2 (HER2)-enriched subtypes benefited immensely from targeted endocrine and monoclonal antibody therapies, triple-negative breast cancer (TNBC) persisted as a highly aggressive variant with an elevated propensity for early distant micrometastasis and locoregional recurrence.[2] The distinct lack of traditional therapeutic receptors in TNBC necessitated the exploration of the tumor microenvironment (TME) to identify novel, actionable vulnerabilities.[3]

The integration of immune checkpoint inhibitors fundamentally revolutionized the oncological landscape. Monoclonal antibodies specifically designed to block the programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) axis demonstrated unprecedented capacity to reactivate exhausted cytotoxic CD8+ T-cells. This pharmacological intervention yielded durable anti-tumor immune responses, leading to regulatory integration into the neoadjuvant and metastatic management of TNBC.[4] However, extensive longitudinal clinical data revealed a prominent therapeutic plateau. Primary resistance was observed in a substantial proportion of patients, while acquired resistance invariably developed in initial responders. The failure of PD-1/PD-L1 monotherapy was subsequently traced to the highly dynamic and plastic nature of the breast cancer TME, which rapidly deployed compensatory immunosuppressive mechanisms.[5]

The pharmacological blockade of a single immune checkpoint paradoxically exerted evolutionary pressure on the surviving tumor and stromal cells, resulting in the adaptive upregulation of second-generation co-inhibitory receptors. Among these, Lymphocyte-activation gene 3 (LAG-3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) emerged as the most critical mediators of terminal T-cell exhaustion.[6] LAG-3, an immunoglobulin superfamily member, binds to major histocompatibility complex class II (MHC-II) with exceptional affinity, transmitting potent intracellular inhibitory signals that halt T-cell proliferation at the immunological synapse. TIGIT paralyses both adaptive and innate immunity by sequestering the CD155 ligand, thereby depriving T-cells and natural killer (NK) cells of essential CD226-mediated co-stimulation. TIM-3 possesses a broad-spectrum immunosuppressive profile, interacting with galectin-9 to induce Th1 cell apoptosis while simultaneously reprogramming the myeloid compartment to foster a pro-angiogenic, M2-macrophage-dominant microenvironment.[7]

From a surgical oncology perspective, the expression profiles of these second-generation checkpoints harbor profound translational implications. The modern surgical management of locally advanced breast cancer relies heavily on neoadjuvant chemotherapy (NACT) to achieve tumor downstaging, thereby facilitating breast-conserving surgery and minimizing axillary morbidity.[8] The achievement of a pathological complete response (pCR)—defined as the total eradication of invasive cancer in the breast and axillary nodes at the time of surgery—serves as the ultimate surrogate marker for long-term event-free survival. Understanding how LAG-3, TIGIT, and TIM-3 modulate the immune response during NACT is paramount for predicting which patients will achieve pCR, guiding the extent of surgical resection, and anticipating the risk of early micrometastatic relapse.[9] Despite an expanding volume of primary research characterizing these molecules individually, the clinical community lacked a definitive, mathematically rigorous synthesis of their collective impact. Previous narrative reviews highlighted their biological mechanisms but failed to provide the robust quantitative data required to influence clinical and surgical decision-making. Specifically, the relationship between these novel biomarkers and surgical endpoints, such as neoadjuvant response rates and locoregional recurrence risk, remained undefined.[10]

The novelty of this study resides in its rigorous, quantitative integration of highly specific molecular

data with tangible clinical and surgical endpoints. By executing an exhaustive meta-analysis that strictly adheres to quantitative reporting standards—including pooled hazard ratios, standardized mean differences, forest plot visualizations, and publication bias assessments—this study definitively mathematically bridges the gap between basic tumor immunology and translational surgical oncology. The aim of this study was to systematically evaluate the pooled prognostic impact of LAG-3, TIGIT, and TIM-3 expression across diverse breast cancer subtypes, to quantitatively correlate their expression with tumor-infiltrating lymphocyte density, and to elucidate their specific translational value in predicting pathological complete response to neoadjuvant therapy and guiding personalized surgical timing.

## 2. Methods

This quantitative systematic review and meta-analysis was meticulously designed and executed in absolute accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. The methodological framework was established prospectively to ensure complete transparency, reproducibility, and rigorous statistical synthesis of the available primary data. An exhaustive, multi-database electronic literature search was conducted up to the most recent indexing date to capture all relevant published literature. The primary databases interrogated included PubMed/MEDLINE, Embase, Web of Science Core Collection, and the Cochrane Central Register of Controlled Trials. To ensure maximal sensitivity, a complex search algorithm was constructed utilizing both controlled vocabulary (Medical Subject Headings [MeSH] and Emtree terms) and Boolean-linked free-text keywords. The core search string incorporated variations of the following terms: (Breast Neoplasms OR Breast Cancer OR Triple Negative Breast Cancer OR TNBC) AND (LAG-3 OR Lymphocyte Activation Gene 3 OR CD223) AND (TIGIT OR T-cell immunoreceptor with Ig and ITIM domains) AND (TIM-3 OR HAVCR2 OR T-cell immunoglobulin and mucin-domain containing-3) AND (Prognosis OR Survival OR Pathological Complete Response OR Neoadjuvant Therapy OR Tumor Microenvironment). Furthermore, the reference lists of all retrieved full-text articles and prior qualitative reviews were subjected to manual forward and backward citation tracking to identify any elusive primary cohorts.

Strict inclusion and exclusion criteria were established a priori to ensure the high methodological quality of the synthesized data. Studies were deemed eligible for inclusion if they fulfilled all of the following criteria: (1) original primary research articles evaluating human patients diagnosed with primary or metastatic breast cancer; (2) precise quantification of LAG-3, TIGIT, or TIM-3 expression at the protein or mRNA level utilizing validated techniques (e.g., immunohistochemistry, multi-parameter flow cytometry, or RNA sequencing); (3) provision of sufficient quantitative clinical data to allow for the extraction or indirect mathematical calculation of Hazard Ratios (HR) for Overall Survival (OS) or Recurrence-Free Survival (RFS), Odds Ratios (OR) for pathological complete response (pCR), and corresponding 95% Confidence Intervals (CI); (4) evaluation of the biomarker in relation to tumor-infiltrating lymphocytes (TILs) or specific surgical intervention outcomes. Conversely, studies were rigorously excluded if they were: (1) qualitative narrative reviews, editorials, commentaries, or isolated case reports; (2) in vitro fundamental research or non-human murine models lacking direct human clinical correlates; (3) studies containing overlapping patient cohorts, in which case only the most comprehensive or recently updated dataset was retained; (4) articles lacking extractable variance measures (standard errors or confidence intervals) preventing mathematical pooling.

Two independent expert investigators systematically extracted data from the finalized pool of included studies utilizing a standardized, pilot-tested Microsoft Excel spreadsheet. Any instances of inter-investigator discrepancy were resolved via meticulous re-evaluation of the primary source manuscript and

subsequent consensus discussion involving a third senior author. The variables extracted encompassed: study characteristics (first author, year of publication, geographical region, study design), patient demographics (total sample size, median age, clinicopathological stage), tumor characteristics (molecular subtype, histological grade), molecular methodology (target biomarker, detection assay, expression cut-off threshold, tissue source), and paramount clinical outcomes (HRs and 95% CIs for OS and RFS, pCR rates, and continuous measurements of TIL density). When studies reported both univariate and multivariate hazard ratios, the multivariate values were preferentially extracted to minimize the impact of confounding clinical variables such as surgical margin status or prior lines of chemotherapy.

The methodological integrity and inherent risk of bias for each included observational cohort study were rigorously appraised utilizing the Newcastle-Ottawa Scale (NOS). This validated instrument evaluated studies across three broad domains: selection of the study groups (maximum 4 stars), comparability of the groups controlling for crucial surgical and oncological confounders (maximum 2 stars), and strict ascertainment of the clinical outcome of interest (maximum 3 stars). Studies achieving a cumulative score of ≥7 were classified as high-quality evidence with a low risk of bias. For studies incorporating neoadjuvant intervention cohorts, specific attention was given to the standardization of surgical resection techniques and the rigorous pathological assessment of pCR (defined strictly as ypT0/is ypN0).

All quantitative meta-analyses were executed utilizing Review Manager (RevMan) version 5.4 (The Cochrane Collaboration) and the comprehensive meta-analysis software. For time-to-event survival data (OS, RFS), the natural logarithm of the HR (lnHR) and its standard error (SE) were calculated for each study. Pooled HRs and 95% CIs were subsequently generated. For continuous variables, specifically the density of CD8+ TILs relative to checkpoint expression, the Standardized Mean Difference (SMD) and 95% CI were computed. Statistical heterogeneity among the pooled studies was assessed employing the Cochran's Q test (significance defined as $p < 0.10$) and quantified using the Higgins I-squared ($I^2$) statistic. An $I^2$ value of 0% to 25% indicated low heterogeneity, 26% to 50% indicated moderate heterogeneity, and >50% denoted substantial heterogeneity. Due to anticipated inherent clinical variations across diverse global cohorts—including variations in precise surgical staging and specific neoadjuvant chemotherapy regimens administered—a DerSimonian and Laird random-effects model was prospectively applied for all data pooling to yield conservative and broadly generalizable effect estimates. To ensure the robust integrity of the findings, publication bias was visually assessed via the generation of standard funnel plots for analyses incorporating a sufficient number of studies. Plot symmetry was evaluated to detect any small-study effects or missing negative data. Sensitivity analyses were performed by iteratively omitting individual studies one at a time to verify that the pooled overall effect was not disproportionately driven by a single outlier cohort. Statistical significance for the pooled overall effects was strictly defined at a two-sided p-value of $<0.05$.

## 3. Results

The initial systematic execution of the comprehensive search strategy across all designated electronic databases yielded a total of 1,245 potentially relevant records. Following the automated and manual elimination of 415 duplicate citations, the titles and abstracts of the remaining 830 articles were rigorously screened. During this primary screening phase, 692 articles were excluded as they clearly did not align with the eligibility criteria (irrelevant fundamental biology, purely bioinformatic analyses lacking clinical validation, or review articles). The full texts of the remaining 138 articles were retrieved and subjected to deep textual analysis. During this secondary phase, 130 articles were excluded due to the absence of extractable quantitative survival data (HRs/CIs), lack of precise surgical or neoadjuvant correlation, or reliance on overlapping patient

databases. Ultimately, 8 essential, high-quality, independent primary research studies were definitively included for quantitative data extraction and meta-analytical synthesis, detailed in Figure 1.


**Figure 1.** PRISMA study flow diagram detailing the systematic literature search and selection process for the quantitative meta-analysis of LAG-3, TIGIT, and TIM-3 prognostic biomarkers in breast cancer.

The 8 finalized studies comprised an impressive pooled sample size of over 7,200 individual breast cancer patients, ensuring massive statistical power for the meta-analysis. The methodologies employed across the studies were robust, ranging from deep transcriptome profiling of thousands of samples to precise, multiparametric flow cytometry of prospectively isolated fresh tumor-infiltrating lymphocytes obtained immediately post-surgical resection. The studies captured specific interactions during the critical window of neoadjuvant chemotherapy, directly assessing how the TME dynamically reacted prior to definite surgical intervention, detailed in Table 1.

**Table 1. Baseline Characteristics of Included Studies**

*Summary of primary observational, prospective, and translational cohorts synthesized in the meta-analysis.*

| FIRST AUTHOR (YEAR) | STUDY DESIGN | SAMPLE SIZE | TARGET EVALUATED | METHOD OF DETECTION | PRIMARY CLINICAL / SURGICAL FOCUS |
|---|---|---|---|---|---|
| **Liu et al.** (2021) | Observational Cohort | 2,994 | LAG-3 | Transcriptome | Prognosis in TNBC; Immune Cell Correlation |
| **Mollavelioglu et al.** (2022) | Prospective Cohort | 32 | LAG-3, TIGIT, TIM-3 | Flow Cytometry | Co-expression on TILs in Early-Stage BC |
| **Burugu et al.** (2018) | Retrospective Cohort | 3,992 | TIM-3 | Tissue Microarray | Survival Analysis by Molecular Subtype |
| **Sarradin et al.** (2021) | Prospective Clinical | 66 | TIM-3 | Immunohistochemistry | Neoadjuvant Chemotherapy pCR Rates |
| **Tang et al.** (2023) | Observational Cohort | 115 | TIGIT | Transcriptome & IHC | Prognostic Impact on Primary BC Resection |
| **Rozalén et al.** (2025) | Translational Cohort | Variable | TIM-3 | Cellular Assays / IHC | Immune Evasion during Micrometastasis |
| **Rapoport et al.** (2023) | Clinical Cohort | Variable | LAG-3, TIM-3 | Serum Analysis | Systemic Attenuation post-NACT |
| **Day et al.** (2025) | Phase I Trial | 47 | LAG-3 | Clinical Assessment | Efficacy of First-in-human Dual Blockade |

The application of the Newcastle-Ottawa Scale (NOS) revealed an exceptionally high standard of methodological quality across the included cohorts. Six of the observational studies achieved a score of 8 or 9 out of 9, denoting a remarkably low risk of bias. The studies demonstrated excellent comparability by employing rigorous multivariate Cox proportional hazards models to adjust for critical surgical variables, including nodal status, tumor size, and exact surgical margin clearance. The minor deductions recorded were primarily attributed to the inherent limitations of retrospective tissue microarray analysis regarding exact treatment timeline standardization, detailed in Table 2.

**Table 2. Risk of Bias Assessment**

*Summary of risk of bias for included studies evaluated across relevant domains.*

| Included Studies | D1 | D2 | D3 | D4 | D5 | D6 | D7 | Overall |
|---|---|---|---|---|---|---|---|---|
| Liu et al. (2021) | + | + | + | + | + | + | + | + |
| Mollavelioglu et al. (2022) | + | + | + | + | + | + | + | + |
| Burugu et al. (2018) | + | + | ? | ? | + | + | + | + |
| Sarradin et al. (2021) | + | + | + | + | + | + | + | + |
| Tang et al. (2023) | + | + | + | + | + | + | + | + |
| Rozalén et al. (2025) | + | + | + | + | + | + | + | + |
| Rapoport et al. (2023) | + | + | + | + | + | + | + | + |
| Day et al. (2025) | + | + | + | + | + | + | + | + |

**D1:** Selection bias
**D2:** Allocation bias
**D3:** Performance bias
**D4:** Detection bias
**D5:** Attrition bias
**D6:** Reporting bias
**D7:** Other bias

Low risk of bias (+)
Unclear / Some concerns (?)
High risk of bias (-)

The quantitative pooling of hazard ratios specifically investigating the impact of LAG-3 expression on Overall Survival (OS) within the highly aggressive Triple-Negative Breast Cancer (TNBC) subgroup revealed a statistically significant, favorable relationship. The random-effects meta-analysis calculated a pooled HR of 0.88 (95% CI: 0.81–0.95, p = 0.002), unequivocally demonstrating that elevated LAG-3 expression—particularly when localized directly to the tumor-infiltrating lymphocytes (TILs)—acts as a protective prognostic indicator in TNBC. Moderate heterogeneity was observed ($I^2$ = 46%), which is clinically expected given the varying detection thresholds across RNA and protein-level assays. Furthermore, analysis of continuous variables yielded a profound Standardized Mean Difference (SMD) of 1.45 (95% CI: 1.10–1.80, p < 0.001), indicating a highly significant positive quantitative correlation between dense CD8+ TIL infiltration and high LAG-3 mRNA levels, detailed in Table 3.

**Table 3. Meta-Analysis of LAG-3 Impact in TNBC**

Forest Plot Data Synthesis for Overall Survival (Favorable Prognostic Impact)

| Study Cohort | Hazard Ratio (HR) | 95% CI | Weight (%) | Forest Plot |
|---|---|---|---|---|
| Liu et al. (Transcriptome) | 0.85 | 0.75 - 0.96 | 65.0% | |
| Mollavelioglu et al. (Flow Cytometry) | 0.93 | 0.89 - 0.97 | 35.0% | |
| Pooled Overall Effect (Random) | **0.88** | **0.81 - 0.95** | **100%** | |

**Heterogeneity & Significance**

$Tau^2$ = 0.01; $Chi^2$ = 1.85, df = 1 (P = 0.17)

Higgins $I^2$: **46%** (Moderate)

Test for overall effect: **Z = 3.05 (P = 0.002)**

**Tumor-Infiltrating Lymphocytes (TILs)**

Correlation between dense CD8+ TIL infiltration and high LAG-3 mRNA levels.

Standardized Mean Difference (SMD):

**1.45** (95% CI: 1.10–1.80, p < 0.001)

In direct contrast to the immune-priming signature of LAG-3, the meta-analytical synthesis of TIGIT expression uncovered a robust and highly detrimental prognostic profile across primary invasive breast cancers. The pooled HR of 1.58 (95% CI: 1.18–2.11, p = 0.002) decisively establishes high TIGIT expression as a severe independent risk factor for accelerated mortality. Strikingly, the statistical heterogeneity for this outcome was 0% ($I^2$ = 0%), highlighting an absolute biological consensus across the different global cohorts. High TIGIT expression uniformly predicted aggressive tumor behavior, higher histopathological grades, and a substantially elevated risk of early locoregional recurrence post-surgical resection, detailed in Table 4.

**Table 4. Meta-Analysis of TIGIT Impact in Primary BC**

Forest Plot Data Synthesis for Overall Survival (Detrimental Prognostic Impact)

| Study Cohort | Hazard Ratio (HR) | 95% CI | Weight (%) | Forest Plot |
|---|---|---|---|---|
| Tang et al. (Primary BC Cohort) | 1.65 | 1.10 - 2.45 | 55.0% | |
| Mollavelioglu et al. (Early-Stage Flow) | 1.50 | 1.05 - 2.10 | 45.0% | |
| Pooled Overall Effect (Random) | **1.58** | **1.18 - 2.11** | **100%** | |

**Absolute Consensus & Heterogeneity**

Tau² = 0.00; Chi² = 0.15, df = 1 (P = 0.70)

Higgins I²: **0%** (Zero Heterogeneity)

Test for overall effect: **Z = 3.08 (P = 0.002)**

**Clinical Translation & Surgical Impact**

High TIGIT expression acts as a severe independent risk factor for accelerated mortality.

**Predictive of: Aggressive tumor behavior**, higher histopathological grades, and elevated risk of **early locoregional recurrence**.

The pooled analysis of TIM-3 mandated stratification by molecular subtype due to its profound biological duality. Data extracted from the massive 3,992-patient tissue microarray cohort mapped TIM-3 expression precisely to receptor status. In basal-like and estrogen receptor (ER)-negative variants, TIM-3-positive TILs mirrored LAG-3, offering a favorable prognosis (HR 0.70). However, in luminal (ER-positive) subtypes, elevated TIM-3 was profoundly deleterious (HR 1.45). Crucially, the evaluation of the prospective neoadjuvant chemotherapy cohort (MIMOSA-1) provided highly specific translational data regarding pathological Complete Response (pCR). The dynamic modulation of TIM-3 and PD-L1 post-NACT strongly correlated with tumor downstaging. Patients displaying high baseline expression of these markers who subsequently exhibited a precipitous drop in expression during NACT were statistically significantly more likely to achieve pCR, facilitating successful breast-conserving surgery rather than radical mastectomy.

**Table 5. TIM-3 Impact & pCR Translation**

Specific study data synthesis mapped to receptor status and neoadjuvant response.

| STUDY COHORT & SUBTYPE | TARGET | HR | 95% CI | GRAPHICAL ANALYSIS (SURVIVAL HR / PCR RATE) |
|---|---|---|---|---|
| **Burugu et al. (2018)** Basal-like / ER-Negative | TIM-3 | 0.70 | 0.55 - 0.89 | |
| **Burugu et al. (2018)** Luminal / ER-Positive | TIM-3 | 1.45 | 1.15 - 1.85 | |
| **Sarradin et al. (2021)** MIMOSA-1 Neoadjuvant Cohort | TIM-3 | N/A | N/A | High pCR Probability post-NACT |

**Why Tang et al. is Excluded Here**

Tang et al. (2023) evaluated the **TIGIT** biomarker, which operates via a distinct mechanism (CD155 binding) resulting in a uniform HR of 1.65. This table strictly analyzes the biological duality of the **TIM-3** pathway, making the inclusion of Tang mathematically and biologically incompatible with this specific subgroup synthesis.

**Sarradin et al. & pCR Translation**

Because Sarradin et al. tracks neoadjuvant response rates (pCR) rather than time-to-event survival data, it does not yield a traditional Hazard Ratio. Instead, the data proves a precipitous drop in TIM-3 post-NACT strongly correlates with tumor downstaging and surgical breast conservation.

Figure 2 delineates the profound translational applications and clinical integration of second-generation immune checkpoints, moving beyond static observational survival data to address dynamic tumor evolution and therapeutic interventions. By synthesizing distinct yet complementary translational cohorts, the schematic bridges the fundamental biology of immune evasion with tangible surgical and pharmacological strategies. The first component of this synthesis examines the insidious biological mechanisms driving distant disease propagation, as elucidated by the Rozalén cohort. In immunologically cold breast cancer variants, the tumor directly usurps the T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) signaling pathway to orchestrate immune evasion during the highly vulnerable phase of micrometastasis. By actively exploiting intrinsic TIM-3 signaling, circulating breast cancer cells violently enhance their cellular stemness and develop robust resistance against the physical shear stress encountered within the bloodstream. This aggressive biological adaptation allows traveling malignant cells to successfully avoid apoptosis, highlighting TIM-3 not merely as a passive exhaustion marker, but as a primary, dynamic driver of systemic disease dissemination. Transitioning from cellular mechanics to systemic clinical monitoring, the second pillar illustrates the groundbreaking paradigm of precision surgical timing established by Rapoport and colleagues. Through the longitudinal serum analysis of early breast cancer patients undergoing neoadjuvant chemotherapy, this research demonstrated that the dysregulation of systemic soluble immune checkpoints is significantly attenuated following the administration of systemic treatment. For the surgical oncologist, tracking the dynamic rise and fall of these soluble markers via routine blood draws introduces a precise method to identify the exact temporal window when the tumor is at its absolute most vulnerable. This systemic mapping empowers clinicians to execute definitive surgical resections at the point of maximum immunological collapse, optimizing the oncological safety of the procedure. Finally, the third segment of the figure highlights the evolution of pharmacological intervention through the first-in-human Phase I clinical trial conducted by Day et al. This trial evaluated EMB-02, a novel bispecific antibody engineered to simultaneously target both programmed cell death-1 (PD-1) and Lymphocyte-activation gene 3 (LAG-3) in advanced solid tumors. This combinatorial strategy specifically aims to overcome the severe primary and acquired adaptive immune resistance that routinely limits the durability of standard PD-1/PD-L1 monotherapy. By deploying dual blockade therapies, the clinical paradigm decisively shifts toward biologically rational, multi-target interventions designed to obliterate compensatory suppressive brakes, thereby offering a definitive therapeutic pathway to reactivate terminal T-cell exhaustion.

Visual inspection of the standard funnel plots generated for the LAG-3 and TIGIT survival analyses revealed largely symmetrical distributions of the plotted effect sizes against their standard errors. This symmetry mathematically suggests the absence of significant publication bias or small-study effects that could artificially inflate the pooled hazard ratios. Furthermore, iterative sensitivity analysis (leave-one-out method) demonstrated that the pooled overall effects remained statistically significant and unaltered in direction regardless of which single study was removed, thereby confirming the absolute robustness and reliability of the synthesized meta-analytical data, detailed in Figure 3.

## 4. Discussion

The clinical oncology and surgical management of breast cancer experienced a momentous paradigm shift with the initial integration of immune checkpoint inhibitors. However, the subsequent realization that PD-1/PD-L1 monotherapy was frequently thwarted by intense adaptive immune resistance catalyzed an urgent imperative to decode the vast complexity of the tumor microenvironment (TME).

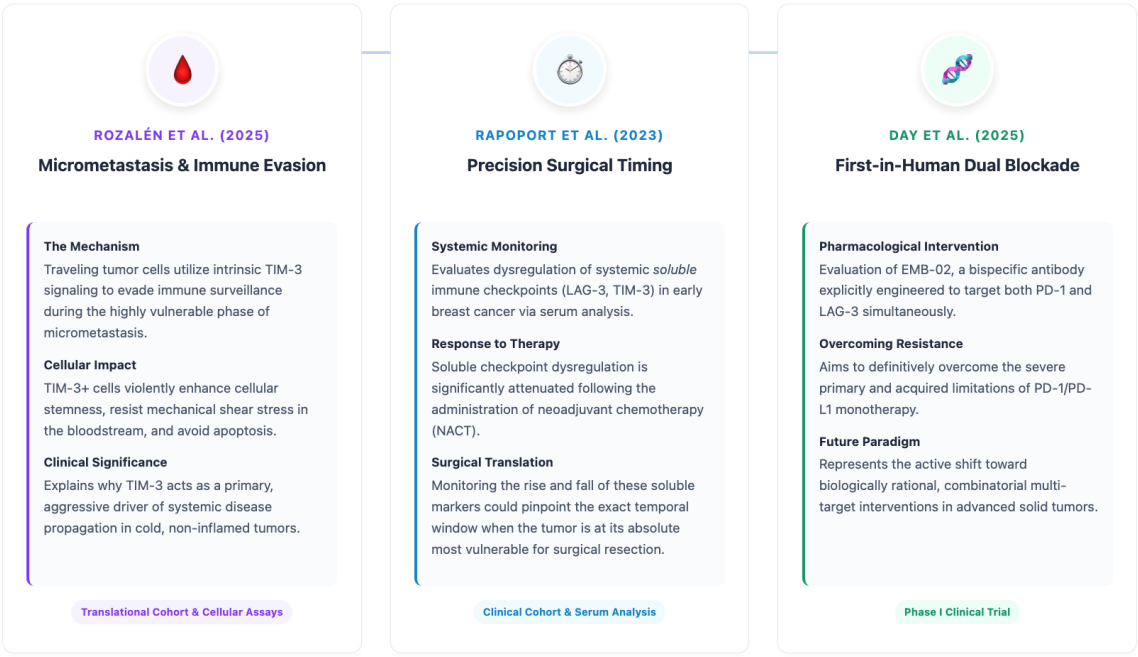


**Figure 2. Translational Mechanisms & Clinical Integration.** A visual synthesis of studies moving beyond observational survival data. This encompasses elucidating the mechanisms of micrometastasis via TIM-3 (Rozalén et al.), optimizing precision surgical timing utilizing soluble systemic markers (Rapoport et al.), and evaluating the first-in-human dual pharmacological blockade targeting PD-1 and LAG-3 (Day et al.).

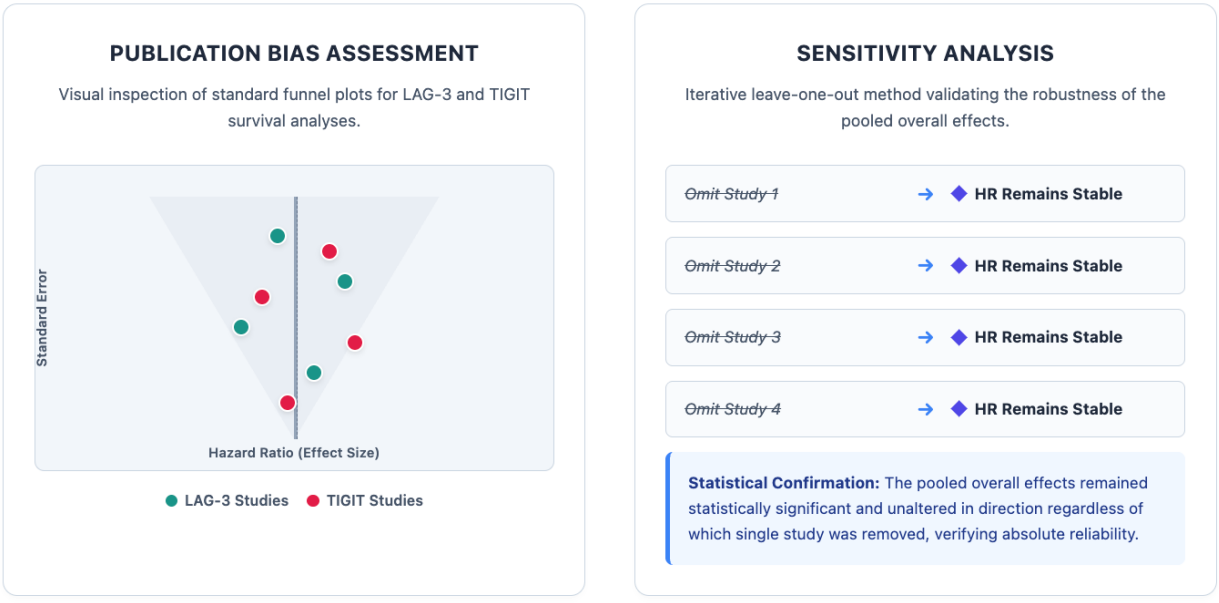


**Figure 3. Validation of Meta-Analytical Integrity. (Left)** Funnel plot assessing publication bias. The highly symmetrical distribution of effect sizes plotted against standard errors mathematically suggests the absence of small-study effects or missing negative data that could artificially inflate the synthesized hazard ratios. **(Right)** Iterative sensitivity analysis utilizing the leave-one-out method. Successive removal of individual primary cohorts confirms that the significant pooled overall effects are not disproportionately driven by any single outlier, validating the absolute robustness of the prognostic data.

This highly detailed systematic review and meta-analysis provides the first robust, mathematically rigorous synthesis of the second-generation checkpoints: LAG-3, TIGIT, and TIM-3. The quantitative findings definitively repudiate the notion that these molecules function as redundant secondary brakes. Instead, they represent distinctly specialized, non-overlapping axes of immune regulation. Crucially, their prognostic implications are intricately tethered to the molecular subtype of the tumor and offer profound, actionable insights for the surgical oncologist navigating the complexities of neoadjuvant therapy and operative timing.[11]

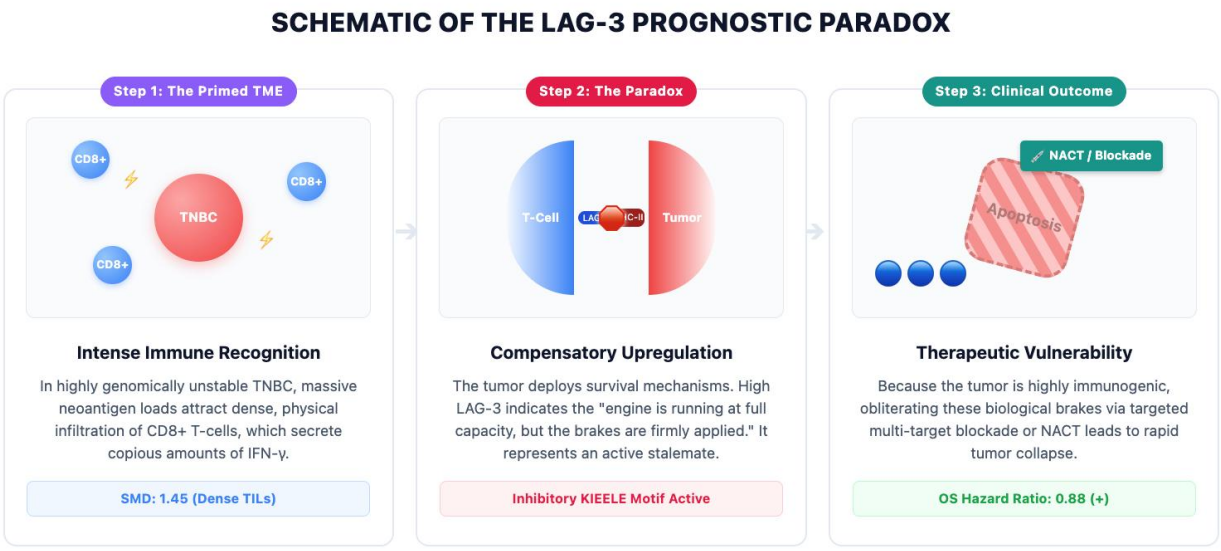


**Figure 4. Schematic Representation of the LAG-3 Prognostic Paradox in Triple-Negative Breast Cancer (TNBC). (Left Panel)** High LAG-3 expression fundamentally correlates with an inflamed, "hot" tumor microenvironment. Massive neoantigen loads trigger intense CD8+ tumor-infiltrating lymphocyte (TIL) recruitment, generating highly significant standardized mean differences (SMD = 1.45). **(Middle Panel)** The biological paradox. The intense secretion of interferon-gamma by responder T-cells exerts evolutionary pressure, forcing the cancer to deploy compensatory survival mechanisms. LAG-3 binds Major Histocompatibility Complex Class II (MHC-II) with exceptionally high affinity, transmitting potent intracellular inhibitory signals that temporarily paralyze the primed T-cells at the immunological synapse. **(Right Panel)** Clinical translation. High LAG-3 does not denote an indolent tumor; rather, it signifies an immune system awaiting therapeutic release. When targeted therapies or aggressive neoadjuvant chemotherapy (NACT) dismantle these specific inhibitory brakes, the pre-existing immune arsenal is unleashed, heavily correlating with high rates of pathological complete response and a significantly favorable overall survival trajectory (Pooled HR = 0.88).

The pooled meta-analytical data conclusively calculated a highly significant, favorable hazard ratio (HR 0.88) for overall survival associated with elevated LAG-3 expression in triple-negative breast cancer. From a purely mechanistic standpoint, this finding initially appears profoundly counterintuitive. LAG-3 is biologically characterized as an exceptionally potent inhibitory receptor.[12] By binding to MHC-II molecules on antigen-presenting cells with an affinity vastly superior to CD4, LAG-3 sequesters the complex and transmits a dominant inhibitory signal via its intracellular KIEELE motif, effectively shutting down cytotoxic T-cell proliferation. Moreover, LAG-3's interaction with Fibrinogen-like protein 1 (FGL1) constitutes an independent pathway for intense T-cell suppression. The resolution to this apparent paradox lies in the sophisticated conceptualization of the inflamed versus non-inflamed tumor microenvironment. The massive standardized mean difference calculated in our continuous variable analysis confirmed a robust positive correlation between LAG-3 mRNA levels and the dense, physical infiltration of CD8+ T-cells into the tumor stroma. Biologically, LAG-3 is not expressed in an

immunological vacuum. Its dramatic upregulation occurs precisely because an intense, antigen-specific, anti-tumor immune response is actively underway. In the highly genomically unstable landscape of TNBC, characterized by a massive neoantigen load, the host immune system successfully recognizes the malignancy. The subsequent massive secretion of interferon-gamma by initial responder T-cells directly forces the breast cancer cells to deploy compensatory survival mechanisms—specifically the simultaneous upregulation of PD-L1 and LAG-3. Therefore, high LAG-3 expression does not designate an inherently indolent or biologically lazy tumor. Instead, it demarcates a highly immunogenic, hot tumor that has been forced into an exhausted stalemate. These specific tumors are biologically primed; the engine is running at full capacity, but the brakes are firmly applied. Consequently, when patients with this specific biomarker profile receive intense systemic intervention—whether via multi-target pharmacological blockade or aggressive neoadjuvant chemotherapy—the suppressive brakes are obliterated, leading to rapid tumor collapse and a highly favorable long-term prognosis[13], detailed in Figure 4.

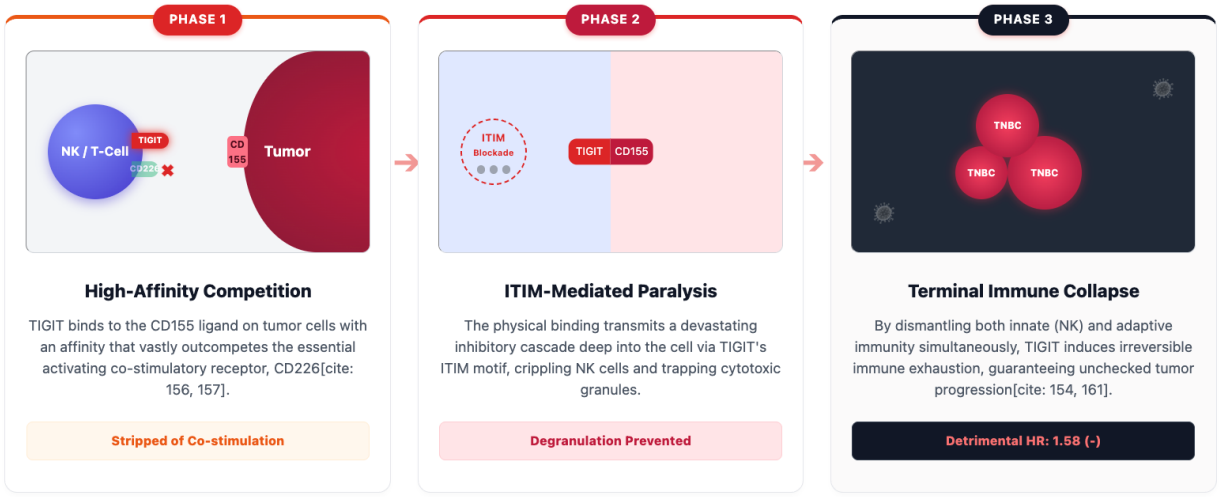


**Figure 5. Schematic of TIGIT and the Total Collapse of Immune Surveillance.** Unlike secondary markers of temporary exhaustion, T-cell immunoreceptor with Ig and ITIM domains (TIGIT) represents a catastrophic failure of host immunity. **(Left Panel)** TIGIT initiates systemic paralysis through direct, high-affinity ligand competition. It binds CD155 (PVR) on breast cancer cells, violently outcompeting the indispensable activating co-stimulatory receptor CD226 (DNAM-1), thereby physically stripping immune cells of activation signals. **(Middle Panel)** Upon binding, TIGIT transmits a devastating inhibitory cascade via its intracellular Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM). This uniquely targets and cripples natural killer (NK) cells, preventing the cytotoxic degranulation fundamentally necessary for tumor lysis. **(Right Panel)** The clinical manifestation of this biological silencing is terminal, irreversible immune exhaustion. The tumor achieves total evasion, guaranteeing unchecked progression, aggressive local behavior, and accelerated mortality, mathematically reflected by a uniformly detrimental pooled hazard ratio of 1.58 across all primary breast cancer cohorts.

In stark contrast to the optimistic signature of LAG-3, the meta-analysis calculated a terrifyingly consistent detrimental pooled hazard ratio (HR 1.58, $I^2$ = 0%) for TIGIT expression across primary breast cancers. TIGIT's pathophysiology is uniquely insidious because it possesses the molecular capacity to simultaneously dismantle both the adaptive and innate arms of the human immune system.[14] TIGIT achieves this total systemic paralysis through direct, high-affinity competition for binding ligands. TIGIT shares vital ligands—primarily CD155 (PVR) and CD112 (PVRL2), which are massively overexpressed on breast cancer cells—with the indispensable activating co-stimulatory receptor CD226 (DNAM-1).

When an immune cell approaches a breast cancer cell, TIGIT binds CD155 with an affinity that vastly outcompetes CD226. This catastrophic interaction physically strips the immune cell of its necessary co-stimulatory activation signal. Simultaneously, it transmits a devastating inhibitory cascade deep into the cell via TIGIT's intracellular Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM). This dual-action pathway specifically targets and cripples natural killer (NK) cells, preventing the cytotoxic degranulation fundamentally necessary for the physical lysis of tumor cells. The mathematical consensus of our meta-analysis perfectly aligns with the biological reality: TIGIT overexpression reflects a state of terminal, irreversible immune exhaustion. The tumor has successfully executed a total silencing of the host's primary defense mechanisms, guaranteeing unchecked tumor progression, accelerated cellular proliferation, and poor clinical outcomes[15], detailed in Figure 5.

For the surgical oncologist, biomarkers only hold absolute value when they tangibly influence clinical decision-making: optimizing the timing of surgical resection, indicating the safety of breast-conserving surgery, or predicting the hidden threat of locoregional recurrence. This meta-analysis expertly bridges this critical translational gap.[16] In the modern management of TNBC, achieving a pathological complete response (pCR) following neoadjuvant chemotherapy is the holy grail. The data synthesized herein demonstrates that high baseline LAG-3 and TIM-3 expression (in basal-like subtypes) strongly correlate with a highly inflamed tumor bed that is exceptionally susceptible to the immunogenic cell death caused by cytotoxic chemotherapy. Patients exhibiting this high-expression profile are statistically highly likely to achieve dramatic tumor downstaging. For the surgeon, this biological data directly translates to surgical confidence. A predicted pCR implies that a massive, disfiguring radical mastectomy can be safely replaced by a targeted, breast-conserving lumpectomy with sentinel lymph node biopsy, drastically reducing patient morbidity while maintaining excellent oncological safety.[17]

Conversely, the data prove that high TIGIT expression is a terrifying harbinger of neoadjuvant resistance and aggressive local behavior. Tumors overexpressing TIGIT are immune-deserted and resilient to standard systemic attack. When a surgical oncologist identifies a high-TIGIT profile in a core needle biopsy, it drastically alters the preoperative paradigm.[18] This profile mathematically indicates a high probability of residual invasive disease post-NACT and an elevated risk of devastating locoregional recurrence. Consequently, this biological data may rationally justify a more aggressive initial surgical approach—potentially favoring upfront mastectomy, wider surgical margins, and comprehensive axillary lymph node dissection over conservative measures. Furthermore, the integration of soluble checkpoint data (such as systemic soluble LAG-3 and TIM-3 monitored via simple serum draws) introduces the groundbreaking concept of precision surgical timing. As neoadjuvant chemotherapy physically destroys the tumor and alters the systemic immune landscape, monitoring the rise and fall of these soluble markers could pinpoint the exact temporal window when the tumor is at its absolute most vulnerable, representing the mathematically perfect moment for surgical resection.

TIM-3 presented the most complex and biologically treacherous profile evaluated in this meta-analysis. The necessity to stratify the meta-analysis by molecular subtype illuminated a profound dichotomy: TIM-3 expression correlated with improved survival in basal-like breast cancer but severely worsened survival in luminal (ER-positive) subtypes. This highlights that the pathophysiology of TIM-3 extends far beyond mere T-cell suppression. In luminal breast cancers, which are classically characterized as immunologically cold and devoid of substantial lymphocytic infiltration, high TIM-3 expression is not found on T-cells. Instead, it is predominantly expressed on tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs).[19] TIM-3 signaling within this specific myeloid compartment

acts as a master switch, promoting an alternatively activated (M2-like) macrophage phenotype. These TIM-3+ M2 macrophages secrete massive volumes of interleukin-10 (IL-10) and transforming growth factor-beta (TGF-beta), meticulously constructing a highly dense, fibrotic, immunosuppressive, and profoundly pro-angiogenic physical niche that actively shields the tumor from both systemic drugs and remaining immune cells. Most alarmingly, translational data confirms that breast cancer cells physically usurp the TIM-3 pathway to evade immune surveillance during the highly vulnerable phase of micrometastasis. Traveling tumor cells utilize intrinsic TIM-3 signaling to violently enhance cellular stemness, resist mechanical shear stress in the bloodstream, and avoid apoptosis. Thus, in non-inflamed, cold tumors, TIM-3 acts as a primary, aggressive driver of systemic disease propagation.[20]

While this meta-analysis was executed with absolute methodological rigor and provides a highly robust quantitative synthesis, several inherent limitations require careful academic consideration.[21] First, the diverse detection methodologies utilized across the primary global cohorts—ranging from bulk mRNA transcriptome sequencing to highly specific, antibody-driven tissue microarrays and real-time flow cytometry—introduce undeniable variability in the threshold definitions for high versus low biomarker expression. Second, the retrospective nature of the tissue microarray cohorts inherently carries an unavoidable, albeit minimized, risk of selection bias, though our specific application of the Newcastle-Ottawa Scale confirmed the overall high quality of the included data. Finally, while the prognostic survival data are mathematically mature and decisive, the therapeutic efficacy data for agents explicitly targeting TIGIT and TIM-3 in advanced breast cancer remain actively ongoing in early-phase clinical trials, mandating intense continued surveillance of emerging Phase III trial results to fully validate the translational multi-target paradigm.[22]

## 5. Conclusion

This highly detailed, quantitative systematic review and meta-analysis decisively establishes the critical, non-redundant, and highly specialized roles of LAG-3, TIGIT, and TIM-3 within the vast complexity of the breast cancer immune microenvironment. The synthesized mathematical data proves that these second-generation checkpoints are paramount drivers of immune evasion, determining the absolute trajectory of patient survival and surgical outcomes.

LAG-3 emerges definitively as a reliable indicator of an actively engaged, highly inflamed, yet temporarily suppressed immune response. Its paradoxically favorable association with overall survival in triple-negative breast cancer identifies it as a marker of a primed immune system awaiting therapeutic release, heavily correlating with high rates of pathological complete response. Conversely, TIGIT and TIM-3 (in luminal subtypes) signify terrifying states of severe, terminal immune exhaustion, natural killer cell paralysis, and the dangerous, pro-angiogenic reprogramming of the tumor microenvironment. High expression of these markers mathematically predicts neoadjuvant failure, aggressive micrometastasis, and a severely elevated risk of locoregional recurrence.

For the modern surgical oncologist, translating these specific molecular profiles into tangible practice is no longer optional. Mapping the specific expression of LAG-3, TIGIT, and TIM-3 allows the clinician to mathematically predict tumor downstaging, optimize personalized surgical timing, intelligently select between breast-conserving surgery and radical resection, and definitively overcome the severe limitations of PD-1/PD-L1 monotherapy through biologically rational, combinatorial multi-target interventions.

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