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Unveiling the immunomodulatory dance: endothelial cells' function and their role in non-small cell lung cancer

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Abstract

The dynamic interactions between tumor endothelial cells (TECs) and the immune microenvironment play a critical role in the progression of non-small cell lung cancer (NSCLC). In general, endothelial cells exhibit diverse immunomodulatory properties, influencing immune cell recruitment, antigen presentation, and regulation of immune checkpoint expression. Understanding the multifaceted roles of TECs as well as assigning specific functional hallmarks to various TEC phenotypes offer new avenues for targeted development of therapeutic interventions, particularly in the context of advanced immunotherapy and anti-angiogenic treatments. This review provides insights into the complex interplay between TECs and the immune system in NSCLC including discussion of potential optimized therapeutic opportunities.

Keywords NSCLC, Endothelium, TEC, Lung, Angiogenesis, Immune response, Vessel, TME, Therapy, Cancer

Introduction

Recent advances in the understanding of the tumor microenvironment (TME) and deep genetic characterization of non-small cell lung cancer (NSCLC) paved the way for the development of targeted therapies and novel treatment strategies. The effectiveness of these advancements, underscored by the application of immuno- and anti-angiogenic therapies, is designed to tackle the complexities inherent in the TME. Nevertheless, NSCLC remains the leading cause of cancer-related mortality worldwide [1].

The lung, being a highly vascularized organ, provides a unique microenvironment with specialized endothelial cells (ECs), that show heightened expression of genes related to immune regulation, particularly those involved in leukocyte cell-to-cell adhesion, T cell activation, and leukocyte migration [2]. High-resolution in-depth characterization of the TME, including the identification and analysis of various cell types and their subpopulations,

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has been made possible through the application of single-cell RNA sequencing (scRNA-seq) techniques [3–8]. These studies have provided valuable insights into the intricate cellular composition and potential interactions within the NSCLC TME.

The significance of tumor endothelial cells (TECs) in promoting tumor growth and their role as a critical therapeutic target in NSCLC have been underscored by the use of anti-angiogenic therapy (AAT). Inhibition of vascular endothelial growth factor (VEGF) and its receptors, such as VEGFR1-3, using monoclonal antibodies like bevacizumab or tyrosine kinase inhibitors (TKIs), has been effective in curbing their pro-angiogenic properties [9–11]. However, despite the initial success of AAT, its effectiveness is often transient, and primary or secondary resistance mechanisms can limit its efficacy over time. This has prompted researchers and clinicians to explore more comprehensive approaches to unveil the complex challenges posed by TECs and their role in tumor progression [12–14]. Emerging evidence suggests that TECs, particularly in the lung but likely also in other organs, play a role in fostering immunogenic features, making them an attractive target for combination therapies with standard as well as targeted anti-tumor treatments [3, 7, 8]. Therefore, a deeper analysis of the tumor endothelium is warranted to elucidate its distinct role within the TME, to identify cancer-specific alterations and to explore potential novel targets to modulate cancer-promoting pathways.

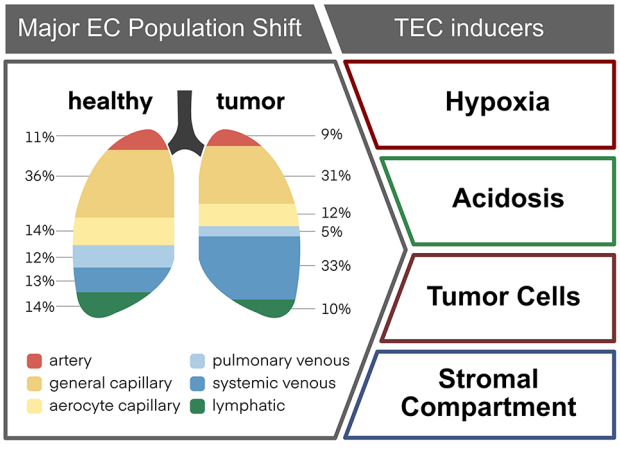

Fig. 1 EC population dynamics in the lung microenvironment. Comparison of major EC populations (artery, general capillary, aerocyte capillary, pulmonary venous, systemic venous, and lymphatic ECs) in healthy lung tissue versus tumor-affected lung tissue reveals a notable shift in their distribution. Environmental cues within the TME, such as hypoxia, acidosis and the local tumor and stromal components, affect EC behavior and induce distinct TEC phenotypes. The figure highlights EC plasticity in the context of the healthy and tumor infiltrated lung, emphasizing the intricate modulation of EC populations in response to microenvironmental stimuli

This review provides a comprehensive examination of the tumor endothelium in NSCLC, focusing on delineating specific subtypes, characterizing their functional consequences, and highlighting putative interactions with other cell types of the TME. This overview lays the ground to discuss potential and promising novel targets and combinational treatment strategies for improved management of NSCLC.

Distinct subtypes of ECs are present in the (tumor) lung

In the healthy adult lung, ECs represent ~ 6% of all cells, whereas in less perfused lung tumors, this proportion is reduced to ~ 3% (Fig. 1) [4–6], primarily due to altered cell-ratios stemming from the significant proportion of neoplastic cells but also challenging conditions such as severe hypoxia or acidic pH as well as the influence of the local (cancer) cell compartment.

Distinct subtypes of ECs encompass the pulmonary microvascular ECs, which regulate gas exchange and promote tumor angiogenesis within the TME (Fig. 2).

Alveolar capillary ECs, vital for gas exchange, undergo phenotypic changes contributing to a hypoxic TME, facilitating tumor growth and metastasis formation. Bronchial venous and arterial ECs modulate bronchial blood flow, potentially impacting lung cancer progression by altering oxygen supply. Lymphatic ECs contribute to fluid homeostasis and immune surveillance, affecting immune responses and the spread of lung cancer cells through the lymphatic system. Understanding the specific roles of these EC subtypes is crucial for identifying novel therapeutic targets to modulate the TME, thereby potentially improving lung cancer treatment outcomes. Discussed EC subtypes are illustrated in Figs. 2 and 3, their canonical marker genes are summarized in Table 1.

Capillary ECs

In the healthy lung, capillary ECs (Caps) make up the majority of the lung endothelium, representing ~ 50% of all ECs. These are further subdivided into general capillary ECs (gCaps), the biggest EC population with ~ 36%, and aerocytes (aCaps) with ~ 14% of all lung ECs [3–6]. The main function of Caps is the maintenance of the blood-air barrier, facilitating the controlled exchange of oxygen and carbon dioxide across millions of alveoli within the bronchial tree [15]. Morphologically, aCaps are large (> 100 µm), porous cells spanning across several alveoli, a cellular architecture exclusively found in lung tissue. Together with smaller (< 40 µm), less porous gCaps, they compose a dense cellular patchwork-structure covering about 86% of the alveolar surface of the lung [15, 20, 21]. Although gCaps are more abundant, considering the size differences, both subtypes collectively cover the total surface area in equal proportions

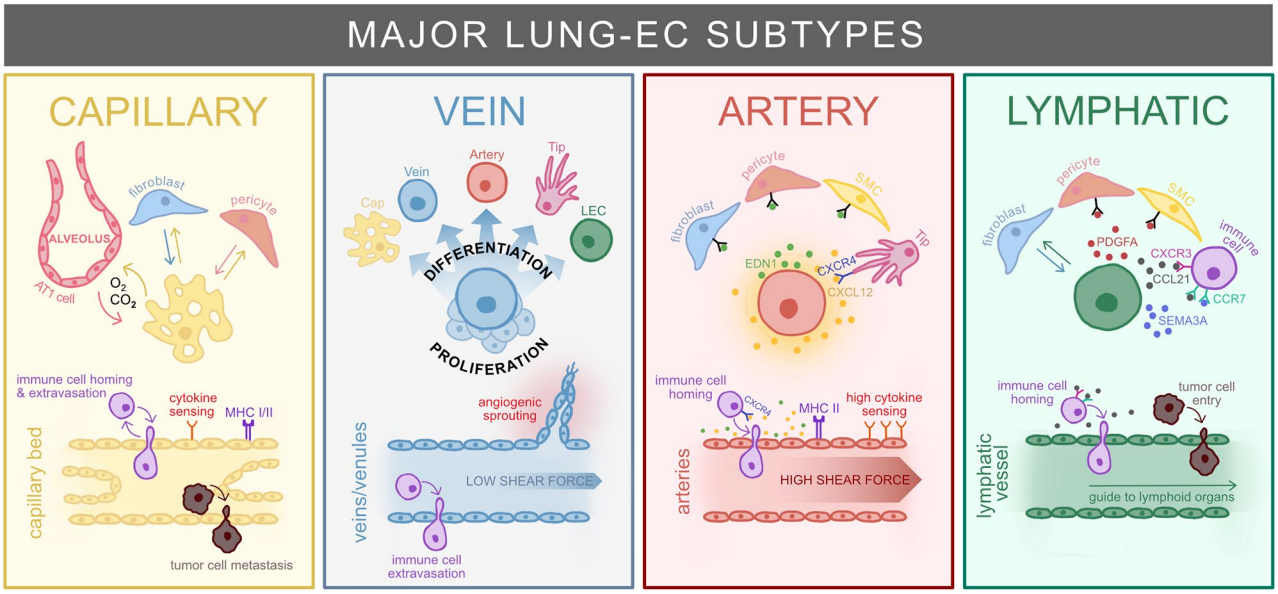

Fig. 2 Four major lung-EC subtypes. EC subtypes and their functional characterization in health and disease. In the healthy lung tissue, different EC subtypes fulfill distinct physiological functions. Capillary ECs (Caps) are specialized cells to execute the oxygen exchange in the alveoli and are involved in immune cell migration. Capillaries are a branched and narrow vascular network where circulating tumor cells can get trapped and form metastases more easily. Vein ECs are highly dynamic and proliferative, they can develop into different EC subtypes (such as Caps, artery, tip or lymphatic ECs (LECs)) and with that are able to re-vascularize damaged tissues. Especially in post-capillary venules increased traffic of immune cell exchange occurs. Artery ECs are the sensory hub within the pulmonary vasculature. They form connections with various cell types and can re-shape their environment due to their vascular remodeling and angiogenesis-inducing capabilities. LECs represent the draining route for immune and tumor cells and are essential for both anti-tumor immune response as well as tumor progression and spread

and execute distinct functions implied by different gene expression profiles [15]. While aCaps primarily facilitate the gas-exchange, they are also associated with leukocyte trafficking and the absence of endothelial-specific Weibel-Palade body contents, including von Willebrand factor (vWF), P-selectin (SELP) or Endothelin-1 (EDN), which are critical components for vessel hemostasis and inflammation [4, 15]. On the other hand, gCaps express gene signatures that are associated with antigen presentation, vasomotor regulation, the transcytosis of lipoproteins (cholesterol), modulation of lipid metabolism, expression of cytokine receptor and participation in the innate immune response [4, 15]. Notably, Caps showed the highest MHC-I and MHC-II expression within the endothelium, although lacking the expression of co-stimulatory factors CD80 and CD86, which indicates a role as semi-professional antigen presenters [3, 4]. In terms of cell-to-cell interaction, aCaps are in close contact with, and often dependent on the presence of alveolar AT1 cells, which specializes them in gas exchange around alveoli [22]. Whereas, gCaps interact with stromal cells, such as pericytes and fibroblasts, thus regulating vessel homeostasis [4, 15]. It could be shown that their sprouting even relies on the presence of fibroblasts and interstitial flow [23].

In lung tumors, the proportions of gCaps and aCaps are comparable to healthy lung tissue, although the total Cap population is reduced [3, 5, 6]. Interestingly, patients with severe chronic obstructive pulmonary disease (COPD), in contrast, have elevated numbers of lung Caps, whereas lethal COVID-19 and idiopathic pulmonary fibrosis patients showed decreased proportions of gCaps, suggesting varying frequencies in different disease phenotypes [6, 24]. However, tissue of lung adenocarcinoma displays a disturbed capillary pattern including a third, "hybrid" intermediate subtype that expresses markers of both aCaps and gCaps, a phenomenon also observed in the lungs of lethal COVID-19-associated acute respiratory distress syndrome [15, 25]. Goveia et al. observed that intermediate Caps represent ~ 56% of all Caps in NSCLC, indicating disturbed EC differentiation [3]. Murine tumors harbor fewer aCaps, which may be attributed to the slow turnover of aCaps in damaged tissues, where repair is almost exclusively restricted to the more proliferative gCaps [15, 16]. In this context, a recent study could identify a distinct capillary phenotype assigned to high expression of *CAR4* ($CAR4^{high}$), induced by influenza infection, that contributes to the regeneration after alveolar damage [26].

Given that the lung is susceptible for metastases, with up to 54% of all metastasizing primary cancers spreading to the pulmonary site [27, 28], circulating tumor cells settle more easily and extravasate through the narrow microvasculature and form pulmonary metastases [29,

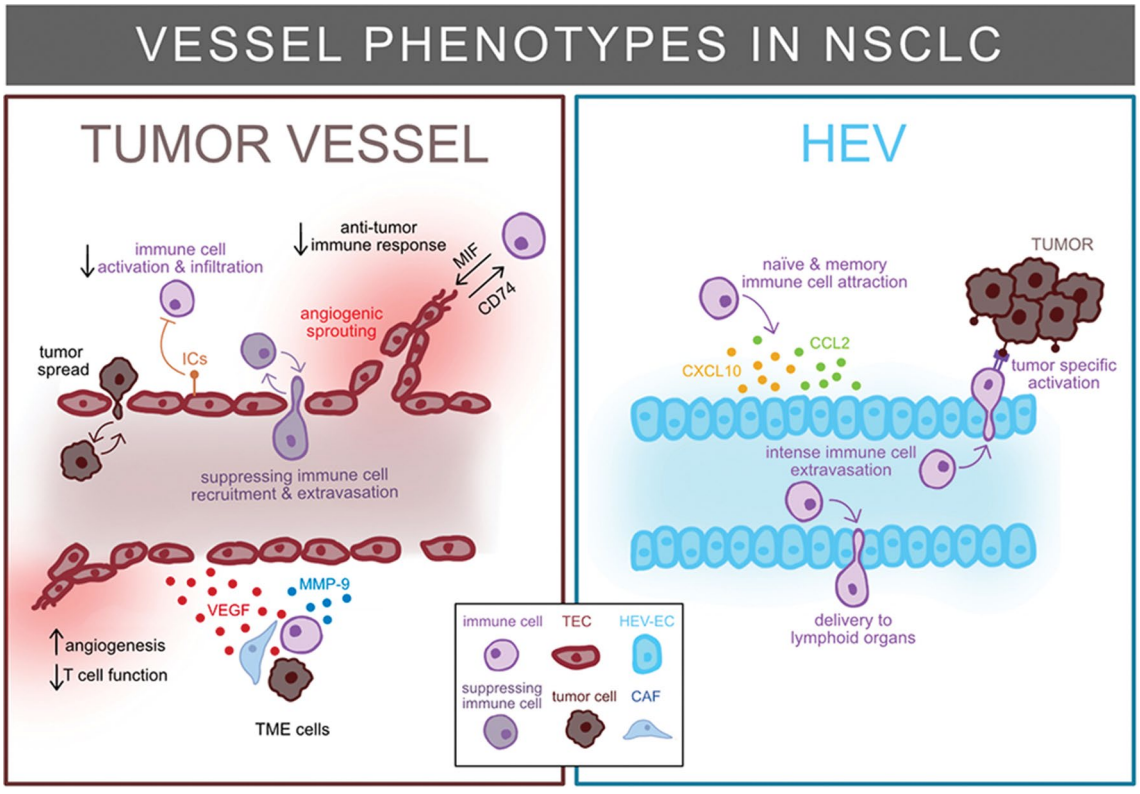

Fig. 3 Vessel phenotypes within the NSCLC TME. Different vessel phenotypes arise in the NSCLC TME that can either promote disease progression (tumor vessels) or enhance the local anti-tumor immune response (high endothelial venules (HEVs)). Tumor vessels are dysfunctional and unable to fulfill functions essential in healthy tissues. They exhibit downregulated immune response mechanisms and increased angiogenesis being not capable to regulate exchange between the circulation and the underlying tissue, which promotes tumor cell spread and infiltration of suppressing immune cells. The elevated requirement of vascularization in the TME results in differentiation into highly proliferative phenotypes such as tip EC. HEVs can develop from vein ECs and form an extraordinary node to boost anti-tumor immune response through recruitment and activation of local immune cells

Table 1 Marker genes for EC subtypes

EC Classification	Subtype	Subdivision	Marker genes	References
Panendothelial			*PECAM1, CDH5, CLDN5, ERG, PODXL, ST6GALNAC3, GALNT18, CAV1, CAV2, CAVIN1, CAVIN2, ITGA5, LAMB2, PRK2, SASH1, LDB2, PTK2, STARD13, FLI1, SOX18, ROBO4, TIE1, S1PR1*	[4, 5, 15, 16]
Healthy lung ECs	capillary		*CA4, HLA-II, PRX, RGCC, SGK1*	[3–5]
		general	*VWFpos/EMCNlow/EDN1, GPIHBP1, APLNR, IL7R, FCN3, BTNL8, CD14, IL18R, LPL, SLC6A*	[3, 4, 15, 16]
		aerocyte	*VWFneg/EMCNhigh/EDNRB, APLN, HPGD, SOSTDC1, TBX2, SPON2, PRKG, CHRM2, S100A3, S100A4, EDA*	[3, 4, 6, 15–17]
	venous		*ACKR1, VCAM1, VWF, CPE, CCL15, CCL23, SELP, NR2F2*	[3–5, 15, 16]
		systemic	*COL15A1pos* *EBF1, EBF3, MEOX1, MEOX2, ZNF385D, TACR1, ROBO1, CYSLTR1, CPXM2, MMP16, PDE7B, PDE2A, SPRY1*	[4]
		pulmonary	*COL15A1neg* *DKK3, PTGS1, PTGIS, C7, PLAT, PROCR, THBD, C1R, EFEMP1, CDH11*	[4]
	artery		*GJA5, DKK2, BMX, EFNB2, SOX17, CXCL12, FBLN5, IL33, SEMA3G, HEY1, LTBP4*	[3–5, 15, 16]
	lymphatic		*PROX1, CCL21, PDPN, LYVE1, TFF3, MMRN1, AKAP12, FABP4, SNCG, GYPC, PPFIBP1, EFEMP1, FLT4, SEMA3A, SEMA3D, TBX1, HOXD3, NR2F1, GPR182*	[3–5, 7, 15, 16]
Tumor ECs	tip		*CXCR4, ANGPT2, FSCN1, PGF, ADM, PDGFB, LAMA4, LAMC1, LAMB1. SPARC, LXN, VIM, MARCKS, MYH9, MYO1B, CD93, MCAM, ITGA5, TCF4, SOX4, SMAD1, COL4A1, COL4A2, COL18A1, IGF2, IGF1R*	[3, 18, 19]

30]. In mouse brain capillaries, it was demonstrated that ECs located near arrested circulating tumor cells undergo cellular remodeling, actively promoting tumor cell extravasation [29]. Furthermore, it is suggested that circulating tumor cells may gain a survival advantage and show higher metastatic potency after encountering mechanical forces present in the capillary bed [31]. While playing a role in mediating tumor metastasis, Caps also contribute to tumor immune surveillance by actively recruiting natural killer (NK) immune cells to the TME *via* expression of CCRL2 [32]. Functioning as antigen-presenting cells, immune-cell recruiters and cytokine-sensors, Caps, with their unique morphology, actively contribute to immune regulation and cancer progression within the TME. Compared to other EC phenotypes, Caps are more susceptible to transcriptional changes upon hyperoxia in the murine lung, and display the highest response to stress and inflammation in COPD patients [33, 34]. Moreover, the expression patterns of Caps in lung tumors are induced by cancer-derived cytokines, such as downregulation of MHC-II, indicating that this EC population may be particularly sensitive to environmental perturbations [3].

Caps exhibit remarkable versatility across various functions in the lung. Not only do they supply tissues with oxygen and nutrients, they also provide the entry-gate for immune and tumor cells, they present antigens and secrete signaling molecules, fostering both tumor surveillance and growth.

Venous ECs

The second largest population in the human lung endothelium is represented by venous ECs, constituting ~ 25% [4–6], which are further subdivided into pulmonary venous ECs ($COL15A1^{neg}$) and systemic venous ECs ($COL15A^{pos}$), accounting for ~ 12% and ~ 13% of ECs, respectively [4, 6]. Pulmonary veins and venules are, in contrast to arteries, thin walled vessels that transport oxygenated blood in the large airways and visceral pleura as part of the systemic circulation and transport oxygen-poor blood in the lung parenchyma for oxygen-exchange as part of the pulmonary circulation [4, 35, 36]. Venous ECs were identified as the main population for angiogenic sprouting and vessel expansion during embryonic development, as well as in a pathological setting [37]. Doing so, venous ECs migrate upstream of the blood flow and differentiate into diverse subpopulations such as tip ECs, arterial ECs and Caps, accounting for ~ 80% of the local ECs as shown in murine models of retinal vasculature [37].

Besides being the source for endothelial expansion, venous ECs administrate different functions based on their location within the lung vasculature. Systemic venous ECs are associated with the expression of *COL15A1*, a gene regulating the adhesion of connective tissue to the basement membrane, along with genes involved in regulating vessel permeability, angiogenesis and leukocyte migration (*vWA1*, *PLVAP*) [4]. In general, postcapillary venules serve as the primary gateway for leukocyte extravasation. In contrast, pulmonary venous ECs are associated with genes involved in prostaglandin synthesis (*PTGS1*, *PTGIS*), coagulation and complement cascade [4]. While shear stress dampens endothelial migration and proliferation, low shear stress in venous vessels promotes sprouting of ECs. Consequently, the origin of excessive growth in pathological settings, such as arteriovenous malformations or oxygen-induced retinopathy, can be ascribed to the venous EC population [4, 37, 38]. Notably, neo-angiogenesis driven by hypoxia-induced VEGF expression arises exclusively from the venous endothelium in oxygen-induced retinopathy [37].

The population of venous ECs within lung tumors exhibits inconsistencies across studies. Goveia et al. and Salcher et al. observed higher numbers of venous ECs in tumor tissues compared to the healthy lung, whereas Sikkema et al. identified no alteration in the overall count, despite substantial changes within its subpopulations [3, 5, 6]. The proportion of pulmonary venous ECs decreased from ~ 48% to ~ 14%, whereas the proportion of systemic venous ECs increased from ~ 52% to ~ 86% of total vein ECs in lung tumors. Of note, vein subclusters were solely depicted by Sikkema et al., albeit with limited numbers of tumor cells compared to healthy cells. Nonetheless, pathological lung conditions, such as COVID-19 or idiopathic pulmonary fibrosis, showed an enrichment of systemic venous endothelium compared to healthy controls, suggesting a pivotal role for this EC subpopulation in damage repair after pulmonary circulation injuries [24]. Consistent with these findings, freshly isolated venous ECs from lung tumors displayed elevated expression of ribosomal genes and immunomodulatory factors (*POST*, *CCL14*, *ACTN1*), identifying them as dynamic phenotypes capable of differentiating into various vascular cell types [3]. Moreover, AATs such as VEGF-inhibitors showed less efficacy in venous ECs and Caps but could induce an high endothelial venule (HEV) phenotype similar to postcapillary veins [3].

With their high proliferative and adaptive potency, dynamic venous ECs may contribute to tumor-induced angiogenesis in large parts, and may potentially be more difficult to target with traditional AATs. The fact that these cells represent a major part of the vasculature in lung tumors underscores the urgency to deeper characterize this population and its function in the NSCLC TME. Their capacity to transition into HEV-EC phenotypes during inflammation, on the other hand, could present a promising mechanism within the anti-tumor response [39].

Artery ECs

The artery endothelium represents ~ 11% of ECs in the healthy adult lung [3–6]. Arteries, subjected to higher shear force and pressure, play pivotal roles in stress response pathways and vascular tone regulation [4, 40]. Schupp et al. identified artery ECs as the most active EC population in terms of secretory capability and expression of signaling molecules. They tightly interact with pericytes, alveolar fibroblasts and smooth muscle cells (SMCs) through the vasoconstrictive EDN1/EDNRA and CXCL12/CXCR4 axis [4]. In the artery wall, arterial ECs are in close proximity to a variety of neighboring cells, which may cause an elevated signaling activity in contrast to more segregated vein ECs or Caps [41]. In pulmonary artery hypertension, especially artery ECs – besides Caps – gain functions as semi-professional antigen presenters by upregulating MHC-II (without CD80/86) and are associated with vascular remodeling [42, 43]. Pulmonary arteries display unique organotypic variations compared to arteries of other organs, emphasizing a specialized adaptive potential shaping their signaling responses. In pulmonary arterial and arteriolar ECs, the activation of p53 induces a vasculo-regenerative program, contrasting its effects in other vascular beds [44–48]. As *TP53* is the second most frequently mutated gene in lung cancer (present in 46% of all cases), this phenomenon may impact cancer progression, highlighting the crucial interplay between anatomical location and functional behavior of ECs [49].

In lung tumors, the proportion of arterial ECs is comparable to healthy tissue, and no specific tumor artery EC function has been identified thus far. In prostate cancer, we previously described a distinct tumor arterial EC phenotype exhibiting a high signaling activity that could be linked to decreased survival and elevated tumor angiogenesis most profoundly through the interaction between artery ECs and tip ECs along the CXCL12/CXCR4 axis [19]. *CXCL12*, a gene associated with tumor aggressiveness, exhibited its highest expression in artery ECs, inducing proliferation and migration in angiogenic tip cells through binding their cognate receptor CXCR4.

Although artery ECs only comprise a small subpopulation, they may possess a potent signaling activity in the NSCLC TME and modulate neighboring cells of various types. This contribution to tumor angiogenesis and the passive influence on immune regulation, as shown in prostate cancer, suggest that the role of tumor arterial ECs might be underestimated, and this EC subtype potentially serves as a signaling hub during tumor progression.

Lymphatic ECs

Lymphatic ECs (LECs) constitute ~ 14% of ECs in the healthy lung and ~ 10% of ECs in lung tumor tissue, primarily building the draining route for interstitial fluid and immune cell transport to the lymph node or collecting vein [3–6]. Like other EC phenotypes, LECs are primarily derived from embryonic venous cells [50–53] and can be identified by elevated expression of lymphangiogenesis markers (*PROX1*, *CCL21*, *VEGFR3*) [54, 55]. Moreover, they are associated with gene expression for extracellular matrix remodeling, angiogenesis, cell migration and MHC-I expression [8, 56]. In the lung, LECs express high levels of CCL21 as well as SEMA3A, signaling molecules required for guiding dendritic cells (DCs) into secondary lymphoid organs and lymphatic vessel maturation by CCR7-mediated recruitment [4, 57]. LECs exhibit high cell-to-cell interaction activity, including most notable cell types, such as antigen-experienced T cells (especially $CD4^+$ T cells), B cells, DCs, vascular ECs as well as alveolar and nonparenchymal fibroblasts. Additionally, LECs contribute to intercellular signaling by secreting PDGFA, connecting them with pericytes and SMCs [4, 58].

Tumor LECs do not exhibit differential gene expression compared to healthy LECs in single-cell analyses [3, 6], nevertheless, the lymphatic system plays a crucial role in tumor progression and dissemination by acting as a passageway to lymph nodes or distant organs. In NSCLC, lymphangiogenesis correlates with an active lung cancer phenotype, elevated metastasis formation and poor overall survival (OS) [3, 59–61].

LECs exhibit a multifaceted role in immune regulation and serve as a transport route for immune and tumor cells, acting as recipients of signals that allow adaption of their cellular functions in a tumor-promoting manner. While vascular ECs have traditionally been the focal point in tumor angiogenesis, LECs are essential contributors to cancer progression and may contribute to metastasis formation in aggressive and high-grade tumors [62]. Thus, in the development of AATs, due consideration should be given to the involvement of LECs to enhance therapy response.

Tumor EC phenotypes

All the above-mentioned EC phenotypes contribute to tumor progression in lung cancer. Apart from that, other EC phenotypes occur that are almost exclusively found in the TME (such as tip, immature and postcapillary venous TECs) with distinct molecular patterns and gene signatures which constitute the dysfunctional endothelium present in tumor lesions (Fig. 3). To understand the molecular differences between normal and tumor vasculature, TECs have been studied for years [63]. Although it is not fully understood how the TME induces a shift from healthy to TEC phenotype, a recent study highlighted loss of endothelial FOXF1 expression, a critical regulator

of vessel stabilizing Wnt/β-catenin signaling, to be profoundly involved in the EC-to-TEC transition [64].

Tip ECs, representing the leading tip cell in growing vessel sprouts, are mainly found in tumor tissue due to highly angiogenic conditions. Their presence was linked to poor clinical outcome in different cancer cohorts, including lung cancer, and displayed the most prominent differences in proportions between the normal and tumor endothelium [3, 65]. In lung cancer, they comprise ~ 8% of all TECs (in healthy lung ~ 0.07% of all ECs), but are considered as a transient cell state, rather than a distinct genetically predefined subpopulation [3]. Tip ECs are identified by the expression of pathways linked to an activated and angiogenic phenotype, such as VEGF, Notch and Wnt/β-catenin signaling, collagen modification, protein secretion, migration and matrix remodeling [3, 65]. Tip ECs inhibit anti-tumor immune response by interacting with immune cells *via* the MIF/CD74 axis, recruiting tumor-associated macrophages (TAMs) or suppressing T cell activation and infiltration [65]. In addition, they increase vascular permeability through negative regulation of cadherin-mediated cell adhesion [65–67]. As a highly angiogenic and tumor-promoting phenotype, tip ECs are a primary target of AAT, such as VEGF inhibitors. VEGFR blockade transitions them into a more mature and quiescent phenotype during vessel normalization [3].

Immature TECs, resembling stalk-like ECs (proliferating ECs following a leading tip cell), show similar gene expressions to tip ECs with additional upregulation of genes required for vessel maturation and barrier integrity (*EGN*, *PLVAP*, *HSPG2*, *APLNR*). They are highly angiogenic and associated with worse OS [3].

In lung tumors, postcapillary venous TECs upregulate markers of HEVs that are normally found in lymphocyte-rich areas in inflamed tissues. HEVs represent the primary gateway for lymphocyte extravasation in cancer and correlate with better clinical outcome, resembling "hot" tumors [68–70]. VEGF-blockage promotes the HEV phenotype in lung cancer ECs, potentially providing an additional beneficial effect in the context of vessel normalization within the NSCLC TME [3].

Interactions of ECs with other cell types of the TME

Increasing availability of scRNA-seq data provides a better understanding of the endothelium in the lung and enables uncovering its highly heterogenous subpopulations. Moreover, these data also help to decipher the complex interaction-network present in the TME, therefore providing detailed insights in the process of oncogenesis on a cellular level. Due to their essential role in forming a barrier between airways and bloodstream – mediating gas exchange and being among the first cells to encounter pathogens in the lung – ECs require intensive and intricate communication with other cell types. They often serve as the initiating point for numerous downstream signaling pathways and act as intermediaries between the air and lung tissue. By representing an important signaling hub, the endothelium is essential for stability and equilibrium in tissue function but vice versa, due to this integral attribute, is also capable to drive the development and progression of cancer. The direct secretion of various signaling molecules and the release of exosomes or extracellular vesicles (EVs) play a pivotal role in steering intercellular communication within the TME [71]. The secretion of VEGFA, one of the main targeted pathways in AAT, is observed in a variety of cell types, including tumor cells, fibroblasts, myeloid cells and pericytes. More precisely, the primary source of VEGFA is provided by tumor cells and myeloid cells, among them macrophages, monocytes, mast cells, DCs and (tumor-associated) neutrophils [65].

EC and stromal cell interaction

As mentioned before, artery ECs engage in cell-to-cell interactions, communicating with surrounding SMCs and pericytes, promoting their differentiation and proliferation [4]. A pathway between ECs and pericytes/SMCs is the EDN1/EDNRA axis, leading to vasoconstriction [4]. Apart from that, TECs exhibit strong associations with cancer-associated fibroblasts (CAFs), presenting up to 5-fold increase in interaction pathways compared to healthy ECs [72, 73]. This is mostly due to their close relationship through endothelial-to-mesenchymal transition, switching cell types *via* TGF-β and SMAD signaling [74, 75]. Furthermore, CAFs themselves are capable of inducing angiogenesis *via* VEGF. When examining intercellular relations between different subsets of ECs and fibroblasts, it was discovered that tip ECs had robust interactions with four out of eleven distinct subsets of fibroblasts. These interactions were primarily mediated by molecules such as PGF, VEGFA, PDGF, and their cognate receptors [73]. Also, CAFs cultured from human oral squamous cell carcinoma were found to secrete small EVs containing VEGF, activating VEGFR2 on ECs in a bevacizumab-resistant manner [76]. An alternative pathway through which CAFs promote angiogenesis is *via* increased expression of WNT2, as observed in colorectal cancer [77].

EC and tumor cell interaction

The main interaction between various types of ECs and tumor cells consists of VEGF signaling, providing vascular supply to hypoxic cancer centers [72]. Traditional mechanisms of tumor cells inducing and enhancing neovessel growth have been long known and intensively described elsewhere [78].

Throughout different cancer therapy approaches, resistance to commonly used drugs remains an unmet medical need. Several resistance mechanisms stem from interactions between TECs and tumor cells. These interactions may lead to treatment failure, in both directions: from ECs to tumor cells, causing resistance to chemotherapy, or from tumor cells to ECs, resulting in resistance to AATs. For example, ECs in prostate cancer were shown to be implicated in docetaxel resistance by expressing basic fibroblast growth factor (FGF2), activating ERG, and initiating downstream Akt/mTOR signaling in tumor cells [79]. Additionally, it was discovered that stearoyl-CoA desaturase-1 (SCD1), expressed by tumor cells, and fatty acid binding protein-4 (FABP4), produced by TECs within the TME, play a crucial role in cooperatively hampering ferroptosis and leading to tumor relapse following treatment with TKIs or chemotherapy [80].

Recent findings suggest that tumor cell-induced angiogenesis activation *via* EVs (like in CAFs) also plays a role in anti-VEGF therapy resistance [81, 82]. Similarly, miRNA-1246, found in tumor EVs, enters ECs and consequently suppresses androgen receptor expression, resulting in the secretion of IL-6. This in turn leads to multiple drug resistance through the autocrine activation of the STAT3 and Akt pathways [83].

High interactive engagement of LECs with cancer and stromal cells of the TME was observed in recent studies [84–86]. For example, LEC-derived CXCL5 in head and neck tumors promotes tumor cell migration and invasion [86]. Other findings suggest that the entry of tumor cells into lymphatic vessels is facilitated by breaks in cell junctions, reducing important extracellular matrix proteins such as fibrillin, collagen and biglycan, thereby increasing intercellular gaps in the endothelium [87, 88]. On the other hand, the secretion of CCL27/28 by tumor cells recruits LECs into the TME through binding of the CCR10 receptor [89].

When it comes to metastasis, the endothelium actively induces tumor cell latency *via* Wnt-signaling alongside other pathways, creating a tumor-suppressive niche. However, proliferative circulating metastatic tumor cells induce a phenotypic shift in ECs towards a primary TEC-like state, promoting local biomass production, systemic immunomodulatory functions, tumor cell proliferation and subsequent metastatic outgrowth [90].

EC and immune cell interaction

It has been well-established that immune cells, particularly myeloid cells, actively engage in promoting angiogenesis [91, 92]. Various components of the innate immune system exhibit pro-angiogenic properties by directly releasing VEGF or expressing MMP-9, a metalloproteinase that can liberate and activate matrix-bound VEGF [91]. To date, no MMP-9 inhibitor is applied in clinical routine, but clinical trials are ongoing [93]. TAMs and neutrophils interfere with neo-angiogenesis, with primarily M2 macrophages, a pro-angiogenic and tumor-promoting phenotype, mediating the sprouting of new vessels [91, 94]. TAMs were found to stimulate tumor vessel growth, showing a significant correlation between *CD68*, a pan-macrophage marker, and *CD105* (endothelin), a marker gene for dysregulated vessel formation [25]. Exosomes play a significant role in the bidirectional macrophage-to-endothelium crosstalk. For example, M2-macrophage derived exosomes containing miR-155-5p and miR-221-5p stimulated angiogenesis by inhibiting *E2F2* – a gene reported to hamper angiogenesis – through microRNA (miRNA) regulation [94].

Neutrophils influence TEC integrity and promote tumor angiogenesis *via* various pathways, like VEGF, MMP-9, oncostatin M or IL-17 [95]. Furthermore, their release of neutrophil extracellular traps, comprising DNA-histone complexes and proteins, contributes to the downregulation of tight junctions on ECs in hepatocellular and gastric carcinoma. This process facilitates tumor cell migration and intravasation, underscoring the multifaceted role of neutrophils in shaping the TME [96, 97]. Moreover, the downregulation of the EC-specific marker endomucin (EMCN) in tumor and metastatic tissues has been shown to promote the formation of a pre-metastatic niche by enhancing the recruitment of tumor-promoting N2 neutrophils through ECs in a TGF-β-dependent manner [98].

DCs can also foster pro- and anti-angiogenic features, depending on their differentiation level or origin [91]. Within tumor tissues, plasmacytoid DCs predominate, which is a subtype markedly demonstrating pro-angiogenic and tumor-promoting properties, as observed in ovarian cancer [99]. Notably, LECs interact with their surrounding immune cell compartment in various ways. Their expression of CCL21, which binds CXCR3 and CCR7 on DCs and B cells, guides these cells to secondary lymphoid organs [4]. The secretion of IFN-γ by tumor-specific $CD8^+$ T cells suppresses their accumulation and response in the TME by upregulating PD-L1 on LECs [100, 101]. Upon cancer therapy with taxanes (e.g. docetaxel), LECs conversely increase lymphangiogenic factors such as TNF-α and VEGFC, and thereby promote permeability and metastasis formation [102, 103].

TECs participate in interactions with adaptive immune cells, with predominant involvement of tip ECs, particularly in relation with mast and B cells, observed in the NSCLC TME [104]. Novel discoveries indicate that TECs possess immunologic properties, with identified ligand-to-receptor pathways facilitating immune cell recruitment or acting as immune checkpoints [4, 105]. Lung artery ECs express CXCL12, a ligand to CXCR4, found on various lymphoid cells, triggering migration, homing

and survival of respective immune cells [4]. CXCL12 was previously identified as a TEC marker in prostate cancer [19]. TECs are known to express immune checkpoints, even the impact of therapeutics on this endothelial immune checkpoint expression has been investigated [106]. Moreover, analysis of scRNA-seq datasets has shown that healthy ECs are among the strongest expressors of PD-L1 within the NSCLC TME, suggesting a yet uncovered role in T cell shutdown [107]. In head and neck carcinoma, TECs were found to express high levels of NECTIN2 and Galectin-9. Their respective receptors are TIGIT and HAVCR2, both primarily found on T cells, NK cells and macrophages, and, in the case of HAVCR2, also on DCs [108]. The interactions between TIGIT-NECTIN2 and HAVCR2-Galectin-9, constituting two immune checkpoint ligand-to-receptor pathways, contribute to immunosuppression by decreased activation of immune cells. Additionally, TECs were observed to express tumor Galectin-1, which leads to an increase in Galectin-9 and PD-L1, suppressing immune cell activation. Therapeutic inhibition of Galectin-1 in mice has shown promising results, improving the response to immune checkpoint inhibition (ICI) [109]. These immunosuppressive TEC characteristics are in line with a loss of CD80/CD86 expression in ECs, rendering them unable to activate T cells *via* CD28 [3].

Of note, circulating ECs – detached ECs in the blood stream – are established biomarkers for diagnosis and prognosis in various cancer entities, including NSCLC, and may also engage in immune checkpoint activity [110]. Aneuploid circulating TECs (CTECs), for example, found in histopathologically PD-L1-negative cancers, expressed high levels of PD-L1 leading to a worse prognosis compared to PD-L1-negative CTECs [111].

Immune response in ECs

The highest immunoregulatory feature within the endothelium is currently attributed to postcapillary venules, although other EC subtypes also substantially contribute to the host's immune response. Upon inflammation, signaling molecules, such as TNF-α or IL-1, lead to EC activation and increased expression of cell surface adhesion molecules such as E-selectin, VCAM-1 and ICAM-1, and contribute to leukocyte attraction and tissue infiltration [112]. Furthermore, ECs are capable of recruiting immune cells *via* the expression of cytokines such as CXCL10 and CCL2 during active inflammation, a capability that can be enhanced by the differentiation of activated ECs into HEVs [105]. Besides their role as immune cell attractors, ECs also present antigens on MHC-I and MHC-II molecules, primarily activating antigen-experienced T cells [3, 105]. While CD80/CD86 expression on murine ECs enables naïve T cell activation in a context dependent manner, their expression on human EC could so far only be observed in vitro [113–123]. However, human ECs express another co-stimulatory ligand, ICOSL, which was shown to interact with naïve T cells *via* CD28, yet its binding is insufficient to elicit their activation [124–126]. This limited ability of co-stimulating naïve T cells demonstrates that the complex nature of immunological functions of ECs is not conserved between species. In the last decade, ECs were also identified to share functions of innate immune cells, including the ability to sense danger molecules such as pathogen-associated molecular patterns and damage-associated molecular patterns and EVs, leading to subsequent immune activation. ECs also exhibit phagocytic functions and express receptors for T cell co-stimulation/inhibition [127–129].

High endothelial venules (HEVs)

HEVs develop in secondary lymphoid organs and are specialized, fully integrated blood vessels adapted for immune cell recruitment, including monocytes, plasmacytoid DCs, neutrophils, B and T cells, and their trafficking [130]. As a first step in homeostatic lymphocyte trafficking, mature ECs of HEVs connect with CD62L (L-selectin) on immune cells through peripheral node addressin (PNAd), which is expressed and maintained in response to LTαβ signals derived from DCs and lymphocytes [131, 132]. HEVs regulate immune response by delivering naïve and memory lymphocytes from the circulation to antigen-presenting cells present in lymphoid organs [133]. In conditions of chronic inflammation (such as autoimmune diseases or cancer), HEVs also develop outside lymphoid organs, referred to as tertiary lymphoid structures (TLSs), which harbor dense T and B cell areas organized in a lymph node-alike anatomy [134]. The new formation of HEVs facilitates tissue-specific lymphocyte recruitment and activation, and is generally associated with prolonged survival in various cancers, including NSCLC [135, 136]. This is also reflected by immunotherapy-induced murine tumor regression due to reduction of $Foxp3^+$ regulatory T cells and recruitment of naïve T cells into the tumor *via* TLSs [137–140]. ScRNA-seq analysis revealed cellular and spatial heterogeneity of HEVs, suggesting that their intricate response to inflammation extends beyond mere lymphocyte recruitment and activation, encompassing additional functions [141].

Endothelial immune dysregulation in cancer and other diseases

Regarding the important role of ECs as immune regulators, endothelial dysfunction causes increased pathogenicity in lung diseases, particularly cancer. Recent studies have uncovered various mechanisms through which ECs contribute to an immunosuppressive environment. Passively, the loss of the endothelial barrier integrity,

observed in lung cancer and facilitated by tumor angiogenesis, results in "leaky" vessels, fostering uncontrolled trafficking of immune and tumor cells through the vasculature [142]. The highly glycolytic tumor endothelium, coupled with elevated VEGF signaling, induces hypoxia and acidosis, impairing effector T cell activity while promoting the recruitment and formation of suppressive immune cells, such as regulatory T cells and myeloid-derived suppressor cells [143–146]. Additionally, TECs in lung cancer actively suppress the anti-tumor immune response through the downregulation of molecules mediating immune cell recruitment (ICAM-1), and antigen presentation (MHC-I/II) as well as pro-inflammatory molecules (IL-6, CCL2, and CCL18). Moreover, they upregulate immune-inhibitory molecules, like PD-L1, and the cell death regulator FasL, inducing apoptosis in $CD8^+$ T cells, with an overall failure to exert the anti-tumor effects necessary to eradicate the tumor [7, 147, 148]. Especially in early stages of lung adenocarcinomas, it has been shown that endothelial PD-L1 expression plays a key role in tumor progression by facilitating regulatory T cell activation [149].

Recent discoveries highlight that autophagy of TECs represents another mechanism of immune evasion in cancer. In a murine melanoma model, impaired autophagosome formation in TECs led to the expansion of effector T cells and elevated inflammatory functions due to enhanced NF-kB and STING signaling. Furthermore, melanoma patients with TECs exhibiting high inflammation and low autophagy showed positive correlations with anti-PD-L1 therapy response [150]. In line with these findings, autophagy of ECs in acute inflammation was identified as negative regulator of leukocyte trafficking, including neutrophil tissue infiltration [151]. Besides cancer, disease severity could be associated with endothelial dysfunction in COVID-19, as ECs of patients in need of intensive care were highly activated and disrupted due to reduction of E-cadherin expression, which resulted in hyperpermeability coupled with substantial immune dysregulation [152]. ECs were shown to be directly but unproductively infected by SARS-CoV-2, yet their over-activated immune response during acute COVID-19 promotes pathogenesis by upregulating ICAM-1 and pro-inflammatory cytokines while losing barrier integrity due to reduced CD31 expression [153, 154]. Autoimmune diseases, such as rheumatoid arthritis, may also be linked to antigen presentation of ECs on MHC-II molecules [155].

EC dysfunction emerges as a hallmark of various diseases, associated with chronic inflammation, including cancer, lethal infections and autoimmune syndromes, thereby limiting the immune response. On the other hand, specialized ECs, such as HEVs, function as local "immune-hubs", crucial for inducing therapy-induced anti-cancer immunity, particularly in patients exposed to anti-checkpoint monoclonal antibodies. Recognizing this multifaceted role of the endothelium as an immune regulator necessitates a re-evaluation of current anti-tumor immunotherapies, emphasizing the inclusion of ECs more prominently in the development of novel treatments and combination therapy approaches.

Targeting the tumor vasculature in NSCLC

AATs, such as bevacizumab, nintedanib and ramucirumab, are approved for first-line or second-line NSCLC combination therapy, and were clinically developed in a pre-immunotherapy era [9–11, 156–159]. Limitations of AATs include the evolvement of primary and secondary resistance mechanisms, leading to short OS benefits. Nevertheless, as resistance to novel ICI remains a therapeutic obstacle, VEGF inhibition has been shown to increase response to ICI therapies [156, 160]. Ongoing trials are exploring novel combination therapies, such as pairing AAT with ICI. The ultimate goal is to neutralize the immunosuppressive activity of VEGF, thereby altering the immunosuppressive milieu within the TME, that at least in part depends on excess levels of VEGF [161].

The emergence of TLSs in the TME, characterized by the clustering of B cells, T cells, DCs and HEVs, contributes to improved response to immunotherapy [162]. Furthermore, resistance to anti-PD1 treatment has been associated with elevated VEGF levels when compared to optimal responders, indicating a potential role of VEGF in immunotherapy resistance [163]. The power of this combinational approach targeting both VEGF and immune-checkpoints is exemplified by the results of the Impower150 trial (ClinicalTrials.gov identifier: NCT02366143) [156]. Here it was shown that the addition of atezolizumab to bevacizumab – targeting PD-L1 and VEGF, respectively – *plus* carboplatin-paclitaxel (ABCP) improved progression-free survival (PFS) and OS in patients with metastatic non-squamous NSCLC, regardless of their PD-L1 expression status when compared to therapy with ICI or AAT *plus* chemotherapy alone.

Novel approaches in combination therapy of advanced NSCLC

In a recent phase II randomized study evaluating the combination of ramucirumab *plus* pembrolizumab (RP) – a VEGFR2 antagonist *plus* a PD-1 inhibitor – in NSCLC patients previously treated with platinum-based chemotherapy and ICI, OS was compared between two study arms (ClinicalTrials.gov identifier: NCT03971474). One arm received ramucirumab *plus* pembrolizumab, while the other received standard of care, consisting of two thirds receiving ramucirumab *plus* docetaxel, and one third docetaxel, pemetrexed or gemcitabine. Hence,

AAT was added to the previous ICI scheme after therapy failure. RP has demonstrated a statistically significant improvement in OS, with a median of 14.5 (13.9 to 16.1) months, compared to 11.6 (9.9 to 13.0) months in the standard of care group [164].

Similarly, the KEYNOTE-495/KeyImPaCT trial provided interim results on biomarker-directed, first-line pembrolizumab-based combination therapies for previously untreated stage IV NSCLC comparing pembrolizumab *plus* lenvatinib/quavonlimab/favezelimab, respectively (ClinicalTrials.gov identifier: NCT03516981). Lenvatinib is a multi-kinase inhibitor targeting VEGFR1–3 (as well as PDGFRA, FGFR1–4, RET and KIT), thus acting as an anti-angiogenic compound, while quavonlimab and favelizumab act as ICI, targeting CTLA-4 and LAG3. In fact, the combination of pembrolizumab and lenvatinib achieved the highest overall response rate with 35%. In comparison, the combinations of pembrolizumab *plus* anti-CTLA-4 quavonlimab and pembrolizumab *plus* anti-LAG3 favezelimab performed less favorably with an overall response rate of 25.6%, 23.3% and 29.4%, respectively [165]. Additionally, it was shown that response rates correlated with chosen biomarkers, such as a high tumor mutational burden and a T cell-inflamed gene expression profile.

Collectively, these studies support the hypothesis of AATs as a foundational strategy in conjunction with immunotherapy, addressing treatment resistance in advanced NSCLC.

Novel therapeutic strategies targeting ECs

Besides traditional VEGF inhibition, a growing number of novel agents targeting neo-vessel formation are being investigated, with many currently undergoing testing at the pre-clinical and clinical levels (Table 2). The conserved protein QKI, essential for vessel-development and sprouting, was shown to play a critical role in vessel formation and function as well as tumor spread as loss of QKI significantly impaired these processes, leading to improved outcomes in NSCLC mouse models [166]. Similarly, TEC-derived cadherin-2 (CDH2) was shown to upregulate VEGF-related signaling pathways, simultaneously downregulating leukocyte rolling and attachment molecules (L-, E-, and P-selectin) as well as EC-specific adhesion molecules [167]. As CDH2 expression is frequently elevated in invasive cancers and implicated in tumor-stromal interactions, it represents a promising therapeutic target for cancer treatment through inhibition strategies [168–171]. However, research is still in early stages and further studies are required to fully evaluate its therapeutic potential.

A new projection seeks to merge ICI and anti-angiogenesis within one single bispecific antibody, AK112 (ivonescimab), simultaneously targeting PD-1 and VEGF. Intriguingly, this combination leads to higher affinity, as pre-clinical studies could demonstrate an improved avidity to PD-1 during the presence of VEGF [172]. Furthermore, it was engineered with alterations to the Fc region to abolish antibody-dependent, cell-mediated and complement-dependent cytotoxicity [173, 174]. In first-line treatment for advanced NSCLC, AK112 *plus* chemotherapy demonstrated encouraging anti-tumor efficacy for patients without driver mutations, as well as for patients with EGFR-activating mutations who had previously not responded to EGFR-TKI therapy. Additionally, AK112 exhibited promise in advanced NSCLC patients who had previously experienced treatment failure with systemic platinum-based chemotherapy and PD-1/L1 inhibitors [175]. A similar study could also confirm better PFS for ivonescimab *plus* chemotherapy than chemotherapy

Table 2 Novel tumor vessel targets across different phases of investigation

Target/Pathway	Mechanism of action	Testing stage	Outcome	References
VEGF + PD-1 inhibition	bispecific antibody AK112 (ivonescimab) with dual inhibition of angiogenesis and ICs	*clinical*	anti-tumor effect in patients with and without driver mutations	[173, 175–177]
Endostatin + ICI	inhibition of angiogenesis and ICI	*pre-clinical and clinical*	reduced tumor growth in NSCLC	[180, 181]
DNMT1 inhibition + ICI	inhibition of angiogenesis and increased immune cell homing	*pre-clinical and early clinical*	improved immune cell migration and reduced angiogenesis	[186]
QKI inhibition	inhibition of metastasis and vessel function	*pre-clinical*	improved outcomes with reduction in metastases	[166]
STING activation	tumor vessel disruption and enhanced drug delivery precision *via* nanoparticle ZnCDA	*pre-clinical*	disrupted tumor vessels and improved drug targeting	[179]
Anti-angiogenesis miRNA transfection	transcriptome modulation to inhibit angiogenesis	*pre-clinical*	—	[83, 182–185]
Aerobic glycolysis inhibition	inhibition of aerobic glycolysis in TECs	*pre-clinical*	reduced immunosuppression	[190]
CDH2 inhibition	inhibition of angiogenesis while improving immune cell homing and EC adhesion	*pre-clinical*	—	[167]

alone in NSCLC patients with progressive disease undergoing EGFR-TKI-treatment [176]. Recently, the HARMONi-2 trial was presented testing AK112 against pembrolizumab as first-line treatment for patients with PD-L1-positive advanced NSCLC [177]. The study demonstrated that AK112 significantly improved PFS, with a median of 11.14 months compared to 5.82 months for pembrolizumab, highlighting its potential as a new standard of care in this context. Also, it was shown that ivonescimab monotherapy could reach comparable efficacy to PD-L1-inhibitors *plus* chemotherapy in advanced NSCLC [173]. All studies showed a tolerable safety profile for AK112 [173–177].

Apart from this, the question remains whether additional signaling pathways or intercellular communication networks between TECs and other cells within the TME, independent of VEGF signaling, can be utilized as therapeutic targets to enhance existing strategies. Many of those efforts made to tackle TECs in the TME still have not progressed beyond a preclinical level, while some interesting observations have already been made. For example, subtypes of TECs have been discovered to mediate nanoparticle transport into solid tumors [178]. This was applied when designing the nano-formulated STING activator ZnCDA, which specifically triggers EC activation, resulting in the disruption of tumor vasculature and enhanced precision in drug delivery. Nanoparticles hold promise for elevating therapeutic effectiveness while reducing the occurrence of unintended side effects [179].

Furthermore, ongoing research continues to explore the anti-angiogenic endostatin pathway and its potential applications in NSCLC. Combination of ICI and recombinant endostatin (rh-endostatin) showed promising results by suppressing tumor growth in murine lung cancer models [180]. This combinational approach has been tested for second-line treatment in advanced NSCLC and yielded greater PFS, OS as well as less adverse events than ICI *plus* chemotherapy [181].

TECs communicate with their surrounding network through the expression of various signaling molecules and the release of miRNAs in EVs. Pro- and anti-angiogenic miRNAs have been identified, suggesting the possibility of using both anti-angiogenic miRNAs and inhibitors of pro-angiogenic miRNAs as anti-tumor treatments [83, 182–184]. However, charge repulsion and high susceptibility to serum RNAse destruction limit the cellular absorption of miRNAs or antagonists [184]. Efforts are underway to overcome this problem, developing viral and non-viral (e.g. lipid-based or EV-based) transfection systems [185]. Another approach targeting TECs involves the use of DNA hypomethylating agents. Inhibition of DNA methyltransferase 1 (DNMT1) results in decreased neo-vessel growth and increased $CD8^+$ T cell extravasation, further enhancing ICI efficacy, due to enhanced expression of cell adhesion molecules and chemokines in ECs [186]. However, a study evaluating the effects of an azacitidine (CC-486)/durvalumab combination (DNA hypomethylating agent *plus* anti-PD-L1 antibody) in poor-responsive, immunologically "cold" tumors (microsatellite stable colorectal cancer, platinum resistant ovarian cancer and estrogen receptor positive, HER2 negative breast cancer) did not find enhanced response rates or other clinical benefits [187]. Additionally, the treatment regimens showed poor tolerance (possibly due to the cytotoxicity of azacitidine [186]) and low demethylation levels in tumor biopsies [187]. Similar studies were terminated due to a lack of efficacy [188]. More studies analyzing the potency of DNMT1-inhibitors combined with ICI in solid tumors are currently ongoing (e.g. ClinicalTrials.gov identifier: NCT02957968, NCT05673200). TECs do not only present altered genetic features compared to healthy ECs, they even foster a different metabolism within the TME [189]. This can also be targeted when designing novel anti-angiogenic drugs. For example, TECs show hyperglycolysis with high levels of GAPDH, which was observed to be reduced by application of osimertinib, a commonly used EGFR-TKI [190]. This suggests that the efficacy of osimertinib is not only mediated through the direct inhibition of EGFR in mutated tumors but also through the dampening of aerobic glycolysis in TECs as an off-target effect, thus leading to reduced immunosuppression which may enhance the efficacy of immunotherapies. Conversely, several studies comparing EGFR-TKIs (erlotinib, osimertinib) *plus* bevacizumab *versus* EGFR TKI monotherapy in NSCLC showed modest improvements in PFS, however no improvements in OS could be generated [191–195].

Conclusion

The intricate interactions between TECs and various components of the TME play a crucial role in tumor progression and therapeutic responses in NSCLC (Fig. 4).

Recent scRNA-seq analyses have revealed the highly heterogeneous endothelium in tumor development, challenging the traditional approach of targeting ECs as a single entity and emphasizing the need to understand distinct EC populations as integral components of a complex, functionally diverse network. The divergent contribution of different EC subtypes to tumor behavior now clearly elucidates the limitations of conventional AAT.

Caps represent a large proportion of lung ECs which are crucial for maintaining the pulmonary function. Additionally, they are sensors and regulators of the immune system and involved in anti-tumor immune response as well as cancer spread. The fact that the lung harbors increased numbers of these specialized Caps compared to other organs, proposes the need to study

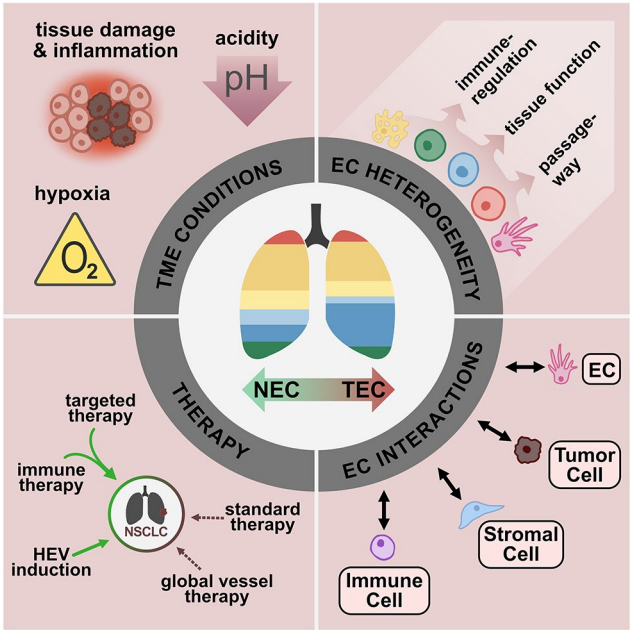

Fig. 4 Dynamic landscape of NEC and TEC in NSCLC. The phenotypic cell state of ECs in lung cancer is influenced by diverse factors which can impact the treatment approaches. Discussed scRNA-seq data revealed a versatility of EC subpopulations, executing essential tasks such as immune regulation, providing tissue functions or serving as passageway for local and peripheral cells in the lung. Challenging environmental conditions present in the TME such as tissue damage and inflammation, acidity and hypoxia induce tumor promoting TEC phenotypes, elevating the EC diversity present in tumor tissue, which may hamper cancer treatment efficacy. The fact that ECs are a highly alert cell population that senses and (re-) shapes the microenvironment via interactions with other ECs or resident cells such as tumor, stromal or immune cells, exemplifies the complexity of targeting tumor vessels in NSCLC. Consequently, alternatives to standard therapies or more globally-focused therapies aiming at tumor vessels must be introduced to yield better outcomes and bypass therapy resistance. Promising candidates comprise combinational approaches using immunotherapy and targeted therapy or the promotion of favorable EC phenotypes such as HEVs

this EC phenotype in the setting of NSCLC more thoroughly. Venous ECs represent the driving proliferative force of vessel formation in health and disease. Together with their dynamic phenotype and highly adaptive potency, they comprise a challenging target using available cancer therapies. Conversely, HEV formation from vein ECs signifies a phenotypical change, favorable during inflammation. Artery ECs extensively interact with their environment, actively driving tumor vascularization as shown in prostate cancer. By sensing environmental perturbations, they can reshape the TME *via* their capability to activate vascular remodeling and angiogenesis. LECs represent a double-edged sword within solid tumors. Influenced by the TME, they adopt tumor promoting features that facilitate tumor cell migration and invasion, while being essential for a proper immune response against cancer by recruiting and guiding immune cells.

While healthy tissues clearly distinguish functional features of EC subpopulations, the EC hierarchical organization is diminished or even lost in tumors. Caps, for example, exhibit disturbed differentiation in NSCLC, leading to an ambiguous functional characterization within the TME. The altered milieu in lung tumors harbors challenging conditions such as hypoxia, inflammation or an acidic environment, giving rise to peculiar EC phenotypes that either contribute to the promotion of lung tumor growth (e.g., tip/immature ECs) or support anti-tumor immune responses (HEV ECs). Hypoxia and tissue damage activate a regenerative EC phenotype in Caps and vein ECs, leading to elevated proliferation and angiogenesis. Chronic inflammation can promote the development of TLSs, where HEVs establish a local node assisting the anti-tumor immune defense. Fully understanding the mechanisms of TLS formation to therapeutically drive HEV development would present a promising approach within vascular-focused cancer therapy. Bidirectional communication between ECs and tumor cells promotes resistance to standard therapies, further complicating treatment outcomes. Additionally, EC-mediated immune regulation within the TME highlights their critical role in fostering an immunosuppressive microenvironment, impacting disease progression and therapeutic responses.

Targeted therapies, particularly the combination of AATs with ICIs, present a promising avenue for enhancing treatment efficacy in NSCLC. Recent clinical trials demonstrated the potential of combinational approaches in improving patient outcomes, while ongoing clinical trials explore the possible advantages of novel combinational approaches, including the use of bispecific antibodies and the integration of nanoparticles for precise drug delivery and enhanced therapeutic efficacy. Furthermore, the identification of alternative signaling pathways and intercellular communication networks involving ECs within the TME highlights potential therapeutic targets beyond VEGF modulation. Tailored approaches targeting specific EC subtypes and their interactions hold promise for developing more effective and personalized treatment regimens. Overall, continued investigation into the multifaceted role of ECs and their adaptive potential, dynamic states and interactions with the TME is crucial for the development of innovative therapeutic strategies to overcome resistance and improve patient outcomes in the management of NSCLC.

Abbreviations

AAT	Anti-Angiogenic Therapy
aCap	aerocyte Capillary Endothelial Cell
CAF	Cancer-Associated Fibroblast
Cap	Capillary Endothelial Cell
DC	Dendritic Cell
EC	Endothelial Cell
EV	Extracellular Vesicle

gCap	general Capillary Endothelial Cell
HEV	High Endothelial Venule
ICI	Immune Checkpoint Inhibition
LEC	Lymphatic Endothelial Cell
miRNA	microRNA
NK	Natural Killer Cell
NSCLC	Non-Small Cell Lung Cancer
OS	Overall Survival
PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Cell Death Ligand 1
PFS	Progression-Free Survival
TKI	Tyrosine Kinase Inhibitor
scRNA-seq	single-cell RNA sequencing
SMC	Smooth Muscle Cell
TAM	Tumor-Associated Macrophage
TEC	Tumor Endothelial Cell
TLS	Tertiary Lymphoid Structure
TME	Tumor Microenvironment
VEGF	Vascular Endothelial Growth Factor

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