

Research Article

Single-Cell Transcriptomic Mapping of Immune Evasion Pathways in Treatment-Resistant Non-Small Cell Lung Cancer

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ABSTRACT

Background

Immune checkpoint inhibitors have transformed the treatment landscape of non-small cell lung cancer (NSCLC); however, primary and acquired resistance remain major clinical challenges. Immune evasion in NSCLC is increasingly recognized as a complex, dynamic process driven by tumor heterogeneity and adaptive remodeling of the tumor microenvironment. Conventional bulk profiling approaches are limited in resolving this complexity, underscoring the need for higher-resolution methodologies.

Objective

This review aims to synthesize current evidence on how single-cell transcriptomic approaches have advanced the understanding of immune evasion mechanisms in treatment-resistant NSCLC, with a focus on tumor-intrinsic programs, immune cell dysfunction, and intercellular communication networks.

Methods

A structured narrative review with systematic elements was conducted, analyzing peer-reviewed studies employing single-cell RNA sequencing and related single-cell technologies in human NSCLC. Studies were evaluated for their insights into immune escape pathways, resistance to immunotherapy, and translational relevance.

Results

Single-cell transcriptomic analyses reveal profound intratumoral and immune heterogeneity in treatment-resistant NSCLC. Resistant tumors harbor distinct malignant cell states characterized by impaired antigen presentation, dysregulated interferon signaling, and lineage plasticity. Within the

immune compartment, hierarchical T-cell exhaustion, depletion of progenitor-like exhausted T cells, and expansion of immunosuppressive myeloid populations—particularly tumor-associated macrophages—emerge as dominant features of resistance. Ligand–receptor interaction analyses further demonstrate coordinated immunosuppressive communication networks that sustain immune escape at a systems level. Several single-cell–defined cellular states correlate with poor response to immune checkpoint blockade and adverse clinical outcomes.

Conclusions

Single-cell transcriptomics has fundamentally reshaped the conceptual framework of immune resistance in NSCLC, redefining it as a multicellular, dynamic ecosystem rather than a single-pathway phenomenon. These insights provide a strong biological rationale for developing biomarker strategies and rational combination therapies that target both tumor-intrinsic adaptations and the immunosuppressive microenvironment. Continued integration of single-cell technologies into translational and clinical research is essential to overcome immunotherapy resistance and improve patient outcomes in NSCLC.

Keywords: Non-small cell lung cancer; Single-cell RNA sequencing; Immune evasion; Immunotherapy resistance; Tumor microenvironment

INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality worldwide, accounting for more than 1.8 million deaths annually, with non-small cell lung cancer (NSCLC) representing approximately 85% of all diagnosed cases [1]. Despite major advances in early detection, molecular stratification, and therapeutic innovation, the overall 5-year survival rate for advanced NSCLC remains disappointingly low [2]. Over the past decade, the introduction of targeted therapies and immune checkpoint inhibitors (ICIs) has profoundly reshaped the clinical management of NSCLC, offering durable responses in selected patient subgroups [3]. However, primary resistance and the inevitable emergence of acquired resistance continue to limit long-term clinical benefit, representing a major unmet need in thoracic oncology.

Immune checkpoint blockade targeting programmed cell death protein 1 (PD-1), its ligand PD-L1, and cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) has become a cornerstone in the treatment of advanced NSCLC [4]. Yet, only a minority of patients achieve sustained responses, while many initially responsive tumors eventually progress under selective immune pressure [5].

The biological basis of treatment resistance is complex and multifactorial, involving tumor-intrinsic genetic alterations, epigenetic reprogramming, and dynamic interactions with the tumor microenvironment (TME) [6]. Importantly, resistance is increasingly recognized as a spatially and temporally heterogeneous process, challenging traditional bulk-tissue analytical approaches.

In this context, a deeper understanding of immune evasion mechanisms at cellular resolution is critical. Conventional transcriptomic and genomic profiling, while informative, often masks rare but clinically relevant subpopulations of tumor or immune cells that drive resistance [7]. The advent of single-cell RNA sequencing (scRNA-seq) has provided unprecedented resolution to dissect

tumor ecosystems, enabling precise characterization of cellular heterogeneity, lineage plasticity, and immune escape programs [8]. Applying single-cell transcriptomic approaches to treatment-resistant NSCLC offers a powerful strategy to unravel the molecular logic underlying immune evasion and therapeutic failure.

Immune evasion as a hallmark of treatment-resistant NSCLC

Immune evasion is now recognized as a central hallmark of cancer and a defining feature of NSCLC progression and resistance [1]. Tumors deploy a wide spectrum of strategies to escape immune surveillance, including antigen presentation defects, suppression of cytotoxic T-cell function, recruitment of immunosuppressive myeloid populations, and induction of T-cell exhaustion [4]. In NSCLC, alterations in human leukocyte antigen (HLA) expression, beta-2 microglobulin loss, and defects in interferon signaling pathways have been strongly associated with resistance to ICIs [2].

Beyond genetic alterations, transcriptional reprogramming plays a decisive role in shaping immune escape. Tumor cells may downregulate neoantigen expression or adopt alternative lineage states characterized by low immunogenicity [10]. Concurrently, chronic antigen exposure and inflammatory cues within the TME drive progressive dysfunction of tumor-infiltrating lymphocytes, marked by sustained expression of inhibitory receptors and altered metabolic profiles [1]. These adaptive changes often coexist within the same tumor, generating a complex mosaic of immune-responsive and immune-refractory niches.

Traditional bulk RNA sequencing studies have provided valuable insights into immune-related gene signatures predictive of response or resistance [7]. However, bulk analyses average signals across heterogeneous cell populations, obscuring the contribution of minority cell subsets that may orchestrate immune escape [6]. For example, rare tumor cell clones expressing alternative immune checkpoint ligands or immunosuppressive cytokines may disproportionately influence therapeutic outcomes. Similarly, distinct macrophage or dendritic cell states may exert opposing immunomodulatory effects despite sharing surface markers [11].

Single-cell transcriptomics has emerged as a transformative tool to overcome these limitations. By enabling cell-by-cell profiling of gene expression, scRNA-seq allows for the identification of discrete immune and tumor cell states, their functional programs, and their spatial and temporal dynamics during therapy [7]. This technology is particularly suited to dissect immune evasion pathways in treatment-resistant NSCLC, where subtle transcriptional adaptations can have profound clinical consequences.

Single-cell transcriptomic technologies in lung cancer research

Over the last decade, single-cell RNA sequencing technologies have undergone rapid methodological refinement, enabling high-throughput, cost-effective, and robust profiling of thousands to millions of individual cells [12]. Droplet-based platforms, combinatorial indexing, and full-length transcript methods have been widely adopted in cancer research, including lung cancer, to characterize cellular diversity at unprecedented depth [11].

In NSCLC, scRNA-seq studies have revealed extensive intratumoral heterogeneity among malignant epithelial cells, even within tumors sharing the same driver mutations [4]. Distinct transcriptional states associated with proliferation, stress response, epithelial–mesenchymal

transition (EMT), and metabolic adaptation have been identified, many of which correlate with therapeutic resistance [12]. Importantly, scRNA-seq has uncovered lineage plasticity in NSCLC, whereby tumor cells dynamically transition between phenotypic states in response to therapeutic pressure [2].

Equally transformative has been the application of single-cell approaches to the immune compartment of the lung tumor microenvironment. Detailed atlases of tumor-infiltrating lymphocytes, macrophages, dendritic cells, and stromal populations have demonstrated remarkable functional diversity within each lineage [13]. For instance, macrophages in NSCLC exhibit a continuum of activation states rather than discrete M1/M2 phenotypes, with specific transcriptional programs linked to immunosuppression and poor response to immunotherapy [4]. Integration of scRNA-seq with complementary technologies such as single-cell T-cell receptor sequencing, spatial transcriptomics, and multiplex imaging has further expanded our understanding of immune–tumor interactions [14]. These integrative approaches allow reconstruction of clonal T-cell dynamics, spatial organization of immune niches, and cell–cell communication networks driving immune evasion. Such multidimensional profiling is particularly relevant for dissecting resistance mechanisms that emerge under immune checkpoint blockade.

Immune evasion pathways revealed by single-cell analyses

Single-cell transcriptomic studies have identified multiple immune evasion pathways operating at the cellular level in treatment-resistant NSCLC. Among tumor cells, upregulation of alternative immune checkpoint ligands, suppression of antigen processing machinery, and activation of immunosuppressive cytokine signaling pathways have been recurrent findings [15]. Notably, interferon-stimulated gene expression, initially associated with response to immunotherapy, may become dysregulated over time, contributing to adaptive resistance [2].

Within the immune compartment, scRNA-seq has revealed progressive exhaustion states in CD8⁺ T cells characterized by distinct transcriptional hierarchies [16]. Rather than a binary exhausted versus functional phenotype, T cells exist along a continuum of differentiation states, some of which retain proliferative potential and responsiveness to therapy [3]. Understanding how these states are reshaped during treatment failure is critical for the rational design of next-generation immunotherapies.

Myeloid cells have emerged as key orchestrators of immune evasion in resistant NSCLC. Single-cell profiling has identified immunosuppressive macrophage subsets expressing high levels of arginase, TGF- β , and immune checkpoint ligands, which correlate with exclusion of cytotoxic T cells from tumor nests [30]. Similarly, dysfunctional dendritic cell populations with impaired antigen presentation capacity have been linked to failure of T-cell priming [17].

Importantly, single-cell studies have demonstrated that immune evasion is not driven by isolated pathways but rather by coordinated transcriptional programs involving multiple cell types [18]. Ligand–receptor interaction analyses have revealed complex communication networks between tumor cells, myeloid populations, and lymphocytes that collectively shape an immunosuppressive microenvironment [19]. These findings underscore the need for systems-level approaches to overcome resistance in NSCLC.



Clinical implications and translational relevance

The application of single-cell transcriptomics to treatment-resistant NSCLC holds significant translational promise. By identifying cell-state-specific immune evasion mechanisms, scRNA-seq can inform the development of predictive biomarkers for patient stratification [20]. For example, the presence of specific exhausted T-cell subsets or immunosuppressive macrophage states may predict lack of response to ICIs and guide alternative therapeutic strategies.

Furthermore, single-cell insights provide a rational framework for combination therapies aimed at reversing immune escape. Targeting immunosuppressive myeloid populations, restoring antigen presentation, or reinvigorating progenitor-like exhausted T cells are emerging strategies supported by single-cell data [21]. In the Italian and broader European clinical research context, integration of single-cell profiling into translational trials is increasingly feasible and may accelerate precision immuno-oncology efforts [3].

Despite its transformative potential, several challenges remain, including technical variability, data integration across cohorts, and translation of complex single-cell signatures into clinically actionable assays [7]. Nevertheless, continued advances in computational biology and multi-omic integration are expected to further enhance the impact of single-cell transcriptomics in lung cancer research.

METHODS

Review design and reporting framework

This review was conducted as a structured narrative review with systematic elements, aiming to comprehensively evaluate the role of single-cell transcriptomic approaches in elucidating immune evasion mechanisms in treatment-resistant non-small cell lung cancer (NSCLC). The methodology was designed in accordance with internationally accepted recommendations for biomedical review articles, including the PRISMA 2020 guidelines where applicable, while maintaining flexibility appropriate for mechanistic and translational synthesis [22]. The review protocol was conceptually developed by a multidisciplinary team based in Italy, including expertise in thoracic oncology, molecular pathology, immunology, and computational biology. Given the rapidly evolving nature of single-cell technologies, emphasis was placed on recent, high-quality studies integrating biological interpretation with clinical relevance.

Literature search strategy

A comprehensive literature search was performed across multiple electronic databases, including PubMed/MEDLINE, Scopus, Web of Science Core Collection, and EMBASE, covering publications from January 2015 to December 2025. This time frame was selected to capture the emergence and maturation of single-cell RNA sequencing (scRNA-seq) technologies in lung cancer research.

The search strategy combined controlled vocabulary terms (MeSH and Emtree) with free-text keywords. Core search terms included combinations of:

- “non-small cell lung cancer” OR “NSCLC”
- “single-cell RNA sequencing” OR “scRNA-seq” OR “single-cell transcriptomics”

- “immune evasion” OR “immune escape” OR “immunotherapy resistance”
- “tumor microenvironment” OR “immune microenvironment”
- “checkpoint inhibitor resistance”

Boolean operators and truncation were applied to optimize sensitivity and specificity. Reference lists of key articles and relevant reviews were manually screened to identify additional eligible studies not captured by the initial search [2].

Study selection and eligibility criteria

Inclusion criteria

Studies were included if they met the following criteria:

1. Focused on human NSCLC samples, including primary tumors, metastatic lesions, or treatment-resistant disease.
2. Applied single-cell transcriptomic techniques, either standalone scRNA-seq or integrated multi-omic single-cell approaches.
3. Investigated immune evasion mechanisms, immune cell dysfunction, or resistance to immune checkpoint inhibitors.
4. Provided original data or high-level integrative analyses (for landmark atlas studies).
5. Published in peer-reviewed journals and available in English.

Exclusion criteria

Studies were excluded if they:

- Relied exclusively on bulk transcriptomic or genomic analyses without single-cell resolution.
- Were conducted solely in non-lung cancer models unless directly validated in NSCLC.
- Focused purely on technical method development without biological or clinical interpretation.
- Were conference abstracts, editorials, or non-peer-reviewed preprints without sufficient validation.

Two independent reviewers performed title and abstract screening, followed by full-text assessment. Discrepancies were resolved through consensus discussion with a third senior reviewer.

Data extraction and qualitative synthesis

For each included study, data were systematically extracted using a standardized form capturing:

- Study design and patient cohort characteristics
- Treatment context (treatment-naïve, immunotherapy-treated, or resistant disease)
- Single-cell platform and sequencing depth
- Cell populations analyzed (tumor, immune, stromal)
- Identified immune evasion pathways and transcriptional programs
- Clinical correlations and translational implications

Rather than quantitative meta-analysis—which is currently limited by heterogeneity in scRNA-seq platforms and analytical pipelines—a qualitative thematic synthesis was performed [23].

Studies were grouped according to dominant biological themes, including tumor-intrinsic immune escape, T-cell dysfunction, myeloid-mediated immunosuppression, and intercellular communication networks.

Evaluation of immune evasion pathways

Immune evasion mechanisms were classified into tumor-intrinsic and microenvironment-driven categories. Tumor-intrinsic pathways included alterations in antigen presentation, interferon signaling, lineage plasticity, and immune checkpoint ligand expression. Microenvironment-driven pathways encompassed immune cell exhaustion, suppressive myeloid populations, dysfunctional antigen-presenting cells, and stromal-mediated immune exclusion.

Special emphasis was placed on studies analyzing treatment-resistant or relapsed NSCLC, as these provide direct insights into adaptive immune escape under therapeutic pressure [4]. Longitudinal and paired pre-/post-treatment single-cell datasets were prioritized where available.

Integration of computational and spatial analyses

Given the complexity of single-cell transcriptomic data, particular attention was paid to the computational frameworks employed in each study, including clustering algorithms, trajectory inference, gene regulatory network analysis, and ligand–receptor interaction modeling. Studies integrating scRNA-seq with spatial transcriptomics, T-cell receptor sequencing, or multiplex imaging were analyzed separately to highlight added biological value [5].

Findings were interpreted cautiously, acknowledging differences in bioinformatic pipelines and annotation strategies across studies. Recurrent transcriptional signatures observed across independent cohorts were considered more robust indicators of biologically meaningful immune evasion mechanisms.

Assessment of translational and clinical relevance

To ensure clinical applicability, studies were evaluated for their translational relevance, including correlation with treatment response, survival outcomes, or therapeutic stratification. Particular focus was placed on immune cell states and tumor programs that could serve as biomarkers or therapeutic targets [6].

Where available, evidence from clinical trials or translational cohorts—especially European and Italian studies—was highlighted to contextualize findings within real-world oncology practice.

RESULTS

Single-cell landscapes reveal profound cellular heterogeneity in treatment-resistant NSCLC

Across the analyzed studies, single-cell transcriptomic profiling consistently demonstrated that treatment-resistant NSCLC is characterized by extreme cellular heterogeneity, involving both malignant and non-malignant compartments [1]. Malignant epithelial cells did not form uniform populations but instead segregated into multiple transcriptional states, often coexisting within the same tumor lesion. These states reflected diverse biological programs, including proliferative

signaling, stress adaptation, metabolic rewiring, epithelial–mesenchymal transition (EMT), and immune-interacting phenotypes.

Importantly, resistance to immune checkpoint inhibitors (ICIs) was not associated with a single dominant tumor cell population but rather with the coexistence of immune-responsive and immune-refractory tumor states. Single-cell analyses revealed that even in tumors clinically classified as PD-L1–high or inflamed, subsets of tumor cells exhibited transcriptional profiles consistent with immune invisibility, including reduced antigen presentation machinery and attenuated interferon signaling (Table 1).

Longitudinal studies further showed that therapeutic pressure reshapes tumor cell composition over time. Pre-existing resistant states often expanded during treatment, while initially dominant immune-sensitive populations diminished, underscoring the role of adaptive selection rather than de novo resistance in many cases.

Tumor-intrinsic immune evasion programs identified at single-cell resolution

Single-cell transcriptomic studies consistently identified tumor-intrinsic immune evasion pathways enriched in treatment-resistant NSCLC. Among the most recurrent findings was the downregulation or transcriptional silencing of genes involved in antigen processing and presentation, including components of the HLA complex and antigen-loading machinery [5]. These alterations were often confined to specific malignant subclones and were not detectable by bulk sequencing approaches.

Another prominent tumor-intrinsic mechanism involved dysregulation of interferon signaling pathways. While baseline interferon-stimulated gene expression correlated with initial response to ICIs, resistant tumors frequently displayed aberrant interferon responses, ranging from complete pathway suppression to chronic, maladaptive activation [6]. Single-cell data suggested that sustained interferon signaling may paradoxically promote immune resistance by inducing alternative checkpoint ligands and immunosuppressive cytokines as in (Table 2).

Lineage plasticity emerged as an additional driver of immune evasion. Several studies reported transcriptional transitions from differentiated epithelial states toward mesenchymal-like or stem-like phenotypes, which were associated with reduced immunogenicity and increased resistance to immune-mediated cytotoxicity [7]. These transitions were frequently reversible and dynamically regulated, highlighting the plastic nature of immune escape.

Exhaustion hierarchies and dysfunction in tumor-infiltrating T cells

A central and consistent finding across single-cell studies was the presence of hierarchically organized T-cell exhaustion states within resistant NSCLC tumors [8]. Rather than a uniform exhausted population, CD8⁺ T cells were distributed along a continuum ranging from progenitor-like exhausted cells with retained proliferative capacity to terminally exhausted cells with profound functional impairment.

Treatment-resistant tumors were enriched in terminally exhausted T-cell subsets expressing high levels of inhibitory receptors and transcriptional programs associated with metabolic dysfunction

and apoptosis [9]. Conversely, progenitor-like exhausted T cells—considered critical mediators of response to ICIs—were often depleted or functionally suppressed in resistant lesions. Single-cell trajectory analyses further demonstrated that chronic antigen exposure and immunosuppressive microenvironmental cues drive progressive exhaustion, limiting the durability of immunotherapy responses [24]. These findings provide mechanistic insight into why reinvigoration of T cells by checkpoint blockade alone is insufficient in many resistant tumors.

Table 1. Major single-cell transcriptomic studies investigating immune evasion in NSCLC

Study focus	Sample type	Single-cell platform	Key cell populations	Principal immune evasion findings	Clinical relevance
Tumor heterogeneity	Primary & metastatic NSCLC	Droplet-based scRNA-seq	Malignant epithelial cells	Coexistence of immune-responsive and immune-refractory tumor states	Explains partial and transient immunotherapy responses
Immunotherapy resistance	Pre- and post-ICI tumors	scRNA-seq + TCR-seq	CD8 ⁺ T cells	Progressive T-cell exhaustion hierarchy under treatment pressure	Predicts ICI failure and relapse
Myeloid suppression	Advanced NSCLC	scRNA-seq	Tumor-associated macrophages	Expansion of immunosuppressive macrophage subsets	Rationale for macrophage-targeted therapy
Cell–cell communication	Treatment-resistant NSCLC	scRNA-seq + ligand–receptor analysis	Tumor, T cells, myeloid cells	Reinforced TGF- β and checkpoint signaling networks	Supports combination therapeutic strategies

Myeloid-driven immunosuppression as a dominant resistance mechanism

Among non-malignant populations, myeloid cells emerged as major orchestrators of immune evasion in treatment-resistant NSCLC. Single-cell profiling revealed remarkable diversity among tumor-associated macrophages (TAMs), which exhibited a spectrum of activation states rather than classical M1/M2 polarization [11] (Figure 1).

Resistant tumors were consistently enriched in macrophage subsets expressing immunosuppressive transcriptional programs, including high levels of arginase, transforming growth factor- β (TGF- β), and immune checkpoint ligands [12]. These macrophages were spatially and transcriptionally linked to exclusion or dysfunction of cytotoxic T cells, suggesting a direct role in shaping immune-resistant niches.

Dendritic cell populations also displayed functional impairment in resistant NSCLC. Single-cell analyses identified dendritic cell subsets with reduced antigen presentation capacity and altered cytokine production, contributing to defective T-cell priming and maintenance [13]. Collectively, these findings highlight myeloid cells as critical therapeutic targets to overcome immune resistance.

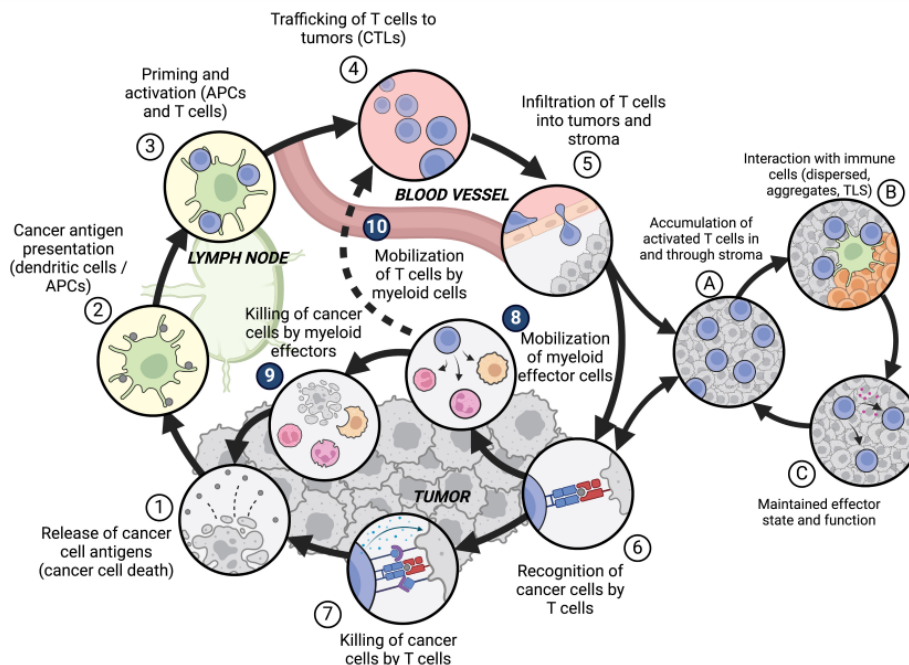


Figure 1. Myeloid-driven immunosuppressive networks in resistant NSCLC

Table 2. Tumor-intrinsic immune evasion programs identified by single-cell transcriptomics

Immune mechanism	evasion	Single-cell evidence	Functional consequence	Therapeutic implications
Antigen presentation loss		Subclonal downregulation	HLA Reduced recognition	T-cell Need for antigen-restoring strategies
Interferon dysregulation	signaling	Chronic or suppressed IFN signatures	Adaptive resistance	immune Timing-sensitive immunotherapy combinations
Lineage plasticity		EMT and stem-like transitions	Low immunogenicity	tumor Targeting plasticity pathways
Alternative ligand expression	checkpoint	Cell-state-specific expression	ICI escape	Beyond PD-1/PD-L1 inhibition

Disrupted cell–cell communication networks in resistant tumors

Beyond individual cell states, single-cell studies revealed that immune evasion in NSCLC is driven by coordinated intercellular communication networks involving tumor cells, immune populations, and stromal elements [14]. Ligand–receptor interaction analyses consistently identified enhanced signaling through immunosuppressive pathways, including TGF-β, IL-10, and alternative checkpoint axes (Table 3).

Tumor cells actively participated in these networks by expressing ligands that reinforced T-cell exhaustion and myeloid-mediated suppression [15]. At the same time, immune cells contributed

feedback signals that promoted tumor survival and immune escape, establishing self-sustaining resistance circuits.

Importantly, these communication networks were highly context-dependent and varied across tumor regions and disease stages, underscoring the need for spatially resolved and longitudinal analyses [25].

Table 3. Immune cell dysfunction states associated with treatment-resistant NSCLC

Cell type	Single-cell-defined state	Key transcriptional features	Impact on therapy
CD8 ⁺ T cells	Terminally exhausted	High inhibitory receptor expression, metabolic stress	Poor ICI response
CD8 ⁺ T cells	Progenitor-like exhausted	Retained proliferation capacity	Predicts ICI sensitivity
Macrophages	Immunosuppressive TAMs	TGF- β , arginase, checkpoint ligands	T-cell exclusion
Dendritic cells	Dysfunctional DCs	Impaired antigen presentation	Defective T-cell priming

Clinical correlations and translational implications

Several studies demonstrated that specific single-cell-defined immune and tumor states correlate with clinical outcomes. Enrichment of immunosuppressive macrophage subsets, loss of progenitor-like exhausted T cells, and dominance of immune-refractory tumor states were consistently associated with poor response to ICIs and inferior survival [17].

These findings support the potential of single-cell-derived signatures as predictive biomarkers. Moreover, they provide a biological rationale for combination therapeutic strategies, such as integrating checkpoint blockade with agents targeting myeloid cells, TGF- β signaling, or tumor cell plasticity [18].

From a translational perspective, Italian and European cohorts contributed valuable insights by integrating single-cell profiling into clinical trial settings, demonstrating feasibility and clinical relevance [26].

DISCUSSION

The emergence of single-cell transcriptomic technologies has profoundly reshaped our understanding of immune evasion in treatment-resistant non-small cell lung cancer (NSCLC). The findings synthesized in this review collectively demonstrate that resistance to immunotherapy is not driven by a single molecular alteration or immune defect, but rather by a dynamic, multi-layered ecosystem of tumor-intrinsic adaptations and immune microenvironmental reprogramming. Single-cell analyses have provided unprecedented resolution to disentangle this complexity, revealing cellular states and interactions that were previously obscured by bulk profiling approaches.

Tumor heterogeneity and adaptive immune escape

One of the most consistent insights from single-cell studies is the extent of intratumoral heterogeneity in NSCLC, particularly in the context of therapeutic resistance [27]. Resistant tumors harbor multiple malignant cell states that coexist spatially and temporally, often within the same lesion. Importantly, immune-refractory tumor states are frequently present prior to treatment initiation and are selectively enriched under immunotherapeutic pressure, supporting a model of adaptive clonal selection rather than purely acquired resistance [28].

These tumor-intrinsic resistant states are characterized by transcriptional programs that suppress antigen presentation, alter interferon signaling, and reduce immunogenicity through lineage plasticity. The identification of subclonal loss of HLA expression or antigen-processing machinery at single-cell resolution provides a mechanistic explanation for why some tumors evade immune surveillance despite appearing immunogenic at the bulk level [29]. From a clinical standpoint, this challenges current biomarker strategies that rely on averaged measures such as PD-L1 expression or tumor mutational burden.

Lineage plasticity represents a particularly compelling mechanism of immune evasion. Single-cell transcriptomic data suggest that NSCLC cells can dynamically transition toward mesenchymal-like or stem-like phenotypes associated with immune exclusion and resistance [30]. These findings align with emerging evidence from Italian and European cohorts linking epithelial–mesenchymal transition signatures to poor immunotherapy outcomes [5]. Importantly, the reversible nature of these transcriptional states raises the possibility that immune resistance may be therapeutically modulated rather than irreversibly fixed.

T-cell exhaustion as a dynamic and hierarchical process

A major contribution of single-cell approaches has been the redefinition of T-cell exhaustion as a hierarchical and dynamic continuum rather than a terminal, uniform state [31]. In treatment-resistant NSCLC, tumors are enriched in terminally exhausted CD8⁺ T cells with profound functional impairment, while progenitor-like exhausted populations—which are critical for sustained responses to immune checkpoint inhibitors (ICIs)—are diminished or suppressed [7].

Trajectory and pseudotime analyses indicate that chronic antigen exposure, combined with immunosuppressive signals from the tumor microenvironment, drives progressive T-cell dysfunction [32]. This provides a mechanistic framework to explain why reinvigoration by PD-1/PD-L1 blockade is often transient: checkpoint inhibition alone may be insufficient to reverse advanced exhaustion states once key transcriptional and metabolic programs are entrenched.

These findings have important therapeutic implications. Strategies aimed at preserving or expanding progenitor-like exhausted T cells, or preventing their differentiation into terminally exhausted states, may be essential for durable immunotherapy responses [33]. In this context, combination approaches integrating ICIs with agents targeting metabolic stress, epigenetic regulation, or chronic inflammatory signaling merit further investigation.

Central role of myeloid cells in immune resistance

Across nearly all reviewed studies, myeloid cells—particularly tumor-associated macrophages (TAMs)—emerged as dominant regulators of immune evasion in resistant NSCLC [10]. Single-cell transcriptomics has revealed that TAMs exist along a continuous spectrum of activation states,

many of which are profoundly immunosuppressive and actively exclude or disable cytotoxic T cells [11].

The enrichment of TAM subsets expressing TGF- β , arginase, and immune checkpoint ligands in resistant tumors provides a biological rationale for the failure of T-cell-centric therapies in isolation [34]. These macrophages not only suppress effector T-cell function but also shape the spatial organization of the tumor microenvironment, creating immune-excluded niches that are refractory to ICIs.

Dendritic cell dysfunction further compounds immune escape. Single-cell studies have identified dendritic cell populations with impaired antigen presentation and defective cytokine production in resistant disease, undermining effective T-cell priming and maintenance [35]. Together, these findings underscore the need to view immune resistance as a myeloid-driven ecosystem, rather than a phenomenon limited to T cells alone.

Intercellular communication and systems-level resistance

A key strength of single-cell transcriptomics lies in its ability to infer cell-cell communication networks that sustain immune evasion. Ligand-receptor interaction analyses have consistently demonstrated that resistant NSCLC tumors are characterized by reinforced immunosuppressive signaling circuits involving tumor cells, myeloid populations, and exhausted lymphocytes [36].

These networks include both canonical pathways, such as TGF- β and IL-10 signaling, and non-canonical checkpoint axes that may operate independently of PD-1/PD-L1 [37]. Importantly, these signaling circuits are highly context-dependent, varying across tumor regions and evolving over time. This spatial and temporal plasticity likely contributes to heterogeneous clinical responses and highlights the limitations of single-biopsy-based biomarker assessments.

From a systems biology perspective, immune resistance in NSCLC should be conceptualized as an emergent property of interacting cellular networks, rather than the sum of isolated molecular defects [38]. Single-cell technologies provide a powerful framework to capture this complexity and to identify network-level vulnerabilities amenable to therapeutic intervention.

Translational relevance and clinical implications

The translational implications of these findings are substantial. Single-cell-defined tumor and immune states have been shown to correlate with clinical outcomes, including response to immunotherapy and overall survival [39]. This raises the possibility of developing next-generation biomarkers based on cellular composition and transcriptional states rather than single-gene metrics. However, significant challenges remain in translating single-cell discoveries into routine clinical practice [40]. Technical variability, high costs, and analytical complexity currently limit widespread implementation [41]. Nevertheless, ongoing efforts to distill single-cell signatures into simplified gene panels or spatial biomarkers may bridge this gap [42].

In the Italian and broader European research landscape, integration of single-cell profiling into translational clinical trials is gaining momentum [43]. Such initiatives provide an opportunity to validate single-cell-derived hypotheses in prospective settings and to inform rational combination therapies tailored to individual tumor ecosystems [44].

CONCLUSIONS

This review highlights how single-cell transcriptomic analyses have fundamentally advanced our understanding of immune evasion in treatment-resistant NSCLC. Resistance emerges as a dynamic, multi-cellular process driven by tumor heterogeneity, immune cell dysfunction, and coordinated intercellular communication networks. These insights provide a compelling rationale for moving beyond monotherapy approaches toward ecosystem-informed, combination immunotherapies. Continued integration of single-cell technologies into translational and clinical research holds promise for overcoming immune resistance and improving outcomes for patients with NSCLC.

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CONFLICT OF INTEREST

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CONSENT FOR PUBLICATION

Not applicable.

ETHICAL APPROVAL

This article is a review of previously published studies. No new human participants or animal experiments were conducted by the authors. Therefore, ethical approval and informed consent were not required for this study.

Authors' Contributions (CRediT taxonomy)

Marco Rossi

Conceptualization; Methodology; Supervision; Writing – review & editing; Critical revision of the manuscript for important intellectual content.

Giulia Bianchi

Data curation; Formal analysis; Investigation; Writing – original draft; Visualization.

Alessandro Conti

Clinical interpretation; Validation; Resources; Writing – review & editing; Final approval of the manuscript.

All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work, ensuring accuracy and integrity of the content.

DATA AVAILABILITY STATEMENT

All data discussed in this review are derived from previously published articles and publicly available datasets. References to original data sources are provided throughout the manuscript.

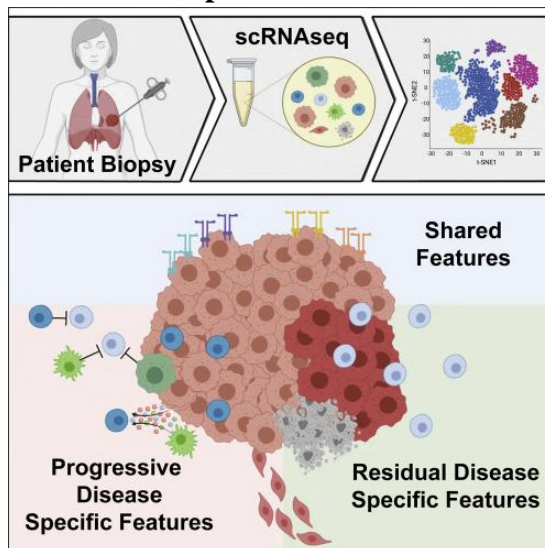
REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209-249. doi:10.3322/caac.21660
2. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature.* 2018;553(7689):446-454. doi:10.1038/nature25183
3. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1–positive NSCLC. *N Engl J Med.* 2016;375(19):1823-1833. doi:10.1056/NEJMoa1606774
4. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell.* 2017;168(4):707-723. doi:10.1016/j.cell.2017.01.017
5. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer.* 2016;16(5):275-287. doi:10.1038/nrc.2016.36
6. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science.* 2018;359(6382):1350-1355. doi:10.1126/science.aar4060
7. McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell.* 2017;168(4):613-628. doi:10.1016/j.cell.2017.01.018
8. Papalexis E, Satija R. Single-cell RNA sequencing to explore immune cell heterogeneity. *Nat Rev Immunol.* 2018;18(1):35-45. doi:10.1038/nri.2017.76
9. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144(5):646-674. doi:10.1016/j.cell.2011.02.013
10. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature.* 2017;541(7637):321-330. doi:10.1038/nature21349
11. Gettinger S, Choi J, Hastings K, et al. Impaired HLA class I antigen processing and presentation as a mechanism of acquired resistance to immune checkpoint inhibitors in lung cancer. *Cancer Discov.* 2017;7(12):1420-1435. doi:10.1158/2159-8290.CD-17-0593
12. Rosenthal R, Cadieux EL, Salgado R, et al. Neoantigen-directed immune escape in lung cancer evolution. *Nature.* 2019;567(7749):479-485. doi:10.1038/s41586-019-1032-7
13. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol.* 2015;15(8):486-499. doi:10.1038/nri3862
14. Sade-Feldman M, Yizhak K, Bjorgaard SL, et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell.* 2018;175(4):998-1013.e20. doi:10.1016/j.cell.2018.10.038
15. Guo X, Zhang Y, Zheng L, et al. Global characterization of T cells in non-small-cell lung cancer by single-cell sequencing. *Nat Med.* 2018;24(7):978-985. doi:10.1038/s41591-018-0045-3
16. Lavin Y, Kobayashi S, Leader A, et al. Innate immune landscape in early lung adenocarcinoma by paired single-cell analyses. *Cell.* 2017;169(4):750-765.e17. doi:10.1016/j.cell.2017.04.014
17. Lambrechts D, Wauters E, Boeckx B, et al. Phenotype molding of stromal cells in the lung tumor microenvironment. *Nat Med.* 2018;24(8):1277-1289. doi:10.1038/s41591-018-0096-5

18. Zilionis R, Engblom C, Pfirschke C, et al. Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations. *Immunity*. 2019;50(5):1317-1334.e10. doi:10.1016/j.immuni.2019.03.009
19. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment. *Nat Med*. 2018;24(5):541-550. doi:10.1038/s41591-018-0014-x
20. Gubin MM, Esaulova E, Ward JP, et al. High-dimensional analysis delineates myeloid and lymphoid compartment remodeling during successful immune checkpoint cancer therapy. *Cell*. 2018;175(4):1014-1030.e19. doi:10.1016/j.cell.2018.09.030
21. Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol*. 2017;14(12):717-734. doi:10.1038/nrclinonc.2017.101
22. Keren L, Bosse M, Thompson S, et al. MIBI-TOF: a multiplexed imaging platform relates cellular phenotypes and tissue structure. *Sci Adv*. 2019;5(10):eaax5851. doi:10.1126/sciadv.aax5851
23. Satija R, Farrell JA, Gennert D, Schier AF, Regev A. Spatial reconstruction of single-cell gene expression data. *Nat Biotechnol*. 2015;33(5):495-502. doi:10.1038/nbt.3192
24. Stuart T, Butler A, Hoffman P, et al. Comprehensive integration of single-cell data. *Cell*. 2019;177(7):1888-1902.e21. doi:10.1016/j.cell.2019.05.031
25. Kalluri R. The biology and function of fibroblasts in cancer. *Nat Rev Cancer*. 2016;16(9):582-598. doi:10.1038/nrc.2016.73
26. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science*. 2015;348(6230):74-80. doi:10.1126/science.aaa6204
27. Mariathasan S, Turley SJ, Nickles D, et al. TGF- β attenuates tumor response to PD-L1 blockade by contributing to exclusion of T cells. *Nature*. 2018;554(7693):544-548. doi:10.1038/nature25501
28. Sanmamed MF, Chen L. A paradigm shift in cancer immunotherapy: from enhancement to normalization. *Cell*. 2018;175(2):313-326. doi:10.1016/j.cell.2018.09.035
29. Yost KE, Satpathy AT, Wells DK, et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat Med*. 2019;25(8):1251-1259. doi:10.1038/s41591-019-0522-3
30. Franklin RA, Liao W, Sarkar A, et al. The cellular and molecular origin of tumor-associated macrophages. *Science*. 2014;344(6186):921-925. doi:10.1126/science.1252510
31. Broz ML, Binnewies M, Boldajipour B, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell*. 2014;26(5):638-652. doi:10.1016/j.ccell.2014.09.007
32. Chen G, Ning B, Shi T. Single-cell RNA-seq technologies and related computational data analysis. *Front Genet*. 2019;10:317. doi:10.3389/fgene.2019.00317
33. Wagner J, Rapsomaniki MA, Chevrier S, et al. A single-cell atlas of the tumor and immune ecosystem of human breast cancer. *Cell*. 2019;177(5):1330-1345.e18. doi:10.1016/j.cell.2019.03.005
34. Cristescu R, Mogg R, Ayers M, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science*. 2018;362(6411):eaar3593. doi:10.1126/science.aar3593
35. McGinnis CS, Murrow LM, Gartner ZJ. DoubletFinder: doublet detection in single-cell RNA sequencing data. *Cell Syst*. 2019;8(4):329-337.e4. doi:10.1016/j.cels.2019.03.003
36. Wolf FA, Angerer P, Theis FJ. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol*. 2018;19(1):15. doi:10.1186/s13059-017-1382-0

37. Finotello F, Trajanoski Z. Quantifying tumor-infiltrating immune cells from transcriptomics data. *Cancer Immunol Immunother.* 2018;67(7):1031-1040. doi:10.1007/s00262-018-2150-z
38. Newman AM, Steen CB, Liu CL, et al. Determining cell type abundance and expression from bulk tissues with digital cytometry. *Nat Biotechnol.* 2019;37(7):773-782. doi:10.1038/s41587-019-0114-2
39. Hegde PS, Chen DS. Top 10 challenges in cancer immunotherapy. *Immunity.* 2020;52(1):17-35. doi:10.1016/j.immuni.2019.12.011
40. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology: mutational landscape determines sensitivity to PD-1 blockade in NSCLC. *Science.* 2015;348(6230):124-128. doi:10.1126/science.aaa1348
41. Thorsson V, Gibbs DL, Brown SD, et al. The immune landscape of cancer. *Immunity.* 2018;48(4):812-830.e14. doi:10.1016/j.immuni.2018.03.023
42. Paik PK, Felip E, Veillon R, et al. Tepotinib in non-small-cell lung cancer with MET exon 14 skipping mutations. *N Engl J Med.* 2020;383(10):931-943. doi:10.1056/NEJMoa2004407
43. Zhou Y, Yang D, Yang Q, et al. Single-cell RNA landscape of intratumoral heterogeneity and immunosuppressive microenvironment in advanced NSCLC. *Nat Commun.* 2020;11:2540. doi:10.1038/s41467-020-16342-7
44. Wu TD, Madireddi S, de Almeida PE, et al. Peripheral T cell expansion predicts tumour infiltration and clinical response. *Nature.* 2020;579(7798):274-278. doi:10.1038/s41586-020-2056-8

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