American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Review Article



# Causes and Consequences of Lung Cancer Heterogeneity

**Aparna Padhye***

*Department of Thoracic/Head and Neck, MD Anderson Cancer Center, USA*

***Corresponding author:** Aparna Padhye, Department of Thoracic/Head and Neck, MD Anderson Cancer Center, USA, Email: aparnapadhye10@gmail.com

***To Cite This Article:*** *Aparna P. Causes and Consequences of Lung Cancer Heterogeneity. Am J Biomed Sci & Res. 2022 17(5) AJBSR.MS.ID.002384,* DOI: 10.34297/AJBSR.2022.17.002384

**Received:** December 13, 2022; **Published:** December 15, 2022

## Abstract

Lung cancer is the foremost cause of cancer related deaths in the U.S. The relatively poor outcome in lung cancer patients stems from the fact that the tumor is not considered in the context of its microenvironment while treating the patients. Lung cancer is a heterogeneous disease composed of genetically and phenotypically different tumor cells as well as a heterogeneous microenvironment that constantly interact with each other. Currently, treatment of lung cancer is guided by the genetic alterations identified in the tumor specimen, which is not representative of the whole tumor. Genome wide studies from patient derived lung tumors suggest presence of significantly high numbers of genetic alterations in a single tumor. Epigenetic variations, such as DNA methylation, histone modifications and microRNAs, have also been suggested to play a role in lung cancer heterogeneity. Such cellular variations lead to differential interactions between the tumor cells and the microenvironment and therefore the tumor is constantly evolving. In order to gain a better understanding of lung cancer progression and design effective treatment strategies, studying tumor in context of its microenvironment becomes very important.

**Keywords:** Lung Cancer; Tumor Heterogeneity; Genetic Heterogeneity; Epigenetics; Tumor Microenvironment

**Abbreviations:** ITH: Intra Tumoral Heterogeneity; SCLC: Small Cell Lung Cancer; NSCLC: Non-Small Cell Lung Cancer; ADC: Adenocarcinoma; SCC: Squamous Cell Carcinoma; LCC: Large Cell Carcinoma: CE: Clonal Evolution; TME: Tumor Microenvironment; HATs: Histone Acetyl Transferases; HDACs: Histone Deacetylases; CSCs: Cancer Stem Cells; ECM: Extracellular Matrix; CAFs: Cancer Associated Fibroblasts; TILs : Tumor Infiltrating Lymphocytes; HGF: Hepatocyte Derived Growth Factor; FGF2: Fibroblast Derived Growth Factor

## Heterogeneity in Lung Cancer

Lung cancer continues to account for almost one-quarter of all cancer-related mortality in the U.S. About two-thirds of the patients present with metastatic disease with a 5-year survival rate of 5% and very limited curative options. Patients with early-stage, localized disease have an improved 5-year survival rate (57%), however, the morbidity is exacerbated due to a recurrence rate of approximately 50 percent in such patients who eventually develop resistance to therapeutic agents [1]. Lung cancer presenting at an advanced stage has a very high mutational burden and for a long time, clinical and translational efforts were largely focused on identifying new mutations in lung cancer for developing novel targeted therapies [2]. Current diagnosis and treatment strategies for lung cancer are based on the genetic defects detected from a small biopsy specimen and are used to classify patients for therapeutic strategies. It is important to note that this approach has significantly improved patient outcomes relative to the conventional cytotoxic chemotherapies like cisplatin and carboplatin [3]. The identification of driver mutations in patients allowed combination of chemotherapy with targeted therapies which significantly improved the response rate and progression-free survival. However, these therapies eventually become ineffective and result in recurrence of the disease [4].

While precision medicine largely focusses on genetic and molecular profiling of tumor cells, identification of various biomarkers to predict disease progression does not necessarily translate into successful clinical outcomes. The complexity of

tumors and the heterogeneity within is now a well-established concept. The term tumor heterogeneity broadly encompasses tumor cell heterogeneity (intratumoral heterogeneity ITH), tumor microenvironment heterogeneity, and inter patient heterogeneity. Lung cancer is not just a composition of epithelial cancer cells with different mutations but an ecosystem consisting of phenotypically distinct tumor cells, surrounded by cellular or non-cellular components that are dynamically interacting and causing tumor evolution. One of the most outstanding examples of clinical application of this concept has been utilization of immunotherapy. The development and success of immune checkpoint inhibitors in many susceptible tumors has led to the approval of anti-PD1 drugs either as single agent or combination with chemotherapy for first-line treatment for many lung cancer patients. However, only a small percentage of lung cancer patients respond upfront and eventually develop resistance [5-7].

The greatest challenge faced by lung cancer patients continues to be either inherent or acquired form of resistance to any therapeutic regimen. Despite extensive research efforts and advancements in the understanding of lung cancer progression, tumor growth control is only short-term and achieving complete/long-lasting cures from current therapies seems unattainable. This modest translation of experimental research into clinical outcome begs the question if current decision-making approaches are accounting for all aspects contributing to tumor progression. An untapped therapeutic potential of the other existing components of lung tumors needs to be exploited. Nuanced observations and evolving understanding of the effect of tumor subpopulations adopting distinct cellular states, extracellular matrix modulating the response to therapy, cancer-associated fibroblasts aiding in metastatic process and vascular compartment affecting drug delivery are moving to the forefront and have implications on the sensitivity to therapies. With so many moving parts in a tumor, it is critical to utilize more advanced, functional *in vitro* and *in vivo* models, over traditional lung cancer cell lines cultured on plastic, for insights into the mechanistic basis of lung cancer progression and development of improved therapeutic strategies.

In this review, we systematically outline the distinctive features of heterogeneity in lung cancer, survey the major approaches that model heterogeneity, and comment on strategies to incorporate the knowledge acquired from laboratory and clinical research to facilitate the discovery of more curative therapeutic modalities in lung cancer patients.

## Genetic Heterogeneity in Lung Cancer

Lung cancer is broadly classified into small cell (SCLC) and non-small cell lung cancer (NSCLC) based on histopathological characteristics. SCLC occurs in about 20% of the patients while the majority of the burden comes from NSCLC (80%), which is further classified as adenocarcinoma (ADC, ~50%), squamous cell carcinoma (SCC, ~40%) and large cell carcinoma (LCC, by exclusion of ADC and SCC). SCLC and LCC are distinct set of neuroendocrine malignancies and have an independent route of clinical management [8,9]. ADCs arise in more distal airways whereas SCCs arise in more proximal airways and are more strongly associated with smoking. There are distinct biomarkers determining the origin of lung tumors which dictates the treatment decisions. Further molecular characterization of lung cancer is primarily based on mutations in EGFR, KRAS, p53, c-MET, EML4-ALK and their associated signaling pathways [10,11]. However, lung cancer cells within a single tumor carry multiple mutations which contributes to genetic heterogeneity. For a long time, the single oncogene paradigm which identified the founder events, such as Kras or EGFR mutations, determined the treatment strategy. However, ADC and SCCs are characterized by high mutational burden and co-occurrence of multiple genomic alterations may have important biological implications on tumor evolution. The significance of co-mutations mediating phenotypic diversity has recently challenged the single oncogene paradigm but the biological effects remain largely uncaptured [12].

One of the earliest models that attempts to explain ITH is the clonal evolution (CE) model which is primarily gene centric [13]. In the classical view of CE, a single initiating cell gains mutational hits which divides to form other tumor cells. As the tumor progresses, different groups of cells acquire different genetic aberrations. The different tumor subpopulations undergo Darwinian evolution through natural selection and the subpopulations with greatest cellular fitness, attributed to acquisition of genomic alterations, dominate over the others providing a growth advantage to the tumor. As more and more mutations accumulate in the cancer cells, the tumor evolves to a more aggressive form. Thus, the tumor is composed of heterogeneous populations of cancer cells with different genetic backgrounds with variable invasive and metastatic potential, and responses to therapy. In order to support the CE model and trace the of evolution of clones in cancer, advanced techniques like multiregional sequencing and genome wide exome sequencing have been applied to patient derived samples from different cancers, including lung cancer [14-16]. Multi-region whole exome sequencing of patient derived lung cancer samples suggested that lung cancer follows a branching evolution. A study led by deBruin et al. [15] identified regionally separated driver mutations showing branched evolution with driver mutations arising before and after sub clonal diversification. Another study by Zhang et al. [14] suggested that single region sequencing may be sufficient to identify the gene mutations associated with lung cancers. However, limitations with these studies were the small sample size [25 and 11 patients respectively]. As the sample size gets larger, the chances of detecting more molecular defects in the genome increase. Therefore, these studies do not represent the entire population of NSCLC patients or the entire tumor within a single patient.

Although these studies have limitations, identification of oncogenes and tumor suppressors remained a focus of research and management of patients guiding personalized medicine [2]. It has beyond doubt improved patient outcomes when compared to generalized chemotherapy-based treatments. The progress, however, has been limited to specific groups of patients like those carrying EGFR, ALK, KRAS mutations [17]. Availability of easier, cheaper and faster genome sequencing for detection of

mutations is one of the reasons for focusing on genetic aspect of lung cancer for designing therapies. However, the defects identified maybe presented as therapeutic challenges but may not always be translationally significant. In addition, these studies do not account for the possible interactions these genetic defects may cause between the different tumor subpopulations and the components of the tumor microenvironment [9]. It is challenging to account for TME components at the time of diagnosis, but understanding tumor as a whole will provide better insight to its progression. Targeting only mutations identified in cancer cells eventually leads to failure of therapy because the tumor continues to evolve spatially and temporally due to emergence of resistance mechanisms.

## Non-Genetic Heterogeneity

Although epithelial cells in lung cancer acquire multiple genetic hits which largely influence the functional characteristics, there is still marked phenotypic plasticity [18-21]. It has been suggested that cells can exist in different phenotypic states which are regulated by intrinsic and extrinsic factors. Intrinsic factors include the fluctuations in gene expressions and other cellular processes, pre-existing differentiation states, genetic interactions between different mutations and epigenetic modifications. If these fluctuations cross a specific threshold, they can cross over to a different phenotypic state [22,23]. Extrinsic factors include the dynamic interactions with the TME which actively modulate the tumor progression. In the following sections we highlight the role of epigenetic modifications and TME in generating heterogeneity in lung cancer.

## Epigenetic Heterogeneity

Epigenetic regulation of gene expression is well recognized for maintaining the normal cellular functions and homeostasis in the tissues. These are heritable changes in gene expression without any genetic changes in the DNA [24]. The epigenetic mechanisms include DNA methylation, histone modifications and non- coding RNAs like microRNAs. Alterations in these epigenetic mechanisms can lead to a dysregulation in the homeostasis of the normal cellular state. The dysregulation can produce variations in the phenotype of the cancer cell while maintaining the same genetic background. Accumulation of epigenetic alterations overtime has been associated with progression from pre-neoplastic to neoplastic stage [25,26].

Alterations in DNA methylation status have been identified in lung cancer. Promoter regions of the tumor suppressor genes are more frequently hypermethylated in lung cancer, and these genes are involved in some crucial cellular functions like proliferation, apoptosis, adhesion, motility, cell cycle and DNA repair. Genes most commonly found hypermethylated are: p16INK4a, RASSF1A, APC, RARβ, CDH1, CDH13, DAPK, FHIT and MGMT [27]. Analysis of patient samples in clinical studies represents a snapshot of the methylation status in the tumor and in a specific region of the tumor. Genome wide hypomethylation also occurs in lung cancers which leads to an oncogenic activation. This generally occurs in later stages in tumor progression. However, gene specific hypomethylation is found in MAGEA, TKTL1, BORIS, DDR1, TMSB10, TP73, ZNF711, G6PD AMD 14-3- 3σ [27]. Histone modifications occur in concert with DNA methylation and are responsible for regulation of chromatin conformation and gene expression. Modifications occur at the histone tails which include acetylation, deacetylation, methylation, phosphorylation and ubiquitylation. There are different enzymes catalyzing these reactions, and the most common are Histone acetyl transferases (HATs) and Histone deacetylases (HDACs). Generally, HDACs are overexpressed in lung cancers and thus cause transcriptional silencing of tumor suppressor genes. Lower cellular levels of histone modifications are associated with poorer clinical outcomes [28]. MicroRNAs are small non-coding RNAs (~22 nucleotides) that bind to the 3' untranslated regions of messenger RNA (mRNA), causing degradation of the mRNA or inhibition of protein translation resulting in decreased gene expression. Numerous microRNAs are frequently dysregulated in lung cancer at different stages of the disease [28]. A single microRNA can have multiple target genes and a single gene can be targeted by many microRNAs. This can lead to heterogeneity in the tissue as the regulation depends on the temporal and spatial characteristics of the tumor.

Epigenetic studies have mainly focused on identifying the dysregulations associated with lung cancer in the clinic primarily for biomarker development. However, similar to the large-scale studies for identifying new mutations in lung cancer, only correlative studies for epigenetic alterations may not be the best approach. Firstly, these epigenetic alterations occur commonly in many cancers and it is difficult to establish exclusivity. Secondly, there is limited data on epigenetics from normal lung for comparison. Thirdly, no single alteration will capture all the tumors on account of heterogeneity in lung cancers and since epigenetic marks are dynamic in nature, they are context dependent. Epigenetic alterations are also believed to maintain a small subpopulation of cells known as cancer stem cells (CSCs) [29]. The cancer stem cell model posits that cancers are maintained by a small subpopulation of cells that have stem cell like properties similar to stem cells that populate the normal tissues. These CSCs have almost unlimited proliferative capacity and differentiate into cells of different lineages giving rise to a hierarchical organization in the tumor [30,31]. The descendant cells have limited proliferative capacity and form the bulk of the tumor. Since they are of different lineages, they contribute to the heterogeneity in the tumor. These cancer stem cells are believed to promote tumor progression and metastasis and have inherent resistance to therapies. CSCs have been reported to exist in lung cancer and associated markers include CD133, CD44, expression and/or activity of the cytoplasmic enzyme aldehyde dehydrogenase ALDH and presence of cells known as side populations. Embryonic stem cell pathways such as Hedgehog, Notch and WNT have also been reported to be altered in lung cancer [32]. However, lack of sensitivity and specificity of these markers and substantial experimental evidence stems the controversy of significance of CSCs.

## Microenvironment Heterogeneity

Tumors are complex ecosystems surrounded by a microenvironment consisting of immune cells, fibroblasts, endothelial cells, extracellular matrix, chemokines and cytokines. They are continuously evolving which results in a heterogeneous mix of phenotypically different cells. Spatial and temporal variability of the TME with respect to the tumor also plays a role in heterogeneity. At a given time, each part of a tumor is exposed to different components of the TME. Thus, there may be additional stable phenotypic states of cancer cells which may not have been present in the normal tissue contributing to intra tumor heterogeneity. The bidirectional interaction between the cancer cells and TME can either promote or inhibit tumorigenesis [9]. The abnormal stroma surrounding the cancer cells can induce stress responses and genomic instability [33], epithelial to mesenchymal transition [34], cause vascular mimicry [35] and promote tumorigenesis through tissue reorganization [36]. Some correlative clinical studies, *in vitro* and animal studies have demonstrated the significance of studying stroma and its association with lung cancer prognosis. However, most of these studies have limitations like studying a single component or a unidirectional effect. Here, we briefly iterate what is known about the tumor microenvironment in lung cancer.

Extracellular matrix (ECM) was considered just a supportive framework for tumors for a long time. However, it plays a more dynamic role in modulating the process of tumor progression [37]. ECM is composed of large variety of proteins, glycoproteins, proteoglycans, and polysaccharides [37]. It maintains tissue architecture and function through dynamic bidirectional interactions [38-40] and disruption in this interaction can cause tumor formation and progression. On the other hand, it has also been demonstrated that normalization of matrix can reverse the malignant phenotype in cancer cells [41]. Normal lung tissue is characterized by limited ECM. However, progression from premalignant to malignant lesions is characterized by dysplastic reaction which is associated with accumulation of and alteration in the ECM [42,43]. In SCLC, ECM proteins have been shown to protect against apoptosis, enhance tumorigenesis, and confer chemoresistance through beta-1 integrin mediated tyrosine kinases activation [44]. Thomas et al. [45] showed that there was a positive correlation between the expression of metalloproteinases and metastasis in NSCLC. SPARC/osteonectin synthesized by tumor stroma showed a strong association with intratumor hypoxia and acidity indicating a link between cellular metabolism and induction of supportive stroma that favors cancer cell migrations and invasion, leading to poor prognosis Integrin mediated adhesive interactions with ECM have been implicated in cancer progression and invasion [46]. ECM can also modulate the phenotypic characteristics of the tumor cells by modulating the levels of microRNA- 200 and switching the cells between metastasis-prone and metastasis-incompetent phenotype [47]. Recently, it has also been shown that the interactions between cancer cells and the matrix in lung cancer are regulated by microRNA-200 through integrin beta-1 and collagen-1 interaction, leading to invasion and metastasis [48].

Fibroblasts are one of the principal cellular components of the TME and have a significant role in deposition of ECM [49]. Activated fibroblasts are found in cancers and are known as cancer associated fibroblasts (CAFs). Role of CAFs have been studied in lung cancers in the last decade [50-57]. Although CAFs are mostly considered pro-tumorigenic, there is some evidence indicating their anti-tumor effects. CAFs were shown to impart resistance to EGFR tyrosine kinase inhibitors through HGF production [51], but a recent study suggested that there is an increased autophagy in lung cancer cells in response to erlotinib treatment when co-cultured with CAFs [58]. Other groups suggest the supportive role of CAFs by demonstrating enhanced motility of NSCLC cells [50], increased expression of MMP-2 on CAFs with a cross-talk between CAFs and ECM, correlation of poor prognosis with increased CAFs [53] and increased plasticity of cancer cells in presence of CAFs [52].

The microenvironment in lung cancer is rich in resident and circulating immune cells. Clinical correlative studies have shown that infiltrating immune cells can determine the prognosis of lung cancer. Using human lung tumor xenografts, it has been shown that inflammatory cells, tumor infiltrating lymphocytes (TILs), within the stroma are functional and can suppress tumor growth through exogenous cytokines like IL-12 [59]. Increased infiltration and invasion of macrophage and mast cells [60], dendritic cells [61] and lymphocytes [62,63] within the tumor have been shown to correlate with better clinical outcome suggesting that stromal immune component may have an anti-tumor effect in NSCLC. Additionally, using mouse models of lung cancer, a specific gene expression signature was identified in the tumor associated macrophages suggesting that tumor cells affect the immune cells as much as the other way around and could be potentially used as surrogate tissue for patient stratification and predicting clinical outcome [64]. Recent data suggests that there is a heterogeneity in immune cell infiltrates between primary and metastatic sites in lung cancer [65]. Comparison of CD4+ and CD8+ cells within tumor cell islets and stromal compartment showed that there were fewer immune cells in tumor clusters. This was also true for metastatic lesions of the corresponding primary tumor. The differential pattern of immune cell infiltration in primary and metastatic tumor suggests a weakened immune response at the metastatic sites.

In response to the structural changes, many metabolic alterations also occur during lung cancer progression such as release of cytokines, chemokines, growth factors, differential alterations in vasculature and hypoxia. The cross talk between stromal and cancer cells potentially occurs through soluble factors like cytokines and chemokines secreted by them. Many such factors have been identified in lung cancer. One of the earliest chemokines identified in lung cancer was SDF-1/CXCL12-CXC chemokine receptor 4 axis, which appeared to regulate metastasis [66]. Many others have

shown that multiple paracrine signaling occurs in lung cancer, in order to create a more suitable microenvironment for tumor progression. CXCR4 and CCR7 have been implicated in metastasis, IL-7R provides signals to lymphocytes and is associated with shorter survival in lung cancer patients [67]. Hepatocyte derived growth factor (HGF) from fibroblasts induces chemotherapeutic resistance to EGFR receptor tyrosine kinase inhibitors [51]. Tumor sub-clones in SCLC communicate via fibroblast derived growth factor (Fgf-2) in order to promote metastasis [68]. Secreted factors from stromal component affect tumorigenesis and alter tumor cell secretome [69]. This suggests that there is an active exchange between cancer cells and stromal components which modify one another contributing to ITH.

Rapidly dividing tumor cells and the recruitment of heterotypic cells supporting tumor growth can cause a strain on the oxygen supply in the tumor microenvironment. Hypoxic TME is known to support tumorigenesis by regulating other aspects of the stroma like ECM remodeling, epithelial-to-mesenchymal transition, promoting angiogenesis and imparting resistance to therapy [70]. However, evidence from another study suggests that hypoxic TME could also be anti-tumorigenic [71]. In an attempt to map the heterogeneity in the hypoxic TME, Cui et al. [71] injected nude mice with lung cancer cell subcutaneously and injected 18F-fluoromisonidazole and hypoxia marker pimonidazole hydrochloride intravenously. Series of PET scans, autoradiography and microscopy helped in the visualization of heterogeneity in hypoxic microenvironment. The study also suggested that cancer cells had shorter life span in a hypoxic *in vivo* environment, which is in contradiction to what is observed *in vitro*. Nevertheless, this study suggested that the cancer cells are spatially and temporally exposed to differential levels of oxygen which could alter their phenotypic characteristics thus contributing to tumor heterogeneity. Thus, phenotypes of tumor cells are shaped by an integration of genetic, epigenetic, and microenvironmental inputs.

## Clinical Significance of Heterogeneity and Future Directions

With technological advances in personalized medicine, great strides have been achieved in the outcome of lung cancer patients. Personalized medicine targeting specific mutations is a main treatment strategy adopted in the clinic. However, lung cancer heterogeneity plays an important role in the development of drug resistance. Thus, understanding the biology of lung cancer progression in context of heterogeneity due to both genetic and non-genetic factors is essential to improve therapeutic strategies. This knowledge would help revisit the approach of lung cancer diagnosis and classification with a treatment strategy that would take into account the complexity of tumors.

Resistance to small molecule inhibitors is frequently attributed to accumulation of additional mutations or activation of a bypass pathway. One of the most successful targeted therapies in lung cancer is the use of EGFR tyrosine kinase inhibitors which include gefitinib and erlotinib. However, about 50% of the patients treated with EGFR inhibitors acquire T790M mutations [72,73]. These patients are responsive to osimertinib, and as a first-line therapy the drug is able to prevent emergence of resistance due to T790M mutation [74]. MET and ERBB2 amplification have been reported to cause primary resistance to third-generation EGFR inhibitors, including osimertinib [74]. This is a key example of presence of co-mutations driving genetic heterogeneity in tumors ultimately leading to therapeutic resistance. It is vital to determine whether such co- mutations present are secondary drivers or merely passenger mutations. Molecular subtype of lung cancer is driven by ALK fusion accounts for a small percentage of patients, of which ~60% respond to targeted inhibitors such as crizotinib, ceritinib, alectinib, and brigatinib. These tumors also demonstrate abundant genomic heterogeneity which may account for differences in treatment response with targeted ALK inhibitors [74]. Many clinical trials have explored novel drugs for different driver mutations by restricting to specific molecular subtypes [74]. Large scale screening studies are also being conducted to identify novel drivers of lung cancer progression, metastasis and therapeutic resistance [75]. This approach certainly provides a detailed insight into the molecular mechanisms in lung cancer. Nevertheless, both cell-intrinsic and cell-extrinsic factors should be evaluated in the consideration for improvement of clinical outcome.

Targeting phenotypically distinct subpopulations by identifying therapeutic vulnerabilities allows for a robust control of tumor growth and this concept has been extensively applied in KRAS mutation driven lung cancers. Due to undruggable nature of KRAS oncoproteins, downstream effector pathways are targeted, which include MAPK pathway members [75]. Despite the availability of specific drugs targeting these pathways, clinical efficacy has remained poor as single agent or in combination with chemotherapy [76,77]. The underlying explanation is due to the presence of profound tumor heterogeneity. Cancer subpopulations with pre-existing resistance or emergence of acquired resistance to drugs eventually leads to the failure of therapy in the clinic [78]. Components of TME, especially CAFs and immune cells, also impart resistance to therapy by modulating cancer cell characteristics [79]. Cancer cells escape detection by the host immune system and in order to elicit a response, cancer drugs are usually designed to eradicate the cancer cells. In recent years, the importance of engaging the host immune system in cancer treatment has been recognized. Lung cancer cells escape immune detection many mechanisms. There can be decreased tumor antigen presentation, recruitment of tumor suppressor cells, and engagement of checkpoint pathway inhibiting antitumor immunity [80]. The checkpoint pathways include cytotoxic T-lymphocytes antigen-4 (CTLA-4) and programmed death receptor-1 (PD-1). Efforts are directed to block these pathways in order to elicit an immune response against the tumor cells. Anti-CTLA-4 include ipilimumab which has shown efficacy in melanoma, is currently in clinical trials for lung cancer [81]. Anti-PD-1 agent nivolumab improved

the overall survival in lung cancer patients after platinum-based chemotherapy when compared to docetaxel [82]. Adoptive cell therapy is also being investigated where immune cells are isolated from peripheral blood followed by *ex vivo* expansion of tumor suppressor cells and autologous administration in the host [83]. The significance of immunotherapy lies in the fact that it is effective regardless of any heterogeneity between the tumor cells. In addition, combining chemotherapy (paclitaxel) with immunotherapy (ipilimumab), targeted therapy (EGFR inhibitors) with immunotherapy (ipilimumab) and different checkpoint inhibitors (anti CTLA-4 and anti PD-1) has proven extremely effective and in fact are standard of care in NSCLC [84]. Although CAFs and ECM seem attractive targets for therapy to prevent tumor progression, due to limited preclinical, effective targeting has not been achieved. Therapies targeting vascular endothelial growth factor (VEGF) like bevacizumab, in combination with chemotherapy have been shown to have some benefit over chemotherapy alone [7]. However, due to heterogeneity in vasculature around the tumor tissue, the response to these agents is limited and not very consistent. Instead, the idea of reverting blood supply to normal conditions is being explored which can improve drug delivery and reduce TME heterogeneity [85]. These examples underscore the importance of understanding and targeting tumor heterogeneity for sustained benefit.

Many unanswered questions that are worth exploring include: whether genetically different subsets of lung cancer have their own unique microenvironment and regulation, how does the targeted therapy affect the microenvironment and how does it influence the progression of the disease, how is the microenvironment different between primary, metastatic and recurrent tumor. To effectively understand these roles in context of all compartments of the TME, better modelling of the human disease is important. It can be achieved by using existing *in vitro* (primary cell cultures, co-cultures, 3-D systems) techniques and animal models (genetically engineered mouse models (GEMMs), syngeneic, xenografts, patient derived xenografts) in combination with clinical studies to boost the translation of experimental findings to the clinic. Efforts in the clinic must also be directed towards improving non-invasive imaging and diagnostic techniques to represent the heterogeneity in stroma for targeting the stroma in addition to the cancer cells. There are limitations in all the models, but effectively combining these can closely recapitulate the human disease for understanding the biology and improving patient outcome.

## References


1. Siegel RL, Miller KD, Fuchs HE, Jemal A (2022) Cancer statistics, 2022. CA Cancer J Clin 72(1): 7-33.
2. Bass AJ, Thorsson V, Shmulevich I (2014) Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513(7517): 202-209.
3. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, et al. (2002) Comparison of Four Chemotherapy Regimens for Advanced Non–Small-Cell Lung Cancer. N Engl J Med 346(2): 92-98.
4. Testa U, Castelli G, Pelosi E (2018) Lung Cancers: Molecular Characterization, Clonal Heterogeneity and Evolution, and Cancer Stem Cells. Cancers (Basel) 10(8): 248.
5. Schiller JH (2018) A New Standard of Care for Advanced Lung Cancer. N Engl J Med 378(22): 2135- 2137.
6. Hellmann MD, Paz-Ares L, Bernabe Caro R, Zurawski B, Sang-We K, et al. (2019) Nivolumab plus Ipilimumab in Advanced Non–Small- Cell Lung Cancer. N Engl J Med 381(21): 2020-2031.
7. Ready N, Hellmann MD, Awad MM, Otterson GA, Gutierrez M, et al. (2019) First-Line Nivolumab Plus Ipilimumab in Advanced Non– Small-Cell Lung Cancer (CheckMate 568): Outcomes by Programmed Death Ligand 1 and Tumor Mutational Burden as Biomarkers. J Clin Oncol 37(12): 992-1000.
8. Davidson MR, Gazdar AF, Clarke BE (2013) The pivotal role of pathology in the management of lung cancer. J Thorac Dis 5(Suppl 5): S463-S478.
9. Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong KK (2014) Non-small-cell lung cancers: a heterogeneous set of diseases. Nat Rev Cancer 14(8): 535-546.
10. Aisner DL, Marshall CB (2012) Molecular Pathology of Non–Small Cell Lung Cancer: A Practical Guide. Am J Clin Pathol 138(3): 332-346.
11. West L, Vidwans SJ, Campbell NP, Shrager J, Simon GR, et al. (2012) A Novel Classification of Lung Cancer into Molecular Subtypes. PLoS One 7(2): e31906.
12. Skoulidis F, Heymach JV (2019) Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. Nat Rev Cancer 19(9): 495-509.
13. Fischer AH, Young KA, DeLellis RA (2004) Incorporating pathologists' criteria of malignancy into the evolutionary model for cancer development. J Cell Biochem 93(1): 28-36.
14. Zhang J, Fujimoto J, Zhang J, Wedge DC, Song X, et al. (2014) Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. Science 346(6206): 256-259.
15. de Bruin EC, McGranahan N, Mitter R, Salm M, Wedge DC, et al. (2014) Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science 346(6206): 251-256.
16. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, et al. (2012) Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. N Engl J Med 366(10): 883-892.
17. Carrera PM, Ormond M (2015) Current practice in and considerations for personalized medicine in lung cancer: From the patient's molecular biology to patient values and preferences. Maturitas 82(1): 94-99.
18. Padhye A, Ungewiss C, Fradette JJ, Rodriguez BL, Albritton JL, et al. (2019) A novel ex vivo tumor system identifies Src-mediated invasion and metastasis in mesenchymal tumor cells in non-small cell lung cancer. Sci Rep 9(1): 4819.
19. Peng DH, Rodriguez BL, Diao L, Gaudreau PO, Padhye A, et al. (2021) Th17 cells contribute to combination MEK inhibitor and anti-PD-L1 therapy resistance in KRAS/p53 mutant lung cancers. Nat Commun 12(1): 2606.
20. Padhye A, Konen JM, Rodriguez BL, Fradette JJ, Ochieng JK, et al. (2021) Targeting CDK4 overcomes EMT-mediated tumor heterogeneity and therapeutic resistance in KRAS-mutant lung cancer. JCI insight 6(17): e148392.
21. Konen JM, Rodriguez BL, Padhye A, Ochieng JK, Gibson L, et al. (2021) Dual Inhibition of MEK and AXL Targets Tumor Cell Heterogeneity and Prevents Resistant Outgrowth Mediated by the Epithelial-to-Mesenchymal Transition in NSCLC. Cancer Res 81(5): 1398-1412.
22. Marusyk A, Almendro V, Polyak K (2012) Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer 12(5): 323-334.
23. Marusyk A, Janiszewska M, Polyak K (2020) Intratumor Heterogeneity: The Rosetta Stone of Therapy Resistance. Cancer Cell 37(4): 471-484.

24. Holliday R (2006) Epigenetics: A Historical Overview. Epigenetics 1(2): 76-80.

25. Cheng Y, He C, Wang M, Ma X, Mo F, et al. (2019) Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. Signal Transduct Target Ther 4(1): 62.

26. Dawson MA, Kouzarides T (2012) Cancer Epigenetics: From Mechanism to Therapy. Cell 150(1): 12-27.

27. Brzeziańska E, Dutkowska A, Antczak A (2013) The significance of epigenetic alterations in lung carcinogenesis. Mol Biol Rep 40(1): 309-325.

28. Barlési F, Giaccone G, Gallegos-Ruiz MI, Loundou A, Span SW, et al. (2007) Global Histone Modifications Predict Prognosis of Resected Non–Small-Cell Lung Cancer. J Clin Oncol 25(28): 4358-4364.

29. Shackleton M, Quintana E, Fearon ER, Morrison SJ (2009) Heterogeneity in Cancer: Cancer Stem Cells versus Clonal Evolution. Cell 138(5): 822-829.

30. Dick JE (2008) Stem cell concepts renew cancer research. Blood 112(13): 4793-4807.

31. Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414(6859): 105-111.

32. Alamgeer M, Ganju V, Watkins DN (2013) Novel therapeutic targets in non-small cell lung cancer. Curr Opin Pharmacol 13(3): 394-401.

33. Radisky DC, Bissell MJ (2006) Matrix metalloproteinase-induced genomic instability. Curr Opin Genet Dev 16(1): 45-50.

34. Polyak K, Weinberg RA (2009) Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 9(4): 265-273.

35. Hendrix MJC, Seftor EA, Hess AR, Seftor REB (2003) Vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma. Nat Rev Cancer 3(6): 411-421.

36. Bissell MJ, Kenny PA, Radisky DC (2005) Microenvironmental Regulators of Tissue Structure and Function Also Regulate Tumor Induction and Progression: The Role of Extracellular Matrix and Its Degrading Enzymes. Cold Spring Harb Symp Quant Biol 70: 343-356.

37. Hynes RO (2009) The Extracellular Matrix: Not Just Pretty Fibrils. Science 326(5957): 1216-1219.

38. Mierke CT (2021) Bidirectional Mechanical Response Between Cells and Their Microenvironment. Front Phys 9: 749830.

39. Alcaraz J, Otero J, Jorba I, Navajas D (2018) Bidirectional mechanobiology between cells and their local extracellular matrix probed by atomic force microscopy. Semin Cell Dev Biol 73: 71-81.

40. Nelson CM, Bissell MJ (2006) Of Extracellular Matrix, Scaffolds, and Signaling: Tissue Architecture Regulates Development, Homeostasis, and Cancer. Annu Rev Cell Dev Biol 22(1): 287-309.

41. Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, et al. (1997) Reversion of the Malignant Phenotype of Human Breast Cells in Three-Dimensional Culture and In Vivo by Integrin Blocking Antibodies. J Cell Biol 137(1): 231-245.

42. Gkretsi V, Stylianou A, Papageorgis P, Polydorou C, Stylianopoulos T (2015) Remodeling Components of the Tumor Microenvironment to Enhance Cancer Therapy. Front Oncol 5: 214.

43. Fisseler-Eckhoff A, Prebeg M, Voss B, Müller KM (1990) Extracellular Matrix in Preneoplastic Lesions and Early Cancer of the Lung. Pathol Res Pract 186(1): 95-101.

44. Sethi T, Rintoul RC, Moore SM, MacKinnon AC, Salter D, et al. (1999) Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: A mechanism for small cell lung cancer growth and drug resistance *in vivo*. Nat Med 5(6): 662-668.

45. Thomas P, Khokha R, Shepherd FA, Feld R, Tsao MS (2000) Differential expression of matrix metalloproteinases and their inhibitors in non-small cell lung cancer. J Pathol 190(2): 150-156.

46. Caccavari F, Valdembri D, Sandri C, Bussolino F, Serini G (2010) Integrin signaling and lung cancer. Cell Adh Migr 4(1): 124-129.

47. Gibbons DL, Lin W, Creighton CJ, Rizvi ZH, Gregory PA, et al. (2009) Contextual extracellular cues promote tumor cell EMT and metastasis by regulating miR-200 family expression. Genes Dev 23(18): 2140-2151.

48. Ungewiss C, Rizvi ZH, Roybal JD, Peng DH, Gold KA, et al. (2016) The microRNA-200/Zeb1 axis regulates ECM-dependent β1- integrin/FAK signaling, cancer cell invasion and metastasis through CRKL. Sci Rep 6(1): 18652.

49. Kalluri R, Zeisberg M (2006) Fibroblasts in cancer. Nat Rev Cancer 6(5): 392-401.

50. Kim SH, Choe C, Shin YS, Jeon MJ, Choi SJ, et al. (2013) Human Lung Cancer-associated Fibroblasts Enhance Motility of Non- small Cell Lung Cancer Cells in Co-culture. Anticancer Res 33(5): 2001-2009.

51. Wang W, Li Q, Yamada T, Matsumoto K, Matsumoto I, et al. (2009) Crosstalk to Stromal Fibroblasts Induces Resistance of Lung Cancer to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors. Clin Cancer Res 15(21): 6630- 6638.

52. Chen WJ, Ho CC, Chang YL, Chen HY, Lin CA, et al. (2014) Cancer-associated fibroblasts regulate the plasticity of lung cancer stemness via paracrine signalling. Nat Commun 5(1): 3472.

53. Bremnes RM, Dønnem T, Al-Saad S, Al-Shibli K, Sigve Andersen, et al. (2011) The Role of Tumor Stroma in Cancer Progression and Prognosis: Emphasis on Carcinoma-Associated Fibroblasts and Non-small Cell Lung Cancer. J Thorac Oncol 6(1): 209-217.

54. Schliekelman MJ, Creighton CJ, Baird BN, Chen Y, Banerjee P, et al. (2017) Thy-1+ Cancer-associated Fibroblasts Adversely Impact Lung Cancer Prognosis. Sci Rep 7(1): 6478.

55. Hu H, Piotrowska Z, Hare PJ, Chen H, Mulvey HE, et al. (2021) Three subtypes of lung cancer fibroblasts define distinct therapeutic paradigms. Cancer Cell 39(11): 1531-1547.e10.

56. Irvine AF, Waise S, Green EW, Stuart B, Thomas GJ (2021) Characterising cancer-associated fibroblast heterogeneity in non-small cell lung cancer: a systematic review and meta-analysis. Sci Rep 11(1): 3727.

57. Bota-Rabassedas N, Banerjee P, Niu Y, Cao W, Luo J, et al. (2021) Contextual cues from cancer cells govern cancer- associated fibroblast heterogeneity. Cell Rep 35(3): 109009.

58. Li YY, Lam SK, Zheng CY, Ho JCM (2015) The Effect of Tumor Microenvironment on Autophagy and Sensitivity to Targeted Therapy in EGFR-Mutated Lung Adenocarcinoma. J Cancer 6(4): 382-386.

59. Sugiyama Y, Kato M, Chen FA, Williams SS, Kawaguchi Y, et al. (2001) Human Inflammatory Cells Within the Tumor Microenvironment of Lung Tumor Xenografts Mediate Tumor Growth Suppression in Situ that Depends on and Is Augmented by Interleukin-12. J Immunother 24(1): 37-45.

60. Welsh TJ, Green RH, Richardson D, Waller DA, O'Byrne KJ, et al. (2005) Macrophage and Mast-Cell Invasion of Tumor Cell Islets Confers a Marked Survival Advantage in Non–Small-Cell Lung Cancer. J Clin Oncol 23(35): 8959-8967.

61. Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, et al. (2008) Long-Term Survival for Patients with Non–Small-Cell Lung Cancer With Intratumoral Lymphoid Structures. J Clin Oncol 26(27): 4410-4417.

62. Alifano M, Mansuet-Lupo A, Lococo F, Roche N, Bobbio A, et al. (2014) Systemic Inflammation, Nutritional Status and Tumor Immune Microenvironment Determine Outcome of Resected Non-Small Cell Lung Cancer. PLoS One 9(9): e106914.

63. Al-Shibli KI, Donnem T, Al-Saad S, Persson M, Bremnes RM, et al. (2008) Prognostic Effect of Epithelial and Stromal Lymphocyte Infiltration in Non–Small Cell Lung Cancer. Clin Cancer Res 14(16): 5220-5227.

64. Stearman RS, Dwyer-Nield L, Grady MC, Malkinson AM, Geraci MW (2008) A Macrophage Gene Expression Signature Defines a Field Effect in the Lung Tumor Microenvironment. Cancer Res 68(1): 34-43.

65. Müller P, Rothschild SI, Arnold W, Hirschmann P, Horvath L, et al. (2016) Metastatic spread in patients with non-small cell lung cancer is associated with a reduced density of tumor-infiltrating T cells. Cancer Immunol Immunother 65(1): 1-11.

66. Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, et al. (2003) The Stromal Derived Factor– 1/CXCL12–CXC Chemokine Receptor 4 Biological Axis in Non–Small Cell Lung Cancer Metastases. Am J Respir Crit Care Med 167(12): 1676-1686.

67. Shimizu K, Okita R, Nakata M (2013) Clinical significance of the tumor microenvironment in non-small cell lung cancer. Ann Transl Med 1(2): 20.

68. Kwon YJ, Lee SJ, Koh JS, Kim SH, Lee WH, et al. (2012) Genome-Wide Analysis of DNA Methylation and the Gene Expression Change in Lung Cancer. J Thorac Oncol 7(1): 20-33.

69. Zhong L, Roybal J, Chaerkady R, Zhang W, Choi K, et al. (2008) Identification of Secreted Proteins that Mediate Cell-Cell Interactions in an *In vitro* Model of the Lung Cancer Microenvironment. Cancer Res 68(17): 7237-7245.

70. Foster JG, Wong SCK, Sharp TV (2014) The hypoxic tumor microenvironment: driving the tumorigenesis of non-small-cell lung cancer. Futur Oncol 10(16): 2659-2674.

71. Cui YL, Wang X, Li XF (2015) 18F-fluoromisonidazole PET reveals spatial and temporal heterogeneity of hypoxia in mouse models of human non-small-cell lung cancer. Futur Oncol 11(20): 2841-2849.

72. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, et al. (2013) Analysis of Tumor Specimens at the Time of Acquired Resistance to EGFR-TKI Therapy in 155 Patients with EGFR-Mutant Lung Cancers. Clin Cancer Res 19(8): 2240-2247.

73. Oxnard GR, Arcila ME, Sima CS, Riely GJ, Chmielecki J, et al. (2011) Acquired Resistance to EGFR Tyrosine Kinase Inhibitors in EGFR- Mutant Lung Cancer: Distinct Natural History of Patients with Tumors Harboring the T790M Mutation. Clin Cancer Res 17(6): 1616-1622.

74. Ramalingam SS, Vansteenkiste J, Planchard D, Cho BC, Gray JE, et al. (2019) Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. N Engl J Med 382(1): 41-50.

75. Collisson EA, Campbell JD, Brooks AN, et al. Comprehensive molecular profiling of lung adenocarcinoma. Nature 511(7511): 543-550.

76. Jänne PA, van den Heuvel MM, Barlesi F, Cobo M, Mazieres J, et al. (2017) Selumetinib Plus Docetaxel Compared with Docetaxel Alone and Progression-Free Survival in Patients With KRAS-Mutant Advanced Non–Small Cell Lung Cancer: The SELECT-1 Randomized Clinical Trial. JAMA 317(18): 1844-1853.

77. Blumenschein GR, Smit EF, Planchard D, Kim DW, Cadranel J, et al. (2015) A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC). Ann Oncol 26(5): 894-901.

78. Ibiayi DJ, Shaw AT (2018) Tumour heterogeneity and resistance to cancer therapies. Nat Rev Clin Oncol 15(2): 81-94.

79. Junttila MR, de Sauvage FJ (2013) Influence of tumour micro-environment heterogeneity on therapeutic response. Nature 501(7467): 346-354.

80. Scagliotti GV, Bironzo P, Vansteenkiste JF (2015) Addressing the unmet need in lung cancer: The potential of immuno-oncology. Cancer Treat Rev 41(6): 465-475.

81. Chae YK, Arya A, Iams W, Cruz MR, Chandra S, et al. (2018) Current landscape and future of dual anti-CTLA4 and PD-1/PD-L1 blockade immunotherapy in cancer; lessons learned from clinical trials with melanoma and non-small cell lung cancer (NSCLC). J Immunother Cancer 6(1): 39.

82. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, et al. (2015) Nivolumab versus Docetaxel in Advanced Nonsquamous Non– Small-Cell Lung Cancer. N Engl J Med 373(17): 1627-1639.

83. Rohaan MW, Wilgenhof S, Haanen JBAG (2019) Adoptive cellular therapies: the current landscape. Virchows Arch 474(4): 449-461.

84. Walsh RJ, Soo RA (2020) Resistance to immune checkpoint inhibitors in non-small cell lung cancer: biomarkers and therapeutic strategies. Ther Adv Med Oncol 12: 1758835920937902.

85. Robertson-Tessi M, Gillies RJ, Gatenby RA, Anderson ARA (2015) Impact of Metabolic Heterogeneity on Tumor Growth, Invasion, and Treatment Outcomes. Cancer Res 75(8): 1567-1579.