THERAPEUTIC ADVANCES in
Medical Oncology

Review

Unraveling the tumor immune microenvironment of lung adenocarcinoma using single-cell RNA sequencing

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Ther Adv Med Oncol

2024, Vol. 16: 1–21

DOI: 10.1177/17588359231210274

***Abstract*:** Tumor immune microenvironment (TIME) and its indications for lung cancer patient prognosis and therapeutic response have become new hotspots in cancer research in recent years. Tumor cells, immune cells, various regulatory factors, and their interactions in the TIME have been suggested to commonly influence lung cancer development and therapeutic outcome. The heterogeneity of TIME is composed of dynamic immune-related components, including various cancer cells, immune cells, cytokine/chemokine environments, cytotoxic activity, or immunosuppressive factors. The specific composition of cell subtypes may facilitate or hamper the response to immunotherapy and influence patient prognosis. Various markers have been found to stratify the patient prognosis or predict the therapeutic outcome. In this article, we systematically reviewed the recent advancement of TIME studies in lung adenocarcinoma (LUAD) using single-cell RNA sequencing (scRNA-seq) techniques, with specific focuses on the roles of TIME in LUAD development, TIME heterogeneity, indications of TIME in patient prognosis and therapeutic response during immunotherapy and drug resistance. The main findings in TIME heterogeneity and relevant markers or models for prognosis stratification and response prediction have been summarized. We hope that this review provides an overview of TIME status in LUAD and an inspiration for future development of strategies and biomarkers in LUAD treatment.

Keywords: heterogeneity, LUAD, lung adenocarcinoma, mutation, NSCLC, prognosis, RNA sequencing, single-cell transcription, tumor immune microenvironment

Received: 9 April 2023; revised manuscript accepted: 9 October 2023.

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Introduction

Lung cancer is the most common type of malignancy around the world in terms of morbidity and mortality, mainly including non-small cell lung cancer (NSCLC) and small cell lung cancer. Lung adenocarcinoma (LUAD) is the most common type of NSCLC in histology.[1] Current precision medicine focuses on the targeted therapy and immunotherapy of LUAD based on a series of biomarkers from gene alterations to transcriptional or protein level changes.[2] The development of stratification biomarkers greatly facilitated the application of various personalized therapeutic strategies in clinical practice.[2] Many patients benefit from the new strategies, and their lives are prolonged with better quality. However, there are still many unsolved issues in targeted therapy and immunotherapy. One of the most prominent obstacles in therapy is cancer heterogeneity, leading to unpredictable outcomes in some patients and progression in others.[3] Since genetic alterations are cursory stratification of patients, more precise and accurate biomarkers are needed for patient stratification, especially in immunotherapy. Tumor immune microenvironment (TIME) represents a type of emerging biomarker and has been extensively investigated in the last a few years.

TIME is a complex ecosystem composed of cancer cells, various types of immune cells, extracellular matrix, and biomolecules.[4,5] During LUAD development, cancer cells produce a series of

biological effects, including inhibition of immune cell function, induction of immune escape, and regulation of immune response, so that they can evade immune surveillance and attack. Meanwhile, immune cells will also be affected by the cancer microenvironment, leading to immune cell inhibition, inactivation, exhaustion, and even necrosis, so that they cannot effectively clear tumor cells.[6,7] Therefore, an in-depth understanding of the characteristics and regulatory mechanisms of the LUAD immune microenvironment is of great significance for the development of novel immunotherapy strategies.[4] Recent studies have shown that the immune microenvironment plays a crucial role in tumor progression and can be a predictive indicator of cancer prognosis and response to therapy.[6,8–10]

Single-cell sequencing is a new technique that allows researchers to analyze the genetic and molecular features of individual cells. Technically, high-throughput cell capture techniques, such as microfluidic, oil droplet wrapping, and barcode, have been used to isolate and label 500–10,000 single cells at a time to obtain the 3′end transcriptome information of each cell. It has the advantages of high cell flux, low cost, and short capture cycle.[9] This technology is mainly used for the identification of cell subtypes and markers and to realize the division of cell populations and the detection of gene expression differences between cell populations. In addition, this technology can also predict cell differentiation and disease development trajectory, which is playing an increasingly important role in the current fields of studies in disease mechanism, immunity, cancer therapeutic strategy, and tissue development.[10] The single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool to characterize the heterogeneity of immune cells and tumor cells within LUAD. The technique allows for the analysis of gene expression profiles of individual cells, enabling researchers to identify rare cell populations and understand intercellular communication within the TIME, which may drive the development of new therapeutic strategies.[11]

In cancer treatment, chemotherapy, targeted therapy, and immunotherapy are important strategies. However, it is still inevitable to develop drug resistance or poor therapeutic response during treatment. Research suggests that tumor heterogeneity is an important reason for drug resistance. Single-cell sequencing technology can be used to reveal heterogeneity, analyze drug resistance mechanisms, screen potential beneficial populations, explore new therapeutic targets, and establish effective combined therapeutic plans.[12]

The scRNA sequencing techniques mainly involve the following advantages. First, it can precisely analyze the cell heterogeneity and provide the gene expression profile of a single cell, allowing researchers to analyze the gene expression characteristics of each single cell and reveal the inter-cell heterogeneity, and to better understand the function and development process of cells. Second, it can help to discover new cell types and subpopulations with small differences, especially in complex tissue samples. Third, it has broad application areas, including cancer research, embryonic development research, immunology, and neuroscience, etc. Fourth, many analytical methods and tools have been developed, which can better reveal the hidden information from data. However, there are also some technical deficiencies in scRNA sequencing. First, current data have a high noise level and high technical variability, which may introduce false positive or false negative results. In addition, technical differences and batch effects can also influence the interpretation and comparison of data. Second, compared to conventional high-throughput sequencing, the cost of single-cell sequencing is higher, mainly due to the complexity of processing individual cells and the high sequencing coverage depth required. Third, the experimental operation is complicated. It requires special experimental operation steps, such as cell capture, cleavage and extraction, etc., which has high technical requirements for experimental personnel. Fourth, the analysis of data is complex, requiring the use of special analysis tools and algorithms, such as alignment, de-batch effect, de-noise, cell clustering, and trajectory inference, etc., which requires high professional knowledge of data analysis.[12]

Many studies have used scRNA-seq to investigate the TIME of LUAD.[12,13] These studies have identified various immune cell types, including T cells, B cells, natural killer cells, and myeloid-derived suppressor cells (MDSCs), and some unique subsets of immune cells that are specifically associated with LUAD.[14–16] These studies provided valuable insights into the complex interactions between the immune system and LUAD and could potentially lead to the development of novel prognostic and therapeutic markers and strategies for the immunotherapy of LUAD.[14–16]

This review will focus on the components and dynamic status, especially the heterogeneity of LUAD TIME, and its indications for patient prognosis, therapeutic outcomes and resistance in immunotherapy.

The components of TIME and their roles in LUAD development

LUAD is a prevalent type of NSCLC that arises from glandular cells in the lungs. Despite recent advances in cancer treatment, LUAD remains challenging to manage, primarily due to its complex immune microenvironment. TIME is a very complex and dynamic ecosystem, which is the 'soil' for tumor survival. The cell components mainly include tumor cells, immune cells, and support cells. Under the influence of chemokines from tumor cells, fibroblasts, or inflammatory cells, immune cells in the blood circulation migrate to the tumor site through a transendothelial process.[12,17] Within the tumor tissue, immune cells locally proliferate, differentiate, function, die, and partially migrate back to the circulatory system. In these cells, cells associated with acute inflammation (including neutrophils, basophils, and eosinophils), cells associated with innate immunity [including macrophages, natural killer (NK) cells, and dendritic cells (DCs)], and cells derived from adaptive immune responses [including cluster-of-differentiation (CD)8+T cells, Th1-/Th2 cells, and B cells] can be found[12,17] (Figure 1).

The main cellular components and their roles in TIME are illustrated in Figure 1. Tumor-associated macrophages (TAM) are a relatively abundant subset of cells that outnumber other types of immune cells in many tumors. TAM has a highly plastic phenotype and function, with two major subtypes identified, including M1 TAM, which is induced by toll-like receptor ligands such as lipopolysaccharide and interferon gamma (IFN-γ), and preferentially expresses pro-inflammatory cytokines and inducible nitric oxide synthase, and M2-type TAM, which is induced by interleukin (IL)−4 or IL-13, and expresses arginase 1, CD206, CD163, IL-4R, transforming growth factor (TGF)−β1, and platelet-derived growth factor.[18,19] Some studies have shown that

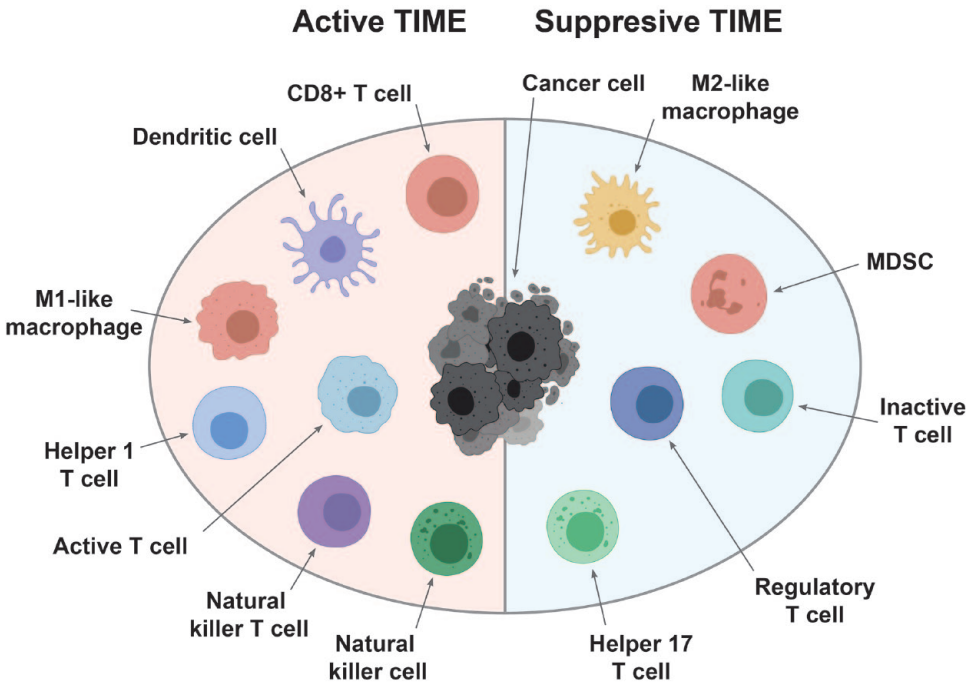

Figure 1. Illustration of cellular components and their roles in TIME. Cells with potential anti-tumor activity or pro-inflammatory activity (active TIME) include but are not limited to CD8+ T cells, dendritic cells, M1-like macrophage, helper 1 T cell (Th1), active T cells, natural killer T cells, and natural killer cells. Cells with potential immune suppressive or pro-tumor activity (suppressive TIME) include but are not limited to M2-like macrophage, MDSC, inactive T cells, regulatory T cell (Treg), and helper 17 T cell (Th17).
MDSC, myeloid-derived suppressor cell; TIME, Tumor immune microenvironment.

M1 TAM can enhance anti-tumor Th1 response and antagonize the inhibitory activity of regulatory immune cells, whereas M2-type TAM mainly plays a role in promoting angiogenesis, tumor growth and metastasis.[18,19] NK cells are cytotoxic effector lymphocytes in the innate immune system and their primary function is to help control infections and tumors. The two main mechanisms by which NK cells recognize tumor cells are that they can recognize cells with downregulated expression of major histocompatibility complex class I (MHC-I) molecules, which is a phenomenon of immune tolerance shown in multiple cancer types, or bind to stress-inducing ligands expressed on tumor cells, such as major histocompatibility complex class I polypeptide-related sequence A (MICA) or MICB.[20,21] The primary function of dendritic cells (DCs) is to act as a bridge between innate and adaptive immune responses. Physiologically, DCs engulf and process non-autoantigens. When they receive activation signals, they move to secondary lymphoid structures in the lymph nodes, where they activate primary B or T cells. DC phenotypes are quite plastic; in that, they can produce a variety of pro-inflammatory or immunosuppressive cytokines and express a range of activating or inhibiting receptors, depending on their environment.[22,23] Tertiary lymphatic structure (TLS) is a highly organized lymphoid aggregate that develops in an inflammatory pathological state. In cancer, TLS usually occurs in the infiltrating margins and/or interstitium of the tumor, similar to other chronic infectious or autoimmune diseases.[24,25]

CD4+ T helper cells can be divided into several subtypes, including Th1, Th2, Th17, follicular helper T cell (Tfh), and regulatory T cell (Treg). Each subgroup plays a specific role in the anti-tumor immune response. Th1-directed responses inhibit tumor growth and are generally associated with favorable clinical outcomes. In fact, Th1 cells enhance the anti-tumor function of cytotoxic T cells in situ by producing several cytokines, including IL-2 and IFN-γ. Tfh cells interact with B cells in TLS to help produce antibodies.[26,27] The role of other tumor-infiltrating CD4+ T-cell subsets (Th2, Th17, and Treg) is not well-understood, but is often associated with poor prognosis in different tumors.[28,29] Many studies have shown that Tregs in tumors inhibit anti-tumor immune responses through two main mechanisms, including the production of inhibitory cytokines (such as IL-10, TGF-β, IL-35) and the inhibition of the development and maturity of DCs.[30,31] In contrast, CD8+ T cells play very important roles in anti-tumor immune responses because they are responsible for recognizing and eliminating tumor cells. Due to genomic instability, tumor cells often express mutant proteins on their surface. Many of these are neoantigens that induce tumor-specific immune responses.[32–34] Activated CD8+ T cells are responsible for tumor cell recognition and lysis, the mechanisms of which have been described in detail, involving the release of cytotoxic particles. Interestingly, in most tumors, invasive cytotoxic T cells express inhibitory receptors (e.g. programed death-1 (PD-1), Tim-3, and Lag-3) whose function in physiological situations is to suppress the immune response when bound to their ligands.[32–34] In fact, many tumor cells can take advantage of this inhibitory mechanism and express multiple ligands (e.g. PD-L1, PD-L2) to help them escape T-cell attacks.[32–34] In tumor and other inflammatory conditions, B cells enhance T-cell response by producing antibodies, stimulant cytokines, and chemokines, acting as local antigen-presenting cells, and organizing the formation of TLS that sustain immune response, which makes B cells generally anti-tumor.[35,36]

The above cells play important roles in the development of LUAD. The cells that promote LUAD mainly include B cells, Treg cells, and TAM (M2 type) cells. The B cells that produce IL-10 have tumor-promoting activity and immunosuppressive characteristics.[19,20] Treg cells promote tumor growth by inhibiting anti-tumor responses.[13,14] TAM (M2 type) contributes to tumor growth, immune suppression, and cancer cell invasion.[4,5] The cells that inhibit LUAD mainly include CD8+ T cells, DC cells, TAM (M1), and NK cells. CD8+*T* cells exert direct cytotoxic effects on cancer cells by secreting granzymes and perforin.[10–12] DC cells capture tumor antigens, and type I DC initiates CD8+ T cell response, whereas type II DC is responsible for initiating CD4+ T-cell response.[6,7] TAM (M1 type) promotes inflammation and secretes tumor necrosis factor (TNF)-α and nitric oxide to kill tumor cells and further activates T-cell-mediated immune responses.[4,5] NK cells exhibit cytotoxic activity against infected and mutated cells, secreting cytokines and chemokines such as tumor necrosis factor α, IFN- γ, and interact with other immune cells.[8,9]

The heterogeneity of TIME in LUAD

Heterogeneity is one of the main characteristics of cancer. During the development of cancer, multiple patterns of evolution exist in cell division and proliferation, and the daughter cells show various alterations in epigenetics, genetics, transcriptomics, and proteomics, resulting in distinct characteristics in tumor growth rate, invasion ability, sensitivity to drugs, and prognosis.[37] The heterogeneity of cancer has been widely investigated in terms of genetic alterations. In LUAD, diverse intratumor heterogeneity and intertumor heterogeneity have been revealed, resulting in distinct outcomes in surgical, targeted, and immunotherapy.[38] In the era of immunotherapy, stratification of patients by genetic alterations is not sufficient to instruct the establishment of therapeutic strategies, as the huge discrepancy in prognosis and therapeutic efficacy cannot be explained by genetic alterations. TIME has attracted attention from both researchers and clinicians as it has provided a promising tool for effective stratification besides genetic alterations. The scRNA-seq facilitates the study of TIME heterogeneity.[39]

During tumorigenesis and subsequent metastasis, malignant cells become gradually diversified, and tumors may be infiltrated by a variety of immune-related components, including various immune cells, cytokine/chemokine environments, cytotoxic activity, or immunosuppressive factors.[39] The TIME is composed of all these components and is common in almost all solid tumors. It varies spatially or temporally, and its dynamic changes can be observed with tumor development, metastasis, and therapeutic interventions. The heterogeneity of antitumor immunity is closely related to disease progression and therapeutic responsiveness, especially in the field of immunotherapy.[39]

Diverse tumor and immune cell types and corresponding markers have been revealed in the study of TIME in LUAD. The main findings from studies on TIME heterogeneity are summarized in Table 1.[40–50] Early reports focused on the heterogeneity of key pathways or immune-related molecules. For example, genes of the γ-interferon (IFN-γ) signaling pathway, especially major histocompatibility complex (MHC) class II molecules, such as human leukocyte antigen (HLA), were found to be heterogeneously expressed and coregulated with other genes in single cancer cells across different LUAD nodules.[40] Most later studies focused more on the heterogeneity of diverse cell types and markers. The heterogeneity and evolution of LUAD were defined by comprehensive profiling of cell types within the tumor in one representative study.[41] Many cells in the LUAD tissue, including epithelial cells, endothelial cells, cancer cells, and club cells have been revealed, and many immune cells, including macrophages, mast cells, T cells, B cells, NK cells, plasma cells, and dendritic cells have also been found. Different nodules exhibited a distinct ratio of cell components and expression profiles, representing the alterations of immunity and pathways. The trajectory analysis of multiple nodules helped to determine the stage and progression of the tumor, exhibiting characteristic trajectory of development across different cancer nodules.[41] Another study further investigated intratumor heterogeneity and found that tumor cells within early-stage LUAD had heterogeneous gene expression signatures by looking at the differential expression profile from functional enrichment analysis.[42] In addition to tumor cells, immune cells within tumors also exhibited substantial heterogeneity. It was reported that 27 cell subtypes of T cells, B cells, fibroblasts, and myeloid cells were present in LUAD, and different CD4+ and CD8+ T-cell clusters exhibited differences in pathway activities.[43] Similarly, 15 main types of cells and 57 cell subgroups were identified from the multiple LUAD scRNA-seq datasets in LUAD, and a series of potential biomarkers were revealed in M2b, exhausted CD8+ T, endothelial cells, fibroblast in TIME.[48]

The scRNA techniques helped to investigate the mechanism of LUAD development in terms of heterogeneity. One study found that the transcriptome features of cancer samples were significantly different from matched para-cancer samples. Cells from adjacent normal samples clustered more closely with those of the LUADs than with more distant normal tissues. In cancer samples, the proportion of B cells increased and the abundance levels of NK cells decreased. These findings reveal spatial heterogeneity in the transcriptomic characteristics of the TIME in early LUADs.[51] The study also found spatial heterogeneity (only mutations in tumor samples) and intratumoral heterogeneity in KRAS mutations and cell lineage features. Similarly, spatial heterogeneity of lymph cells, including subtypes of CD4+ and CD8+ T cells, was also revealed. This was also supported by the findings that the

Table 1. Summary of the main findings and the significance of studies on heterogeneity in TIME of LUAD.

Ref No.	Publication Year	Total number of cells analyzed	Total number of patients involved	Protocol used for scRNA-seq library preparation	Pipeline used to analyze the data	Main findings*	Significance of the study
Ma *et al.*[40]	2019	77 (LC-PT-45), 49(LC-MBT-15)	2 patient-derived xenografts (PDXs) samples and 2 LUAD cell lines	SMART-seq	Raw reads were mapped to GRCh37 with RSEM and normalized using SCnorm.	IFN-γ signaling corresponds to acquired resistance	Development of prognostic metrics based on heterogeneity
He *et al.*[41]	2021	55713 cells	5 multiple nodules LUAD patients	10X genomics	Reads were mapped to GRCh38. Cell number was determined by 'knee' method. Seurat was used to perform analysis.	Heterogeneity and evolution and changes in TIME in LUAD early development.	TIME mechanism of early tumor development
He *et al.*[42]	2021	125,674 cells	7 stage-I/II LUAD samples	10X genomics	NA	The LUAD cells can be categorized into subtypes by distinct gene expression signatures	Substantial heterogeneity within early-stage LUAD harboring EGFR mutations
Wang *et al.*[43]	2021	9993 single cells	3 patients, 12 samples	10X genomics	Reads were mapped to GRCh38 using CellRanger (3.0.1) pipeline.	8 main cell types and 27 cell subtypes with distinct signaling and expression profile are identified.	Immunological heterogeneity in the TME of LUAD patients
Wu *et al.*[44]	2021	40,362 cells for GSE127465, and 208,506 cells for GSE131907	5 LUAD patients	inDrop	The Seurat package (version 3.0) was used to perform scRNA-seq analysis	Twenty myeloid cell types were detected, 13 of which had distinct enrichment patterns	TIME infiltration landscape may facilitate the development of immune therapy.
Zheng *et al.*[45]	2022	3583 cells from the tumor cores of three human LUAD samples	1 for GSM3304007, 1 for GSM3304011 and 1 for GSM3304013,	10X genomics	Run CellRanger aggr to aggregate multiple libraries from a single experiment. Genome_ build: GRCh38	A risk scoring model involving CCL20 mutation status was established based on eight independent prognostic genes	A prognostic model that could be used to guide clinical practice

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Table 1. (Continued)

Ref No.	Publication Year	Total number of cells analyzed	Total number of patients involved	Protocol used for scRNA-seq library preparation	Pipeline used to analyze the data	Main findings*	Significance of the study
Wang *et al.*[46]	2022	72,475 immune cells	19 pathologically diagnosed NSCLC patients (10 LUAD and 9 LUSC, 40 samples)	self-developed method	Raw gene expression matrices were generated using CellRanger (version 3.0.1).	Revealed the roles of several immune cells between LUAD and LUSC.	The comprehensive depiction of the immune heterogeneity of immune cells
Song *et al.*[47]	2022	208,506 cells.	58 specimens from 44 LUAD patients	10X genomics	The single-cell data were filtered and dimensionally reduced using the R 'Seurat' and the 'dplyr' packages.	The presence of abundant transition-state CD8+ T cells during tumor differentiation	Heterogeneity and functional exhaustion of infiltrating CD8+ T cells in LUAD
Fan *et al.*[48]	2022	24,550 single cells	19 normal and 53 LUAD samples	microwell-seq, 10X genomics and Smart-seq2	The analysis and quality control (QC) process used the Seurat R package	Identified 15 main types of cells and 57 cell subgroups, and revealed a series of potential biomarkers in TME.	This study provided insights into the heterogeneity of LUAD
Yao *et al.*[49]	2022	13,989 cells	6 patients	10X genomics	We finally retrieved the singlet cells and merged them again into the working Seurat object	NecroLRS was positively correlated with neutrophil enrichment, inflammatory immune response.	Emerging mechanisms of necroptosis-induced TIME alteration
Sun *et al.*[50]	2022	61,867 individual cells	4 glioma samples, 10 LUAD samples	BD Rhapsody, CytoSeq	Clean data were mapped to the human genome (Ensembl version 91) utilizing STAR. Seurat package was used for cell normalization and regression.	Lung-to-brain metastases reprogramed cells into immune suppressed state	A comprehensive understanding of the TIME heterogenicity in brain metastasis.

IFN-γ, interferon gamma; LUAD, lung adenocarcinoma; NSCLC, non-small cell lung cancer; RSEM, RNA-Seq by Expectation Maximization; STAR, Spliced Transcripts Alignment to a Reference; TIME, Tumor immune microenvironment.

closer to the tumor area, the less proportion were found for the M2-like macrophages C5, monocytes (classical and non-classical) and mast cells, which were depleted in the tumor area, while M2-like macrophages C1, proliferating myeloid cells and cDC2 cells were enriched in the tumor.[51]

In another study, the authors delineated the dynamic evolution from preneoplasia to invasive LUAD by integrating scRNA sequencing and spatial transcriptomics.[52] It was found that the UBE2C+ cancer cell subpopulation increased during LUAD invasion and was significantly elevated in invasive LUAD, where it was spatially distributed in the peripheral cancer area of invasive LUAD and represented a more malignant phenotype. In addition, analysis of the TME cell subpopulation revealed a sustained decrease in mast cells, monocytes, and lymph endothelial cells involved in the entire course of LUAD invasion, accompanied by a significant increase in NK cells and MALT B cells during the early stages of invasive LUAD, and the increase of Tregs and secretory B cells in late invasive LUAD.[52]

The molecular and cellular reprograming in metastatic LUAD was also investigated by scRNA sequencing.[53] The majority of T cells and myeloid cells were found in the primary lesion. Compared with normal tissue, T cells and B cells were enriched in early lesions, whereas NK cells and myeloid cells were reduced in late lesions. Compared with normal lymph nodes, there were more myeloid cells in metastatic lymph nodes, indicating that the process of metastasis was accompanied by more myeloid cell infiltration. The brain metastatic samples contained immune cells (T cells, B cells, NK cells), oligodendrocytes, and myeloid cells (microglia), of which oligodendrocytes were only present in brain metastasis. Furthermore, many angiogenic genes were upregulated, whereas many genes related to immune activity were downregulated in tumor tissues and brain metastases. Myofibroblasts originated only from tumor tissues (including primary and metastatic sites), which can promote tissue remodeling, angiogenesis, and tumor progression.[53]

Temporal and spatial heterogeneity has been widely reported in studies of LUAD TIME. The heterogeneity is composed of immune cells, tumor cells, and molecular alterations and is characterized by distinct components at various stages of tumor development. Heterogeneity may influence therapeutic response and prognosis and could be a potential target of therapy that is worth future investigation.

The prognostic TIME markers for LUAD

The prognostic factors of LUAD in immunotherapy have been widely studied at multiple levels. Gender, Eastern Cooperative Oncology Group Performance Status (ECOG-PS), body mass index, metastatic status, combined drug use, blood neutrophil-to-lymphocyte ratio, blood lactate dehydrogenase level, blood C-reactive protein(CRP) level, PD-L1 expression level, vascular endothelial growth factor(VEGF) status, tumor mutational burden (TMB), the status of driver mutations and large fragment alterations, and circulating tumor DNA (ctDNA) status have all been suggested as prognostic factors for the immunotherapy of LUAD.[54]

The prognosis of LUAD in immunotherapy is also the focus of many studies investigating TIME. Markers related to TIME have been comprehensively studied, and several types of markers have been discovered, including differentially expressed genes (DEGs) or specific cell markers, risk score from the combination of multiple factors and cell property (cell components or cell subtype ratio) related markers. The prognostic markers and the indications for prognosis in these studies are summarized in Table 2.[36,44,45,47–49,55–89] DEGs and their combined prognostic model were the most common types of prognostic markers in these studies. For example, one study identified nine cancer-specific DEGs (CBFA2T3, CR2, SEL1L3, TM6SF1, TSPAN32, ITGA6, MAPK11, RASA3, and TLR6) and established a prognostic risk model in combination with clinical factors.[62] The risk model was validated in tumor and normal tissues by RNA-sequencing and scRNA-seq and revealed that high-risk patients were associated with poor prognosis, including advanced stages and low survival rates.[62] Another similar study identified eight independent prognostic genes. CCL20 mutational status was also found to affect the prognosis and differentiation of LUAD and led to a poor histologic grade of tumor cells. A combined risk score involving the eight independent prognostic genes, clinicopathological information, and CCL20 mutation status was established as a nomogram with good predictive performance and high accuracy.[45]

Table 2. Summary of prognostic markers and the indications for LUAD prognosis in studies on TIME.

Ref No.	Publication year	Prognostic markers	Indications for prognosis	Subject profile	Therapies involved
Song *et al.*[36]	2022	Nine-gene signature	Prognostic prediction	Both early and advanced stage LUAD	ICIs (drugs not specified)
Wu *et al.*[44]	2021	MSC1 and MSC2 (MSC subtype)	Overall survival	Both early and advanced stage LUAD	ICIs (drugs not specified)
Zheng *et al.*[45]	2022	A risk scoring model (8 genes);CCL20	risk score model; poor prognosis (CCL20 mutations)	both early and advanced stage LUAD	ICIs (drugs not specified)
Song *et al.*[47]	2022	Exhausted CD8+ T lymphocyte (ETL) subset; four hub genes	ETL subset and four hub genes are prognostic	Both early and advanced stage LUAD	Surgery or ICI (PD-1 blocking)
Fan *et al.*[48]	2022	M0 macrophage and T cell activation	Correlated to a better prognosis	Both early and advanced stage LUAD	ICIs (drugs not specified)
Yao *et al.*[49]	2022	Necroptosis-related LncRNA Risk Scoring (NecroLRS)	NecroLRS is prognostic	Both early and advanced stage LUAD	Surgery or ICI (drugs not specified)
Mansuet-Lupo *et al.*[55]	2016	Intratumoral CD8+ T-cell and mDC densities	Independent markers of overall survival	Resectable LUAD	Surgery
Pocha *et al.*[56]	2020	TIL numbers within tumor islands; surfactant metabolism-related genes	Prognostic in LUAD with brain metastases	LUAD with brain metastasis	Chemotherapy, WBRT, surgery
Wu *et al.*[57]	2020	A risk score model	A robust and independent prognostic biomarker	Both early and advanced stage LUAD	Anti-PD-L1 ICIs (drugs not specified)
Li *et al.*[58]	2020	An immunoscore model	Prognostic immunoscore in LUAD was established	Both early and advanced stage LUAD	Surgery and ICIs (drugs not specified)
Zeng *et al.*[59]	2020	15 co-expressed stemness related genes	Potential biomarkers for prognostic indicators	Both early and advanced stage LUAD	Surgery, adjuvant therapy, ICIs (drugs not specified)
Zhao *et al.*[60]	2021	A risk score model (8 genes)	Contributed to patient prognosis stratification	Both early and advanced stage LUAD	Surgery, TKI therapy, ICIs (drugs not specified)
Wang *et al.*[61]	2021	TTN mutations	TTN-mutant is prognostic	late-stage LUAD	ICIs (drugs not specified)
Zhong *et al.* [62]	2021	Nine hub genes	prognostic risk model	Early-stage LUAD	Surgery
Bischoff *et al.*[63]	2021	CP^2E and N^3MC microenvironment	Prognostically unfavorable (CP^2E);a favorable prognosis (N^3MC)	Early-stage LUAD	Surgery
Gong *et al.*[64]	2021	A ceRNA network of SNHG6-hsa-miR-30e-5p-CYSLTR1	associated with the prognosis	Both early and advanced stage LUAD	anti-PD-1 ICIs (drugs not specified)

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Table 2. (Continued)

Ref No.	Publication year	Prognostic markers	Indications for prognosis	Subject profile	Therapies involved
Zhou *et al.*[65]	2021	CENPA, CENPH, CENPM, CENPN, and CENPU	A group of potential prognostic markers	Early-stage LUAD	Surgery, radiotherapy, and chemotherapy
Zhou *et al.*[66]	2021	A risk signature (6 genes)	Patients with a higher risk score had shorter PFS	Advanced lung cancer (T1–4, N1–2, M0)	Surgery, chemotherapy, targeted therapy
Ly *et al.*[67]	2021	A CD4+ LAIR2+ Treg gene signature	LAIR2 expression was adversely prognostic	early-stage LUAD (I-II)	Surgery, chemotherapy, targeted therapy
Wang *et al.*[68]	2022	CXCL7, XCL17, B cell infiltration	CXCL7, XCL17 expression and B cell infiltration are prognositc	both early and advanced stage LUAD	Surgery, chemotherapy, targeted therapy, ICIs (drugs not specified)
Shinohara *et al.*[69]	2022	T-score, I-score and S-score; PI3K pathway alteration	T, I, S scores and PI3K pathway alterations are prognostic	Resectable LUAD	Surgery, chemotherapy, targeted therapy, anti-PD-1 ICIs (drugs not specified)
Noyes *et al.*[70]	2022	clonal shrinkage of tumor-infiltrating CD8 +*T* cells	Reduced survival	Transgenic mouse model	No therapy applied
Guan *et al.*[71]	2022	Molecular subtype; ten gene risk model	Longest OS (C3);strong prediction power in prognosis (risk model)	Early-stage LUAD	Surgery, chemotherapy
Zhu *et al.*[72]	2022	RTN1 expression	Better prognosis	Both early and advanced stage LUAD	Surgery, chemotherapy, targeted therapy, ICIs (drugs not specified)
Jin *et al.* [73]	2022	A 5-gene risk model	An excellent prognostic performance	Both early and advanced stage LUAD	Surgery, chemotherapy, targeted therapy, ICIs (drugs not specified)
Luo *et al.*[74]	2022	Nine differentiation-related genes	Significant survival-predicting power	Early-stage LUAD	Surgery, chemotherapy, targeted therapy
Liu *et al.*[75]	2022	Gln metabolism-based model	Played a significant role in predicting prognosis in lung cancer	Both early and advanced stage LUAD	nivolumab or pembrolizumab
Wang *et al.*[76]	2022	ACAP1 expression	ACAP1 was predictive of prognosis	Both early and advanced stage LUAD	ICIs (drugs not specified)
Zhong *et al.*[77]	2022	High expression of XBP1	Good prognosis	Both early and advanced stage LUAD	Surgery, chemotherapy, radiotherapy, ICIs (drugs not specified)

(Continued)

Table 2. (Continued)

Ref No.	Publication year	Prognostic markers	Indications for prognosis	Subject profile	Therapies involved
Liu *et al.*[78]	2022	SLC7A5 high expression	Indicates poor prognosis and was an independent prognostic factor	Both early and advanced stage LUAD	Chemotherapy, radiotherapy, ipilimumab
Hao *et al.*[79]	2022	MYC signaling status	Significant differences in prognosis	Early-stage LUAD	Surgery, chemotherapy
Shi *et al.*[80]	2022	Two clusters (C1 and C2); Four hub chromatin regulators	Two clusters (C1/C2) and hub CRs can predict prognosis and outcome	Both early and advanced stage LUAD	ICIs (drugs not specified)
Li *et al.*[81]	2022	The four-gene prognostic signature	The four-gene signature could be used for predicting outcomes	Both early and advanced stage LUAD	ICIs (drugs not specified)
Ma *et al.*[82]	2022	61 cuproptosis-related lncRNAs prognostic lncRNAs	Exhibited a satisfactory performance predicting LUAD patients' survival	Both early and advanced stage LUAD	Chemotherapy and ICIs (drugs not specified)
Li *et al.*[83]	2022	Risk score (a 17-gene signature)	Prognostic risk prediction based on the overall survival time of LUAD patients	Both early and advanced stage LUAD	Surgery, adjuvant therapy, targeted therapy
Van Hiep *et al.*[84]	2022	HB-EGF expression	High HB-EGF expression was significantly correlated with poor overall survival	Both early and advanced stage LUAD	Surgery, adjuvant therapy, ICIs (drugs not specified)
Zhang *et al.*[85]	2022	High proportion of tip-like endothelial cells	Poor prognosis in multiple cancer types	Both early and advanced stage LUAD	Surgery, chemotherapy, targeted therapy, ICIs (drugs not specified)
Zhang *et al.*[86]	2023	A risk model consisting of nine gene signatures based on T-cell marker genes	The model can predict the survival and treatment outcomes	Early-stage LUAD	Surgery, adjuvant therapy, ICIs (drugs not specified)
Zhang *et al.*[87]	2023	High cancer-specific fibroblasts (CSF) proportion	Associated with poor prognosis	Early-stage LUAD	Surgery, adjuvant therapy, ICIs (drugs not specified)
Liu *et al.*[88]	2023	12 genes; m7G cluster-B; NUDT4 and WDR4	Predict survival and outcomes	Both early and advanced stage LUAD	CTLA-4 and PD-1 ICIs (drugs not specified)
Zhang *et al.*[89]	2023	A risk model consisting of 11 genes	High-risk group had a worse prognosis	Both early and advanced stage LUAD	PD-1/PD-L1 or CTLA4 ICIs (drugs not specified), or both

CRs, chromatin regulators; ICIs, immune checkpoint inhibitors; LUAD, lung adenocarcinoma; OS, overall survival; TIME, Tumor immune microenvironment.

Specific cell markers were also suggested to be prognostic in TIME. One study suggested that tumor-associated regulatory T cell expression of immunoglobulin-like receptor 2 (LAIR2) was prognostic in LUAD. The study found that LAIR2 was preferentially produced by activated CD4+ T cells and enhanced in vitro tumor invasion into collagen. A CD4+ LAIR2+ Treg gene signature was prognostically significant in the The Cancer Genome Atlas (TCGA) dataset.[67] Another study reported that patients with low gene transcription of CXC ligand (CXCL) 7 and high expression of CXCL 17 had a better prognosis in LUAD. Immune cell infiltration, specifically B-cell infiltration was significantly correlated with LUAD microenvironment mediated by cysteine(C)-non cysteine(X)-cysteine(C) (CXC) chemokines.[68] In addition, Arf-GAP with coiled-coil ankyrin (ANK) repeat and PH domain-containing protein 1 (ACAP1) expression appeared to be positively associated with the infiltrating level of immune cells in TIME and the expression of immune checkpoint molecules.[76] ACAP1 was highly correlated with T-cell activation and immune response. Since overexpression of ACAP1 was found to cause attenuation in cell proliferation, migration, invasion, and promoted apoptosis, low ACAP1 expression was suggested to be correlated with unsatisfactory OS and disease-specific survival in LUAD patients.[76]

Immune cell properties were also found to be correlated with LUAD prognosis. One study reported that heterogeneous carcinoma cell transcriptomes reflecting two distinct microenvironmental patterns, namely N3MC pattern (normal-like myofibroblasts, non-inflammatory monocyte-derived macrophages, NK cells, myeloid dendritic cells, and conventional T cells) and CP2E pattern (cancer-associated myofibroblasts, proinflammatory monocyte-derived macrophages, plasmacytoid dendritic cells, and exhausted CD8+ T cells). It was found that the immune-activated CP2E microenvironment was prognostically unfavorable, whereas the inert N3MC microenvironment was associated with a favorable prognosis.[63] Another study pointed out that CD8+ T cells infiltrating the TIME of LUAD are critical for establishing antitumor immunity because the cell differentiation trajectory showed the presence of abundant transition-state CD8+ T cells during the differentiation of naive-like CD8+ T cells into cytotoxic CD8+ T cells and exhausted CD8+ T cells. The higher the proportion of the exhausted CD8+ T lymphocyte (ETL) subset, the shorter the OS of LUAD patients.[47]

The prognosis of LUAD patients can be predicted by a series of TIME markers, including DEGs, specific cell markers, risk score models, and cell components or subtypes. It appeared from the above reports that the subtypes, composition of immune cells and molecular alterations in cancer tissue reflected the cancer progression. These components may be new biomarkers for LUAD prognosis.

The TIME markers for LUAD therapeutic response

Many biomarkers have been investigated and suggested as indicators for therapeutic response in immunotherapy. The state of PD-L1 expression and the status of TMB were suggested as predictive biomarkers. The status of HLA/MHC expression was suggested as a marker of antigen presentation. The status of the oncogenic driver genes (e.g. EGFR, ALK, KRAS, MET) and co-mutations (e.g. STK11, KEAP1, SMARCA4) was also suggested to stratify the patient response.[90]

The markers related to the TIME were also suggested to stratify the patient response in immunotherapy. There are three types of markers on response stratification, including DEGs or specific cell markers, risk scores from combined multiple markers, and cell property markers. The studies on therapeutic response and details of the markers are summarized in Table 3.[40,44,57,61,64,69,75,76,78,81,82,86,88,89,91–99] Several DEGs have been found to predict patient response. In one study, four immune-related genes, including PTPRC, CCR2, SLAMF1, and HLA-DQA2 were identified as the signature for better outcomes. The four-gene signature was suggested to be used for outcome prediction in LUAD patients. The risk score was calculated by combining their expression levels and coefficients. Further analyses revealed that patients who had a higher risk score were also accompanied by a lower immune infiltration level and a worse response.[81] Similarly, a risk model consisting of nine gene signatures (CASZ1, CCDC85B, CCL20, MAPK1IP1L, MYO6, RHOQ, ST13, TLE1, TMEM11) based on T-cell marker genes was established. High-risk groups presented discriminative immune-cell infiltrations and immune-suppressive states. The authors suggested that the treatment outcomes can be accurately predicted by the risk model.[86] In another study,

Table 3. Summary of therapeutic markers and the indications for LUAD responses in studies on TIME.

Ref No.	Publication year	Therapeutic markers	Indications for therapeutic response	Subject profile	Therapies involved
Li *et al.*[92]	2019	Cytotoxic CD8+ T cells and dendritic cells, Foxp3+ Tregs, and M2-like polarization of macrophages	Tumor shrinkage	Transgenic mouse model	Oral gavage: vehicle, gefitinib or osimertinib
Ma *et al.*[40]	2019	The downregulation of genes in IFN-γ signaling pathways	Acquired resistance phenotype	Two PDX samples, both early and advanced stage LUAD	One male:treatment-naïve one female: standard chemotherapy and erlotinib treatment
Li *et al.*[92]	2020	Loss of the histone chaperone Asf1a	Sensitized tumors to anti-PD-1 treatment.	Mouse model and cell lines	Anti-PD-1 antibody (29F.1A12)
Li *et al.*[93]	2019	Treg-specific deletion of ST2	Increased infiltration of CD8+ T cells into tumors, and decreases tumor burden.	Genetic mouse model	Interferon
Wu *et al.*[57]	2020	A riskScore (gene and cell signature) model	predicting immunotherapeutic outcomes	Both early and advanced stage LUAD	Anti-PD-L1 immunotherapy (no specific drugs mentioned)
Guo *et al.*[94]	2020	Thymocyte selection-associated high mobility group box (TOX) expression	Increase of immune infiltration levels in most of the immune cells	Both early and advanced stage LUAD	ICIs (drugs not specified)
Marinelli *et al.*[95]	2020	KEAP1, PBRM1, SMARCA4 and STK11	Potentially associated with reduced efficacy of immunotherapy	Metastatic NSCLC patients	ICIs (drugs not specified)
Sage *et al.*[96]	2020	siRNA-mediated knockdown of AC008750.1	Impaired NKG7 expression and NK cell anti-tumor capacity	Both early and advanced stage LUAD	ICIs (drugs not specified)
Rubio-Perez *et al.*[97]	2021	CSF CD8+ T cell infiltration	Predicted the response to ICI	LUAD with brain metastasis	ICIs (drugs not specified)
Zheng *et al.*[98]	2021	A high-resolution TME cell atlas (TME stratification analysis)	Predicted potential resistance regulators of PD-1/PD-L1 blockade	Non-metastatic LUAD	Combination of natural products with ICIs (drugs not specified)

(Continued)

Table 3. (Continued)

Ref No.	Publication year	Therapeutic markers	Indications for therapeutic response	Subject profile	Therapies involved
Wang *et al.*[61]	2021	TTN mutations	Potential predictive marker for patients with LUAD to accept ICIs	Late-stage LUAD	ICIs (drugs not specified)
Wu *et al.*[44]	2021	MSC1 and MSC2 (MSC subtype)	Immune blockade therapy responses	Both early and advanced stage LUAD	ICIs (drugs not specified)
Gong *et al.*[64]	2021	A ceRNA network of SNHG6-hsa-miR-30e-5p-CYSLTR1	Associated with the prognosis of and immunotherapy response	Both early and advanced stage LUAD	Anti-PD-1 ICIs (drugs not specified)
Yu *et al.*[99]	2022	Colocalization of CTGF:LRP6	Associated with LUAD progression	Both early and advanced stage LUAD	Anti-CTGF (connective tissue growth factor) therapy
Shinohara *et al.*[69]	2022	TIME score	TIME score predicted the efficacy of ICI	Resectable LUAD	Anti-PD-1 ICIs (drugs not specified)
Liu *et al.*[75]	2022	Low levels of Gln metabolism (Gln metabolism-based model)	Predicting prognosis and immunotherapy efficacy	Both early and advanced stage LUAD	Nivolumab or pembrolizumab
Wang *et al.*[76]	2022	ACAP1 expression	Predictive of prognosis and immunotherapy response	Both early and advanced stage LUAD	ICIs (drugs not specified)
Liu *et al.*[78]	2022	SLC7A5 high expression	High SLC7A5 expression indicated poor immunotherapy efficacy	Both early and advanced stage LUAD	chemotherapy, radiotherapy, ipilimumab
Li *et al.*[81]	2022	A four-gene prognostic signature	Predicting outcomes and immune landscapes for LUAD patients	Both early and advanced stage LUAD	ICIs (drugs not specified)
Ma *et al.*[82]	2022	61 cuproptosis-related LncRNAs prognostic lncRNAs (CLPS)	A predictor for the prognosis and therapeutic responses	Both early and advanced stage LUAD	Chemotherapy and ICIs (drugs not specified)
Zhang *et al.*[86]	2023	A risk model consisting of nine gene signatures	Predictive for survival and treatment outcomes	Early-stage LUAD	ICIs (drugs not specified)
Liu *et al.*[88]	2023	m7G cluster-B	Predictive for survival and response to immunotherapy	Both early and advanced stage LUAD	CTLA-4 and PD-1 checkpoint inhibitors (drugs not specified)
Zhang *et al.*[89]	2023	A risk model consisting of 11 genes	Low-risk group was more likely to benefit from immunotherapy	Both early and advanced stage LUAD	PD-1/PD-L1 or CTLA4 ICIs (drugs not specified), or both

15 N7-methylguanosine (m7G)-related genes (CYFIP1, DCP2, DIF3D, EIF4E, EIF4G3, EIF4E2, LARP1, METTL1, NCBP1, NCBP2, NUDT3, NUDT4, NUDT11, SNUPN, WDR4,) were found to be highly expressed in tumor samples. m7G cluster-B was shown to have a lower immune infiltration level and predicted poor responses to immunotherapy. NUDT4 and WDR4 were identified as independent risk factors.[88]

Specific immune cell properties and cell markers in TIME were also shown to predict the treatment response. One study suggested that the T-cell receptor (TCR) clonotypes of the cerebrospinal fluid, specifically CD8+ T-cell infiltration, can provide a non-invasive alternative to predict the response to immune checkpoint inhibitors in LUAD patients with brain metastasis.[97] Another study confirmed the colocalization of ligand–receptor interaction CTGF:LRP6 among malignant cell subtypes as an indicator predicted to be associated with LUAD progression.[99] SLC7A5 high-expression was shown to be involved in the activation of multiple oncogenic pathways, including mTORC1, cell cycle, DNA damage repair, response to reactive oxygen, angiogenesis, epithelial–mesenchymal transition, and various growth factors. Its high expression was found to be an indicator of poor immunotherapy efficacy and was an independent prognostic factor. High SLC7A5 expression also indicated higher sensitivity to inhibitors of the mTORC1 pathway, cell cycle, and angiogenesis.[78]

It can be summarized that DEGs, specific cell markers, risk score models, and cell property markers are potential indicators for therapeutic response. These reported markers mostly focus on the response to immunotherapy. More investigations on TIME-related markers are needed in other therapies, including surgery, chemotherapy, radiotherapy, targeted therapy, etc.

The TIME-related drug resistance

The resistance to immunotherapy in LUAD has been investigated, and specific genetic alterations have been suggested as biomarkers for poor response to immunotherapy. EGFR mutations were suggested as biomarkers of poor response to single-agent immune checkpoint inhibitors (ICIs), and combined strategies may be needed for patients with EGFR mutations.[100,101] Co-mutations of KRAS and STK11 in LUAD were associated with an inactive immune microenvironment, mechanistically led to the poor outcomes of KRAS/STK11-mutated patients receiving single-agent immunotherapy, with a relevant proportion of primary resistance.[102,103] There were also reports on the poor effectiveness of ICIs in LUAD with ALK rearrangement.[100,101] Meanwhile, hyperprogressive disease (HPD) after immunotherapy in LUAD has also been reported. Biomarkers linked to HPD in LUAD included MDM2/4 amplification, EGFR mutations, and BRCA2 mutations.[104]

The TIME-related drug resistance may involve the dysregulation of anti-tumor activity of Treg cells, exhausted T cells, dendritic cells, MDSCs, and macrophages.[104] Expanded or activated Treg cells may have strong immune suppression, thus hampering the functions of effector T cells, including CD4+ T and CD8+ T cells.[105,106] T-cell exhaustion refers to the progressive decline in T-cell function or dysfunction due to continuous stimulation of TCR under continuous antigen exposure. T-cell exhaustion ranges from highly proliferative T cells with stem-like properties to T cells that have completely lost effector function and the ability to replicate. Exhausted T cells are characterized by reduced cytokine production and inhibited expression of receptors such as PD-1, CTLA4, and LAG3 (lymphocyte activation gene 3 protein), TIGIT [T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains] and TIM3 (T-cell immunoglobulin and mucin domain-containing protein 3).[104] Dendritic cells are a type of antigen-presenting cells that uptake tumor antigens and play a crucial role in generating the anti-tumor response by T cells. PD-1 on T cells, PD-L1 on DC cells and activation of TGF-β in DCs all contribute to the inhibition of DC activity.[107–109] MDSCs are a group of immunosuppressive cells, which may be involved in regulating immune responses. MDSCs may contribute to tumor angiogenesis, metastasis, and prognosis.[110] The roles of MDSCs in LUAD immunotherapy are still not clear, but they might be related to impaired activity of effector T cells, inducing Treg cell expansion, NK cell function reduction, and cytokine secretion.[111] Macrophages have also been reported to participate in the response to immunotherapy. M2 TAMs were suggested to be associated with pro-tumorigenic properties, and their presence was correlated with a poor prognosis in various tumors.[112] In contrast, M1 macrophages were suggested to promote anti-tumor

immunity. It was suggested that tumor growth might be inhibited by the depletion of M2 TAMs in LUAD.[104,113] The spatiotemporal evolution of TAMs was shown to be associated with LUAD progression and tumor response to immunotherapy. It was also found that PD-1 was expressed in a highly plastic tumor-promoting subtype of TAMs that develops during tumor progression, and protected tumor cells.[114]

Apart from immune cells, other biomarkers were also suggested to influence drug resistance. Heterogeneous expression and coregulation with other genes were found in IFN-γ signaling pathway genes in LUAD, and the downregulation of these genes might correspond to an acquired resistance phenotype.[40] Another study established a model containing 23 circadian-related genes, and used it in predicting the immunotherapeutic outcomes in independent cohorts.[115] In addition, patients with high expression of JAG1 were found to correlate with immunosuppressive phenotype, leading to immunotherapy resistance.[116]

Both molecular alterations and TIME-related immune cell components and activities were found to be markers for drug resistance. They may help to predict resistance before any therapy is implemented, and regulation of key components may also potentially help to overcome resistance. Markers on resistance also need to be developed in other therapeutic strategies, apart from immunotherapy.

Conclusion

The TIME of LUAD is composed of tumor cells, immune cells, support cells, and regulatory factors with great heterogeneity. Immune cells include those associated with acute inflammation, innate immunity, and adaptive immune responses. These cells can be divided by their roles into immune active cells, such as CD8+ T, Th1, NK, DC, M1 TAM cells, and immune suppressive cells, such as M2 TAM, MDSC, Treg, Th17, and exhausted T cells. The dynamic status of these cells, and their interaction with other components in TIME, influence the immune response to surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy and mediate the resistance to immunotherapy. Many prognostic and response models composed of DEGs or specific cell markers, risk scores and cell property markers have been summarized in this review. These past findings should be translated into future actionable strategies based on consensus on markers or models in TIME for clinical practice. Future studies may focus on the clinical validation of markers or models in prospective cohorts.

Declarations

Ethics approval and consent to participate

This research is a review and ethics approval and consent to participate were therefore waived.

Consent for publication

Not applicable.

Author contributions

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Acknowledgements

None.

Funding

This study was supported by the key project of "Intelligent Robot", Real-time accurate control of tumor radiation therapy robot system with moving target location (Project number: 2023YFB4706200), funded by the National Key Research and Development Plan.

Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The datasets generated and/or analyzed during the current study were all included in the manuscript.

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