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RELEVANCE OF ANNEXIN A1 AND CD70 IMMUNO-ONCOLOGICAL MARKERS IN SMALL CELL LUNG CANCER

PhD thesis

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List of Abbreviations

ANOVA: analysis of variance	GAPDH: glyceraldehyde-3-phosphate dehydrogenase
ANXA1: annexin A1	GSK3 β : glycogen synthase kinase 3 beta
ASCL1: achaete-scute homolog 1	H&E: hematoxylin and eosin
AT2: alveolar type 2	HIF1: hypoxia-inducible factor 1
AURKA: aurora kinase A	HR: hazard ratio
CAF: cancer-associated fibroblast	HRP: horseradish peroxidase
CAR-T: chimeric antigen receptor T cell	ICI: immune checkpoint inhibitor
CKK-8: Cell Counting Kit-8	IF: immunofluorescence
CD: cluster of differentiation	IgG: immunoglobulin G
CVCL: Cellosaurus Cell Line	IHC: immunohistochemistry
CS: citrate synthase	INSM-1: insulinoma-associated protein 1
CTLA-4: cytotoxic T-lymphocyte-associated protein 4	LDHa: lactate dehydrogenase A
DAPI: 4',6-diamidino-2-phenylindole	L-DOPA: L-3,4-dihydroxyphenylalanine
DC: dendritic cell	mAb: monoclonal antibody
DFS: disease-free survival	MAPK: mitogen-activated protein kinase
DLL3: delta-like ligand 3	MDSC: myeloid-derived suppressor cells
DMEM: Dulbecco's Modified Eagle Medium	MHC: major histocompatibility complex
DMSO: dimethyl sulfoxide	NADH: nicotinamide adenine dinucleotide
EGFR: epidermal growth factor receptor	NCAM: neural cell adhesion molecule
EMT: epithelial-mesenchymal transition	NCCN: National Comprehensive Cancer Network
ERK: extracellular signal-regulated kinase	NCI: National Cancer Institute
FADH2: reduced flavin adenine dinucleotide	NEUROD1: neurogenic differentiation 1
FFPE: formalyn-fixed paraffin-embedded	NK cell: natural killer cell
FPR: formyl peptide receptor	NSCLC: non-small cell lung cancer

pAb: polyclonal antibody	SLFN11: schlafen family member 11
PD-1: programmed cell death protein 1	SMARCA: SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A
PD-L1: programmed cell death-ligand 1	SqCLC: squamous cell lung carcinoma
PFS: progression-free survival	TAM: tumor-associated macrophage
PNEC: pulmonary neuroendocrine cell	TCA: tricarboxylic acid
POU2F3: POU class 2 homeobox 3	TMA: tissue microarray
PVDF: polyvinylidene difluoride	TMB: tumor mutational burden
RB: retinoblastoma	TME: tumor microenvironment
RFU: relative fluorescence unit	TPS: tumor proportion score
RRID: Research Resource Identifier	YAP1: yes-associated protein 1
SCLC: small cell lung cancer	
shRNA: short hairpin RNA	
SIRP α : signal regulatory protein alpha	

1.Introduction

1.Small Cell Lung Cancer

Lung cancer is the leading cause of cancer-related mortality worldwide. The broadest classification of lung cancer is based on microscopic morphology, distinguishing non-small cell lung cancer (NSCLC) from small cell lung cancer (SCLC) (Figure 1). NSCLC accounts for approximately 85% of cases and includes tumors with diverse morphological features, such as adenocarcinoma, squamous cell carcinoma and large cell carcinoma, whereas SCLC represents about 10–15% of cases and is characterized by small, relatively uniform blue cells [125, 140, 143, 151]. In addition to the “pure” form, the World Health Organization (WHO) defines a “combined” SCLC subtype, accounting for 20–30% of cases and features an NSCLC component, although this distinction has no impact on current treatment strategies [127, 143]. The NSCLC-SCLC grouping also highlights SCLC from other types of lung cancer with its outstandingly bad prognosis, with its five-year survival rate below 7% [125, 139, 143, 151]. At least 70% of patients are first diagnosed with SCLC when distant metastasis is present [140, 151]. Clinical symptoms, such as dyspnea, cough, hemoptysis and paraneoplastic syndromes become apparent due to the location and bulk of the tumor [80, 140, 151]. SCLC is one of the most strongly linked malignancies with smoking, also reflected in its epidemiological incidence [125, 127, 140, 151]. SCLC is officially recognized as a recalcitrant cancer under the Recalcitrant Cancer Research Act of 2012 [143, 151]. This federal initiative classified recalcitrant cancers (specifically SCLC and pancreatic cancer) as those associated with a 5-year survival rate under 20% and a mortality rate of 30 000 deaths in the United States yearly, and aimed to promote focused research efforts to accelerate research and improve outcomes [150].

Besides imaging modalities (X-ray, CT, brain MRI, PET-CT) and laboratory studies, a biopsy or cytology should be obtained with bronchoscopy, mediastinoscopy or guided transthoracic needle biopsy to confirm diagnosis. Diagnostic procedures cannot delay treatment for more than a week due to aggressivity of the disease [47, 140].

SCLC is further subclassified as a neuroendocrine tumor of the lung, belonging to the poorly differentiated, high-grade neuroendocrine carcinoma group [140]. As first called „lymphosarcoma” and later „oat cell carcinoma”, SCLC consists of small, blue cells with scant cytoplasm, finely granular „salt and pepper” chromatin and inconspicuous

nucleoli [127]. Crush artifacts and nuclear molding are common; therefore, cytology is an important modality to reduce the impact of these. Hallmarks of the histological presentation also include very high mitotic rate and extensive necrosis [127, 143].

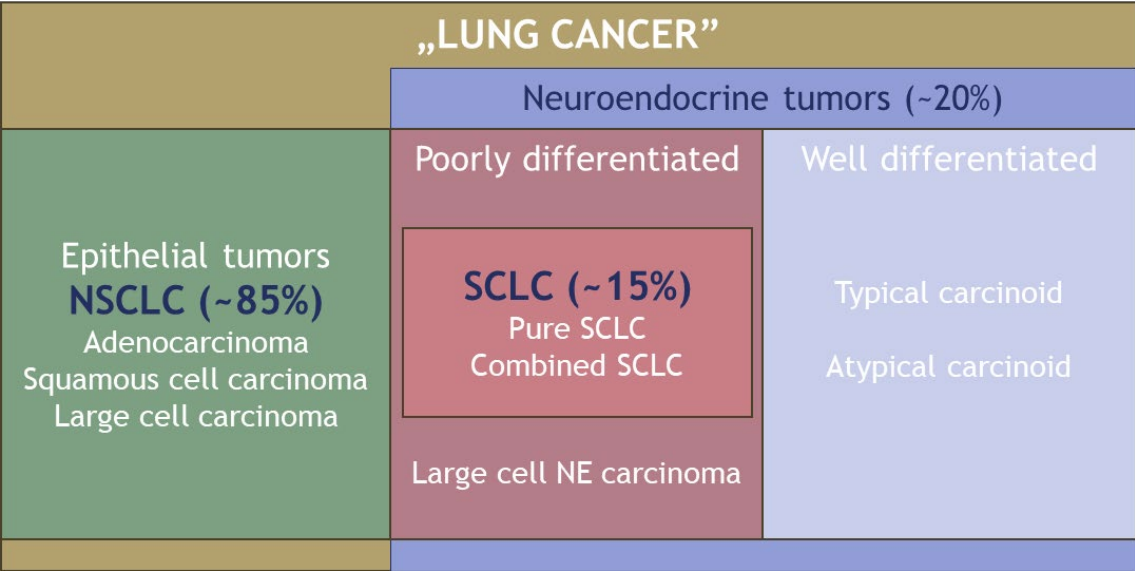


Figure 1. Place of SCLC in the classification of lung tumors. *The broadest categorization distinguishes NSCLC and SCLC [71, 80, 143], while 20% of primary lung neoplasms are neuroendocrine tumors, [156] the majority of which are SCLC, representing ~15% of all lung cancers. For detailed classification, see the 2021 World Health Organization (WHO) Classification of Thoracic Tumors (5th Edition) [109].*

The cellular origin of SCLC is mainly the pulmonary neuroendocrine cells (PNECs), but it can also arise from basal cells, club cells, cuboidal alveolar type 2 cells and Tuft cells, also known as brush cells [28, 127]. Moreover, “transformed” SCLC may derive from NSCLC or other epithelial cancers, such as prostate cancer, further complicating etiological origin [151]. Expression of neuroendocrine differentiation markers, such as neural cell adhesion molecule (NCAM), chromogranin, synaptophysin, insulinoma-associated protein 1 (INSM-1) are variable in SCLC, or they can be even negative, which is also a basis of further subclassification, as discussed later [127]. More frequently expressed markers are low molecular weight cytokeratins, thyroid transcription factor 1 and p16, while high-molecular weight cytokeratins, napsin and retinoblastoma 1 (RB1) are almost always negative [28, 127, 140].

Clinical significance of SCLC is pronounced with the difficulties in therapeutic advancement. Basis of therapy in first-line setting is a chemotherapy regimen of a

platinum-based combination (preferably cisplatin) with etoposide applied to all patients [143, 151]. For over three decades, no improvement was made in therapy. Clinically, the most important distinction depends on the extent of the disease, which classifies patients into limited-stage (LS) or extensive-stage (ES) (Figure 2). This classification determines whether a patient is eligible for radiotherapy at definitive, tolerable doses combined with chemotherapy—specifically, whether the disease can be contained within a radiation treatment field [49, 125, 151]. Only a minority of LS patients, about 5% of all patients, who do not have nodal involvement are offered surgery with adjuvant chemoradiotherapy [27, 92] explaining the low availability of tumor samples for research purposes. In summary, for most patients the only treatment option has been chemotherapy for decades. As a high-grade cancer, SCLC is characterized by great initial response to chemotherapy but has a high risk of recurrence as therapy-resistant disease, with sudden increase in molecular heterogeneity [31, 143]. Therefore, finding novel therapeutic modalities is critical.

On the other hand, treatment of NSCLC has been revolutionized in the last two decades with targeted therapies and immunotherapies resulting in significantly improved survival rates [71]. From the first approval of epidermal growth factor receptor (EGFR) inhibitors in the 2000s to the development of novel mutation-specific agents—such as adagrasib and sotorasib targeting KRAS G12C—multiple targeted treatment options are now available for patients with actionable genomic alterations [71, 134]. Therefore, biomarker testing is routinely done in NSCLC for EGFR and anaplastic lymphoma kinase (ALK) alterations and programmed cell death protein 1 (PD-L1) expression. In advanced stages, expanded molecular profiling may include KRAS, ROS1, BRAF, NTRK1/2/3, MET, RET, ERBB2, and NRG1 alterations to identify actionable driver mutations and guide the selection of personalized treatment strategies [71, 131]. Molecularly defined subgroups can receive first-line targeted therapies, such as the EGFR inhibitor osimertinib and the ALK inhibitor alectinib, which have largely replaced chemotherapy in these settings [71].

In SCLC, the most common genetic alterations are loss of function mutations of P53 (75-90%) and RB1 (50%), which are currently not actionable, but predispose cells for genomic instability and accumulation of oncogenic mutations [27], making SCLC an extremely heterogeneous disease genetically, that rapidly develops therapy resistance but

rarely contains targetable oncogenic drivers [151]. The first advancement in SCLC therapy has been the addition of immune checkpoint inhibitors (ICIs) to the first-line treatment of ES disease, resulting in a modest increase in overall survival (OS) with around two months [63, 95, 119, 133]. Accelerated approval, granted for pembrolizumab and nivolumab, has been later withdrawn because long-term follow-up studies failed to prove a superior OS of treated patients [34, 145]. Briefly, current guidelines include programmed cell death ligand-1 (PD-L1) inhibitors, durvalumab or atezolizumab in combination with chemotherapy for ES-SCLC patients and consolidation therapy with durvalumab in LS-SCLC [25, 49, 140, 151].

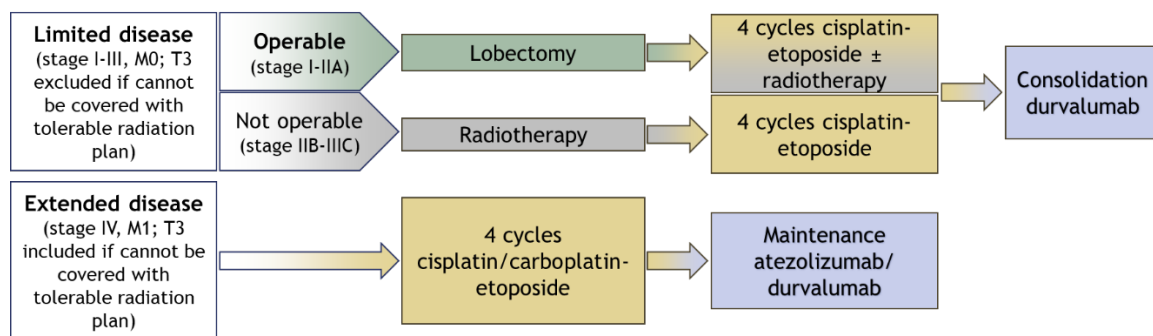


Figure 2. Simplified therapeutic guideline for first-line treatment of SCLC. *Current guidelines now incorporate PD-L1 inhibitors (durvalumab or atezolizumab) in combination with platinum-based chemotherapy for first-line treatment of both limited-stage and extensive-stage diseases. For details, see the National Comprehensive Cancer Network (NCCN) Guidelines [27, 49].*

A further great challenge to SCLC therapy is the lack of reliable predictive biomarkers. While smoking status, tumor mutation burden (TMB) and TPS (tumor proportion score measuring PD-L1 expression) can be used to predict ICI response in NSCLC [85], they have not proven effective for patient stratification in SCLC [80, 125]. Therefore, several clinical trials are currently in progress to identify novel immunotherapeutic strategies and refine predictive biomarkers. Recent research emphasizes the complex tumor microenvironment (TME) and pronounced heterogeneity of SCLC as key determinants of therapeutic resistance, and any new insight into these mechanisms may have relevance in this aggressive malignancy. [84, 125, 141].

1.2. Emerging molecular subclassification of SCLC

One of the major limitations in SCLC treatment over the past decades has been the recognition of the disease as a single entity, with a uniform therapeutic approach based solely on the tumor's extent. This contributed to the differences in contrast with NSCLC tumors, where characteristic histological subtypes, and later, mutually exclusive targetable mutations were identified [134]. Demand for subclassification to enable a personalized approach in SCLC, appeared in the 1970s, when SCLC cell lines were established and characterization identified two groups with potential clinical relevance [16, 28, 53]. The „classic” subtype showed floating growth in culture, forming spherical clusters, while the „variant” subtype grew either in loosely adherent clumps or as a tightly adherent single-cell layer in culture [134]. The morphologically distinct groups also corresponded with different neuroendocrine marker expression levels: the „classic” subtype expressed NE markers at high levels, while the „variant” subtype did not [53, 134]. Moreover, radioresistance and amplification of the proto-oncogene MYC were also associated with the “variant” subtype [53].

Since then, comprehensive, multimodal research on SCLC has focused on finding novel molecular subclasses with possibly different therapeutic vulnerabilities. Although no consensus has been reached, at least four molecular subtypes are widely recognized, based on the expressions of different dominant transcriptional drivers, broadly corresponding to neuroendocrine marker expression and immune phenotype (Figure 3). Tumors with high neuroendocrine marker expression („NE-high”) have achaete-scute homolog 1 (ASCL1, “SCLC-A”) or neuronal differentiation 1 (NEUROD1, “SCLC-N”) as dominant transcriptional drivers, while „NE-low” tumors have POU class 2 homeobox 3 (POU2F3, “SCLC-P”) or yes-associated protein 1 (YAP1, “SCLC-Y”) [6, 27, 52, 127, 134]. SCLC-P is a distinct subtype, originating from the Tuft cells [5, 127], while other types primarily originate from pulmonary neuroendocrine cells and other lung epithelial cells [127]. Recently, rather than representing a distinct subtype, YAP1 is increasingly recognized as a key regulator of SCLC plasticity, neuroendocrine dedifferentiation, and chemoresistance, and its expression may be dominant in tumor subpopulations across all molecular subtypes [6, 27, 143].

Regarding immunophenotype, as categorized in studies on the TME, NE-high tumors are considered “immune desert”, while NE-low and non-NE tumors are

considered “immune-oasis”, suggesting a potentially better outcome with immunotherapies in NE-low tumors [33, 34, 103, 141]. Among NE-low tumors, an “inflamed” subtype (SCLC-I) has been proposed as a fourth subtype, replacing the YAP1-defined subtype in the classification. It is characterized by the highest cytotoxic immune infiltration and immune checkpoint expression, while ASCL1, NEUROD1, and POU2F3 are absent [27], and YAP1 is usually increased [28, 141, 143]. This subtype has also been associated with improved responses to immunotherapy [151].

To view the molecular subtypes as even less rigid categories, it is important to note that, due to the high mutagenicity of SCLC, subtype transitions may occur throughout tumor development [28] and treatment [27, 151]. For instance, a widely recognized sequence involves a gradual shift from a NE-high state to a non-NE state, specifically transitioning from SCLC-A to SCLC-N and ultimately to SCLC-I, driven by the upregulation of MYC and activation of Notch signaling pathways [28, 122, 144]. Moreover, local expressions of immunomodulatory genes modulate immune infiltration [34, 143]. Such intratumoral diversity enables different molecular subtypes and “inflamed” subsets to coexist within a single tumor, making therapeutic development particularly challenging, as targeting vulnerabilities of one subtype alone fails to capture the biological complexity of SCLC [143].

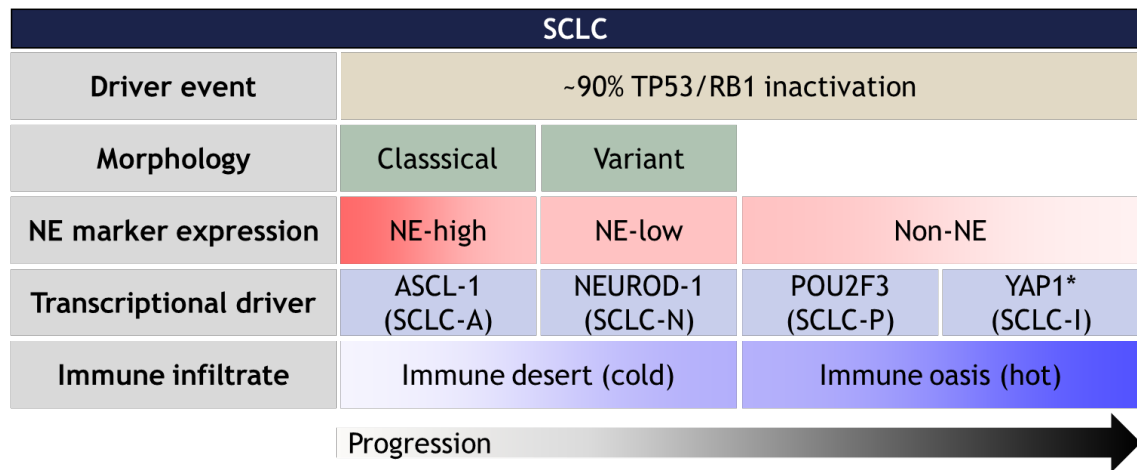


Figure 3. Simplified emerging subclassification of SCLC. *SCLC comprises at least four molecular subtypes corresponding to NE marker expression and immune infiltration states, from NE-high “immune-desert” to NE-low or non-NE “immune-oasis” phenotypes. Marked plasticity and subtype switching, both during tumor evolution and under therapeutic pressure, create intratumoral heterogeneity that limits the efficacy of*

targeted treatments. *: Recent classifications suggest an “Inflamed” subtype, which is associated with advanced stage, the highest immune infiltration and usually elevated YAP1. However, the YAP1 subtype was part of the original four–transcription factor–based classification and was included in our analysis. Reviewed in detail elsewhere [28, 47, 52, 103, 151].

1.3 Tumor microenvironment of SCLC

The tumor microenvironment (TME) is the complex network within a tumor, which includes cancer cells, immune cells, stromal cells, blood vessels, and the extracellular matrix, all interacting via signaling molecules to influence tumor growth, progression, and eventually response to therapy [13]. Through numerous mutually exacerbating mechanisms, SCLC is characterized by an adverse TME with poor vasculature, hypoxia and acidity that all have been linked to therapy resistance [4, 40]. Characterization of molecular subtypes indicates that the continuum of immune infiltration states is broadly inversely proportional to neuroendocrine marker expression. Accordingly, SCLC-N represents the most immunologically “cold” state, characterized by very low T-cell infiltration and reduced major histocompatibility complex I (MHC-I) and PD-L1 expression [88, 143]. At the other end of the spectrum, SCLC-I shows an immune-inflamed, “hot” phenotype, whereas other subtypes may display an “immune-excluded” pattern, with T-cells confined to the stroma and remaining at the periphery of the tumor nests [88]. Immunotherapeutic strategies aim to boost the anti-tumor response of immunogenic tumors by activating effector immune cells within the TME [151]. Therefore, components of the TME can be predictive of immunotherapy response, but none of them have proven clinically reliable in SCLC yet [125].

The TME in SCLC has potent immuno-inhibitory mechanisms, such as lower monocyte, dendritic cell (DC), natural killer (NK) cell and neutrophil granulocyte infiltration as compared to normal lung tissue [27]. Further mechanisms include downregulated major histocompatibility complex (MHC) I and II, lower PD-L1 expression, attraction of regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), M2-polarized macrophages [88], poor tumor infiltration of effector T-cells [27, 127], and upregulation of immune evasion molecules, such as CD47 [41]. Cancer-associated fibroblasts (CAFs) have been identified as key elements of the SCLC TME, by promoting transition to a non-NE fate [31]. In situ analyses confirmed that NE-low

tumors contain markedly higher CD45⁺ and CD3⁺ immune infiltration than NE-high tumors, with immune cells accumulating predominantly in the surrounding stroma rather than within the tumor nests [33]. Favorable TME features, such as increased immune infiltration, higher CD45⁺ and CD8⁺ T-cell densities, and elevated MHC-I expression have been shown prognostic in SCLC [27, 81, 127]. Consistently, biomarker analysis of tumor samples from large clinical trials showed that higher CD8⁺ infiltration and lower infiltration of tumor associated macrophages (TAMs) contributed to better survival in IMPOWER133 and CASPIAN trials, while higher expression of MHC molecules resulted in better response to anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA4) therapy [125, 143].

Previous work by Dora et al. aimed to characterize the TME of SCLC using immunohistochemistry (IHC), gene expression profiling, and pathway enrichment analyses in a cohort of limited-stage SCLC patients, and identified molecular clusters corresponding to NE-high and NE-low tumors, as well as immune-oasis and immune-desert phenotypes [34]. They demonstrated that M2-polarized macrophages are the most abundant cell population in the SCLC TME, and that local overexpression of immunomodulatory and inflammatory genes shapes localized immune microenvironments within the tumor, thus, despite increased immune infiltration, immunosuppressive molecules may be locally overexpressed [20]. Among these, annexin A1 (ANXA1) and the immunoregulatory ligand cluster of differentiation 70 (CD70) emerged as key molecules, with ANXA1 overexpressed in NE-low tumors irrespective of immune infiltration and CD70 elevated in NE-low immune-oasis tumors. ANXA1 expression was also higher in M2 macrophage marker CD163-high tumors of the cohort [34]. Together, these findings indicate that ANXA1 and CD70 might play central roles in shaping the immunosuppressive TME of SCLC, supporting their relevance for further mechanistic and translational studies.

1.4. Annexin A1 in physiology and pathology

Annexins are a superfamily of proteins ubiquitous in eukaryotic species, with the Annexin A subgroup including 12 members found in vertebrates. Annexin A1 (ANXA1), like all annexins, has the ability to bind phospholipids in a calcium-dependent manner and has several cell biological roles both closely related to membranes, such as vesicle trafficking, membrane repair and in other functions such as proliferation and angiogenesis

[51]. The diverse roles of annexin family members can be attributed to the differential regulation of gene expression and post-translational modifications, leading to variable expression levels across tissues and cell types, a phenomenon particularly evident for ANXA1 [55].

The best-characterized role of ANXA1 is physiological involvement in anti-inflammatory processes, it was first discovered to inhibit eicosanoid generation by phospholipase A2, acting as a glucocorticoid mimic [51]. In the innate immune system, ANXA1 inhibits leukocyte adhesion and extravasation. However, its complex role in the adaptive immune system is far from understood, greatly depending on specific cell types and activation states [51]. It is also described to play a role in resolving inflammation in ischemia-reperfusion injury and infectious diseases [55]. These effects are mediated through specific bindings of externalized ANXA1 to formyl peptide receptors (FPRs), a property not found in other annexins [55]. However, pro-inflammatory properties have also been reported, such as involvement in necroptosis and conflicting roles in autoimmune diseases, such as in multiple sclerosis (Figure 4) [51, 77].

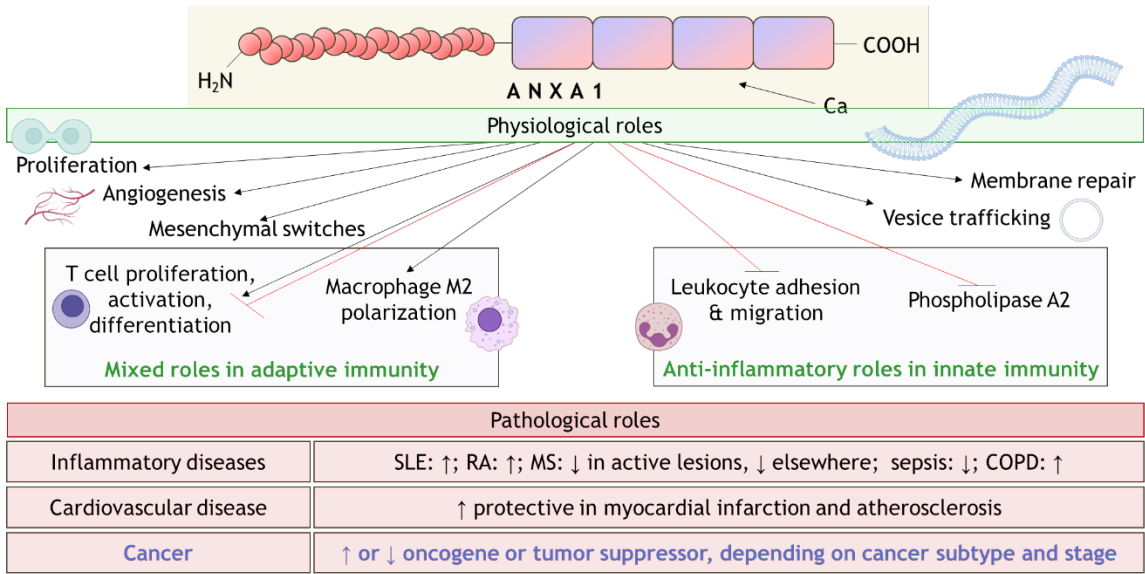


Figure 4. Context-dependent roles of ANXA1 in physiology and disease. *Receptor-mediated pathways (e.g., via FPR receptors) are not shown for simplicity; ANXA1 functions are also modulated by genetic alterations, post-translational modifications and secretion [51, 55, 77].*

Changed expression of ANXA1 in both directions was observed in several cancers with complex effects that can vary not even with tissue type but also with cancer subtype

and tumor stage. ANXA1 has been described as both a tumor promoter and a suppressor in lung adenocarcinoma [30, 58], bladder cancer [45], different types of breast cancer [29, 104] and gliomas [45, 137]. Still, there is more evidence linking upregulated ANXA1 expression to poorer prognoses in cancers, such as melanoma [12], hepatocellular carcinoma [91], gastric cancer [24, 45] and in general, in lung cancer. Previous experiments with knockdown of high ANXA1 expression in cancer cell lines have demonstrated reduced viability and proliferation in glioma [163] and squamous cell lung cancer cells [37]. Additionally, recent research has shown that an anti-ANXA1 antibody directly inhibited the growth of breast, pancreatic, ovarian, and colorectal cancer cell lines [2]. It can be concluded, although evidence is heterogeneous, that aberrant regulation of ANXA1 is involved in cancerogenesis [46].

ANXA1 was highlighted as a potential target in SCLC by Dora et al., showing increased expression in the NE-low subtype independent of overall immune infiltration. Moreover, elevated ANXA1 expression also correlated with higher M2 polarized macrophage marker CD163 expression, implying a link between ANXA1 and an immunosuppressive microenvironment [34]. Additionally, it was previously shown to facilitate immune evasion by regulating EGFR signaling, promoting mesenchymal switches and migration of fibroblasts and endothelial cells in pancreatic cancer [111].

Together, these findings justified a detailed characterization of ANXA1 in SCLC. For our *in vitro* functional studies, we selected SCLC cell lines based on published gene expression data by Dora et al. [34], choosing the NE-low lines with the highest ANXA1 expression, which are also characterized by YAP1 or POU2F3 as their dominant transcriptional drivers.

1.5. Hallmarks of the malignant phenotype

1.5.1. Warburg effect

The Warburg effect, also known as metabolic shift, is a hallmark of 70-80% of human cancers. In this process, cancer cells prefer aerobic glycolysis over oxidative phosphorylation, despite the presence of oxygen and functioning mitochondria [154]. This seemingly disadvantageous, “low-yield high-speed” adenosine triphosphate (ATP) production enables rapid generation of ATP and metabolic intermediates in tumor cells. This metabolic switch may be attributed to oncogenic mutations in transcription factors hypoxia-inducible factor 1 (HIF-1), the proto-oncogene identified in progression of

SCLC and NE-low subtypes, MYC [14] and the tumor suppressor gene mutated in 90% of SCLCs, P53 [65]. HIF-1 and MYC upregulate glycolytic enzymes, while loss of P53 relieves repression of glycolysis contributing to a rate of glycolysis about 10-40 times higher in tumor cells than in normal cells [154]. These events lead to increased glucose uptake, which phenomenon is utilized in a key diagnostic method of lung lesions, ¹⁸F-fluorodeoxyglucose positron emission tomography (FDG-PET) scans, where FDG is trapped in tumor cells by hexokinase-dependent phosphorylation [40].

Upregulated glycolytic enzymes include lactate dehydrogenase A (LDHa), which catalyzes the final step of glycolysis, converting pyruvate to lactate [65]. Accumulation of lactic acid contributes to acidification of the TME, which has been shown to promote angiogenesis, metastasis formation, therapy resistance, and immune evasion by affecting leukocytes of the TME [120]. Knockdown of LDHa was described to inhibit cancer cell proliferation [120] and to improve the TME by increasing CD8⁺ T cell infiltration [155].

A key self-promoting factor of the Warburg effect is low citrate concentration resulting from high metabolic turnover and dependence on glycolysis. Citrate is an intermediate of the tricarboxylic acid cycle (TCA cycle, Krebs cycle), produced by the enzyme citrate synthase (CS), from oxaloacetate and acetyl-CoA, finally producing reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH₂) through oxidative phosphorylation in the mitochondria [66]. Lower citrate levels not only promote glycolysis by relieving inhibition of glycolytic enzymes, but also activate oncogenic pathways such as phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), hypoxia-inducible factor 1 α (HIF-1 α), and Wnt/ β -catenin signaling, all of which contribute to tumor aggressiveness, epithelial–mesenchymal transition (EMT), and dedifferentiation [65]. In line with these, there is evidence for multifaceted effects of citrate in tumor biology [66, 90], for instance, treatment with citrate promoted apoptosis and synergized with cisplatin in A549 lung adenocarcinoma cells [129].

Simultaneous knockdown of HIF-1 and ANXA1 resulted in stopped proliferation in a gastric cancer cell line [132], suggesting a possible link between ANXA1 and metabolic dysregulation.

1.5.2. Epithelial-mesenchymal transition

Epithelial to mesenchymal transition (EMT) is both a physiological and pathological process, through which epithelial cells lose their polarity and adhesion

properties to gain migratory and invasive capabilities characteristic of mesenchymal cells [75]. This is seen in changed expression of EMT effectors: epithelial markers such as E-cadherin, cytokeratins and occludins become downregulated, while mesenchymal markers, such as vimentin, N-cadherin, fibronectin, alpha-smooth muscle actin (α -SMA) are upregulated (Figure 5) [152]. Physiological EMT occurs during development and wound healing, while the pathological form occurs during cancer invasion and metastasis formation. The Wnt/ β -catenin pathway is one of the many inducers of EMT and broadly a driver of cancer progression. In the absence of Wnt ligands, β -catenin is constantly degraded by a multiprotein complex and the ubiquitin proteasome system. When Wnt ligands bind to their receptors, the multiprotein complex is recruited to the cell membrane and disrupted, therefore, β -catenin accumulates and translocates into the nucleus, where it activates transcription of EMT effectors: genes of survival, proliferation, migration and differentiation [168]. Vimentin is a type III intermediate filament protein, mainly found in mesenchymal cells, and a key regulator of cellular integrity, attachment and migration, strongly linked to metastasis [152].

The diverse role of ANXA1 is depicted in the literature regarding EMT as well, promoting it in oral squamous cell carcinoma and pancreatic cancer [111, 114, 159] and suppressing it in nasopharyngeal carcinoma and breast cancer, for instance [101, 165].

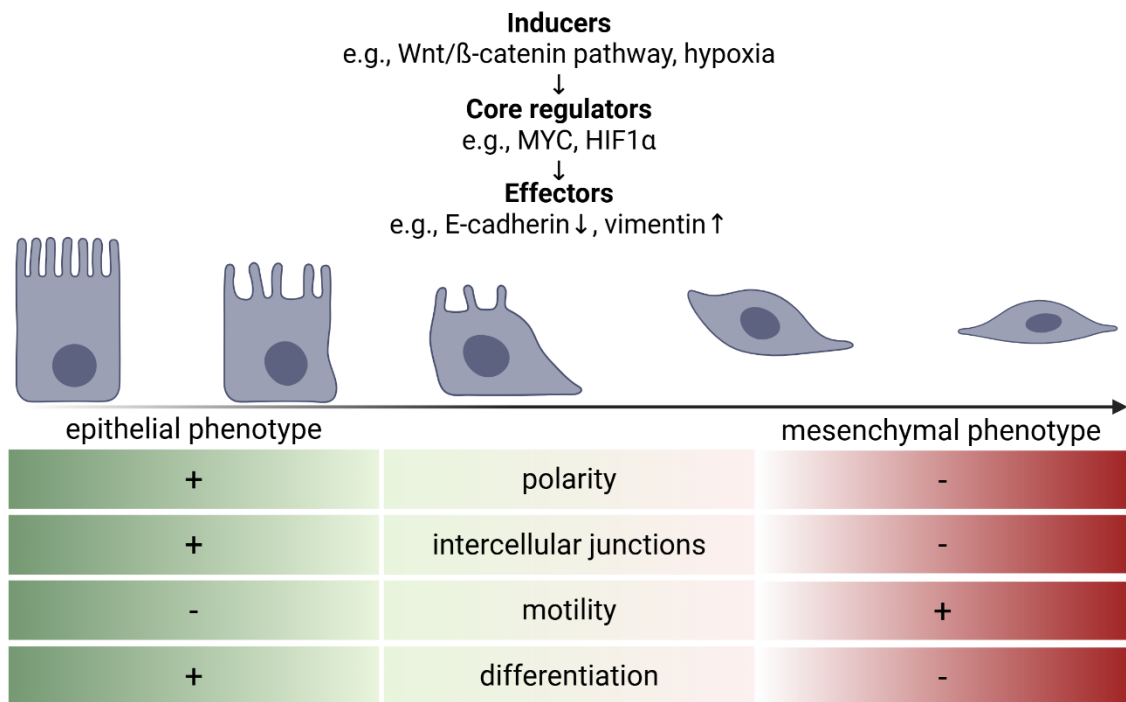


Figure 5. Simplified summary of the epithelial-mesenchymal transition. *Inducers such as signaling pathways activate core transcriptional regulators, which in turn control downstream effector molecules responsible for cytoskeletal reorganization and cell–cell adhesion changes. β -catenin and vimentin were analyzed in this study as representative mediators of EMT. [75, 152] Created with BioRender.com.*

1.5.3. Immune checkpoints: PD-1/PD-L1, CD47, CD70-CD27

Immune checkpoints physiologically act as switches for immune responses to maintain self-tolerance and prevent autoimmunity by inhibitory and co-stimulatory molecules, expressed on immune cells (Figure 6). Tumor cells can dysregulate this fine-tuned process in the TME by expressing these regulatory molecules to suppress anti-tumor immune responses [116]. Currently, PD-1 and PD-L1 inhibitors stand out as the most effective immunotherapeutic agents, that revolutionized treatment of many types of cancer in the 2010s, NSCLC being one of the most responsive tumor types [19, 130].

Programmed cell death 1 (PD-1) is an inhibitory molecule expressed on T cells after T cell receptor (TCR) stimulation. Its specific programmed death ligands, PD-L1 and PD-L2 are constitutively expressed by antigen-presenting cells (APCs) and can be induced in other cell types in response to pro-inflammatory cytokines. The PD-1/PD-L1 axis is a key physiological inhibitor of T cell hyperactivation by induction of T cell

exhaustion or apoptosis to maintain immune homeostasis. Cancer cells often upregulate PD-L1 to escape anti-tumor immunity and induce a TME that aids tumor growth and invasion (Figure 6A) [158].

In one of the largest international cohorts of surgically resected SCLC specimens, PD-L1 expression was generally low, predominantly present in tumor stroma, and was associated with better prognosis [81]. Literature supports that ANXA1 affects PD-L1 expression and T cell mediated killing in breast cancer and NSCLC [50, 164, 173].

The cell surface protein CD47 is ubiquitously expressed in four isoforms across various tissues. Its canonical physiological role is to support homeostasis of aging cells, erythrocytes, platelets, hematopoietic stem cells, and to facilitate synaptic pruning [1]. Acting as a “marker of self,” CD47 delivers a “don't eat me” signal by interacting with signal regulatory protein alpha (SIRP α) on myeloid cells, thereby inhibiting phagocytosis and serving as an immune checkpoint (Figure 6B). The gradual loss of CD47 expression is a key mechanism enabling efferocytosis, including the senescence of red blood cells. Correspondingly, CD47 upregulation is a recognized strategy for immune evasion in numerous cancers, including SCLC, as well as in atherosclerosis and fibrotic diseases [97, 121].

Inhibitors of the CD47-SIRP α axis are being investigated in clinical trials as potential next generation immunotherapeutics, particularly in NSCLC, where CD47 upregulation is associated with worse prognosis and EGFR mutations, which tend to be resistant to PD-1 blockade [72, 82]. Regarding SCLC, CD47 expression was detected in more than 80% of surgically resected SCLC tumors and was correlated with worse OS and disease-free survival (DFS) [81]. In a study investigating immunologic profiles of neuroendocrine lung neoplasms, SCLC was characterized by high CD47 expression, which correlated with poorer survival outcomes despite exhibiting a more immunogenic profile [41].

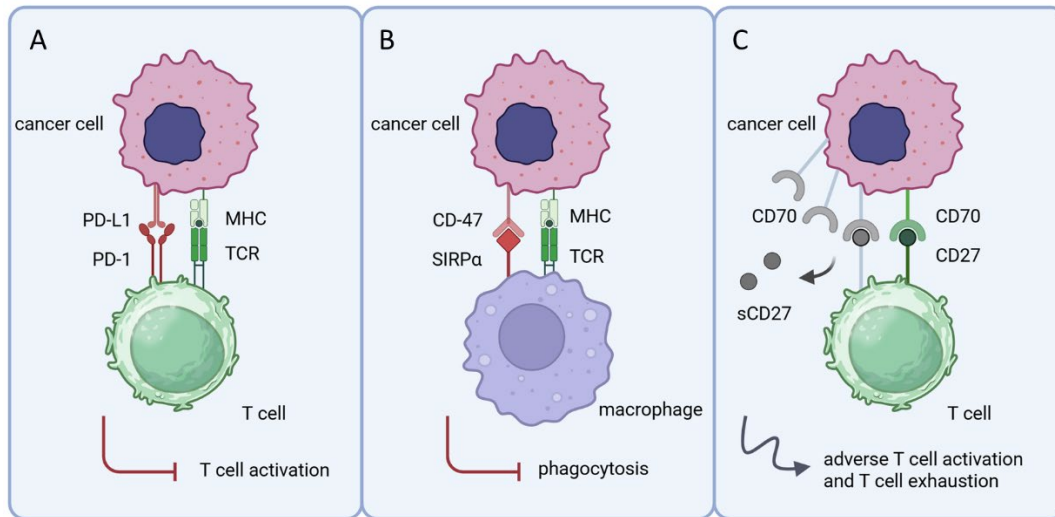


Figure 6. Schematic overview of immune checkpoints assessed in this study. Upregulation of the PD-1/PD-L1 axis **(A)** inhibits T cell activation, CD47–SIRPα signaling **(B)** inhibits phagocytosis, and the CD70/CD27 axis **(C)** drives pathological T cell activation and exhaustion, all contributing to tumor immune evasion by suppressing the anti-tumor immune response. [1, 44, 158]. Created with BioRender.com.

The CD70-CD27 ligand-receptor interaction is a co-stimulator of the immune response (Figure 6C). Physiologically, CD70 is only expressed transiently in activated T and B cells, mature dendritic cells, and constitutively on a subset of gut lamina propria APCs. Its specific receptor, CD27, is found on naïve T cells, memory T and B cells and a subset of NK cells [44, 70]. The ligand-receptor interaction physiologically induces signaling of immune cell proliferation, survival, differentiation and functions as a regulator in hematopoiesis [44].

Hematological malignancies were the first to be identified as upregulating CD70 and/or CD27 [44]. In contrast, cancer cells of solid tumors have only been associated with CD70 upregulation, CD27 expression can be increased within the TME of these cancers. Immune escape mechanisms of the aberrant CD27-CD70 pathway include induction and reduced apoptosis of regulatory T cells, apoptosis and exhaustion of effector T cells (Figure 6C), and depletion of NK cells. Involvement in M2 macrophage polarization [54], and promotion of migration and invasion by CD70⁺ CAFs have been also suggested [69]. Stromal CD27 has been associated with the cancer stem cell phenotype and EMT in NSCLC [110] and increased soluble CD27 plasma levels with worse prognosis [70].

Single-cell data from the Human Protein Atlas showed that both CD70 and CD27 are expressed in healthy lung tissue [32] and Dora et al. identified CD70 upregulation in both NE-low and immune oasis SCLC tumors in a cohort of 32 early-stage patients, highlighting its theranostic potential and context-dependent role in this malignancy [34].

2. Objectives

Therapy resistance and sensitivity SCLC remain poorly understood, making it essential to identify reliable biomarkers for currently approved treatments and to discover new characteristics that can aid in patient stratification, enable personalized therapies, and potentially serve as therapeutic targets. Based on prior findings [34, 35], upregulation of ANXA1 and the CD70-CD27 axis might be key factors of an immunosuppressive and tumor growth promoting microenvironment. Therefore, we aimed to comprehensively characterize ANXA1 in SCLC using cell line models, predictive algorithms and patient data [115]. Additionally, we evaluated the *in situ* expression of ANXA1 and the CD70-CD27 axis in SCLC and analyzed their potential effect on prognosis (Figure 7) [32].

To address Objectives 1 and 2 outlined below, we established an *in vitro* model where ANXA1 expression is stably silenced in three NE-low, ANXA1-high SCLC cell lines as a platform for our experiments.

- 1) **Functional effects of ANXA1 expression in SCLC.** To evaluate the impact of ANXA1 silencing on proliferation, chemosensitivity to standard cytotoxic agents; malignant phenotypic traits, specifically metabolic shift and EMT; and immune evasion-related mechanisms as indicated by PD-L1 and CD47 immune checkpoint expression.
- 2) **Potential roles of ANXA1 in SCLC subtype switches and TME composition.** To investigate whether ANXA1 silencing affects dominant transcriptional driver expression in the three NE-low ANXA1-high cell lines, and if differences occur in the cellular composition of the TME of ANXA1-high and ANXA1-low tumors.
- 3) **ANXA1 and the CD70/CD27 axis in the clinical context of SCLC.** To assess the *in situ* expression of ANXA1 and the CD70–CD27 axis in SCLC patient cohorts, including association of CD70 and CD27 expression with immune cell infiltration, and descriptive analysis of the spatial co-localization of ANXA1, CD70 and CD27 with TME–specific markers, and to evaluate the potential prognostic significance of ANXA1, CD70, and CD27.

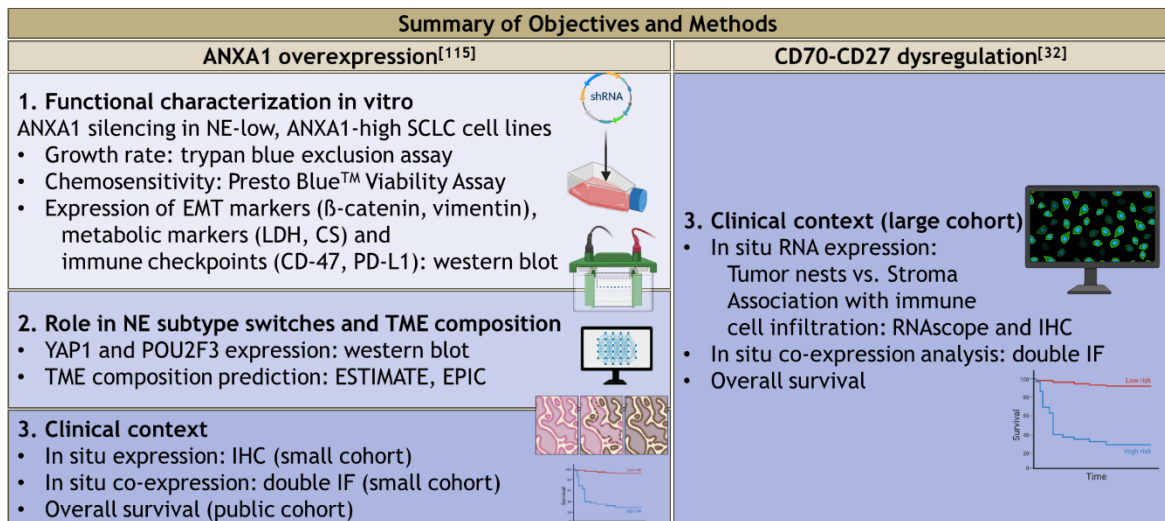


Figure 7. Overview of the endpoints investigated in this thesis. [32, 115] *Created with BioRender.com.*

3. Methods

3.1. Cell Lines, Transduction, and Culture Conditions

In vitro experiments were carried out as previously described [115]. Three NE-low, ANXA1-high SCLC cell lines, NCI-H841 (RRID: CVCL_1595), SW1271 (RRID: CVCL_1716), and NCI-H1048 (RRID: CVCL_1453) were obtained from the American Type Culture Collection (Manassas, VA, USA) and handled according to standard protocols. Cell line identity was ensured through detailed documentation of origin, passage number, and experimental use. Morphological characteristics were regularly monitored by microscopy, and cells were routinely tested for Mycoplasma contamination. ANXA1 was silenced using a lentiviral shRNA vector (shANXA1; MISSION® TRC2 pLKO.1-puro-CMV-tGFP, TRCN0000296381; target sequence: 5'-CTATGATCAGAAGACTTTAAT-3'). A non-targeting control shRNA construct (SHC202V) was used as control for potential effects of lentiviral transduction and puromycin selection. Both constructs were purchased from Sigma-Aldrich®. Although the use of multiple independent shRNA constructs could further strengthen target specificity, knockdown efficiency was confirmed at the protein level, and consistent phenotypic changes across all three cell lines support a target-specific effect. Transductions were performed according to manufacturer's instructions with 8 µg/mL Polybrene (#TR-1003, Sigma-Aldrich®). Cells were cultured in low-glucose ulbecco's Modified Eagle Medium (DMEM, #D6046, Sigma-Aldrich®) supplemented with 10% fetal bovine serum (#TMS-016 Sigma-Aldrich®) and 1% penicillin–streptomycin (#30-004-CI Corning®) at 37 °C with 5% CO₂. Puromycin (10 µg/mL, #ant-pr-1, Invivogen®) was used for the selection of transduced cells and was maintained throughout the experiments. Native, shCTRL, and shANXA1 cells were maintained in parallel for experiments for up to two passages. *In vitro* experimental setup is outlined in Figure 8. [115].

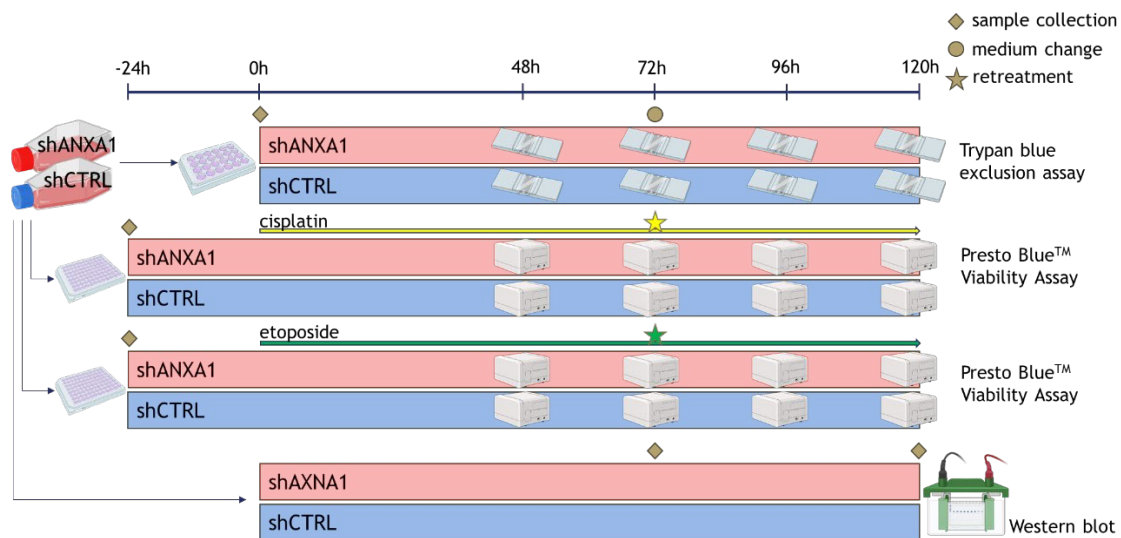


Figure 8. Experimental setup with SCLC cell lines. Created with BioRender.com, adapted from Paál, Dora et al. [115]

3.2. Trypan Blue Exclusion Growth Rate Assay

Cell counting and viability assessment were performed as we previously described [115]. Transduced shCTRL and shANXA1 cells were seeded in 24-well plates (#130186, Thermo Fisher Scientific™) in technical triplicates at densities of 50,000 cells/well for SW1271 and H1048 and 25,000 cells/well for H841. Cells were detached with TrypLE™ Express Enzyme (1X, no phenol red; #12604021, Gibco), centrifuged at 200 relative centrifugal force (rcf) for 5 min, and counted using a hemocytometer and 0.4% trypan blue (1:1; #SV30084.01, Cytiva HyClone™) at 48, 72, 96, and 120 h.

3.3. Assessment of Chemosensitivity and Growth Rate with Presto Blue Viability Assay™

Growth rate assay and chemosensitivity tests were carried out as we described [115]. Cells were seeded in five 96-well plates (#353377, Corning®) at 5,000 cells per well, with randomized layouts and technical triplicates for each experiment. After overnight incubation to allow attachment, three plates designated for 72 h, 96 h, and 120 h time points were treated the following day with etoposide (#12092, Cayman Chemical®) or cisplatin (#S1166, Avidin Ltd.®) at clinically relevant concentrations of 5, 10, and 20 μ M [4, 18, 93, 138]. Each plate included untreated controls as negative references for normalization. As a positive control, 0.1% staurosporine (#S6942, Sigma-Aldrich®) was applied to shANXA1 cells. Etoposide was dissolved in 0.1% dimethyl sulfoxide (DMSO, #D2650, Sigma-Aldrich®), which also served as the vehicle control in etoposide assays,

while cisplatin was dissolved directly in culture medium. Plates for the 96 h and 120 h time points were re-treated at 72 h. Cell viability was assessed using the Presto Blue Viability Assay™ (#A13261, Invitrogen™) by measuring resazurin reduction-induced fluorescence once per plate at treatment (0 h) and at 48, 72, 96, and 120 hours post-treatment. Measurements were performed at room temperature using a Varioskan™ Lux multimode microplate reader (Thermo Fisher Scientific™) with excitation at 560 nm and emission at 590 nm. Results were normalized to corresponding non-treated controls.

3.4. Western Blot Analysis

Western blotting was performed as previously described [115, 157] using the following antibodies: annexin A1 (D5V2T) XP® rabbit mAb (#32934, Cell Signaling®), citrate synthase rabbit mAb (#ab129095, AbCam®), LDHa (C45B) rabbit mAb (#3582, Cell Signaling®), vimentin rabbit pAb (#bs-0756, Bioss®), β -catenin (D10A8) XP® rabbit mAb (#8480, Cell Signaling®), YAP1 rabbit pAb (#PA1-46189, Invitrogen), POU2F3 rabbit pAb (#av32537, Sigma Aldrich®), CD47 rabbit pAb (#ab175388, Abcam), PD-L1 E1L3N XP® rabbit mAb (#13684), β -actin (13E5) rabbit mAb (#4970, Cell Signaling®), and GAPDH (D16H11) XP® rabbit mAb (#5174, Cell Signaling®). For details and antibody dilutions, we refer to Paál, Dora et al. [115].

Cells were detached with TrypLE™ Express, centrifuged at 130 rcf for 5 min, and lysed on ice in radioimmunoprecipitation assay (RIPA) buffer (#9806, Cell Signaling®) supplemented with Halt™ protease inhibitor cocktail (#78429, Thermo Fisher™). Lysates were sonicated (3×15 s) and centrifuged twice at 10,000 rcf, 4 °C for 15 min. Protein concentration was determined by bicinchoninic acid (BCA) assay (#23225, Thermo Fisher™). Equal amounts of protein were mixed with lysis buffer and 1/4 volume Laemmli buffer containing β -mercaptoethanol (#16010747, Bio-Rad®).

Samples were separated on 4–20% Criterion™ TGX™ Precast Midi Gels (#5671095, Bio-Rad®) and transferred onto polyvinylidene fluoride (PVDF) membranes (#1704273, Bio-Rad®) using the Trans-Blot® Turbo RTA Midi 0.2 μ m PVDF Transfer Kit. Membranes were blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline with Tween (TBS-T, 0.05% Tween-80) for 2 h at room temperature, then incubated overnight at 4 °C with primary antibodies. After washing in TBS-T, membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (#7074, Cell Signaling®) for 2 h at room temperature, followed by additional washes.

Chemiluminescence detection was performed using an enhanced chemiluminescence kit (#1705061, Bio-Rad®) and imaged with Chemidoc™ XRS+ or MP+ systems. Densitometric analysis was conducted using Image Lab™ 6.0 (Bio-Rad®). Protein expression was normalized to β -actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and presented relative to the native group for annexin A1 and to the shCTRL group for other targets.

3.5. Tissue samples and tissue microarray slides

For ANXA1 studies, nine early-stage SCLC samples were provided by the Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest. For CD70 studies, 190 SCLC patients treated between 1975 and 2013 at the National Korányi Institute of Pulmonology, Budapest, were included, with follow-up data until April 2022 [115].

Tissue microarrays (TMAs) were made from the formalin-fixed paraffin-embedded (FFPE) blocks as described earlier [8, 36]. The conventional microscope slides prepared as described above were stained with hematoxylin and eosin (H&E) using an automated Tissue-Tek® Prisma staining platform (Sakura Finetek®) stainer. An experienced thoracic pathologist examined the H&E-stained slides and manually outlined the tumor area, from where two 1-mm tissue punches were extracted from the FFPE blocks using a manual TMA instrument (Beecher Instruments®) with an MP10 1.0-mm tissue punch. The tissue cores were precisely arranged in an encoded array format within a recipient paraffin block. For each patient, two TMA cores were analyzed. From each TMA block, two separate 4- μ m sections spaced at least 100 μ m apart were used for quantification.

3.6. Immunohistochemistry and Immunofluorescence

Immunohistochemistry (IHC) and immunofluorescence (IF) were performed on conventional microscope slides prepared from ANXA1 FFPE blocks using a Leica Bond RX autostainer. Imaging for all IF experiments and ANXA1 IHC was performed using a Zeiss® LSM 780 confocal microscope with 10 $\times$ and 20 $\times$ objectives. TMA IHC images were acquired using an Olympus® BX53 microscope equipped with a DP74 camera at 10 $\times$ magnification (20MP resolution) [32, 115].

For IHC, slides were first deparaffinized and rehydrated automatically, followed by antigen retrieval in low-pH citrate buffer for 20 minutes to unmask epitopes.

Endogenous peroxidase activity was quenched with 2% hydrogen peroxide, and tissue sections were permeabilized with 0.5% Triton X-100 to enhance antibody penetration. Primary antibody annexin A1 rabbit mAb (#32934, Cell Signaling®) was then applied at 4°C overnight. Detection was carried out using the manual ImmPRESS® HRP Universal Polymer Kit (peroxidase, horse anti-mouse/rabbit IgG, vector) for the ANXA1 samples [115] according to manufacturer instructions, followed by hematoxylin counterstaining to visualize nuclei. Previously published tumoral and stromal CD45 and CD8 immunohistochemistry data from the CD70 cohort, including compartment-specific assessment of immune cell infiltration [33], were used for comparison with RNAscope results.

The ImageJ software package was used to perform cell counting, where microscopical images of sectioned and stained TMA cores were imported and followed by conversion to an 8-bit grayscale format to enhance contrast. The "Threshold" function was used to define and isolate the cells from the background, with adjustments averaged using 100 images manually counted to yield an accuracy of $\pm 5\%$. Adjustments were made to ensure proper identification of individual cells. Subsequently, the "Analyze Particles" feature was utilized to count the cells, with size and circularity parameters set to exclude debris or cell clusters, using the above-mentioned averaging. A macro was then created and applied to all samples. The results were then recorded as the total number of cells per blank [32].

For double IF staining on the ANXA1 samples [115], the following primary antibodies were used: CD45 rabbit mAb (#ab40763 Abcam®), CD68 rabbit mAb (#ab303565 Abcam®), CD3 rabbit pAb (#ab5690 Abcam®), CD19 rabbit mAb (#90176 CellSignaling®), vimentin mouse mAb (#MA5-11883 Invitrogen®) and ANXA1 rabbit mAb (#32934 CellSignaling®), followed by Alexa Fluor® 488-conjugated anti-rabbit and Alexa Fluor® 594-conjugated anti-mouse antibodies. For double IF staining of the CD70 cohort TMA slides [32], primary antibodies were CD70 rabbit mAb (#ab300083 Abcam®), CD27 rabbit mAb (#ab131254 Abcam®), CD68 mouse mAb (#ab201340 Abcam®), CD3 rabbit pAb (#ab5690 Abcam®), CD20 mouse mAb (#14-0202-82 Invitrogen®) and vimentin mouse mAb (#MA5-11883 Invitrogen), followed by Alexa Fluor® 488 anti-rabbit and Alexa Fluor® 546 anti-mouse secondaries. After blocking and permeabilization, slides were incubated overnight with primary antibodies at 4 °C,

followed by incubation with Alexa Fluor conjugated corresponding secondary antibodies for 1 hour at room temperature. Autofluorescence was quenched using TrueVIEW® Autofluorescence Quenching Kit and nuclei were visualized with 4',6-diamidino-2-phenylindole (DAPI) in both studies, followed by detection. Positive controls for protein expression detection were human tonsil and lung adenocarcinoma tissues.

3.7. RNA In Situ Hybridization (RNAScope®)

The RNAScope® in situ hybridization (ISH) assay was performed on the CD70 cohort TMA slides using the RNAScope® Multiplex Fluorescent Kit v2 (Advanced Cell Diagnostics) according to the manufacturer's protocol and previously published methods [32, 36]. Four-micrometer sections of TMAs were first deparaffinized and subjected to antigen retrieval buffer in a pressure steamer to reverse cross-linking and expose RNA targets. This was followed by protease digestion to permeabilize the tissue and facilitate probe penetration.

Target-specific oligonucleotide probes were then hybridized to the tissue sections at 40°C for approximately 2 hours. The probes included a positive control targeting housekeeping genes (3plex-Hs-Positive Control Probe #320861 ACD® Bio) to prove RNA integrity, a negative control probe to assess background fluorescence (3plex-Hs-Negative Control Probe, #320871 ACD® Bio), and the probes specific for human CD70 mRNA (Hs-CD70, #419331 ACD® Bio) and CD27 mRNA (Hs-CD27, #415451 ACD® Bio). Amplification was performed through sequential hybridization steps using three distinct amplifier oligonucleotide probes.

Fluorogenic signals were developed using HRP-conjugated Cyanine™ 3 tyramide, resulting in distinct red fluorescent puncta representing individual RNA molecules. Background fluorescence was quenched with TrueBlack® treatment, and nuclei were visualized with DAPI counterstaining. Quality control was ensured by confirming RNA integrity with the positive control probe and evaluating nonspecific background fluorescence with the negative control probe. Fluorescent signals represented precise localization and quantification of CD70 and CD27 mRNA expression within the tissue context.

Multichannel fluorescence images were processed and exported in ZEN® Blue software. Single-cell RNAScope intensity measurements were analyzed in QuPath software following established protocols [7, 36]. First, DAPI nuclear staining was used

to identify individual cells by defining each cell's radius, creating distinct regions of interest (ROIs). Within each ROI, the number of fluorescent RNA dots was counted according to standard methods.

For CD70, each tumor core, an integrated expression score ranging from 0 to 3 was calculated based on the average dot density and raw fluorescence intensity. Cutoff values to classify expression levels were determined using k-means clustering. For each patient, the mean CD70 score from two representative TMA cores was used for analysis. Tumors with a score of 2.0 or higher were classified as "CD70 high," whereas those with scores below 2.0 were considered "CD70 low."

The intensity distribution of CD27 expression showed a right-skewed pattern, with most cells exhibiting low levels. A Gaussian fit was applied to the histogram, and the peak of the fitted curve, reflecting the mean expression level within the dataset, served as cutoff to classify samples as CD27 high or low expression groups.

To complement mRNA expression analysis, image-based cell quantification was additionally performed on TMA sections to assess overall cellular density. Brightfield images were acquired using an Olympus BX53 microscope, and cell counting was conducted in ImageJ using threshold-based particle analysis following grayscale conversion. Parameters were optimized against manually counted reference images, achieving an estimated accuracy of $\pm 5\%$. This approach enabled the distinction between expression level and cell abundance within the tumor microenvironment [32].

Previously published CD45 and CD8 immunohistochemistry data from the CD70 cohort were used as a reference to compare the RNAscope findings on tumoral and stromal immune cell infiltration [33].

3.8 Survival analysis

Overall survival was analyzed using Kaplan–Meier curves with log-rank tests, and hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using Cox proportional hazards regression. Kaplan–Meier curves were generated using MedCalc® 14 statistical software [32, 115]. ANXA1 analysis was performed using bulk RNA-seq data from the publicly available UCologne2015 cohort (cBioPortal), with patients stratified into ANXA1-high and -low groups based on median expression [17]. In contrast, CD70 was analyzed using RNAscope, and CD27 by IHC, in the study cohort with available clinical data.

3.9. Predictive algorithms

The association between ANXA1 expression and tumor microenvironment composition was investigated *in silico* using the ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumor tissues using Expression data) and EPIC (Estimating the Proportions of Immune and Cancer cells) algorithms applied to bulk RNA-seq data from the UCologne2015 cohort (cBioPortal; $n = 81$), as we previously described [115]. Briefly, ESTIMATE was used to calculate immune, stromal, estimate, and tumor purity scores, while EPIC was applied to predict the relative proportions of immune and stromal cell populations in ANXA1-high versus -low tumors.

3.10. Statistical Analysis

Statistical analyses other than survival analysis were performed using GraphPad Prism[®] 8.0.0 (GraphPad Software, Inc.). Growth rate assays were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Chemosensitivity assays were evaluated using two-way ANOVA with Tukey's post hoc test. Western blot comparisons between shCTRL and shANXA1 groups were conducted using unpaired t-tests or Mann–Whitney tests, depending on normality assessed by the Shapiro–Wilk test. Experiments including native, non-transduced cells were analyzed by one-way ANOVA with Tukey's multiple comparisons test. Data are presented as mean $\pm$ standard error of the mean (SEM) with group sizes of $n = 6$ – 8 . Cell density comparisons based on RNAscope and IHC were performed using the Shapiro–Wilk and Mann–Whitney U tests [32, 115]. The Wilcoxon rank-sum test was applied to compare the results of the predictive algorithms.

4. Results

4.1. Annexin A1 expression is stably silenced in shANXA1 transduced cells

To investigate the functional roles of ANXA1 in SCLC, we evaluated key malignant phenotypic traits *in vitro* using cancer cell lines. The experimental setup for these studies is outlined in Figure 8. As published earlier [34], based on relative expression data of 28 SCLC cell lines from cell line datasets, H841, SW1271, and H1048 exhibited high ANXA1 expression, with H1048 showing the highest levels. Strong baseline ANXA1 expression and the effective and stable knockdown after transduction with shANXA1 constructs were confirmed in these cell lines by Western blot (Figure 9). In H841 and H1048 cells, transduction with the control shRNA vector (shCTRL) did not significantly alter ANXA1 levels. In SW1271 cells, shCTRL transduction resulted in a moderate but significant reduction in ANXA1 expression relative to native cells, indicating a cell line-specific effect of the transduction procedure. Importantly, ANXA1 levels remained significantly higher in shCTRL than in shANXA1 cells (Figure 9). Therefore, shCTRL cells were used as the experimental control, while results obtained in SW1271 should be interpreted taking this limitation into account.

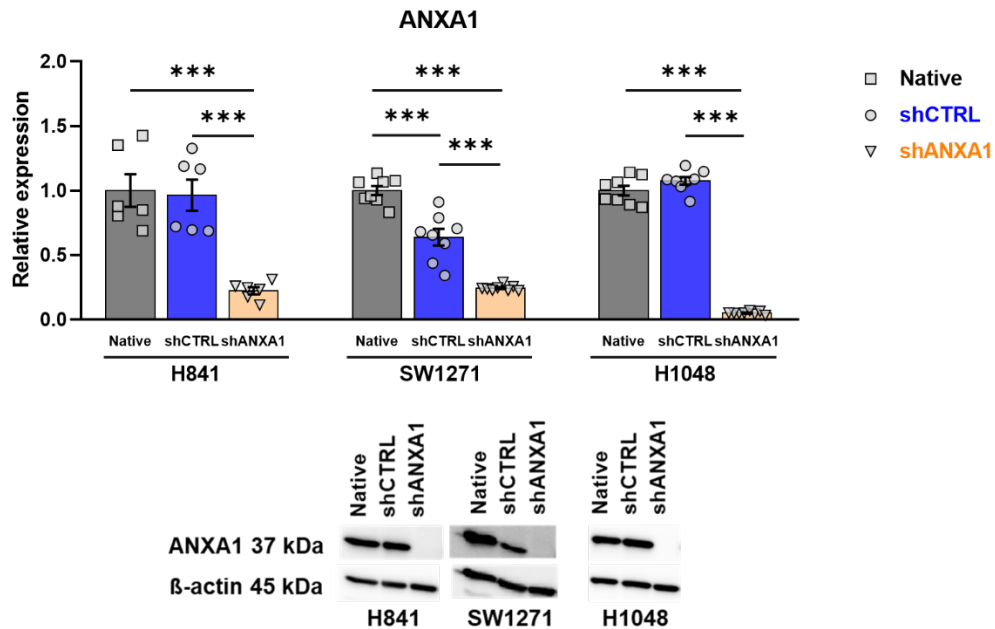


Figure 9. ANXA1 relative protein expression in native, shCTRL and shANXA1 SCLC cell lines. ANXA1 expression was stably silenced in all three shANXA1 cell lines. In SW1271 cells, however, the shCTRL construct was also associated with a significant decrease in ANXA1 expression compared with the native cells, suggesting a cell-line-specific effect of

the transduction procedure. Nevertheless, SW1271 shANXA1 cells still displayed significantly lower ANXA1 expression than shCTRL cells, indicating additional target-specific silencing beyond this baseline effect. Therefore, shCTRL SW1271 cells were used as the experimental control, although findings in SW1271 should be interpreted in light of this limitation. One-way ANOVA, Tukey's post hoc test, $n = 6-8$; *** $p < 0.001$. Data are expressed as mean $\pm$ SEM. Obtained from Paál, Dora et al. [115].

4.2. Functional effects of ANXA1 expression in SCLC

4.2.1. Silencing of ANXA1 differentially affects the growth rates of SCLC cell lines

We performed a trypan blue exclusion assay over 120 hours to assess cell growth rates. These results are presented as growth curves (Figure 10A) alongside bar graphs displaying individual data points (Figure 10B). Silencing ANXA1 affected proliferation differently across all three SCLC cell lines. In H841 cells, proliferation rates were comparable between shANXA1 and shCTRL groups, with no statistically significant differences observed (Figure 10A,B, first panel). In SW1271 cells, shANXA1 knockdown significantly inhibited growth compared to controls, with the difference becoming more pronounced over time (Figure 10A,B, second panel). In contrast, in H1048 cells, ANXA1 silencing led to a marked unexpected increase in proliferation starting at 72 hours and persisting through 120 hours, where the greatest difference was detected (Figure 10A,B, third panel).

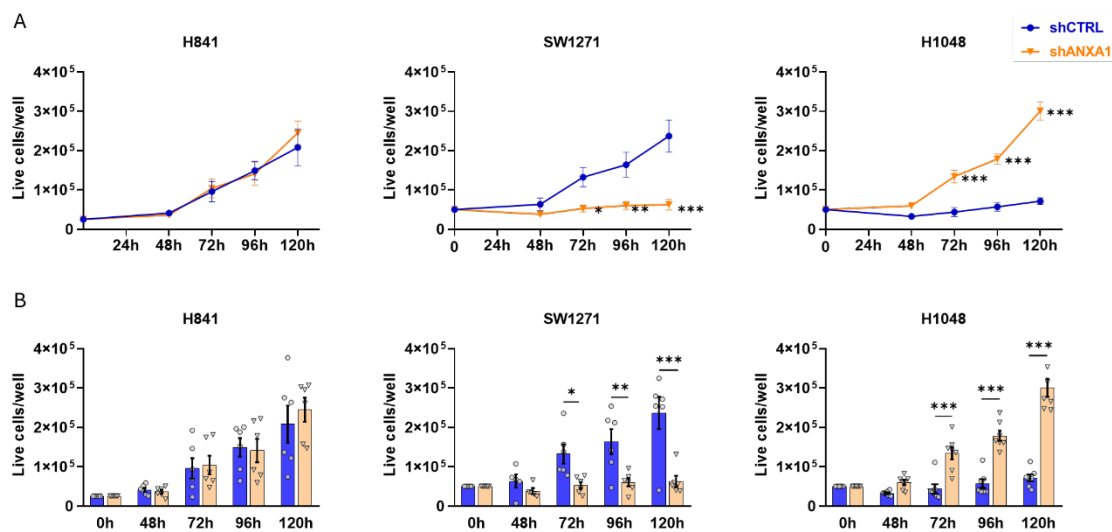


Figure 10. 120-hour growth rate assay. Results shown as growth curve **(A)** and as individual data at time points **(B)**. ANXA1 silencing had no effect on the proliferation of H841 cells (**first panel**), whereas from 72 h and onward, it significantly decreased the growth rate of SW1271 cells (**second panel**) and increased the growth rate of H1048 cells (**third panel**). Trypan blue exclusion assay, $n = 6$, one-way ANOVA, Tukey's post hoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are displayed as mean $\pm$ SEM. Obtained from Paál, Dora et al. [115]

4.2.3 ANXA1 silencing decreases sensitivity to cisplatin and etoposide

To investigate whether ANXA1 silencing affects chemosensitivity in SCLC, we treated the three cell lines with first-line chemotherapy agents cisplatin and etoposide *in vitro*. Based on our growth rate data, treatments were applied over a 120-hour incubation period. Overall, ANXA1 knockdown reduced sensitivity to both drugs across all cell lines.

In H841 cells, despite similar proliferation rates between shCTRL and shANXA1 groups, shANXA1 cells were significantly less sensitive to cisplatin at 5 μ M and 10 μ M concentrations at 72, 96, and 120 hours (Figure 11A, first panel). At 20 μ M cisplatin, both groups showed similarly reduced viability at all time points. For etoposide, shANXA1 cells exhibited significantly decreased sensitivity at 10 μ M at 72 and 96 hours, with a similar trend at 120 hours. No significant differences were observed at 5 μ M or 20 μ M etoposide (Figure 11B, first panel).

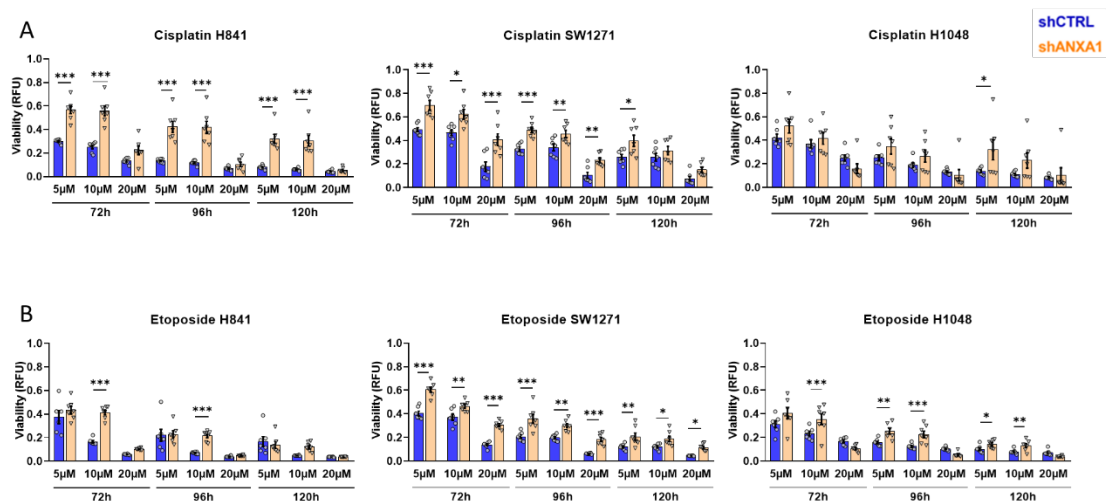


Figure 11. Chemosensitivity assay with cisplatin (A) and etoposide (B). ANXA1 silencing broadly decreased sensitivity to both cisplatin and etoposide in all three cell lines.

ANXA1-silenced H841 cells showed decreased sensitivity to cisplatin at 5 and 10 μ M at all time points, and to etoposide at 10 μ M at 72 and 96 h. At 20 μ M cisplatin and 5 and 10 μ M etoposide, similar trends were observed (**first panel**). ANXA1 silencing significantly increased the viability of SW1271 cells at all tested cisplatin concentrations at 72 and 96 h, as well as at all concentrations and time points following etoposide treatment (**second panel**). Cisplatin treatment in H1048 cells showed high variability, precluding statistical significance; however, a trend toward decreased sensitivity at 5 and 10 μ M was observed across all time points. In contrast, ANXA1-silenced H1048 cells exhibited significantly reduced sensitivity to etoposide at 10 μ M, as well as at 5 μ M at 96 and 120 h. At the highest concentrations of both cisplatin and etoposide, H1048 shANXA1 cells showed a slight decrease in viability across all time points, except at 120 h cisplatin treatment (**third panel**). Presto BlueTM Viability Assay, $n = 6-7$; two-way ANOVA, Tukey's post hoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are displayed as mean $\pm$ SEM. RFU: relative fluorescence intensity unit. Obtained from Paál, Dora et al. [115]

In SW1271 cells, shANXA1 cells showed consistently increased resistance compared to controls at all drug concentrations and time points for both cisplatin and etoposide, with statistically significant differences in almost each condition. The exceptions were the 10 μ M and 20 μ M cisplatin treatments at 120 hours, where the trend persisted but did not reach significance (Figure 9A, second panel).

For H1048 cells, 5 μ M and 10 μ M treatments with both drugs resulted in higher viability in shANXA1 cells than controls at across all time points. Although statistical spread of data limited statistical significance in cisplatin treatments, clear tendencies were observed (Figure 11A, third panel). 5 μ M etoposide treatment at 72h and both 5 μ M and 10 μ M at 96 h and 120 h resulted in significantly increased viability in shANXA1 cells (Figure 11B, third panel). Interestingly, at the highest concentration, 20 μ M, both cisplatin and etoposide showed a tendency to induce greater cell death in shANXA1 cells, notably at 72 and 96 hours for cisplatin and at all time points for etoposide (Figure 11A,B, third panel).

4.2.4. ANXA1 silencing decreases aerobic metabolism in H841 and H1048, and induces a Warburg-like shift in SW1271

To assess the effects of ANXA1 silencing on metabolic changes, namely the Warburg effect, we measured protein levels of citrate synthase (CS) as an indicator of oxidative metabolism and lactate dehydrogenase a (LDHa) as a marker of anaerobic glycolysis. According to our results, there was a significant reduction in citrate synthase expression in H841 and H1048 shANXA1 cells compared to their respective shCTRL controls (Figure 12A). SW1271 cells showed only a slight, non-significant decrease in CS expression upon ANXA1 silencing (Figure 12A).

Regarding LDHa, expression levels remained unchanged in H841 and H1048 shANXA1 cells but were significantly elevated in SW1271 shANXA1 cells as compared to corresponding controls (Figure 12B).

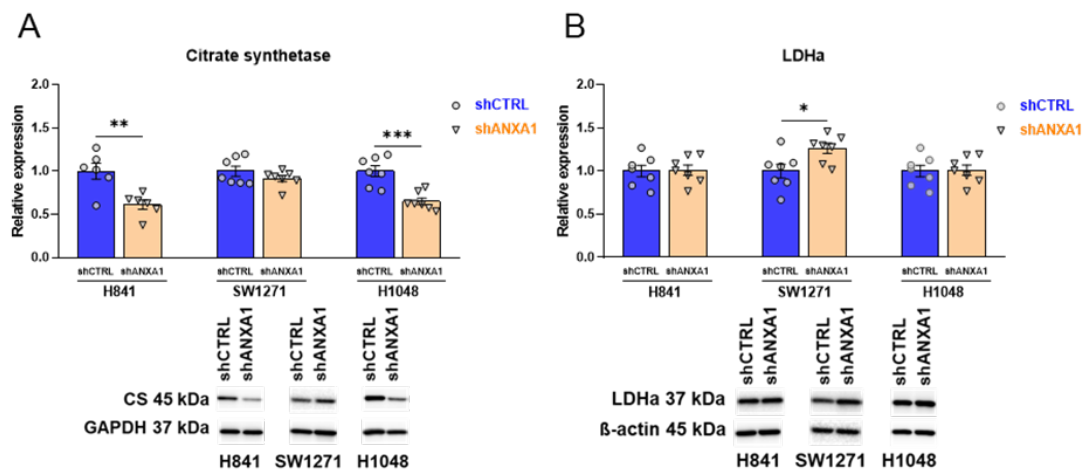


Figure 12. Western blot evaluation of metabolic markers. *Relative protein levels of citrate synthetase (A) and lactate dehydrogenase a (LDHa) (B). ANXA1 silencing resulted in significantly decreased citrate synthase expression in H841 and H1048, which was not accompanied by an increase in LDHa levels. In SW1271 cells, LDHa expression significantly increased after ANXA1 silencing, suggesting a Warburg-like shift. Western blot, n = 6–7; normalized for shCTRL group of each cell line. Unpaired t-test, * p < 0.05, ** p < 0.01, *** p < 0.001. Data are displayed as mean ± SEM. Obtained from Paál, Dora et al. [115]*

4.2.5. ANXA1 silencing differentially affects expression of mesenchymal markers in SCLC cell lines

We evaluated the protein expression of two mesenchymal markers, vimentin and β -catenin, to assess the mesenchymal characteristics as a hallmark of malignancy in cancer cells. Vimentin expression was slightly reduced in H841 and H1048 shANXA1 cells, with the decrease reaching statistical significance only in H1048 shANXA1 cells compared to their shCTRL counterparts (Figure 13A). In contrast, vimentin expression was significantly elevated in SW1271 shANXA1 cells (Figure 11A). β -catenin levels remained unchanged in H841 cells following ANXA1 silencing but showed a trend towards increased expression in SW1271 shANXA1 cells (Figure 13B). Notably, β -catenin expression was significantly decreased in H1048 shANXA1 cells relative to controls (Figure 13B).

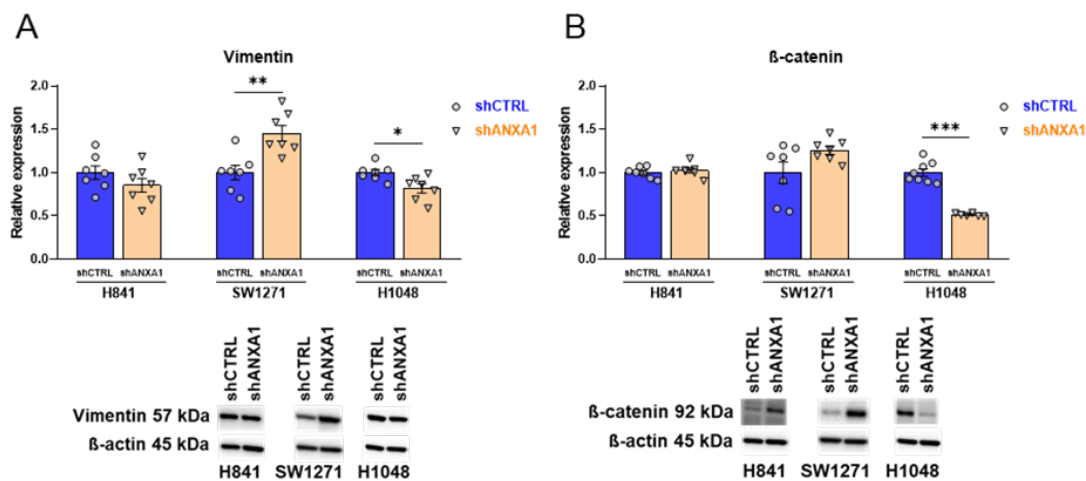


Figure 13. Western blot evaluation of mesenchymal markers. *Relative protein levels of vimentin (A), and β -catenin (B). ANXA1 silencing significantly decreased β -catenin and vimentin expression in H1048 cells. In contrast, SW1271 cells showed the opposite pattern, although the increase in β -catenin did not reach statistical significance. Western blot, $n = 6-7$; normalized for shCTRL group of each cell line. ANXA1 silencing did not affect the expression of these mesenchymal markers in H841 cells. Unpaired t -test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are displayed as mean $\pm$ SEM. Obtained from Paál, Dora et al. [115]*

In summary, expression of mesenchymal markers was not significantly affected by ANXA1 silencing in H841 cells, while it slightly increased in SW1271 shANXA1 cells. In H1048 cells, protein levels of both vimentin and β -catenin significantly

decreased, which may be in line with our observation that these cells exhibited stronger adherence in culture dishes compared to their control counterparts (Figure 14).

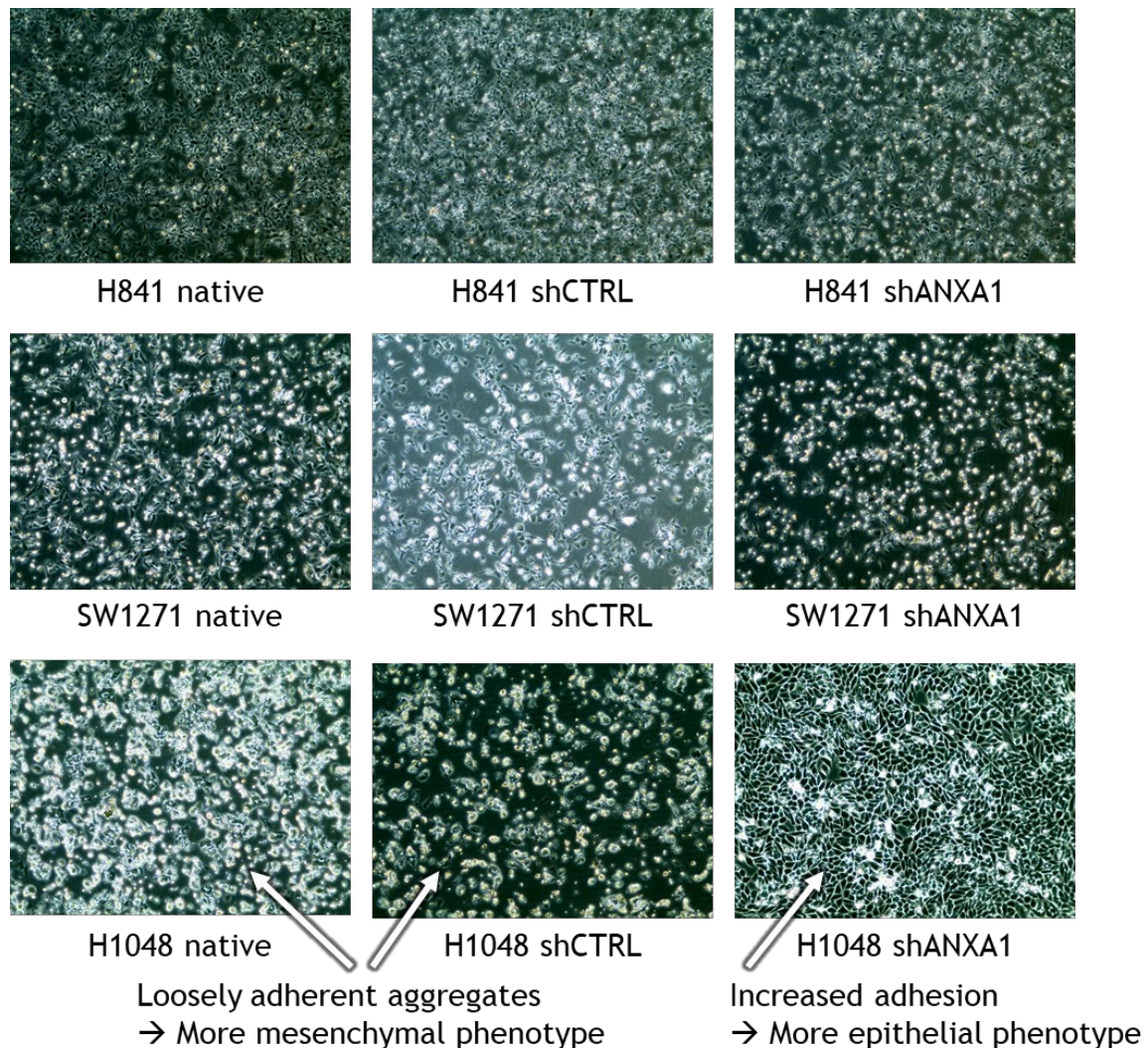


Figure 14. Photos of SCLC native and transduced shCTRL and shANXA1 cells in culture.

The most pronounced phenotypic changes were observed in H1048 cells: ANXA1-silenced cells exhibited increased adhesion to culture dishes and required prolonged incubation for detachment with TrypLE™ Express compared with native and control counterparts.

4.3.2. ANXA1 silencing has no significant effect on CD47 and PD-L1 expression

The expression of CD47, a potential mediator of tumor immune evasion in SCLC, was significantly increased in H841 shANXA1 cells compared to H841 shCTRL cells (Figure 15A), meanwhile, it did not change significantly in ANXA1-silenced SW1271 and H1048 cells (Figure 16A). Protein levels of PD-L1, one of the most successful immunotherapeutic targets for NSCLC and other cancers, showed no significant changes

in expression across the ANXA1-silenced groups in any of the cell lines, except for a slight but statistically non-significant reduction in PD-L1 expression observed in SW1271 shANXA1 cells (Figure 16B).

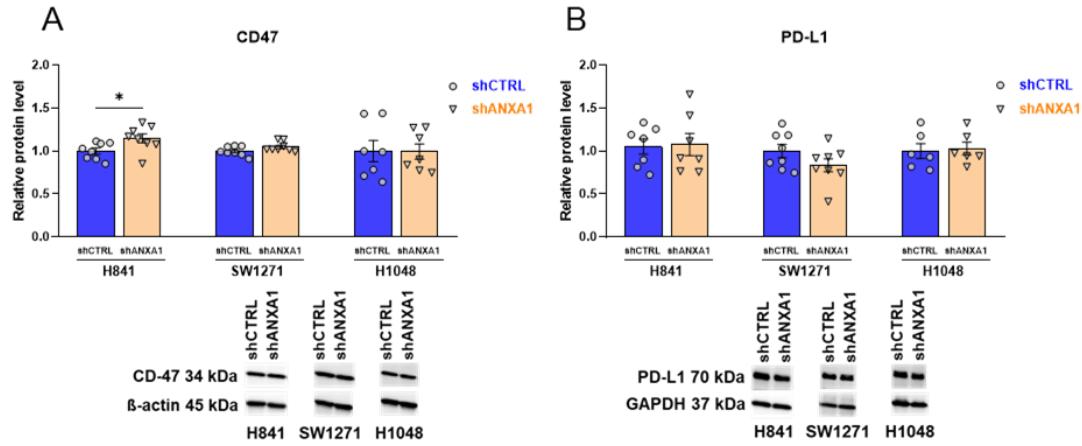


Figure 16. Western blot evaluation of immunomodulatory markers. Relative protein levels of CD47 (**A**), and PD-L1 (**B**). Western blot, $n = 6-8$; normalized for shCTRL group of each cell line. Unpaired t-test, * $p < 0.05$. Data are displayed as mean $\pm$ SEM. Obtained from Paál, Dora et al. [115]

4.3. Potential roles of ANXA1 in SCLC subtype switches and TME composition

4.3.1. ANXA1 silencing significantly affected dominant transcriptional drivers in POU2F3-dominant H1048 cell line

Among the three SCLC cell lines, the dominant transcriptional driver is YAP1 in H841 and SW1271 cell line, whereas it is POU2F3 in H1048, as previously reported [34]. Upon ANXA1 silencing, YAP1 expression showed a slight increase in H841 and SW1271 shANXA1 cells, and a pronounced three-fold increase in H1048 shANXA1 cells compared to their respective shCTRL controls (Figure 15A). POU2F3 expression remained largely unchanged in H841 and SW1271 shANXA1 cells, with a modest decrease in SW1271 shANXA1 cells. In contrast, POU2F3 expression was significantly elevated in H1048 shANXA1 cells relative to controls (Figure 15B).

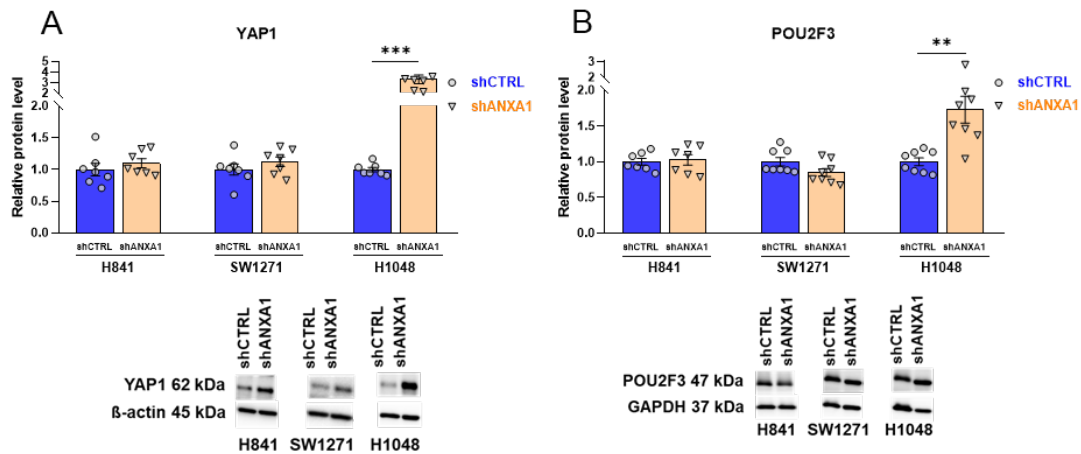


Figure 15. Western blot evaluation of dominant transcriptional drivers. *Relative protein levels of YAP1 (A) and POU2F3 (B). YAP-1 expression showed an approximately threefold increase in ANXA1-silenced H1048 cells, while POU2F3 expression also significantly increased in H1048 shANXA1 cells. No significant changes were observed in the other two cell lines. Western blot, $n = 6-8$; normalized for shCTRL group of each cell line. Unpaired t-test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are displayed as mean $\pm$ SEM. . Obtained from Paál, Dora et al. [115].*

4.3.2. ANXA1-high SCLC is associated with increased immune cell infiltration

In a previous study by our collaborators, increased ANXA1 expression was found in immune-oasis, NE-low SCLC [34]. To further investigate this, open access bulk RNA-seq data of 81 SCLC patients was analyzed and divided to ANXA1-high and ANXA1-low groups. According to ESTIMATE, ANXA1-high tumors exhibited significantly higher immune (Figure 17A), stroma (Figure 17B), and ESTIMATE scores (Figure 17C), along with a significantly lower purity score (Figure 17D), suggesting increased immune and stromal cell infiltration compared with ANXA1-low tumors.

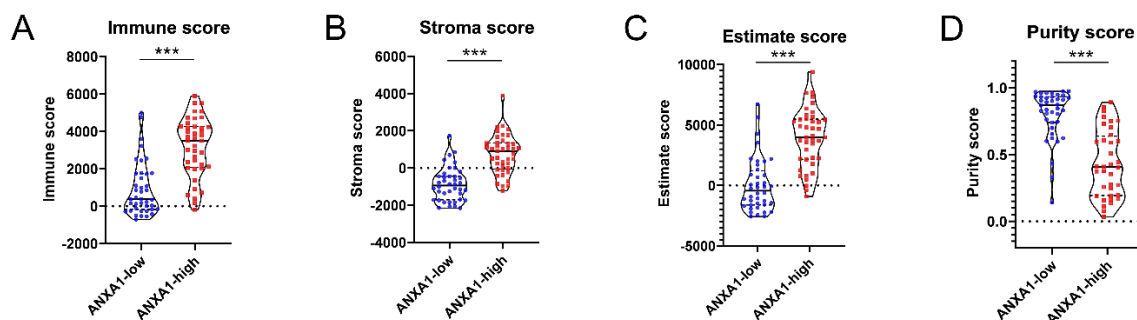


Figure 17. Predictive characterization of the SCLC TME based on ANXA1 expression with the ESTIMATE algorithm. *ANXA1-high tumors showed significantly higher immune score (A) stroma score (B) and estimate score (C), with lower purity score (D), indicating a higher proportion of nontumor cells in ANXA1-high SCLC ($p < 0.001$). Obtained from Paál, Dora et al. [115]*

To evaluate the cellular composition of the SCLC TME based on ANXA1 expression, we applied the EPIC algorithm [126], which predicted higher proportions of CAFs, TAMs and NK cells in ANXA1-high tumors, suggesting a more active TME compared with ANXA1-low cases (Figure 18A,B) [115].

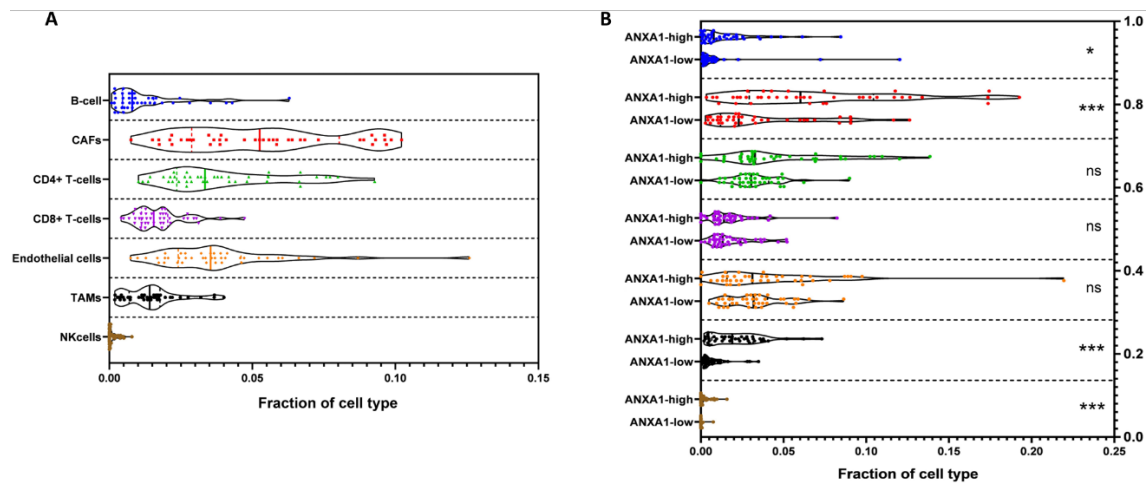


Figure 18. Predictive characterization of the SCLC TME based on ANXA1 expression with the EPIC algorithm. *Immune and stromal cell fractions in SCLC (A). Immune and stromal cell fractions in ANXA1-high versus ANXA1-low SCLC tumors (B). ANXA1-high tumors were predicted to contain significantly higher proportions of CAFs, TAMs and NK cells, along with lower proportion of B-cells. Data are shown as violin plots with medians (solid lines) and quartiles (dashed lines). Wilcoxon's rank-sum test (non-normal by Shapiro–Wilk). ns, non-significant; *, $p < 0.05$; *, $p < 0.001$. Obtained from Paál, Dora et al. [115]*

4.4. ANXA1 and the CD70/CD27 axis in the clinical context of SCLC

4.4.1. ANXA1 protein is expressed on tumor cells and macrophages in SCLC tumor samples

We evaluated ANXA1 expression in tumor samples from nine SCLC patients using IHC. In this limited cohort, two patients showed ANXA1 positivity in tumor cells, with scattered positive non-tumor cells in the stroma (Figure 19A,B). Two additional patients exhibited ANXA1 expression in non-tumor cells only, both in the stromal compartment and in the tumor nests (Figure 19C,D). The remaining five patients did not show detectable ANXA1 expression in either tumor or stromal compartments.

To further characterize the ANXA1-expressing stromal cells, we performed double IF staining combining ANXA1 with markers for pan-leukocytes (CD45), cancer-associated fibroblasts (vimentin), macrophages (CD68), T cells (CD3), and B cells (CD19). We observed frequent co-expression of ANXA1 with CD45 on cells with amoeboid or ramified morphologies restricted to the stroma and absent in tumor nests (Figure 19E, asterisks indicate ANXA1⁺/CD45⁺ leukocytes). No co-expression was detected with vimentin, moreover, ANXA1-positive tumor cells did not co-localize with vimentin-positive CAFs (Figure 19F). However, ANXA1 showed co-expression with CD68 in most TAMs in both stromal areas and tumor nests (Figure 19G), but ANXA1-negative TAMs were also detected (Figure 19G, arrows). No co-expression of ANXA1 was observed with CD3 and CD19 in the samples analyzed (not shown).

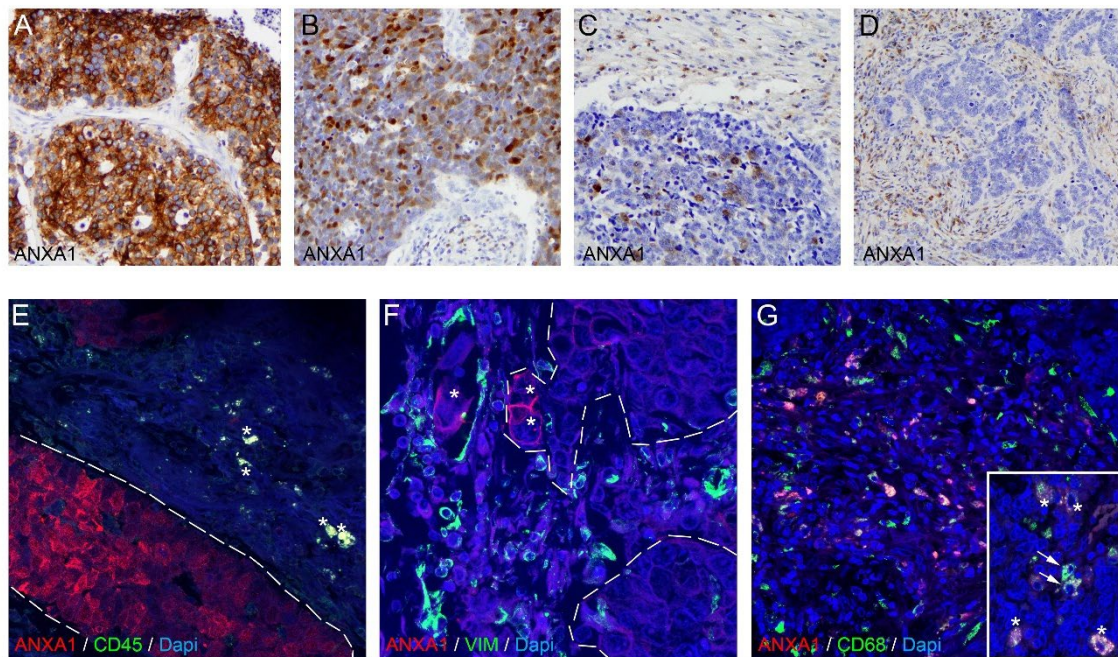


Figure 19. In situ expression of ANXA1 in tumor samples. Representative IHC images show ANXA1 positivity in tumor cells (**A, B**) with scattered stromal ANXA1 expression in non-tumor cells (**C, D**). Double IF confirmed ANXA1 expression in tumor nests (**E**), and co-expression with CD45 is restricted to stromal immune cells (**E, ***). No co-expression was detected with vimentin (**F**), and no co-localization was observed between vimentin⁺ CAFs and ANXA1⁺ tumor cells (**F, ***). TAMs show ANXA1/CD68 co-expression both in the stroma and tumor nests (**G**), while ANXA1⁻ CD68⁺ TAMs are also present (**G, arrows**). Obtained from Paál, Dora et al. [115]

4.4.2. CD70 expression in tumor nests and stroma is not associated with immune cell infiltration, whereas stromal CD27 expression is

RNA expression scores were constructed for each patient using RNAscope assays combined with an area-adjusted quantitative scoring system in QuPath. Representative images show strong (score = 3), moderate (score = 2), and low (score = 1) CD70 expression, predominantly localized in tumor nests but also present in the stroma (Figure 20A-C). Analysis of previously published IHC-based immune cell density data of the same cohort [35] showed no significant correlation between CD70 RNA expression and CD45⁺ immune cell or CD8⁺ T cell infiltration in either stroma or tumor nests (Figure 20D-G).

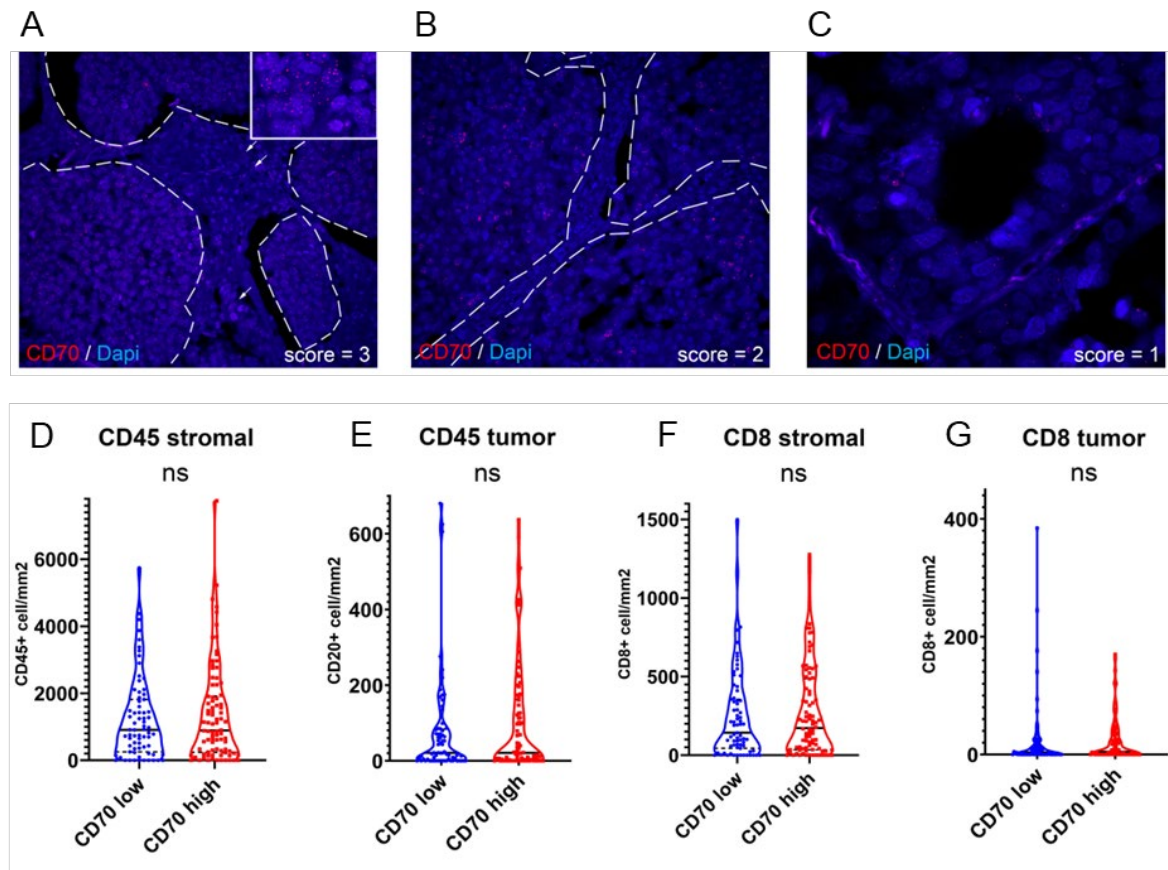


Figure 20. CD70 RNAscope. Representative RNAscope images showing high (**A, B**) and low (**C**) CD70 expression predominantly in tumor nests (areas outlined by dashed lines). CD70 expression was not significantly associated with CD45⁺ or CD8⁺ cell densities in stroma (**D, F**) or tumor nests (**E, G**). Mann-Whitney U test (non-normal by Shapiro-Wilk); $n=31$ for CD45, $n=30$ for CD8. Obtained from Dora et al. [32]

CD27 expression was evaluated with an IHC-based scoring system as described earlier [33]. CD27 showed predominantly stromal expression, supported by RNAscope results as well (Figure 21A,B). Unlike CD70, high CD27 expression was associated with decreased CD45⁺ immune cell and CD8⁺ T cell densities in the stroma (Figure 21C,E) but not in tumor nests (Figure 21D,F). [32].

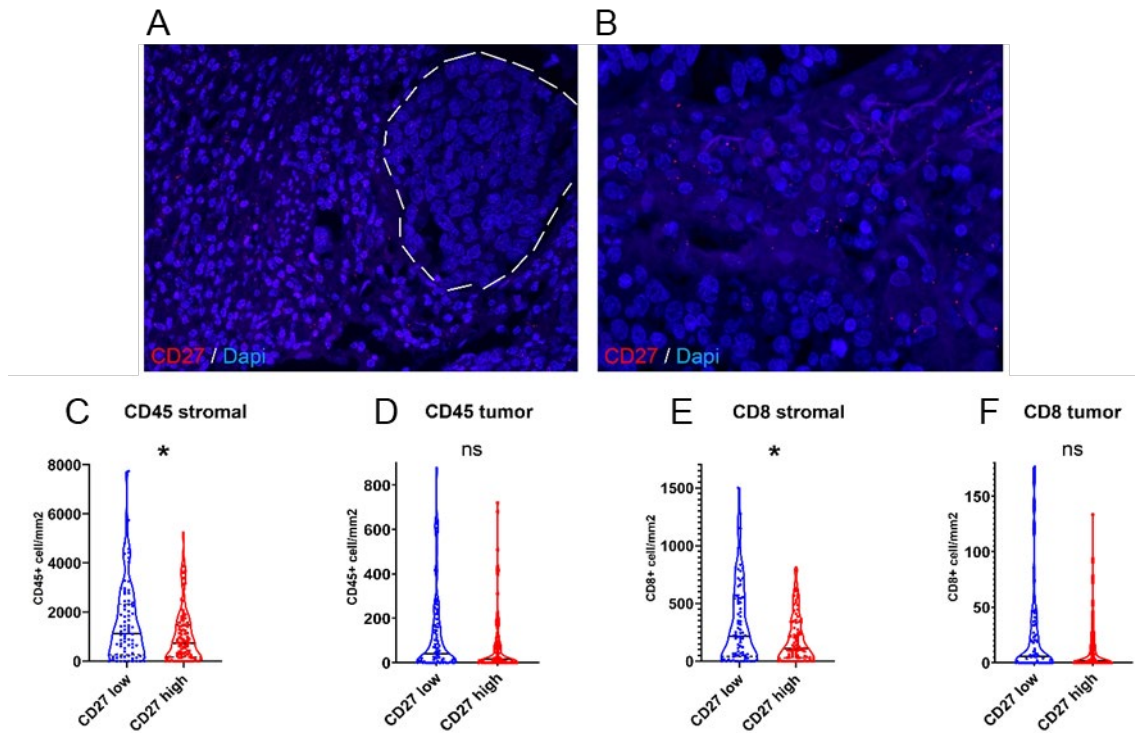


Figure 21. CD27 RNAscope. Representative RNAscope images showing high (A) and low (B) CD27 expression predominantly in stroma (A: tumor nest outlined by dashed line). High CD27⁺ stromal expression was associated with reduced CD45⁺ (C) and CD8⁺ (E) cell densities in the stroma, but not in tumor nests (D, F). Mann-Whitney U test (non-normal by Shapiro-Wilk); n=31 for CD45, n=30 for CD8. Obtained from Dora et al. [32]

4.4.3. CD70 and CD27 show distinct localization across tumor, stromal, and immune cell populations in SCLC

To characterize the expression patterns and associations of the CD70/CD27 axis in the SCLC TME, we performed double IF staining with the EMT marker vimentin, pan-macrophage marker CD68, pan-T-cell marker CD3, and B-cell marker CD20.

CD70 was detected as a diffuse and patchy signal, that was in inverse correlation with vimentin expression in tumor nests of CD70-high patients (Figure 22A), suggesting lower CD70 expression in vimentin-positive, mesenchymal-like tumor cells and higher expression in tumor cells with epithelial phenotypes. In contrast, CD70 was strongly expressed by vimentin-positive cancer-associated fibroblasts (CAFs) in the stromal compartment (Figure 22B, arrow). Among immune cells, most CD68⁺ tumor-associated macrophages (TAMs) expressed CD70 in both stromal and intratumoral regions (Figure 22C,D). In CD70-high tumors, the majority of CD3⁺ T cells were CD70-negative, only a

small subset of them co-expressed CD70 (Figure 22E,F). CD20⁺ B-cells showed no CD70 coexpression, even in CD70-high tumors (Figure 22G,H).

As expected, CD27 expression was limited to the TME, and not detected on tumor cells. CD27 colocalized with scattered vimentin⁺ CAFs (Figure 22I), CD3⁺ T-cells (Figure 22J), and a moderate number of CD68⁺ TAMs in both stroma and tumor nests (Figure 22K).

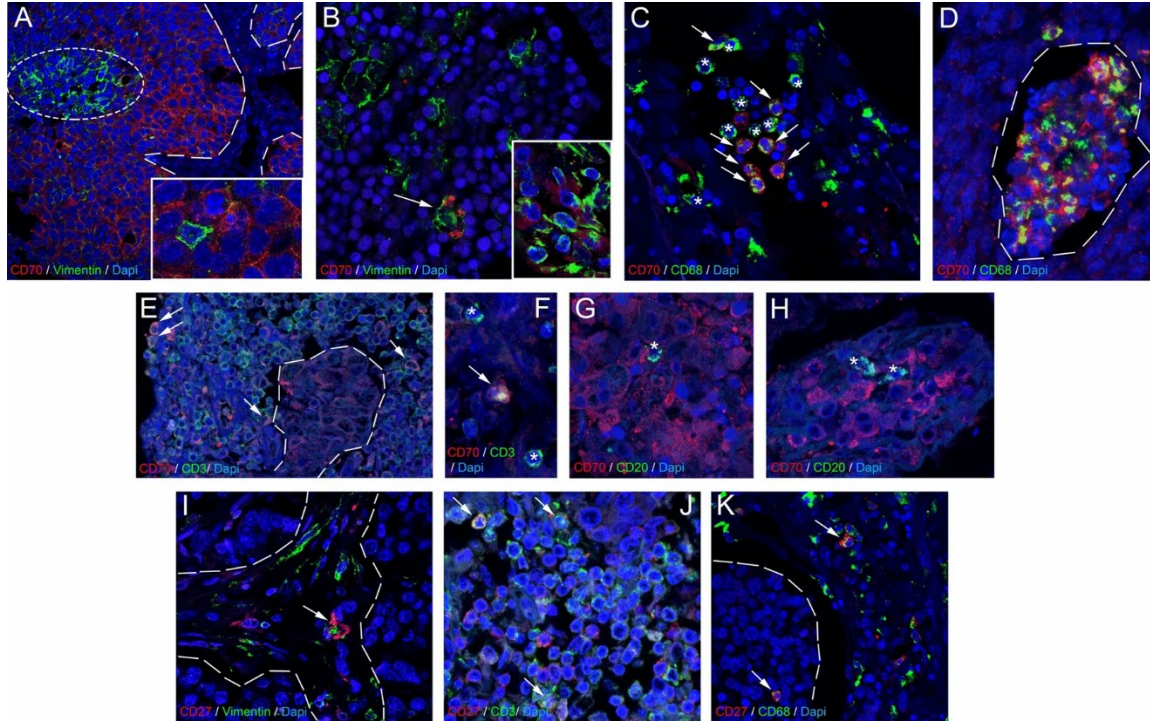


Figure 22. Immunofluorescence detection of CD70 and CD27 co-expression in cells of the TME. Diffuse CD70 positivity observed in tumor nests of CD70-high patients (**A**, **outlined by dashed line**), with a complementary distribution relative to vimentin-positive tumor cells (**A**, **ellipsoid area**). Stromal CAFs showing co-expression of CD70 and vimentin (**B**, **arrow**). TAMs showing co-expression of CD70 and CD68 in both the stroma (**C**, **arrows**) and tumor nests (**D**, **outlined by dashed line**). CD70/CD68⁺ TAMs also present in the stroma (**C**, *****). In CD70-high tumors, a some CD3⁺ T cells expressed CD70 (**E**, **F**, **arrows**, **E**: tumor nest outlined by dashed line). CD20⁺ B cells showed no co-expression with CD70 (**G**, **H**, *****). CD27 is restricted to the stroma (**I**, **J**, **K**), exhibiting occasional co-expression with vimentin in CAFs (**I**, **arrow**), CD3 in T cells (**J**, **arrows**), and CD68 in TAMs (**K**, **arrows**). Obtained from Dora et al. [32]

4.4.4. ANXA1 and CD70 expressions are related to significantly decreased overall survival

Overall survival (OS) was also evaluated with Kaplan-Meier analysis on the publicly available “UCologne2015” ANXA1 cohort and the CD70 cohort’s clinical data. ANXA1-high tumors were associated with significantly decreased OS compared to ANXA1-low tumors (HR 1.81, $p = 0.034$, Figure 23A). Multivariate Cox regression analysis, including the two most relevant confounders—tumor stage and gender—showed that ANXA1 remained a significant predictor of survival, with prognostic power comparable to that of gender (HR 2.05, $p = 0.016$; not shown) [115].

Similarly, high CD70 expression correlated with decreased OS in the whole cohort (HR 1.80, $p = 0.008$; Figure 23B) and in early-stage patients (HR 2.00, $p = 0.008$), as well as in immune-oasis tumors (HR 2.21, $p = 0.035$), but not in immune-desert tumors ($p = 0.115$, not shown). Other clinical parameters, including age, tumor stage, and gender, showed no significant association with ANXA1 or CD70 expression (for details, we refer to Dora et al. [32])

On the other hand, CD27 expression showed no significant association with OS in the SCLC cohort, neither in the entire patient group nor when stratified by immune status, also confirmed by univariate and multivariate analyses (Figure 23C) [32].

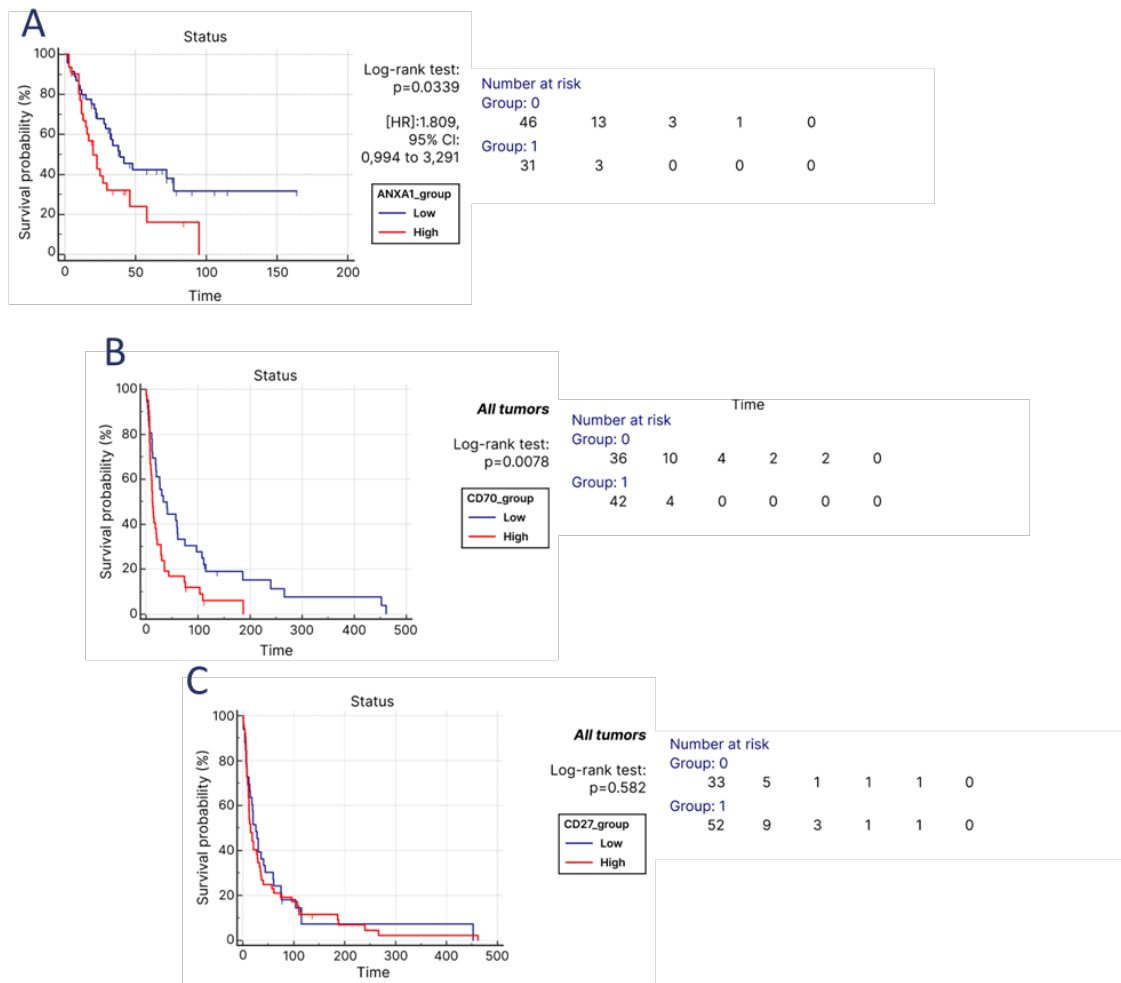


Figure 23. Prognostic impact of ANXA1 expression based on the UCologne dataset (A), CD70 (B) and CD27 (C) expression in the IHC cohort. ANXA1-high patients showed significantly decreased overall survival (OS) ($p = 0.0339$; HR = 1.809) (A). CD70-high patients also showed significantly decreased OS compared to CD70-low patients ($p = 0.0078$; HR = 1.795) (B). CD27 expression had no significant effect on OS in the whole cohort ($p = 0.582$) (C). Kaplan–Meier analysis, log-rank test, hazard ratio from Cox proportional hazards model. Obtained from Paál, Dora et al. and Dora et al. [32, 115]

5. Discussion

A pivotal factor that may have hindered advancement in SCLC therapy for over three decades was the view of this aggressive malignancy as a single entity, resulting in approach with a “one-size-fits-all” therapeutic strategy. Indeed, over 70% of patients are still first diagnosed with extensive-stage disease, where surgery is not optional [140]. Rapid progression and relapses with therapy-resistance are general characteristics of most cases. However, research in the past decade has challenged the oversimplified view of SCLC as a homogeneous disease, and it is becoming clear that SCLC is an extremely heterogeneous cancer with high plasticity. Delineation of molecular subtypes is a promising approach to identify therapeutic vulnerabilities [27, 52, 125], but reaching consensus remains subject of ongoing research.

Previous work demonstrated that a complex and immunosuppressive TME particularly contributes to difficulties in patient stratification and effectiveness of current therapies [88, 143]. Promising characteristics also seem to overlap across subgroups, supporting heterogeneity and plasticity. Gene expression analysis revealed ANXA1 upregulation in NE-low tumors irrespective of immune-infiltration, consistent with a potential immunosuppressive role, while upregulation of CD70 and CD27 is a feature shared by both NE-low and immune oasis tumors [34]. Based on these observations, we aimed to characterize ANXA1 in SCLC using *in vitro* models, *in silico* analyses and patient datasets, and the CD70-CD27 axis in a large SCLC patient cohort to assess the theranostic potential of these molecules and help understand mechanisms driving this poor-prognosis malignancy.

5.1. Biological roles of ANXA1 expression in SCLC cell lines

ANXA1 is known to have conflicting roles both physiologically and pathologically. Particularly in cancer, it has been described as a tumor promoter and a tumor suppressor, showing upregulation and downregulation, depending on cancer type [45, 77]. Until our publication, only two studies investigated ANXA1 in SCLC. Liu et al. connected ANXA1 to SCLC brain metastases, reporting its elevated levels in plasma of affected patients. ANXA1 inhibition by antibody or shRNA knockdown decreased transendothelial migration *in vitro* and brain metastasis *in vivo*, while an ANXA1 mimic promoted these endpoints. They demonstrated these results in co-culture of NCI-H446 cells with brain microvascular endothelial cells and in mice, highlighting the importance

of the TME in the ANXA1-linked mechanisms [96]. Chen et al. supported bad prognostic effects of ANXA1 when investigating its roles in SCLC bone metastases. They found elevated ANXA1 in serum of affected patients. Supporting complexity of ANXA1 functions, they found divergent expression changes of ANXA1 in variants of the SBC SCLC cell line, decreased in the highly bone-metastatic SBC-5 cells, but increased in the less aggressive SBC-3 cells. ANXA1 also promoted proliferation, invasion, migration and bone metastasis in their experiments [20].

In line with the literature, it was anticipated that experiments with different SCLC cell lines would yield heterogeneous results. For instance, ER⁺ breast cancer is associated with loss of ANXA1 expression, while triple-negative breast cancers (TNBCs) usually show upregulation [29]. In NSCLC, Elakad et al. reported that squamous cell carcinoma was associated with longer OS if ANXA1 expression was elevated, and in functional studies, ANXA1 inhibited migration, while promoted proliferation in seven lung squamous cell carcinoma (SqCLC) cell lines. They proposed that metastasis potential associated with migration has a stronger impact on tumor aggressiveness than proliferation, confirming complex pathogenic roles [107]. In lung adenocarcinoma however, ANXA1 upregulation was reported to have an oncogenic effect on both proliferation and invasive capacity [58, 59], while there is evidence for the opposite as well [30, 98].

We investigated some direct effects of ANXA1 expression by shRNA-mediated silencing in three non-NE, ANXA1-high SCLC cell lines, H841 (YAP1⁺), SW1271 (YAP1⁺) and H1048 (POU2F3⁺). Although short tandem repeat (STR) profiling was not performed, all cell lines were obtained from ATCC and handled under strictly controlled conditions with detailed documentation and regular morphological monitoring. The consistency of phenotypic effects across cell lines further supports the reliability of the experimental system. Recent studies have suggested that certain cell lines traditionally classified as SCLC, including SW1271 and H841, may cluster with SMARCA4-deficient thoracic tumors based on molecular profiling. This highlights the biological overlap between SCLC subtypes and SMARCA4-deficient malignancies. Importantly, these models are characterized by non-neuroendocrine features (e.g., YAP1/POU2F3 expression), and therefore may represent a broader spectrum of high-grade thoracic tumors with shared transcriptional programs. Nevertheless, they remain widely used as

models of NE-low SCLC, and are appropriate for studying subtype-specific biology and tumor plasticity [108]. Additionally, in SW1271 cells, the non-targeting control vector significantly reduced ANXA1 expression compared to native cells, suggesting a cell line-specific effect of the transduction procedure. This limits the specificity of the model, as some results may be attributed to transduction-related changes rather than ANXA1 silencing alone. Therefore, we focused on differences between shANXA1 and shCTRL cells, while considering this limitation in SW1271.

Uncontrolled cell proliferation is one of the most obvious hallmarks of cancer, and is particularly important in neuroendocrine tumors, where mitotic count and Ki67 index are used for grading [127, 140]. Our growth rate analysis with trypan blue exclusion assay and Presto Blue viability assay revealed different effects of ANXA1 silencing in the three cell lines, with no significant change in H841, a decrease in SW1271, and a marked increase in H1048. This is in line with the literature, evidence shows that ANXA1 can both inhibit or promote proliferation in tumor cells or have no significant effect [45, 77]. In a 2024 study, a panel of ANXA1-expressing breast, ovarian, pancreatic, colorectal cancer and lung adenocarcinoma cell lines responded to an anti-ANXA1 antibody with suppressed cell proliferation, and the proposed mechanism was through G1 phase cell cycle arrest [10]. ANXA1 inhibition *in vitro* resulted in decreased proliferation in glioma [163, 177], papillary thyroid cancer [175], pancreatic cancer and oral squamous cell carcinoma cells as well [159]. On the other hand, ANXA1 overexpression was associated with suppressed proliferation in oral squamous cell carcinoma cells [159].

In lung cancer, association of ANXA1 with increased proliferation has been demonstrated in multiple studies. Three lung adenocarcinoma cell lines (A549, H1650, H1975) showed decreased proliferation in response to siRNA-mediated ANXA1 silencing, measured with CellTiter® Glo assay, potentially through involvement of the EGFR pathway [26]. Similar results were observed after lentiviral silencing of ANXA1 in A549 and H1299 lung adenocarcinoma cells with CCK-8 assay [39]. Guan et. al proposed that ANXA1 promoted proliferation, invasion and migration of A549 cells via the NF- κ B–miR-26a pathway [58]. ANXA1 enhanced proliferation in SqCLC cell lines but inhibited their migration, whereas ANXA1 knockout increased cell migration while reducing proliferation [37].

We can hypothesize that the different effects of ANXA1 silencing on growth rate reflects context-dependent signaling of ANXA1 in SCLC, due to intrinsic genetic and epigenetic differences as well as non-neuroendocrine subtype heterogeneity among these cell lines. For example, the increased proliferation of H1048 cells following ANXA1 silencing may be due to their initially higher dependence on ANXA1-regulated growth-suppressive pathways, which are relieved when ANXA1 is silenced. In contrast, SW1271 cells might utilize alternative signaling mechanisms or compensatory pathways that, upon ANXA1 loss, result in decreased proliferative capacity, reflecting functional redundancy or distinct oncogenic dependencies. These findings underscore the complexity of ANXA1 in SCLC biology and highlight the importance of cellular context and molecular background in determining its impact on tumor cell behavior [115]. Therefore, our findings are first to demonstrate context-dependent effects of ANXA1 on SCLC cell proliferation.

In our chemosensitivity assay with Presto Blue viability assay, ANXA1 silencing broadly reduced the sensitivity to both cisplatin and etoposide in all three cell lines, when tested at three concentrations comparable to patient plasma levels *in vitro* [4, 18, 93, 138]. This experimental design did not allow for dose–response curve fitting and IC₅₀ determination, however, we could evaluate relative treatment responses across cell lines. Upregulated ANXA1 has been linked to chemoresistance of multiple cancers, including the BT-549 breast cancer cell line to paclitaxel and doxorubicin [149], and the SW480 colon cell line to 5-fluorouracil [112]. However, ANXA1 was downregulated in adriamycin-resistant pumc-91 bladder cancer cell line [172]. A noteworthy concept proposed by a research group in 2016 is that FPR1 functions as a pattern recognition receptor, with ANXA1 serving as its DAMP ligand to guide DCs toward dying tumor cells and trigger an antitumor immune response during anthracycline therapy, therefore in the absence of ANXA1, cancer cells become resistant to treatment with dependence on the TME [153].

Regarding platinum agents, ANXA1 upregulation was shown to induce resistance to oxaliplatin in gastric cancer cells via the PI3K/AKT/mTOR pathway [128] and was identified in three cisplatin-resistant germ cell tumor cell lines as well [42]. In contrast, ANXA1 was downregulated in the cisplatin-resistant ovarian cancer cell line A2780 [74]. Furthermore, an ANXA1-derived peptide showed a synergistic effect with cisplatin in the

SiHa cervical cancer cell line in combination treatment [123]. Clinically, ANXA1 was a predictive biomarker of sensitivity to docetaxel, cisplatin and 5-fluorouracil (TPF) induction chemotherapy in oral squamous cell carcinoma patients [147]. In chemosensitive breast cancer cells, ANXA1 overexpression has been shown to increase resistance to multiple chemotherapeutic agents, including etoposide, melphalan, and adriamycin, suggesting a functional contribution to multidrug resistance [48, 162].

Reduced chemosensitivity in the SCLC cell lines we investigated, may be associated with alterations in cell cycle regulation, apoptotic pathways and expression of drug efflux transporters and EMT mediators influenced by ANXA1 [48]. Since the relative selectivity of chemotherapy relies on higher proliferative activity, the markedly lower growth rate of SW1271 shANXA1 cells compared with shCTRL suggests that the reduced chemosensitivity observed in this context is not a direct effect of ANXA1 silencing, but rather a consequence of escaping cytostatic effects due to slower proliferation [115]. In contrast, the findings in H1048 cells cannot be fully explained by their increased growth rate: while the markedly higher baseline cell counts resulting from rapid proliferation may account for the elevated fluorescence intensity observed at 5 μ M and 10 μ M treatments, at 20 μ M a chemosensitizing effect of ANXA1 silencing could be proposed. Importantly, high chemosensitivity can be an intrinsic feature of certain aggressive tumors driven by rapid division and specific genetic alterations; however, sensitivity is highly context-dependent and modulated by tumor type and a permissive TME. Therefore, from the divergent growth assay results, firm conclusions cannot be drawn, and our observations likely reflect the inherent biological heterogeneity of SCLC [115].

The Warburg effect is often linked to rapid tumor growth, survival under stress, therapy resistance, immune evasion, and invasiveness [89]. Research on metabolic profile of lung cancer mostly focused on NSCLC. However, in a retrospective clinical study of over 300 patients with limited- and extensive-stage SCLC, elevated LDH levels were significantly associated with reduced survival [62], and the MYC-driven subset of SCLC has been described as a metabolically distinct entity, particularly dependent on glycolysis [15]. Our results suggest high metabolic flexibility in the three investigated SCLC cell lines [115]. Silencing ANXA1 significantly decreased CS expression in H841

and H1048 cells, indicating a shift away from oxidative phosphorylation without raising LDH expression, a phenomenon only seen in the SW1271 shANXA1 cells.

While LDH is a recognized tumor-promoting enzyme [120], citrate synthase, like ANXA1, plays cancer-type-specific roles regarding promotion or inhibition of progression [64], it was even shown to increase sensitivity of A549 NSCLC cells to cisplatin [129]. The limited anaerobic shift to maintain redox balance and limit excess lactate production seen in our experiments suggests that instead of a complete shift to anaerobic metabolism, SCLC cells may compensate reduced TCA cycle activity by using alternative pathways to maintain metabolic flux [115]. These include redox-supporting pathways, such as the pentose phosphate pathway [117] or folate-mediated one-carbon metabolism [38] to provide NADPH to counteract oxidative stress. In parallel, anaplerotic processes, most prominently glutaminolysis [3, 73] replenish TCA cycle intermediates to maintain mitochondrial function. Together, these inputs allow the TCA cycle to continue operating through enzymes downstream of citrate synthase, ensuring ongoing energy and biosynthetic capacity. Rohwer et al. reported a critical role of ANXA1 in maintaining metabolism and proliferation in gastric cancer cells upon loss of HIF1 α , a key mediator of hypoxia-driven tumor progression [132]. ANXA1 knockdown impaired citrate utilization by reducing glutamine-driven anaplerosis into the TCA cycle and decreased lactate output, indicating altered glycolysis. Moreover, MYC, an identified regulator of the Warburg effect and glutamine metabolism in SCLC [15, 105] and a proposed mediator of the SCLC NE to non-NE transition [67], was completely lost upon simultaneous ANXA1 and HIF-1 α knockdown in gastric cancer cells, underscoring its role in ANXA1-mediated metabolic adaptation [132]. Collectively, these findings highlight that the effects of ANXA1 are highly context-dependent, as reflected in the heterogeneous responses of EMT and metabolic markers to ANXA1 silencing.

A consistent decrease in EMT after ANXA1 silencing was detected in H1048 cells, as seen in decreased vimentin and β -catenin expressions. Furthermore, morphological observations in culture also suggested reversal of EMT in H1048: ANXA1-silenced cells adhered more tightly to culture dishes and required prolonged TrypLE Express treatment for detachment, in line with a more epithelial-like phenotype with reduced invasive potential (Figure 15). Similar morphological changes were observed in basal-like MDA-MB-231 breast cancer cells after ANXA1 knockdown [29].

ANXA1 has been reported to promote EMT, supported by experimental findings where its overexpression or knockdown led to inverse changes in epithelial markers such as E-cadherin and corresponding proportional changes in mesenchymal markers such as vimentin, as shown in bladder cancer [87], papillary thyroid cancer [175], pancreatic cancer [9], prostate cancer [11, 169], and melanoma [142]. Conversely, other studies have reported the opposite effect in nasopharyngeal carcinoma cells [165] and oral squamous cell carcinoma cells [159], where ANXA1 overexpression reduced mesenchymal markers and/or increased epithelial markers, suggesting a context-dependent role of ANXA1 in EMT regulation. This pattern was recapitulated in the SW1271 cell line, where vimentin expression increased in shANXA1 cells. Deng et al. also showed in NSCLC cell lines H1299 and A549, that ANXA1 inhibits EMT through the inhibition of glycogen synthase kinase 3 beta (GSK3 β) phosphorylation and downregulation of β -catenin [52].

Proposed molecular mechanisms through which ANXA1 modulates EMT include several canonical pathways. ANXA1 has been linked to the induction of EMT-promoting transcriptional regulators, such as Smad via the TGF- β pathway and SNAIL through the PI3K/Akt axis [176]. The Janus kinase / signal transducer and activator of transcription (JAK2/STAT3) pathway has also been implicated: ANXA1 overexpression was shown to reverse EMT in breast cancer cells [101], while it promoted EMT in papillary thyroid cancer [175]. GSK3 β could be a multifaceted downstream effector of ANXA1, as it may either enhance stabilization of SNAIL to promote EMT [146] or downregulate β -catenin to suppress it [30]. In nasopharyngeal carcinoma, ANXA1 was reported to form a heterotrimer with vimentin and S100A9, thereby suppressing EMT [165]. Furthermore, in androgen-independent prostate cancer, ANXA1 was identified as a key mediator of hypoxia-induced EMT [169]. The mechanisms by which ANXA1 contributes to EMT have been reviewed elsewhere [176], including its involvement in MAPK/ERK and NF- κ B signaling and its capacity to enhance invasion and metastasis through extracellular-matrix remodeling via matrix metalloproteinase activation, microRNA-mediated regulation, and extracellular-vesicle-driven cell-matrix communication.

Groves et al. hypothesized that the transition from NE to non-NE states in SCLC may parallel EMT [57]. Transcriptomic analyses revealed a distinct, highly epithelial “A2” subtype within the NE group, while the other NE subtypes (A and N) displayed partial mesenchymal features. In contrast, non-NE subtypes (P and Y) were more

mesenchymal, yet expressed divergent mesenchymal gene programs, underscoring heterogeneity within the mesenchymal state itself. Notably, cisplatin treatment further shifted cell populations toward mesenchymal-like profiles, in line with the recognized role of EMT in therapy resistance. Collectively, these findings highlight the existence of EMT-associated intermediate states and emphasize contribution of EMT to SCLC heterogeneity, progression, and treatment failure [57]. This is in line with the finding in breast cancer, where initially ANXA1 can be downregulated, but upregulated in metastatic disease [147].

Although PD-L1 is a validated target and predictive marker in NSCLC, anti-PD-1/PD-L1 therapies show limited efficacy in SCLC, and PD-L1 status does not predict ICI response, consistent with a distinct immune microenvironment [118]. Given the evidence implicating ANXA1 in tumor immune tolerance and the central therapeutic role of the PD-1/PD-L1 checkpoint, we investigated whether ANXA1 silencing affects PD-L1 expression. We did not observe significant changes in PD-L1 expression upon ANXA1 silencing, suggesting that ANXA1 may not play a major role in regulating PD-L1 expression in SCLC. This implies that the ANXA1–PD-L1 axis may not be a key immunomodulatory pathway in SCLC, in contrast to NSCLC, further underscoring the need to identify alternative immune checkpoints in SCLC, as highlighted by several ongoing studies [34, 115, 125].

In other cancers, however, recent studies have described well-characterized mechanisms through which ANXA1 regulates PD-L1 expression in tumors. Ubiquitin Specific Peptidase 7 (USP-7) has been identified as a target of ANXA1, competing with PD-L1 for deubiquitination, thereby promoting degradation of PD-L1 in esophageal squamous cell carcinoma [160], and similar mechanisms were described with ANXA1-derived peptide A11 in NSCLC, melanoma and breast cancer cell lines [173]. Opposite correlations to this have been also described with different underlying pathways. ANXA1 was shown to stabilize PD-L1 in renal cell carcinoma cell lines and in NCI-H441 lung adenocarcinoma cell line [83, 167] by activation of the PI3K/Akt pathway through FPRs. In a study with melanoma, NSCLC, and breast cancer cell lines, knockdown of ANXA1 decreased PD-L1 levels, in line with the proposed mechanism described by the authors, whereby ANXA1 stabilizes STAT3 through blocking its interaction with PARP1, which in turn drives PD-L1 upregulation [164].

CD47, which delivers a macrophage “don’t-eat-me” signal via SIRP α , is an active immunotherapy target [170], blocking CD47–SIRP α can enhance phagocytic clearance, a concept especially relevant in SCLC where tumor-associated macrophages constitute a major immune compartment in the TME [34], moreover, CD47 is highly expressed in SCLC and its upregulation has been associated with impaired prognosis [41]. Nevertheless, ANXA1 silencing did not alter CD47 expression significantly in the tested cell lines, not supporting a direct signaling dependency between these two proteins at the cellular level [115]. In cervical cancer, transcriptomic and network analyses identified ANXA1 and CD47 as key hub genes with potential immunotherapy relevance [61], however, no studies to date have examined these two genes in detail.

5.2. ANXA1 as a modulator of SCLC subtypes and TME composition

Because of the significance of SCLC subtypes, we investigated whether ANXA1 expression affects YAP1 or POU2F3 expression in non-NE cell lines. In our experiments, YAP1 predominated as the transcriptional driver in the SW1271 and H841 cell lines, whereas POU2F3 was dominant in the H1048 line. Due to possible subtype overlaps and known shifts [67], both markers were assessed across all cell lines. Notably, silencing ANXA1 led to strong upregulation of YAP1 and POU2F3 in H1048 cells, however, no significant changes were observed in the other SCLC lines. These findings suggest an inverse relationship between ANXA1 and non-neuroendocrine subtype markers in H1048 cells, though the excessive heterogeneity of SCLC suggests that this effect is unlikely to be generalized across all tumor subtypes [115]. Previously, YAP1 knockout reduced proliferation in H69AR and SBC5 SCLC cells [135] and inhibited growth in H841 and SW1271 cells [102], suggesting that ANXA1 silencing may indirectly increase proliferation in H1048 via YAP1 induction. The absence of proliferation increase in other shANXA1 cell lines likely reflects their distinct molecular signaling landscapes [115]. Interestingly, ANXA1 was reported to positively correlate with YAP1 expression previously. In breast cancer, YAP1 overexpression resulted in protection against DNA damage in ANXA1 knockdown [148], and in hypopharynx carcinoma, ANXA1 suppressed lymph node metastasis through YAP-dependent regulation [86], supporting a highly context-dependent axis. POU2F3 and ANXA1 have been identified in a multi-omics study among the key genes distinguishing the SCLC-I subtype from other subtypes

[21]. However, their concurrent investigation, including potential gene-expression changes and potential relationship had not been explored prior to our publication.

The *in silico* part of our work using ESTIMATE and EPIC supported previous observations that ANXA1-high SCLC exhibits increased immune-cell infiltration, particularly by TAMs [24, 92]. These findings are presented as supportive evidence and were not a primary focus of the thesis.

Taken together, our findings support the well-established notion, that the effects of ANXA1 are highly context-dependent, now presented in SCLC, varying across cellular backgrounds and pathways. While ANXA1 does not appear to be an ideal therapeutic target in this tumor type, its pleiotropic roles in proliferation, chemosensitivity, EMT and immune signaling highlight its importance in shaping the malignant phenotype. Therefore, studying ANXA1 provides valuable insights into the biological heterogeneity of SCLC and may help to identify alternative vulnerabilities and novel immune checkpoints and regulators relevant to this disease. Future studies could investigate the proposed signaling pathways and gene expression changes, for example PI3K/AKT, JAK/STAT, GSK3 β and MYC expression, to characterize the underlying mechanisms of heterogeneous effects of ANXA1 across SCLC subtypes.

5.3. Clinical relevance of ANXA1 and the CD70/CD27 axis in SCLC

Due to the limited size of the ANXA1 cohort, analyses were restricted to descriptive evaluation based on IHC and double IF. ANXA1 expression was detected in both tumor nests and stromal compartments, with ANXA1–CD45 co-expression confined to the stroma. ANXA1-positive tumor cells did not colocalize with vimentin-positive CAFs, suggesting distinct cellular localization patterns within the TME. In contrast, CD68⁺ tumor-associated macrophages expressed ANXA1 in both stromal and tumor compartments, supporting a potential role in macrophage-enriched microenvironments characteristic of SCLC. These findings are in line with previous reports showing that ANXA1 can influence stromal cell behavior and macrophage polarization, including CAF transformation [22] and M2 macrophage polarization in esophageal squamous carcinoma [99].

The CD70–CD27 axis is an emerging focus in the development of novel immunotherapeutic strategies. Although the CD70-CD27 axis functions as a co-stimulatory pathway, its abnormal overexpression in tumors facilitates immune evasion

by inducing T cell exhaustion, promoting T cell apoptosis, and enhancing regulatory T cell activity [79]. Since CD70 expression is physiologically restricted to activated T cells, B cells, and dendritic cells, targeting CD70 may be a valid therapeutic strategy with high tumor-selectivity, potentially fewer off-target effects and good tolerability [79]. Beside hematological malignancies, several solid tumors were reported to have CD70-high subsets, including pleural mesothelioma, glioblastoma, ovarian cancer, pancreatic cancer, melanoma and colorectal cancer, in most cases, associated with poor prognosis [44]. Recent pan-cancer analysis of gene expression data revealed upregulation of CD70 in renal cell carcinoma, head and neck squamous cell carcinoma, thyroid carcinoma, and lung adenocarcinoma [174]. In nasopharyngeal carcinoma (NPC), CD70 is expressed on tumor cells, while CD27⁺ lymphocytes infiltrate the tumors and are associated with better prognosis, however, high CD70 expression in CD27-high tumors tends to impair prognosis, suggesting a complex interplay between CD70 and CD27 in the TME [106]. In a different study with NPC cells, CD70 blockage restored cytotoxic T cell mediated immunity and acted synergistically with anti-PD-1 therapy [56]. CD70 was also associated with metastasis in NSCLC, pancreatic cancer and osteosarcoma [44], and notably, lung-specific metastasis in breast cancer [94]. In NSCLC, anti-CD70 antibodies were cytotoxic as monotherapy [70], synergistic with cisplatin [68] and cisplatin-docetaxel chemotherapy [43]. CD70 upregulation appears to be linked to EMT, as demonstrated by increased CD70 expression in NSCLC exhibiting a mesenchymal phenotype, with particular overexpression in the sarcomatoid carcinoma subtype, associated with poor prognosis [113]. The EMT-inducing transcription factor ZEB1 was shown to promote CD70 expression, reduce CD3⁺ and CD8⁺ T cell densities, and induce T cell exhaustion [113]. In the context of EMT-driven resistance to EGFR tyrosine kinase inhibitors (TKIs), CD70 upregulation seems to be an early event in metastatic progression. Moreover, drug-tolerant persistent cells in this setting are sensitive to therapies targeting CD70, including antibody-drug conjugates, chimeric antigen receptor T cell (CAR-T) cells, and NK cells [110]. This suggests a key role for CD70 in EMT-associated immune evasion and drug resistance in NSCLC [110]. The expression, distribution, and effects of CD70 and CD27 vary widely across tumors. We aimed to characterize their expression patterns and associations with overall survival in SCLC.

In our cohort, CD70 was primarily expressed in SCLC tumor nests with weaker stromal staining, while CD27 expression was largely stromal. Expression patterns also correlated differently with immune context. CD70 did not associate with immune cell densities in either tumor or stroma, whereas high CD27 expression coincided with reduced stromal CD45⁺ and CD8⁺ T-cell infiltration. Analysis of single-cell RNA datasets in lung adenocarcinoma showed upregulation of both CD70 and CD27, associated with T and B cell functions that contribute to a TME unfavorable to prognosis [23]. In line with a macrophage-rich TME in SCLC [34, 35], we found substantial CD70 expression in CD68⁺ TAMs, with little coexpression on T or B cells. Regarding CD27, staining patterns resembled those reported in NSCLC within T cells, but we also detected expression in TAMs, a feature not previously shown in NSCLC [32].

Detailed assessment showed diffuse CD70 staining in tumor cells, with lower intensity in vimentin-positive clusters but clear expression in vimentin-positive CAFs within the stroma. CD68⁺ TAMs expressed CD70 broadly in both tumor and stromal compartments, while CD3⁺ T cells displayed only limited expression, and CD20⁺ B cells were negative [32]. Similar CAF-associated CD70 expression has been reported in colorectal cancer, where CD70⁺ CAF density predicted poor outcome and promoted tumor cell migration and Treg induction [69, 78]. In glioblastoma, CD70 expression correlated with M2-polarized TAMs, although spatial coexpression was not investigated [54, 124]. Similarly to ANXA1, CD70 likely has context-dependent roles. For instance, CD70 expressed on dendritic cells can stimulate antitumor CD8⁺ T-cell responses in mice with immunogenic melanoma [76], and, in a phase II clinical trial with anti-PD-1 agent sintilimab combined with platinum-etoposide chemotherapy involving ES-SCLC patients, CD70 upregulation was identified in the subgroup experiencing durable clinical benefit, correlating with improved progression-free survival, highlighting the potential role of CD70 as a biomarker for therapeutic response and clinical outcome in this setting [166].

CD27 expression was limited to the TME, colocalizing with vimentin⁺ CAFs, CD3⁺ T cells, and some CD68⁺ TAMs, but not with CD20⁺ B cells. In NSCLC, Human Protein Atlas data show CD27 expression only in stromal compartments without tumor cell positivity in NSCLC as well. Besides hematological malignancies and NSCLC, CD27 expression has been reported in solid tumors, such as renal cell carcinoma,

melanoma [136], nasopharyngeal carcinoma [56], and expression on lymphocytes in lung LCNEC [32, 41].

From a prognostic perspective, our results show that high ANXA1 expression may be linked to poorer prognosis in SCLC [92]. While ANXA1 expression has been predominantly linked to reduced overall survival in multiple solid tumors, such as gastrointestinal cancers and lung adenocarcinoma [2, 91, 114, 171], prolonged survival has also been described in squamous cell lung carcinoma [37] and ovarian cancer [100], supporting the notion that the prognostic relevance of ANXA1 is tumor type-dependent and that universal conclusions cannot be drawn.

High CD70 expression also correlated with worse survival in our SCLC cohort, particularly in early-stage and immune-oasis tumors, whereas CD27 expression showed no prognostic significance. Multivariate analysis confirmed CD70 expression and tumor stage as independent predictors of OS [32]. Using a publicly available Kaplan–Meier Plotter tool which compiled overall survival and RNAseq expression data from 504 patients across multiple cohorts, results are similar in NSCLC, where high CD70 expression correlated with shorter OS ($p = 0.008$, HR 1.49), and high CD27 expression conferred a survival advantage ($p = 0.0085$, HR 0.67) [32, 60]. Our results suggest that CD70 expression may hold relevance for patient stratification in future immunotherapy trials in SCLC.

5.4. Further considerations

Extensive intratumoral heterogeneity and cellular plasticity in SCLC allow multiple molecular subtypes to coexist within a single tumor. This underscores that identifying subtype-specific vulnerabilities—such as those associated with more immune-infiltrated states—is insufficient on its own and will likely require combination strategies [27, 125, 143, 151]. Despite recent progress, chemotherapy remains the backbone of SCLC treatment, and immunotherapy with ICIs provide only modest benefit, typically extending survival by 2–4 months.

The high TMB, the near-universal inactivation of TP53 and RB1, the scarcity of clearly targetable oncogenic drivers, lack of reliable biomarkers and the absence of consistent co-occurring or mutually exclusive mutational patterns all highlight why effective targeted therapy for SCLC remains a distant prospect. This challenge is aggravated by the diverse etiological and cellular origins of SCLC—including smoking-

induced and disease arising in never-smokers and transformation from other cancer types—each of which may require highly refined therapeutic approaches that are not yet defined. Moreover, treatment itself can induce additional genetic and epigenetic changes, driving resistance mechanisms and further enhancing subtype plasticity [125, 143, 151].

Together, these factors explain the substantial complexity of SCLC biology and reinforce the need for continued, in-depth research, where even small-scale insights into underlying mechanisms are valuable.

In this context, ANXA1-associated signaling may also play a role in therapy-induced adaptation, potentially linking stress responses to phenotypic transitions. Nevertheless, comprehensive molecular profiling has begun to uncover subtype-specific vulnerabilities, as reviewed elsewhere [27, 125, 141, 143, 151]. For instance, MYC-driven and RB1-mutant tumors exhibit increased sensitivity to Aurora kinase inhibition, and the AURKA inhibitor alisertib has demonstrated improved efficacy over chemotherapy when incorporated into combination regimens. schlafen family member 11 (SLFN11) is one of the most validated predictive biomarker to date, associated with sensitivity to DNA-damaging chemotherapy and poly(ADP-ribose) polymerase (PARP) inhibition, its clinical utility is now being prospectively evaluated in a phase II trial. POU2F3-high (tuft-cell-like) SCLC has been identified as preferentially responsive to SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily A (SMARCA) inhibitors, and retrospective analyses from IMpower133 and CASPIAN suggest that this subtype may also gain more durable benefit from ICI-containing regimens [151].

Among novel cytotoxic agents, the RNA polymerase II inhibitor lurbinectedin has demonstrated meaningful efficacy in relapsed SCLC, leading to approval in second-line therapy. Current trials are now evaluating its use in combination with ICIs, reflecting an emerging paradigm that integrates transcriptional stress and DNA damage with enhanced immune activation [151].

Novel combinatorial strategies aim to simultaneously exploit tumor-intrinsic vulnerabilities and counteract the pronounced immune escape characteristic of SCLC. Novel immunotherapeutic approaches include targeting emerging immune checkpoints such as lymphocyte activation gene 3 (LAG3) and the B7-H3 immunoregulatory axis. Targeting tumor-selective surface molecules has also become a promising strategy, with

T-cell engagers (TCEs), antibody–drug conjugates (ADCs), radioligand, and CAR-T or CAR-NK cell-based therapies. A key example is tarlatamab, recently approved in the second-line treatment, which is a bispecific TCE, binding delta-like ligand 3 (DLL3, selectively expressed on neuroendocrine SCLC cells) and CD3 (selectively expressed on T cells), showing significant response rates and improved survival so far. In addition, CD47 has emerged as another actionable target now under investigation in early-phase clinical trials. [125, 151].

Early diagnosis remains essential for effective intervention. Recent advances in liquid biopsy with circulating tumor DNA profiling, is a promising method for earlier detection and disease monitoring to track tumor heterogeneity, molecular evolution, and emerging resistance mechanisms, also holding promise for acceleration of research [47, 143, 151].

Together, these advances pave the way for more personalized treatment strategies and improved outcomes in this aggressive malignancy. Further research into the mechanistic roles of ANXA1 is warranted to clarify its contribution to intratumoral heterogeneity, NE to non-NE plasticity, metastasis formation and immune evasion. Moreover, targeting the CD70–CD27 axis in preclinical models could provide additional insight into the functional significance of these pathways and their potential as therapeutic targets in SCLC.

6. Conclusions

Although SCLC has been considered a uniform disease, leading to a “one-size-fits-all” therapeutic strategy and no progress in treatment over several decades, research over the past years has revealed that SCLC is an extremely heterogeneous and highly plastic malignancy. Identifying molecular subtypes has emerged as a promising approach to find therapeutic vulnerabilities, however, consensus on classification and subtype stability remains an active area of research. The adverse immunosuppressive TME of SCLC further complicates patient stratification and contributes to treatment resistance [88, 125, 151].

In this study, we focused on two immune-related factors with potential relevance in SCLC biology: ANXA1 and the CD70–CD27 immune checkpoint axis. We addressed three main aspects: **the functional effects of ANXA1 perturbation** in SCLC, **its association with subtype switches and TME features**, and the **clinical context of ANXA1 and the CD70–CD27** axis in patient samples, using *in vitro* models, *in silico* analyses, and patient cohorts.

ANXA1 is known to exert complex, sometimes conflicting roles in cancer, acting as either a tumor suppressor or a tumor promoter depending on tissue and cancer type [45, 77]. Only two studies had previously assessed ANXA1 in SCLC [20, 96]. The CD70-CD27 axis is an emerging immunoregulatory immune checkpoint pathway in cancer. Its aberrant activation promotes immune evasion by inducing T-cell exhaustion, apoptosis, and regulatory T-cell expansion [44, 136]. Because its physiological expression is restricted to activated immune cells, CD70 represents an attractive therapeutic target with potentially limited off-target toxicity.

In our work, ANXA1 silencing in three non-NE SCLC cell lines produced diverse effects on **functional endpoints**. ANXA1 silencing variably affected proliferation: no change in H841 cells, decreased proliferation in SW1271, and increased proliferation in H1048. Chemosensitivity to cisplatin and etoposide was consistently reduced following ANXA1 silencing. ANXA1 silencing in H1048 led to reduced vimentin and β -catenin expression and more epithelial-like morphology, suggesting EMT reversal and decreased invasive potential. In contrast, EMT marker changes in SW1271 were consistent with a more mesenchymal shift after ANXA1 knockdown. These findings mirror the literature, where ANXA1 can either promote or inhibit EMT in a context-specific manner.

CS and LDHa expressions were also altered in a cell line-specific manner, implying shifts between oxidative phosphorylation and glycolysis. The patterns suggest that rather than switching to fully anaerobic metabolism, SCLC cells may rely on compensatory pathways to maintain TCA cycle flux and redox balance when ANXA1-dependent regulation is disturbed. These observations are consistent with the idea that ANXA1 can influence metabolic adaptation in hypoxic or HIF1 α -compromised settings and intersect with MYC-driven metabolic programs [105, 132].

ANXA1 silencing did not significantly alter PD-L1 or CD47 levels in the SCLC cell lines tested. Therefore, ANXA1 is unlikely to be a major upstream regulator of these checkpoint molecules, and that the ANXA1–PD-L1 or ANXA1–CD47 axes described in other tumors[161, 164, 173] may not be directly transferable to SCLC biology.

SCLC non-NE subtype-specific markers, YAP1 and POU2F3 were markedly upregulated in response to ANXA1 silencing, specifically in H1048, suggesting an inverse relationship between ANXA1 and these transcriptional drivers in this context. No similar changes were observed in the other cell lines, suggesting that ANXA1–driver interactions are not uniform across SCLC and likely depend on the underlying molecular background. Bioinformatic analyses showed that ANXA1-high tumors are associated with increased immune infiltration, particularly with TAMs.

In the clinical context, ANXA1 expression was detected in both stroma and tumor nests, showing co-localization with CD45+ immune cells and C68+ macrophages. Across our CD70 SCLC cohort, CD70 was mainly expressed in tumor nests, CAFs and TAMs, while CD27 expression was confined to stromal cell populations. Regarding prognosis, in a small patient cohort, retrospective analysis showed poorer survival in ANXA1-high SCLC. However, limited sample numbers prevent firm conclusions on prognostic significance.

Across our CD70 cohort, high CD70 expression significantly correlated with worse overall survival, while CD27 expression had no prognostic effect. These findings align with reports in other solid tumors and suggest that CD70 may serve as a clinically relevant biomarker and potential immunotherapy target in SCLC.

Overall, our findings provide the first comprehensive characterization of ANXA1 in SCLC, exerting context-dependent functional effects, influencing proliferation,

therapy response, EMT and metabolic regulation, transcriptional driver activation and immune infiltration.

In conclusion, our results highlight the multifaceted and context-dependent roles of ANXA1 and CD70 in SCLC. ANXA1 contributes to cellular heterogeneity, treatment response, metabolic and EMT plasticity, while CD70 identifies a subgroup of patients with potentially poorer prognosis and may represent a new therapeutic target. As SCLC continues to evolve under treatment pressure, understanding such context-dependent vulnerabilities and immune escape mechanisms will be essential for developing more effective, personalized therapies.

7. Summary

The complex pathogenesis of SCLC involves intricate interactions between multiple molecular pathways, including ANXA1 and the CD70-CD27 axis, which appear to be intertwined through processes such as EMT and immune modulation. These pathways contribute to tumor progression, immune evasion, and metastatic potential, underscoring the multifaceted biology of SCLC.

Our study demonstrates that ANXA1 plays highly context-dependent roles in SCLC, affecting multiple functional endpoints, including proliferation, chemosensitivity, EMT, and immune-related molecule expression. ANXA1 expression is also associated in a cell type-dependent manner with non-NE subtype-specific markers and increased immune cell infiltration. In the clinical context, ANXA1 shows both stromal and intratumoral expression *in situ*, co-localizing with CD45⁺ immune cells and CD68⁺ macrophages, and is associated with poorer survival. We also show that CD70 is a clinically relevant biomarker in SCLC, with high tumor expression predicting worse overall survival and aligning with macrophage-rich TME. In contrast, CD27 expression showed only stromal expression and was associated with no prognostic impact.

Although probably not an ideal therapeutic target itself, uncovering mechanisms through which ANXA1 influences SCLC phenotype, reflects underlying biological heterogeneity and may help reveal alternative vulnerabilities in SCLC. Together, these findings highlight the importance of molecular and microenvironmental context in SCLC biology and support further investigation of ANXA1-linked pathways and the CD70-CD27 axis as potential contributors to SCLC progression, immune evasion, and therapy resistance.

8. References

1. Ahvati, H., R. Roudi, N. Sobhani, and F. Safari, *CD47 as a potent target in cancer immunotherapy: A review*. Biochim Biophys Acta Rev Cancer, 2025. **1880**(2): p. 189294.
2. Al-Ali, H.N., S.J. Crichton, C. Fabian, C. Pepper, D.R. Butcher, F.C. Dempsey, and C.N. Parris, *A therapeutic antibody targeting annexin-A1 inhibits cancer cell growth in vitro and in vivo*. Oncogene, 2024. **43**(8): p. 608-614.
3. Altman, B.J., Z.E. Stine, and C.V. Dang, *From Krebs to clinic: glutamine metabolism to cancer therapy*. Nat Rev Cancer, 2016. **16**(10): p. 619-34.
4. Ando, Y., H. Minami, H. Saka, M. Ando, S. Sakai, and K. Shimokata, *Therapeutic drug monitoring of etoposide in a 14-day infusion for non-small-cell lung cancer*. Jpn J Cancer Res, 1996. **87**(2): p. 200-5.
5. Baine, M.K., C.A. Febres-Aldana, J.C. Chang, A.A. Jungbluth, S. Sethi, C.R. Antonescu, W.D. Travis, M.S. Hsieh, M.S. Roh, R.J. Homer, et al., *POU2F3 in SCLC: Clinicopathologic and Genomic Analysis With a Focus on Its Diagnostic Utility in Neuroendocrine-Low SCLC*. J Thorac Oncol, 2022. **17**(9): p. 1109-1121.
6. Baine, M.K., M.S. Hsieh, W.V. Lai, J.V. Egger, A.A. Jungbluth, Y. Daneshbod, A. Beras, R. Spencer, J. Lopardo, F. Bodd, et al., *SCLC Subtypes Defined by ASCL1, NEUROD1, POU2F3, and YAP1: A Comprehensive Immunohistochemical and Histopathologic Characterization*. J Thorac Oncol, 2020. **15**(12): p. 1823-1835.
7. Bankhead, P., M.B. Loughrey, J.A. Fernandez, Y. Dombrowski, D.G. McArt, P.D. Dunne, S. McQuaid, R.T. Gray, L.J. Murray, H.G. Coleman, et al., *QuPath: Open source software for digital pathology image analysis*. Sci Rep, 2017. **7**(1): p. 16878.
8. Battifora, H., *The multitumor (sausage) tissue block: novel method for immunohistochemical antibody testing*. Lab Invest, 1986. **55**(2): p. 244-8.
9. Belvedere, R., P. Saggese, E. Pessolano, D. Memoli, V. Bizzarro, F. Rizzo, L. Parente, A. Weisz, and A. Petrella, *miR-196a Is Able to Restore the Aggressive Phenotype of Annexin A1 Knock-Out in Pancreatic Cancer Cells by CRISPR/Cas9 Genome Editing*. Int J Mol Sci, 2018. **19**(7).

10. Bist, P., S. Shu, H. Lee, S. Arora, S. Nair, J.Y. Lim, J. Dayalan, S. Gasser, S.K. Biswas, A.M. Fairhurst, et al., *Annexin-A1 regulates TLR-mediated IFN-beta production through an interaction with TANK-binding kinase 1*. J Immunol, 2013. **191**(8): p. 4375-82.
11. Bizzarro, V., R. Belvedere, V. Migliaro, E. Romano, L. Parente, and A. Petrella, *Hypoxia regulates ANXA1 expression to support prostate cancer cell invasion and aggressiveness*. Cell Adh Migr, 2017. **11**(3): p. 247-260.
12. Boudhraa, Z., F. Rondepierre, L. Ouchchane, R. Kintossou, A. Trzeciakiewicz, F. Franck, J. Kanitakis, B. Labeille, J. Joubert-Zakeyh, B. Bouchon, et al., *Annexin A1 in primary tumors promotes melanoma dissemination*. Clin Exp Metastasis, 2014. **31**(7): p. 749-60.
13. Bozyk, A., K. Wojas-Krawczyk, P. Krawczyk, and J. Milanowski, *Tumor Microenvironment-A Short Review of Cellular and Interaction Diversity*. Biology (Basel), 2022. **11**(6).
14. Bragelmann, J., S. Bohm, M.R. Guthrie, G. Mollaoglu, T.G. Oliver, and M.L. Sos, *Family matters: How MYC family oncogenes impact small cell lung cancer*. Cell Cycle, 2017. **16**(16): p. 1489-1498.
15. Cargill, K.R., C.A. Stewart, E.M. Park, K. Ramkumar, C.M. Gay, R.J. Cardnell, Q. Wang, L. Diao, L. Shen, Y.H. Fan, et al., *Targeting MYC-enhanced glycolysis for the treatment of small cell lung cancer*. Cancer Metab, 2021. **9**(1): p. 33.
16. Carney, D.N., A.F. Gazdar, G. Bepler, J.G. Guccion, P.J. Marangos, T.W. Moody, M.H. Zweig, and J.D. Minna, *Establishment and identification of small cell lung cancer cell lines having classic and variant features*. Cancer Res, 1985. **45**(6): p. 2913-23.
17. Cerami, E., J. Gao, U. Dogrusoz, B.E. Gross, S.O. Sumer, B.A. Aksoy, A. Jacobsen, C.J. Byrne, M.L. Heuer, E. Larsson, et al., *The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data*. Cancer Discov, 2012. **2**(5): p. 401-4.
18. Charlier, C., P. Kintz, N. Dubois, and G. Plomteux, *Fatal overdosage with cisplatin*. J Anal Toxicol, 2004. **28**(2): p. 138-40.
19. Chen, H.Z., N.H. Kim, D. Nishizaki, M.K. Nesline, J.M. Conroy, P. DePietro, S. Pabla, S. Kato, and R. Kurzrock, *PD-1 transcriptomic landscape across cancers*

- and implications for immune checkpoint blockade outcome. NPJ Genom Med, 2025. **10**(1): p. 21.
20. Chen, P., J. Min, H. Wu, H. Zhang, C. Wang, G. Tan, and F. Zhang, *Annexin A1 is a potential biomarker of bone metastasis in small cell lung cancer*. Oncol Lett, 2021. **21**(2): p. 141.
 21. Chen, Y., Z. Fang, Y. Tang, Y. Jin, C. Guo, L. Hu, Y. Xu, X. Ma, J. Gao, M. Xie, et al., *Integrative analysis of multi-omics data reveals the heterogeneity and signatures of immune therapy for small cell lung cancer*. Clin Transl Med, 2021. **11**(12): p. e620.
 22. Chen, Y., S. Zhu, T. Liu, S. Zhang, J. Lu, W. Fan, L. Lin, T. Xiang, J. Yang, X. Zhao, et al., *Epithelial cells activate fibroblasts to promote esophageal cancer development*. Cancer Cell, 2023. **41**(5): p. 903-918 e8.
 23. Chen, Z., X. Yang, G. Bi, J. Liang, Z. Hu, M. Zhao, M. Li, T. Lu, Y. Zheng, Q. Sui, et al., *Ligand-receptor interaction atlas within and between tumor cells and T cells in lung adenocarcinoma*. Int J Biol Sci, 2020. **16**(12): p. 2205-2219.
 24. Cheng, T.Y., M.S. Wu, J.T. Lin, M.T. Lin, C.T. Shun, H.Y. Huang, K.T. Hua, and M.L. Kuo, *Annexin A1 is associated with gastric cancer survival and promotes gastric cancer cell invasiveness through the formyl peptide receptor/extracellular signal-regulated kinase/integrin beta-1-binding protein 1 pathway*. Cancer, 2012. **118**(23): p. 5757-67.
 25. Cheng, Y., D.R. Spigel, B.C. Cho, K.K. Laktionov, J. Fang, Y. Chen, Y. Zenke, K.H. Lee, Q. Wang, A. Navarro, et al., *Durvalumab after Chemoradiotherapy in Limited-Stage Small-Cell Lung Cancer*. N Engl J Med, 2024. **391**(14): p. 1313-1327.
 26. Chuang, M.C., J.H. Lung, Y.C. Chen, Y.C. Lin, Y.C. Li, and M.S. Hung, *The Association of Annexin A1 and Chemosensitivity to Osimertinib in Lung Cancer Cells*. Cancers (Basel), 2021. **13**(16).
 27. Cognigni, V., I. Toscani, S. D'Agnelli, F. Pecci, L. Righi, R. Berardi, and M. Tiseo, *Molecular heterogeneity of small cell lung cancer and new therapeutic possibilities: a narrative review of the literature*. Transl Lung Cancer Res, 2025. **14**(4): p. 1441-1455.

28. Das, S. and S. Samaddar, *Recent Advances in the Clinical Translation of Small-Cell Lung Cancer Therapeutics*. Cancers (Basel), 2025. **17**(2).
29. de Graauw, M., M.H. van Miltenburg, M.K. Schmidt, C. Pont, R. Lalai, J. Kartopawiro, E. Pardali, S.E. Le Devedec, V.T. Smit, A. van der Wal, et al., *Annexin A1 regulates TGF-beta signaling and promotes metastasis formation of basal-like breast cancer cells*. Proc Natl Acad Sci U S A, 2010. **107**(14): p. 6340-5.
30. Deng, C., X. Liu, C. Zhang, L. Li, S. Wen, X. Gao, and L. Liu, *ANXA1-GSK3beta interaction and its involvement in NSCLC metastasis*. Acta Biochim Biophys Sin (Shanghai), 2021. **53**(7): p. 912-924.
31. Desai, P., N. Takahashi, R. Kumar, S. Nichols, J. Malin, A. Hunt, C. Schultz, Y. Cao, D. Tillo, D. Nousome, et al., *Microenvironment shapes small-cell lung cancer neuroendocrine states and presents therapeutic opportunities*. Cell Rep Med, 2024. **5**(6): p. 101610.
32. Dora, D., Z. Megyesfalvi, I. Voros, A. Paal, P. Takacs, D. Dobos, B. Lorincz, S.M.Z. Bokhari, K. Aloss, G. Pallag, et al., *Expression of costimulatory molecule CD70 is prognostic in small cell lung cancer*. Cancer Immunol Immunother, 2025. **74**(5): p. 165.
33. Dora, D., C. Rivard, H. Yu, P. Bunn, K. Suda, S. Ren, S. Lueke Pickard, V. Laszlo, T. Harko, Z. Megyesfalvi, et al., *Neuroendocrine subtypes of small cell lung cancer differ in terms of immune microenvironment and checkpoint molecule distribution*. Mol Oncol, 2020. **14**(9): p. 1947-1965.
34. Dora, D., C. Rivard, H. Yu, S.L. Pickard, V. Laszlo, T. Harko, Z. Megyesfalvi, E. Dinya, C. Gerdan, G. Szegvari, et al., *Characterization of Tumor-Associated Macrophages and the Immune Microenvironment in Limited-Stage Neuroendocrine-High and -Low Small Cell Lung Cancer*. Biology (Basel), 2021. **10**(6).
35. Dora, D., C. Rivard, H. Yu, S.L. Pickard, V. Laszlo, T. Harko, Z. Megyesfalvi, C. Gerdan, E. Dinya, K. Hoetzenecker, et al., *Protein Expression of immune checkpoints STING and MHCII in small cell lung cancer*. Cancer Immunol Immunother, 2023. **72**(3): p. 561-578.

36. Dora, D., I. Voros, Z.V. Varga, P. Takacs, V. Teglas, J. Moldvay, and Z. Lohinai, *BRAF RNA is prognostic and widely expressed in lung adenocarcinoma*. Transl Lung Cancer Res, 2023. **12**(1): p. 27-41.
37. Elakad, O., Y. Li, N. Gieser, S. Yao, S. Kuffer, M. Hinterthaler, B.C. Danner, A. von Hammerstein-Equord, P. Strobel, and H. Bohnenberger, *Role of Annexin A1 in Squamous Cell Lung Cancer Progression*. Dis Markers, 2021. **2021**: p. 5520832.
38. Fan, J., J. Ye, J.J. Kamphorst, T. Shlomi, C.B. Thompson, and J.D. Rabinowitz, *Quantitative flux analysis reveals folate-dependent NADPH production*. Nature, 2014. **510**(7504): p. 298-302.
39. Fang, Y., X. Guan, T. Cai, J. Long, H. Wang, X. Xie, and Y. Zhang, *Knockdown of ANXA1 suppresses the biological behavior of human NSCLC cells in vitro*. Mol Med Rep, 2016. **13**(5): p. 3858-66.
40. Farwell, M.D., D.A. Pryma, and D.A. Mankoff, *PET/CT imaging in cancer: current applications and future directions*. Cancer, 2014. **120**(22): p. 3433-45.
41. Ferencz, B., Z. Megyesfalvi, K. Csende, J. Fillinger, V. Poor, A. Lantos, O. Pipek, A. Solyom-Tisza, F. Renyi-Vamos, K. Schelch, et al., *Comparative expression analysis of immune-related markers in surgically resected lung neuroendocrine neoplasms*. Lung Cancer, 2023. **181**: p. 107263.
42. Fichtner, A., H. Bohnenberger, O. Elakad, A. Richter, C. Lenz, C. Oing, P. Strobel, S. Kueffer, D. Nettersheim, and F. Bremmer, *Proteomic profiling of cisplatin-resistant and cisplatin-sensitive germ cell tumour cell lines using quantitative mass spectrometry*. World J Urol, 2022. **40**(2): p. 373-383.
43. Flieswasser, T., A. Van den Eynde, L. Freire Boullosa, J. Melis, C. Hermans, C. Merlin, H.W. Lau, J. Van Audenaerde, F. Lardon, E. Smits, et al., *Targeting CD70 in combination with chemotherapy to enhance the anti-tumor immune effects in non-small cell lung cancer*. Oncoimmunology, 2023. **12**(1): p. 2192100.
44. Flieswasser, T., A. Van den Eynde, J. Van Audenaerde, J. De Waele, F. Lardon, C. Riether, H. de Haard, E. Smits, P. Pauwels, and J. Jacobs, *The CD70-CD27 axis in oncology: the new kids on the block*. J Exp Clin Cancer Res, 2022. **41**(1): p. 12.

45. Foo, S.L., G. Yap, J. Cui, and L.H.K. Lim, *Annexin-A1 - A Blessing or a Curse in Cancer?* Trends Mol Med, 2019. **25**(4): p. 315-327.
46. Fu, Z., S. Zhang, B. Wang, W. Huang, L. Zheng, and A. Cheng, *Annexin A1: A double-edged sword as novel cancer biomarker.* Clin Chim Acta, 2020. **504**: p. 36-42.
47. Fur, G.M., K. Nemes, E. Mago, A.A. Beno, P. Topolcsanyi, J. Moldvay, and L.S. Pongor, *Applied models and molecular characteristics of small cell lung cancer.* Pathol Oncol Res, 2024. **30**: p. 1611743.
48. Ganesan, T., A. Sinniah, T.S. Ramasamy, and M.A. Alshawsh, *Cracking the code of Annexin A1-mediated chemoresistance.* Biochem Biophys Res Commun, 2024. **725**: p. 150202.
49. Ganti, A.K.P., B.W. Loo, M. Bassetti, C. Blakely, A. Chiang, T.A. D'Amico, C. D'Avella, A. Dowlati, R.J. Downey, M. Edelman, et al., *Small Cell Lung Cancer, Version 2.2022, NCCN Clinical Practice Guidelines in Oncology.* J Natl Compr Canc Netw, 2021. **19**(12): p. 1441-1464.
50. Gao, K., X. Li, S. Luo, and L. Zhao, *An overview of the regulatory role of annexin A1 in the tumor microenvironment and its prospective clinical application (Review).* Int J Oncol, 2024. **64**(5).
51. Gavins, F.N. and M.J. Hickey, *Annexin A1 and the regulation of innate and adaptive immunity.* Front Immunol, 2012. **3**: p. 354.
52. Gay, C.M., C.A. Stewart, E.M. Park, L. Diao, S.M. Groves, S. Heeke, B.Y. Nabet, J. Fujimoto, L.M. Solis, W. Lu, et al., *Patterns of transcription factor programs and immune pathway activation define four major subtypes of SCLC with distinct therapeutic vulnerabilities.* Cancer Cell, 2021. **39**(3): p. 346-360 e7.
53. Gazdar, A.F., D.N. Carney, M.M. Nau, and J.D. Minna, *Characterization of variant subclasses of cell lines derived from small cell lung cancer having distinctive biochemical, morphological, and growth properties.* Cancer Res, 1985. **45**(6): p. 2924-30.
54. Ge, H., L. Mu, L. Jin, C. Yang, Y.E. Chang, Y. Long, G. DeLeon, L. Deleyrolle, D.A. Mitchell, P.S. Kubilis, et al., *Tumor associated CD70 expression is involved in promoting tumor migration and macrophage infiltration in GBM.* Int J Cancer, 2017. **141**(7): p. 1434-1444.

55. Gerke, V., F.N.E. Gavins, M. Geisow, T. Grewal, J.K. Jaiswal, J. Nylandsted, and U. Rescher, *Annexins-a family of proteins with distinctive tastes for cell signaling and membrane dynamics*. Nat Commun, 2024. **15**(1): p. 1574.
56. Gong, L., J. Luo, Y. Zhang, Y. Yang, S. Li, X. Fang, B. Zhang, J. Huang, L.K. Chow, D. Chung, et al., *Nasopharyngeal carcinoma cells promote regulatory T cell development and suppressive activity via CD70-CD27 interaction*. Nat Commun, 2023. **14**(1): p. 1912.
57. Groves, S.M., N. Panchy, D.R. Tyson, L.A. Harris, V. Quaranta, and T. Hong, *Involvement of Epithelial-Mesenchymal Transition Genes in Small Cell Lung Cancer Phenotypic Plasticity*. Cancers (Basel), 2023. **15**(5).
58. Guan, X., Y. Fang, J. Long, and Y. Zhang, *Annexin I-nuclear factor-kappaB-microRNA-26a regulatory pathway in the metastasis of non-small cell lung cancer*. Thorac Cancer, 2019. **10**(4): p. 665-675.
59. Guedez, L., S. Jensen-Taubman, D. Bourboulia, C.J. Kwityn, B. Wei, J. Caterina, and W.G. Stetler-Stevenson, *TIMP-2 targets tumor-associated myeloid suppressor cells with effects in cancer immune dysfunction and angiogenesis*. J Immunother, 2012. **35**(6): p. 502-12.
60. Gyorffy, B., *Transcriptome-level discovery of survival-associated biomarkers and therapy targets in non-small-cell lung cancer*. Br J Pharmacol, 2024. **181**(3): p. 362-374.
61. Han, Y.H., D.Y. Ma, S.J. Lee, Y.Y. Mao, S.Y. Sun, M.H. Jin, H.N. Sun, and T. Kwon, *Bioinformatics Analysis of Novel Targets for Treating Cervical Cancer by Immunotherapy Based on Immune Escape*. Cancer Genomics Proteomics, 2023. **20**(4): p. 383-397.
62. Hermes, A., U. Gatzemeier, B. Waschki, and M. Reck, *Lactate dehydrogenase as prognostic factor in limited and extensive disease stage small cell lung cancer - a retrospective single institution analysis*. Respir Med, 2010. **104**(12): p. 1937-42.
63. Horn, L., A.S. Mansfield, A. Szczesna, L. Havel, M. Krzakowski, M.J. Hochmair, F. Huemer, G. Losonczy, M.L. Johnson, M. Nishio, et al., *First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer*. N Engl J Med, 2018. **379**(23): p. 2220-2229.

64. Huang, L., C. Wang, H. Xu, and G. Peng, *Targeting citrate as a novel therapeutic strategy in cancer treatment*. Biochim Biophys Acta Rev Cancer, 2020. **1873**(1): p. 188332.
65. Icard, P., A. Coquerel, Z. Wu, J. Gligorov, D. Fuks, L. Fournel, H. Lincet, and L. Simula, *Understanding the Central Role of Citrate in the Metabolism of Cancer Cells and Tumors: An Update*. Int J Mol Sci, 2021. **22**(12).
66. Icard, P., L. Simula, G. Zahn, M. Alifano, and M.E. Mycielska, *The dual role of citrate in cancer*. Biochim Biophys Acta Rev Cancer, 2023. **1878**(6): p. 188987.
67. Ireland, A.S., A.M. Micinski, D.W. Kastner, B. Guo, S.J. Wait, K.B. Spainhower, C.C. Conley, O.S. Chen, M.R. Guthrie, D. Soltero, et al., *MYC Drives Temporal Evolution of Small Cell Lung Cancer Subtypes by Reprogramming Neuroendocrine Fate*. Cancer Cell, 2020. **38**(1): p. 60-78 e12.
68. Jacobs, J., V. Deschoolmeester, C. Rolfo, K. Zwaenepoel, J. Van den Bossche, C. Deben, K. Silence, H. de Haard, C. Hermans, S. Rottey, et al., *Preclinical data on the combination of cisplatin and anti-CD70 therapy in non-small cell lung cancer as an excellent match in the era of combination therapy*. Oncotarget, 2017. **8**(43): p. 74058-74067.
69. Jacobs, J., V. Deschoolmeester, K. Zwaenepoel, T. Flieswasser, C. Deben, J. Van den Bossche, C. Hermans, C. Rolfo, M. Peeters, O. De Wever, et al., *Unveiling a CD70-positive subset of cancer-associated fibroblasts marked by pro-migratory activity and thriving regulatory T cell accumulation*. Oncoimmunology, 2018. **7**(7): p. e1440167.
70. Jacobs, J., V. Deschoolmeester, K. Zwaenepoel, C. Rolfo, K. Silence, S. Rottey, F. Lardon, E. Smits, and P. Pauwels, *CD70: An emerging target in cancer immunotherapy*. Pharmacol Ther, 2015. **155**: p. 1-10.
71. Jeon, H., S. Wang, J. Song, H. Gill, and H. Cheng, *Update 2025: Management of Non-Small-Cell Lung Cancer*. Lung, 2025. **203**(1): p. 53.
72. Jiang, C., H. Sun, Z. Jiang, W. Tian, S. Cang, and J. Yu, *Targeting the CD47/SIRPalpha pathway in malignancies: recent progress, difficulties and future perspectives*. Front Oncol, 2024. **14**: p. 1378647.
73. Jin, L., G.N. Alesi, and S. Kang, *Glutaminolysis as a target for cancer therapy*. Oncogene, 2016. **35**(28): p. 3619-25.

74. Jin, L., Y. Huo, Z. Zheng, X. Jiang, H. Deng, Y. Chen, Q. Lian, R. Ge, and H. Deng, *Down-regulation of Ras-related protein Rab 5C-dependent endocytosis and glycolysis in cisplatin-resistant ovarian cancer cell lines*. Mol Cell Proteomics, 2014. **13**(11): p. 3138-51.
75. Kalluri, R. and R.A. Weinberg, *The basics of epithelial-mesenchymal transition*. J Clin Invest, 2009. **119**(6): p. 1420-8.
76. Keller, A.M., A. Schildknecht, Y. Xiao, M. van den Broek, and J. Borst, *Expression of costimulatory ligand CD70 on steady-state dendritic cells breaks CD8⁺ T cell tolerance and permits effective immunity*. Immunity, 2008. **29**(6): p. 934-46.
77. Kelly, L., S. McGrath, L. Rodgers, K. McCall, A. Tulunay Virkan, F. Dempsey, S. Crichton, and C.S. Goodyear, *Annexin-A1: The culprit or the solution?* Immunology, 2022. **166**(1): p. 2-16.
78. Komura, M., C. Wang, S. Ito, S. Kato, A. Ueki, M. Ebi, N. Ogasawara, T. Tsuzuki, K. Kasai, K. Kasugai, et al., *Simultaneous Expression of CD70 and POSTN in Cancer-Associated Fibroblasts Predicts Worse Survival of Colorectal Cancer Patients*. Int J Mol Sci, 2024. **25**(5).
79. Kong, F., Q. Ye, and Y. Xiong, *Comprehensive analysis of prognosis and immune function of CD70-CD27 signaling axis in pan-cancer*. Funct Integr Genomics, 2023. **23**(1): p. 48.
80. Krpina, K., S. Vranic, K. Tomic, M. Samarzija, and L. Baticic, *Small Cell Lung Carcinoma: Current Diagnosis, Biomarkers, and Treatment Options with Future Perspectives*. Biomedicines, 2023. **11**(7).
81. Lang, C., A. Lantos, Z. Megyesfalvi, F. Egger, M.A. Hoda, B. Mosleh, T. Klikovits, F. Oberndorfer, G. Timelthaler, B. Ferencz, et al., *Clinical and prognostic implications of CD47 and PD-L1 expression in surgically resected small-cell lung cancer*. ESMO Open, 2022. **7**(6): p. 100631.
82. Lau, A.P.Y., S.S. Khavkine Binstock, and K.L. Thu, *CD47: The Next Frontier in Immune Checkpoint Blockade for Non-Small Cell Lung Cancer*. Cancers (Basel), 2023. **15**(21).
83. Lee, S.K., Y. Hwang, J.H. Han, S. Haam, H.W. Lee, and Y.W. Koh, *Characteristics of the immune microenvironment associated with RRM2*

- expression and its application to PD-L1/PD-1 inhibitors in lung adenocarcinoma.* Am J Cancer Res, 2023. **13**(11): p. 5443-5454.
84. Li, H., P. Zhao, L. Tian, Y. Lu, X. Wang, W. Shao, and Y. Cheng, *Advances in biomarkers for immunotherapy in small-cell lung cancer.* Front Immunol, 2024. **15**: p. 1490590.
 85. Li, J.J.N., K. Karim, M. Sung, L.W. Le, S.C.M. Lau, A. Sacher, and N.B. Leighl, *Tobacco exposure and immunotherapy response in PD-L1 positive lung cancer patients.* Lung Cancer, 2020. **150**: p. 159-163.
 86. Li, L., Z. Wang, T. Lu, Y. Li, M. Pan, D. Yu, and G. Hu, *Expression and Functional Relevance of ANXA1 in Hypopharyngeal Carcinoma with Lymph Node Metastasis.* Onco Targets Ther, 2021. **14**: p. 1387-1399.
 87. Li, P., L. Li, Z. Li, S. Wang, R. Li, W. Zhao, Y. Feng, S. Huang, L. Li, H. Qiu, et al., *Annexin A1 promotes the progression of bladder cancer via regulating EGFR signaling pathway.* Cancer Cell Int, 2022. **22**(1): p. 7.
 88. Li, T. and T. Qiao, *Unraveling tumor microenvironment of small-cell lung cancer: Implications for immunotherapy.* Semin Cancer Biol, 2022. **86**(Pt 2): p. 117-125.
 89. Liberti, M.V. and J.W. Locasale, *Correction to: 'The Warburg Effect: How Does it Benefit Cancer Cells?': [Trends in Biochemical Sciences, 41 (2016) 211].* Trends Biochem Sci, 2016. **41**(3): p. 287.
 90. Lin, C.C., T.L. Cheng, W.H. Tsai, H.J. Tsai, K.H. Hu, H.C. Chang, C.W. Yeh, Y.C. Chen, C.C. Liao, and W.T. Chang, *Loss of the respiratory enzyme citrate synthase directly links the Warburg effect to tumor malignancy.* Sci Rep, 2012. **2**: p. 785.
 91. Lin, Y., G. Lin, W. Fang, H. Zhu, and K. Chu, *Increased expression of annexin A1 predicts poor prognosis in human hepatocellular carcinoma and enhances cell malignant phenotype.* Med Oncol, 2014. **31**(12): p. 327.
 92. Lissa, D., N. Takahashi, P. Desai, I. Manukyan, C.W. Schultz, V. Rajapakse, M.J. Velez, D. Mulford, N. Roper, S. Nichols, et al., *Heterogeneity of neuroendocrine transcriptional states in metastatic small cell lung cancers and patient-derived models.* Nat Commun, 2022. **13**(1): p. 2023.

93. Liston, D.R. and M. Davis, *Clinically Relevant Concentrations of Anticancer Drugs: A Guide for Nonclinical Studies*. Clin Cancer Res, 2017. **23**(14): p. 3489-3498.
94. Liu, L., B. Yin, Z. Yi, X. Liu, Z. Hu, W. Gao, H. Yu, and Q. Li, *Breast cancer stem cells characterized by CD70 expression preferentially metastasize to the lungs*. Breast Cancer, 2018. **25**(6): p. 706-716.
95. Liu, S.V., M. Reck, A.S. Mansfield, T. Mok, A. Scherpereel, N. Reinmuth, M.C. Garassino, J. De Castro Carpeno, R. Califano, M. Nishio, et al., *Updated Overall Survival and PD-L1 Subgroup Analysis of Patients With Extensive-Stage Small-Cell Lung Cancer Treated With Atezolizumab, Carboplatin, and Etoposide (IMpower133)*. J Clin Oncol, 2021. **39**(6): p. 619-630.
96. Liu, Y., Y.S. Liu, P.F. Wu, Q. Li, W.M. Dai, S. Yuan, Z.H. Xu, T.T. Liu, Z.W. Miao, W.G. Fang, et al., *Brain microvascular endothelium induced-annexin A1 secretion contributes to small cell lung cancer brain metastasis*. Int J Biochem Cell Biol, 2015. **66**: p. 11-9.
97. Logtenberg, M.E.W., F.A. Scheeren, and T.N. Schumacher, *The CD47-SIRPalpha Immune Checkpoint*. Immunity, 2020. **52**(5): p. 742-752.
98. Lu, Q.Y., Y. Jin, J.T. Mao, Z.F. Zhang, D. Heber, S.M. Dubinett, and J. Rao, *Green tea inhibits cyclooxygenase-2 in non-small cell lung cancer cells through the induction of Annexin-I*. Biochem Biophys Res Commun, 2012. **427**(4): p. 725-30.
99. Lv, X., M. Liu, J. Chen, X. He, J. Weng, Y. Lin, Y. Wang, R. Zhang, M. Huang, L. Zhao, et al., *GPRC5A+ myCAFs promote ESCC progression via TGF-beta-induced fibroblast activation and ANXA1-mediated M2 macrophage polarization*. Int Immunopharmacol, 2025. **167**: p. 115663.
100. Manai, M., R. Doghri, P. Finetti, K. Mrad, R. Bouabsa, M. Manai, D. Birnbaum, F. Bertucci, L. Charfi, and M. Driss, *Overexpression of Annexin A1 Is an Independent Predictor of Longer Overall Survival in Epithelial Ovarian Cancer*. In Vivo, 2020. **34**(1): p. 177-184.
101. Maschler, S., C.A. Gebeshuber, E.M. Wiedemann, M. Alacakaptan, M. Schreiber, I. Custic, and H. Beug, *Annexin A1 attenuates EMT and metastatic potential in breast cancer*. EMBO Mol Med, 2010. **2**(10): p. 401-14.

102. McColl, K., G. Wildey, N. Sakre, M.B. Lipka, M. Behtaj, A. Kresak, Y. Chen, M. Yang, V. Velcheti, P. Fu, et al., *Reciprocal expression of INSM1 and YAP1 defines subgroups in small cell lung cancer*. *Oncotarget*, 2017. **8**(43): p. 73745-73756.
103. Megyesfalvi, Z., C.M. Gay, H. Popper, R. Pirker, G. Ostoros, S. Heeke, C. Lang, K. Hoetzenecker, A. Schwendenwein, K. Boettiger, et al., *Clinical insights into small cell lung cancer: Tumor heterogeneity, diagnosis, therapy, and future directions*. *CA Cancer J Clin*, 2023. **73**(6): p. 620-652.
104. Moraes, L.A., S. Kar, S.L. Foo, T. Gu, Y.Q. Toh, P.B. Ampomah, K. Sachaphibulkij, G. Yap, O. Zharkova, H.M. Lukman, et al., *Annexin-A1 enhances breast cancer growth and migration by promoting alternative macrophage polarization in the tumour microenvironment*. *Sci Rep*, 2017. **7**(1): p. 17925.
105. Munksgaard Thoren, M., M. Vaapil, J. Staaf, M. Planck, M.E. Johansson, S. Mohlin, and S. Pahlman, *Myc-induced glutaminolysis bypasses HIF-driven glycolysis in hypoxic small cell lung carcinoma cells*. *Oncotarget*, 2017. **8**(30): p. 48983-48995.
106. Nagato, T., H. Komatsuda, R. Hayashi, M. Takahara, N. Ujiie, A. Kosaka, T. Ohkuri, K. Oikawa, R. Sato, R. Wakisaka, et al., *Soluble CD27 as a predictive biomarker for intra-tumoral CD70/CD27 interaction in nasopharyngeal carcinoma*. *Cancer Sci*, 2024. **115**(4): p. 1073-1084.
107. Nair, N.U., A. Das, V.M. Rogkoti, M. Fokkelman, R. Marcotte, C.G. de Jong, E. Koedoot, J.S. Lee, I. Meilijson, S. Hannenhalli, et al., *Migration rather than proliferation transcriptomic signatures are strongly associated with breast cancer patient survival*. *Sci Rep*, 2019. **9**(1): p. 10989.
108. Ng, J., L. Cai, L. Girard, O.W.J. Prall, N. Rajan, C. Khoo, A. Batrouney, D.J. Byrne, D.K. Boyd, A.J. Kersbergen, et al., *Molecular and Pathologic Characterization of YAP1-Expressing Small Cell Lung Cancer Cell Lines Leads to Reclassification as SMARCA4-Deficient Malignancies*. *Clin Cancer Res*, 2024. **30**(9): p. 1846-1858.
109. Nicholson, A.G., M.S. Tsao, M.B. Beasley, A.C. Borczuk, E. Brambilla, W.A. Cooper, S. Dacic, D. Jain, K.M. Kerr, S. Lantuejoul, et al., *The 2021 WHO Classification of Lung Tumors: Impact of Advances Since 2015*. *J Thorac Oncol*, 2022. **17**(3): p. 362-387.

110. Nilsson, M.B., Y. Yang, S. Heeke, S.A. Patel, A. Poteete, H. Udagawa, Y.Y. Elamin, C.A. Moran, Y. Kashima, T. Arumugam, et al., *CD70 is a therapeutic target upregulated in EMT-associated EGFR tyrosine kinase inhibitor resistance*. Cancer Cell, 2023. **41**(2): p. 340-355 e6.
111. Novizio, N., R. Belvedere, E. Pessolano, A. Tosco, A. Porta, M. Perretti, P. Campiglia, A. Filippelli, and A. Petrella, *Annexin A1 Released in Extracellular Vesicles by Pancreatic Cancer Cells Activates Components of the Tumor Microenvironment, through Interaction with the Formyl-Peptide Receptors*. Cells, 2020. **9**(12).
112. Onozawa, H., M. Saito, K. Saito, Y. Kanke, Y. Watanabe, S. Hayase, W. Sakamoto, T. Ishigame, T. Momma, S. Ohki, et al., *Annexin A1 is involved in resistance to 5-FU in colon cancer cells*. Oncol Rep, 2017. **37**(1): p. 235-240.
113. Ortiz-Cuaran, S., A. Swalduz, J.P. Foy, S. Marteau, A.P. Morel, F. Fauvet, G. De Souza, L. Michon, M. Boussageon, N. Gadot, et al., *Epithelial-to-mesenchymal transition promotes immune escape by inducing CD70 in non-small cell lung cancer*. Eur J Cancer, 2022. **169**: p. 106-122.
114. Oshi, M., Y. Tokumaru, S. Mukhopadhyay, L. Yan, R. Matsuyama, I. Endo, and K. Takabe, *Annexin A1 Expression Is Associated with Epithelial-Mesenchymal Transition (EMT), Cell Proliferation, Prognosis, and Drug Response in Pancreatic Cancer*. Cells, 2021. **10**(3).
115. Paal, A., D. Dora, A. Takacs, C. Rivard, S.L. Pickard, F.R. Hirsch, B. Rosko, P. Kiraly, P. Ferdinandy, Z.V. Varga, et al., *Roles of Annexin A1 Expression in Small Cell Lung Cancer*. Cancers (Basel), 2025. **17**(9).
116. Pardoll, D.M., *The blockade of immune checkpoints in cancer immunotherapy*. Nat Rev Cancer, 2012. **12**(4): p. 252-64.
117. Patra, K.C. and N. Hay, *The pentose phosphate pathway and cancer*. Trends Biochem Sci, 2014. **39**(8): p. 347-54.
118. Pavan, A., I. Attili, G. Pasello, V. Guarneri, P.F. Conte, and L. Bonanno, *Immunotherapy in small-cell lung cancer: from molecular promises to clinical challenges*. J Immunother Cancer, 2019. **7**(1): p. 205.
119. Paz-Ares, L., M. Dvorkin, Y. Chen, N. Reinmuth, K. Hotta, D. Trukhin, G. Statsenko, M.J. Hochmair, M. Ozguroglu, J.H. Ji, et al., *Durvalumab plus*

- platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial*. Lancet, 2019. **394**(10212): p. 1929-1939.
120. Perez-Tomas, R. and I. Perez-Guillen, *Lactate in the Tumor Microenvironment: An Essential Molecule in Cancer Progression and Treatment*. Cancers (Basel), 2020. **12**(11).
 121. Polara, R., R. Ganesan, S.M. Pitson, and N. Robinson, *Cell autonomous functions of CD47 in regulating cellular plasticity and metabolic plasticity*. Cell Death Differ, 2024. **31**(10): p. 1255-1266.
 122. Pongor, L.S., C.W. Schultz, L. Rinaldi, D. Wangsa, C.E. Redon, N. Takahashi, G. Fialkoff, P. Desai, Y. Zhang, S. Burkett, et al., *Extrachromosomal DNA Amplification Contributes to Small Cell Lung Cancer Heterogeneity and Is Associated with Worse Outcomes*. Cancer Discov, 2023. **13**(4): p. 928-949.
 123. Prates, J., J.B. Moreli, A.D. Gimenes, J.M. Biselli, S.C.G. Pires D'Avila, S. Sandri, S.H.P. Farsky, F.C. Rodrigues-Lisoni, and S.M. Oliani, *Cisplatin treatment modulates Annexin A1 and inhibitor of differentiation to DNA 1 expression in cervical cancer cells*. Biomed Pharmacother, 2020. **129**: p. 110331.
 124. Pratt, D., S. Pittaluga, M. Palisoc, P. Fetsch, L. Xi, M. Raffeld, M.R. Gilbert, and M. Quezado, *Expression of CD70 (CD27L) Is Associated With Epithelioid and Sarcomatous Features in IDH-Wild-Type Glioblastoma*. J Neuropathol Exp Neurol, 2017. **76**(8): p. 697-708.
 125. Qin, K., C.M. Gay, L.A. Byers, and J. Zhang, *The current and emerging immunotherapy paradigm in small-cell lung cancer*. Nat Cancer, 2025. **6**(6): p. 954-966.
 126. Racle, J. and D. Gfeller, *EPIC: A Tool to Estimate the Proportions of Different Cell Types from Bulk Gene Expression Data*. Methods Mol Biol, 2020. **2120**: p. 233-248.
 127. Raso, M.G., N. Bota-Rabassedas, and Wistuba, II, *Pathology and Classification of SCLC*. Cancers (Basel), 2021. **13**(4).
 128. Ren, J., Z. Hu, G. Niu, J. Xia, X. Wang, R. Hong, J. Gu, D. Wang, and C. Ke, *Annexin A1 induces oxaliplatin resistance of gastric cancer through autophagy by targeting PI3K/AKT/mTOR*. FASEB J, 2023. **37**(3): p. e22790.

129. Ren, J.G., P. Seth, H. Ye, K. Guo, J.I. Hanai, Z. Husain, and V.P. Sukhatme, *Citrate Suppresses Tumor Growth in Multiple Models through Inhibition of Glycolysis, the Tricarboxylic Acid Cycle and the IGF-1R Pathway*. Sci Rep, 2017. **7**(1): p. 4537.
130. Rezazadeh-Gavgani, E., R. Majidazar, P. Lotfinejad, T. Kazemi, and A. Shamekh, *Immune Checkpoint Molecules: A Review on Pathways and Immunotherapy Implications*. Immun Inflamm Dis, 2025. **13**(4): p. e70196.
131. Riely, G.J., D.E. Wood, D.S. Ettinger, D.L. Aisner, W. Akerley, J.R. Bauman, A. Bharat, D.S. Bruno, J.Y. Chang, L.R. Chirieac, et al., *Non-Small Cell Lung Cancer, Version 4.2024, NCCN Clinical Practice Guidelines in Oncology*. J Natl Compr Canc Netw, 2024. **22**(4): p. 249-274.
132. Rohwer, N., F. Bindel, C. Grimm, S.J. Lin, J. Wappler, B. Klinger, N. Bluthgen, I. Du Bois, B. Schmeck, H. Lehrach, et al., *Annexin A1 sustains tumor metabolism and cellular proliferation upon stable loss of HIF1A*. Oncotarget, 2016. **7**(6): p. 6693-710.
133. Rudin, C.M., M.M. Awad, A. Navarro, M. Gottfried, S. Peters, T. Csoszi, P.K. Cheema, D. Rodriguez-Abreu, M. Wollner, J.C. Yang, et al., *Pembrolizumab or Placebo Plus Etoposide and Platinum as First-Line Therapy for Extensive-Stage Small-Cell Lung Cancer: Randomized, Double-Blind, Phase III KEYNOTE-604 Study*. J Clin Oncol, 2020. **38**(21): p. 2369-2379.
134. Rudin, C.M., J.T. Poirier, L.A. Byers, C. Dive, A. Dowlati, J. George, J.V. Heymach, J.E. Johnson, J.M. Lehman, D. MacPherson, et al., *Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data*. Nat Rev Cancer, 2019. **19**(5): p. 289-297.
135. Saito, H., Y. Tenjin, T. Yamada, S. Kudoh, N. Kudo, M. Sanada, Y. Sato, A. Matsuo, Y. Orita, and T. Ito, *The role of YAP1 in small cell lung cancer*. Hum Cell, 2022. **35**(2): p. 628-638.
136. Sam, I., N. Ben Hamouda, M. Alkatrib, C. Gonnin, P.J. Siska, S. Oudard, C. Lebbe, and E. Tartour, *The CD70-CD27 Axis in Cancer Immunotherapy: Predictive Biomarker and Therapeutic Target*. Clin Cancer Res, 2025. **31**(14): p. 2872-2881.

137. Schittenhelm, J., K. Trautmann, G. Tabatabai, C. Hermann, R. Meyermann, and R. Beschorner, *Comparative analysis of annexin-1 in neuroepithelial tumors shows altered expression with the grade of malignancy but is not associated with survival*. Mod Pathol, 2009. **22**(12): p. 1600-11.
138. Schulz, M., S. Iwersen-Bergmann, H. Andresen, and A. Schmoldt, *Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics*. Crit Care, 2012. **16**(4): p. R136.
139. Semanova, E.A., R. Nagel, and A. Berns, *Origins, genetic landscape, and emerging therapies of small cell lung cancer*. Genes Dev, 2015. **29**(14): p. 1447-62.
140. Sen, T., Y. Dotsu, V. Corbett, S. Puri, U. Sen, T.A. Boyle, P. Mack, F. Hirsch, R. Aljumaily, A.R. Naqash, et al., *Pulmonary neuroendocrine neoplasms: the molecular landscape, therapeutic challenges, and diagnosis and management strategies*. Lancet Oncol, 2025. **26**(1): p. e13-e33.
141. Shen, Y., Z. Liu, Y. Chen, X. Shi, S. Dong, and B. Wang, *Candidate Biomarker of Response to Immunotherapy In Small Cell Lung Cancer*. Curr Treat Options Oncol, 2025. **26**(2): p. 73-83.
142. Shin, J., I.S. Song, J.H. Pak, and S.W. Jang, *Upregulation of annexin A1 expression by butyrate in human melanoma cells induces invasion by inhibiting E-cadherin expression*. Tumour Biol, 2016. **37**(11): p. 14577-14584.
143. Simpson, K.L., D.G. Rothwell, F. Blackhall, and C. Dive, *Challenges of small cell lung cancer heterogeneity and phenotypic plasticity*. Nat Rev Cancer, 2025. **25**(6): p. 447-462.
144. Solta, A., B. Ernhofer, K. Boettiger, Z. Megyesfalvi, S. Heeke, M.A. Hoda, C. Lang, C. Aigner, F.R. Hirsch, K. Schelch, et al., *Small cells - big issues: biological implications and preclinical advancements in small cell lung cancer*. Mol Cancer, 2024. **23**(1): p. 41.
145. Spigel, D.R., D. Vicente, T.E. Ciuleanu, S. Gettinger, S. Peters, L. Horn, C. Audigier-Valette, N. Pardo Aranda, O. Juan-Vidal, Y. Cheng, et al., *Second-line nivolumab in relapsed small-cell lung cancer: CheckMate 331(☆)*. Ann Oncol, 2021. **32**(5): p. 631-641.

146. Sun, R., J. He, Q. Xiang, Y. Feng, Y. Gong, Y. Ning, C. Deng, K. Sun, M. Zhang, Z. Cheng, et al., *NTF4 plays a dual role in breast cancer in mammary tumorigenesis and metastatic progression*. Int J Biol Sci, 2023. **19**(2): p. 641-657.
147. Sun, W., T. Zhao, T.O. Aladelusi, W. Ju, Z. Zhang, L. Zhong, and D. Zhu, *Decreased Annexin A1 expression enhances sensitivity to docetaxel, cisplatin and 5-fluorouracil combination induction chemotherapy in oral squamous cell carcinoma*. J Oral Pathol Med, 2021. **50**(8): p. 795-802.
148. Swa, H.L., W.P. Blackstock, L.H. Lim, and J. Gunaratne, *Quantitative proteomics profiling of murine mammary gland cells unravels impact of annexin-1 on DNA damage response, cell adhesion, and migration*. Mol Cell Proteomics, 2012. **11**(8): p. 381-93.
149. Tang, L., Y. Chen, H. Chen, P. Jiang, L. Yan, D. Mo, X. Tang, and F. Yan, *DCST1-AS1 Promotes TGF-beta-Induced Epithelial-Mesenchymal Transition and Enhances Chemoresistance in Triple-Negative Breast Cancer Cells via ANXA1*. Front Oncol, 2020. **10**: p. 280.
150. Teicher, B.A., *Perspective: Opportunities in recalcitrant, rare and neglected tumors*. Oncol Rep, 2013. **30**(3): p. 1030-4.
151. Thomas, A., C. Mohindroo, and G. Giaccone, *Advancing therapeutics in small-cell lung cancer*. Nat Cancer, 2025. **6**(6): p. 938-953.
152. Usman, S., N.H. Waseem, T.K.N. Nguyen, S. Mohsin, A. Jamal, M.T. Teh, and A. Waseem, *Vimentin Is at the Heart of Epithelial Mesenchymal Transition (EMT) Mediated Metastasis*. Cancers (Basel), 2021. **13**(19).
153. Vacchelli, E., Y. Ma, E.E. Baracco, L. Zitvogel, and G. Kroemer, *Yet another pattern recognition receptor involved in the chemotherapy-induced anticancer immune response: Formyl peptide receptor-1*. Oncoimmunology, 2016. **5**(5): p. e1118600.
154. Vaupel, P. and G. Multhoff, *Revisiting the Warburg effect: historical dogma versus current understanding*. J Physiol, 2021. **599**(6): p. 1745-1757.
155. Verma, S., S. Budhu, I. Serganova, L. Dong, L.M. Mangarin, J.F. Khan, M.A. Bah, A. Assouvie, Y. Marouf, I. Schulze, et al., *Pharmacologic LDH inhibition redirects intratumoral glucose uptake and improves antitumor immunity in solid tumor models*. J Clin Invest, 2024. **134**(17).

156. Vocino Trucco, G., L. Righi, M. Volante, and M. Papotti, *Updates on lung neuroendocrine neoplasm classification*. Histopathology, 2024. **84**(1): p. 67-85.
157. Voros, I., Z. Onodi, V.E. Toth, T.G. Gergely, E. Saghy, A. Gorbe, A. Kemeny, P. Leszek, Z. Helyes, P. Ferdinandy, et al., *Saxagliptin Cardiotoxicity in Chronic Heart Failure: The Role of DPP4 in the Regulation of Neuropeptide Tone*. Biomedicines, 2022. **10**(7).
158. Waldman, A.D., J.M. Fritz, and M.J. Lenardo, *A guide to cancer immunotherapy: from T cell basic science to clinical practice*. Nat Rev Immunol, 2020. **20**(11): p. 651-668.
159. Wan, Y.M., J. Tian, L. Qi, L.M. Liu, and N. Xu, *ANXA1 affects cell proliferation, invasion and epithelial-mesenchymal transition of oral squamous cell carcinoma*. Exp Ther Med, 2017. **14**(5): p. 5214-5218.
160. Wang, C., C. Ju, D. Du, P. Zhu, J. Yin, J. Jia, X. Wang, X. Xu, L. Zhao, J. Wan, et al., *CircNF1 modulates the progression and immune evasion of esophageal squamous cell carcinoma through dual regulation of PD-L1*. Cell Mol Biol Lett, 2025. **30**(1): p. 37.
161. Wang, H., J.F. Sun, C. Wang, Z.S. Jiang, and Z.S. Tong, *Co-Expression of MHC-II and ANXA1: Mediators of PD-1/PD-L1 Therapy Resistance in Breast Cancer*. Cancer Rep (Hoboken), 2025. **8**(8): p. e70291.
162. Wang, Y., L. Serfass, M.O. Roy, J. Wong, A.M. Bonneau, and E. Georges, *Annexin-I expression modulates drug resistance in tumor cells*. Biochem Biophys Res Commun, 2004. **314**(2): p. 565-70.
163. Wei, L., L. Li, L. Liu, R. Yu, X. Li, and Z. Luo, *Knockdown of Annexin-A1 Inhibits Growth, Migration and Invasion of Glioma Cells by Suppressing the PI3K/Akt Signaling Pathway*. ASN Neuro, 2021. **13**: p. 17590914211001218.
164. Xiao, D., T. Zeng, W. Zhu, Z.Z. Yu, W. Huang, H. Yi, S.S. Lu, J. Feng, X.P. Feng, D. Wu, et al., *ANXA1 Promotes Tumor Immune Evasion by Binding PARP1 and Upregulating Stat3-Induced Expression of PD-L1 in Multiple Cancers*. Cancer Immunol Res, 2023. **11**(10): p. 1367-1383.
165. Xiao, Y., C. Ouyang, W. Huang, Y. Tang, W. Fu, and A. Cheng, *Annexin A1 can inhibit the in vitro invasive ability of nasopharyngeal carcinoma cells possibly*

- through Annexin A1/S100A9/Vimentin interaction. PLoS One, 2017. **12**(3): p. e0174383.
166. Xie, M., M. Bao, X. Dong, L. Wu, L. Liu, J. Zhao, X. Chu, Y. Wu, X. Ji, Y. Fang, et al., *Harnessing local and system immune profiling delineating differential responders to first-line sintilimab (anti-PD-1 antibody) combined with chemotherapy in extensive-stage small cell lung cancer: an exploratory biomarker analysis of a phase II study*. Signal Transduct Target Ther, 2025. **10**(1): p. 168.
 167. Xiong, W., B. Zhang, H. Yu, L. Zhu, L. Yi, and X. Jin, *RRM2 Regulates Sensitivity to Sunitinib and PD-1 Blockade in Renal Cancer by Stabilizing ANXA1 and Activating the AKT Pathway*. Adv Sci (Weinh), 2021. **8**(18): p. e2100881.
 168. Xue, W., L. Yang, C. Chen, M. Ashrafizadeh, Y. Tian, and R. Sun, *Wnt/beta-catenin-driven EMT regulation in human cancers*. Cell Mol Life Sci, 2024. **81**(1): p. 79.
 169. Yang, F., J. Cai, H. Zhan, J. Situ, W. Li, Y. Mao, and Y. Luo, *Suppression of TRPM7 Inhibited Hypoxia-Induced Migration and Invasion of Androgen-Independent Prostate Cancer Cells by Enhancing RACK1-Mediated Degradation of HIF-1alpha*. Oxid Med Cell Longev, 2020. **2020**: p. 6724810.
 170. Yang, H., Y. Xun, and H. You, *The landscape overview of CD47-based immunotherapy for hematological malignancies*. Biomark Res, 2023. **11**(1): p. 15.
 171. Ying, X., L. Chen, J. Xie, Y. Hu, Q. Wu, L. Cao, and H. Yu, *ANXA1 (Annexin A1) regulated by MYC (MYC proto-oncogene) promotes the growth of papillary thyroid carcinoma*. Bioengineered, 2021. **12**(2): p. 9251-9265.
 172. Yu, S., Q. Meng, H. Hu, and M. Zhang, *Correlation of ANXA1 expression with drug resistance and relapse in bladder cancer*. Int J Clin Exp Pathol, 2014. **7**(9): p. 5538-48.
 173. Yu, Z.Z., Y.Y. Liu, W. Zhu, D. Xiao, W. Huang, S.S. Lu, H. Yi, T. Zeng, X.P. Feng, L. Yuan, et al., *ANXA1-derived peptide for targeting PD-L1 degradation inhibits tumor immune evasion in multiple cancers*. J Immunother Cancer, 2023. **11**(3).
 174. Zhang, X., B. Hong, W. Yu, Q. Miao, W. Wang, Y. Wang, Z. Sun, Y. Bo, J. Zhang, H. Zhang, et al., *Comprehensive pan-cancer analysis reveals CD70 as a*

- promising therapeutic target and biomarker in clear cell renal cell carcinoma. Int J Biol Macromol*, 2025. **307**(Pt 3): p. 142079.
175. Zhao, X., W. Ma, X. Li, H. Li, J. Li, H. Li, and F. He, *ANXA1 enhances tumor proliferation and migration by regulating epithelial-mesenchymal transition and IL-6/JAK2/STAT3 pathway in papillary thyroid carcinoma. J Cancer*, 2021. **12**(5): p. 1295-1306.
 176. Zheng, L., L. Li, B. Wang, S. Zhang, Z. Fu, A. Cheng, and X. Liang, *Annexin A1 affects tumor metastasis through epithelial-mesenchymal transition: a narrative review. Transl Cancer Res*, 2022. **11**(12): p. 4416-4433.
 177. Zhu, X., G. Shi, J. Lu, X. Qian, and D. Wang, *Potential regulatory mechanism of TNF-alpha/TNFR1/ANXA1 in glioma cells and its role in glioma cell proliferation. Open Life Sci*, 2022. **17**(1): p. 208-220.

9. Bibliography of the candidate's publications

Publications underlying the thesis:

1. Dora, D., Megyesfalvi, Z., Vörös, I., Paál, Á., Takacs, P., Dobos, D., Lőrincz, B., Bokhari, S. M. Z., Aloss, K., Pallag, G., Rivard, C., Yu, H., Hirsch, F. R., Görbe, A., Varga, Z. V., Lohinai, Z., & Dome, B. (2025). Expression of costimulatory molecule CD70 is prognostic in small cell lung cancer. *Cancer immunology, immunotherapy: CII*, 74(5), 165. <https://doi.org/10.1007/s00262-025-04006-2>
D1, IF: 5,1
2. Paál, Á.*, Dora, D.*, Takács, Á., Rivard, C., Pickard, S. L., Hirsch, F. R., Roskó, B., Kiraly, P., Ferdinandy, P., Varga, Z. V., Lohinai, Z., & Görbe, A. (2025). Roles of Annexin A1 Expression in Small Cell Lung Cancer. *Cancers*, 17(9), 1407. <https://doi.org/10.3390/cancers17091407> *: shared first authorship
Q1, IF: 4,4

Additional publications:

1. Daradics, N., Horvath, G., Tretter, L., Paál, A., Fulop, A., Budai, A., & Szijarto, A. (2022). The effect of Cyclophilin D depletion on liver regeneration following associating liver partition and portal vein ligation for staged hepatectomy. *PloS one*, 17(7), e0271606. <https://doi.org/10.1371/journal.pone.0271606>
Q1, IF: 3,7
2. Pálóczi, J., Paál, Á., Pigler, J., Kiss, B., Rhoden, A., Varga, Z. V., Ferdinandy, P., Eschenhagen, T., & Görbe, A. (2023). Organ-specific model of simulated ischemia/reperfusion and hyperglycemia based on engineered heart tissue. *Vascular pharmacology*, 152, 107208. <https://doi.org/10.1016/j.vph.2023.107208>
Q1, IF: 3,5
3. Sayour, N. V., Paál, Á. M., Ameri, P., Meijers, W. C., Minotti, G., Andreadou, I., Lombardo, A., Camilli, M., Drexel, H., Grove, E. L., Dan, G. A., Ivanescu, A., Semb, A. G., Savarese, G., Dobrev, D., Crea, F., Kaski, J. C., de Boer, R. A., Ferdinandy, P., & Varga, Z. V. (2024). Heart failure pharmacotherapy and cancer: pathways and pre-clinical/clinical evidence. *European heart journal*, 45(14), 1224–1240. <https://doi.org/10.1093/eurheartj/ehae105>
D1, IF: 35,7

4. Kovács, S. A., Kovács, T., Lánckzy, A., Paál, Á., Hegedűs, Z. I., Sayour, N. V., Szabó, L., Kovács, A., Bianchini, G., Ferdinandy, P., Ocana, A., Varga, Z. V., Fekete, J. T., & Győrffy, B. (2025). Unlocking the power of immune checkpoint inhibitors: Targeting YAP1 reduces anti-PD1 resistance in skin cutaneous melanoma. *British journal of pharmacology*, 182(16), 3903–3922. <https://doi.org/10.1111/bph.70052>
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