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LUNG CANCER:
An Introduction

Ariad Pharmaceuticals, Inc.

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INTRODUCTION

In the United States, lung and bronchus cancer was estimated to be the leading cause of cancer deaths in both men and women in 2015; in fact, it is expected to cause more deaths than the next 3 most common cancers combined (prostate, colon/rectum, and pancreas cancers in men, and breast, colon/rectum, and pancreas cancers in women). In total, lung and bronchus cancer is estimated to cause 158,000 deaths every year, or about 27% of all cancer deaths in the United States. Globally, there were 1.8 million new cases of lung cancer and 1.6 million deaths attributable to lung cancer in 2012.[1,2] Lung cancer is primarily a disease of smokers, although a small, but clinically important proportion of nonsmokers and light former smokers are affected by the disease.[3] In total, smoking contributes to about 80% of lung cancer deaths in women and 90% of lung cancer deaths in men.[2] There are 2 main classifications of lung cancer: small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), the latter of which accounts for the majority of lung cancers in the United States.[4]

Although advances are continuously being made in lung cancer, the fact remains that it is associated with very poor 5-year relative survival rates (data for patients diagnosed between 2005 and 2011).[23] In fact, as a class, the various tumor types composing NSCLC as a clinical entity are relatively insensitive to chemotherapy and radiation therapy.[4] The five-year relative survival rate is better than that seen in small-cell lung cancer (SCLC), but remains poor at only about 22.3%.[27]

More than half of people with NSCLC remain undiagnosed until the cancer has metastasized, a situation that is associated with particularly poor 5-year relative survival rates of only about 4.7%.[27] A plateau has been reached in the efficacy of conventional chemotherapies, with 1-year survival rates of only 30% to 40% and 2-year survival rates of 10% to 15% in fit patients.[6] Thus, new approaches to treatment are needed. These new approaches have focused on modalities that specifically address so-called “driver” mutations, including—but not limited to—mutations in anaplastic lymphoma kinase (ALK) that are known to contribute to cancer cell proliferation, growth, survival, and the development of new vasculature in the tumor bed.[7] These therapies are collectively known as targeted therapy. Other recent advances include leveraging the immune system to attack cancers. These therapies fall, collectively, under the category of “immuno-oncology.”[8]

This backgrounder serves as a broad introduction to the:

- Anatomy and physiology of the lung
- Basic concepts in tumor growth and cell signaling
- Key signaling pathways involved in lung cancer
- Lung cancer pathophysiology and epidemiology
- Lung cancer diagnosis
- Pathologic analysis of lung cancers
- Treatment of lung cancers

It is also meant to serve as a general resource while you explore the Brigatinib Learning System and to provide an enduring reference to key concepts that you must understand in order to sell brigatinib effectively for its approved indications in the competitive market for the treatment of refractory ALK-positive NSCLC.

This backgrounder only provides topline information on key concepts; please note that the Brigatinib Learning System will dive into many of the topics covered in this backgrounder in much greater detail.

CHAPTER 1

Anatomy and Physiology: The Lung and Other Relevant Organ Systems

1.0. Introduction

Lung cancer affects the lungs and the surrounding structures of the respiratory, lymphatic, and circulatory systems. **Metastases** may affect other tissues, such as the bone, brain, liver, and other lung.[9] For this reason, it is important to understand these systems so you know how lung cancer grows and spreads through the body, as well as how it affects the patient.

Objectives

After completing this chapter, you will be able to:

- Name the major anatomic structures of the respiratory system
- Identify the major components of the lymphatic and circulatory system surrounding the lungs
- Describe key structures of the brain, bone, and liver

1.1 Overview of the Respiratory System

The primary functions of the respiratory system are to supply the body with oxygen and dispose of carbon dioxide.[10]

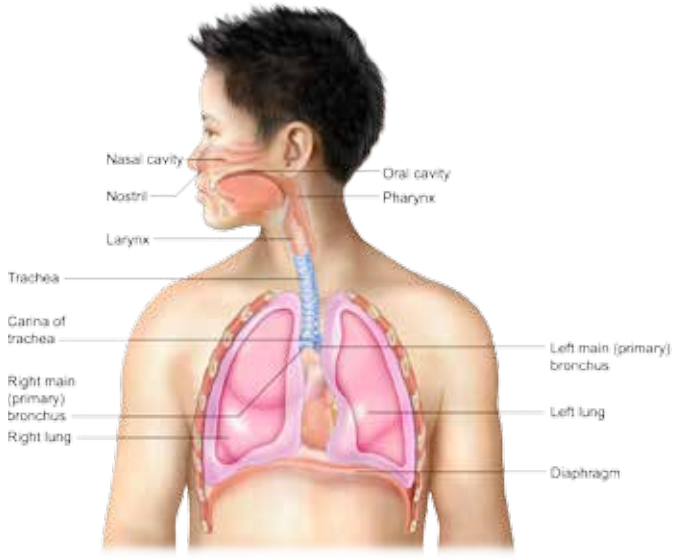
The respiratory system is divided into 2 broad categories **(Figure 1.1A)**[10]:

- The upper respiratory system consists of all structures from the nose to the larynx
- The lower respiratory system includes the larynx and all of the structures below it, including the trachea, bronchi, and lungs

Sometimes, the respiratory muscles, such as the diaphragm, are also included as part of the lower respiratory system.

The remainder of this chapter will focus on the bronchi and lungs, because that is where primary lung cancer occurs.

Figure 1.1A. *The major respiratory organs*[10]



Adapted from Marieb et al, 2014.

Orientation and Anatomy

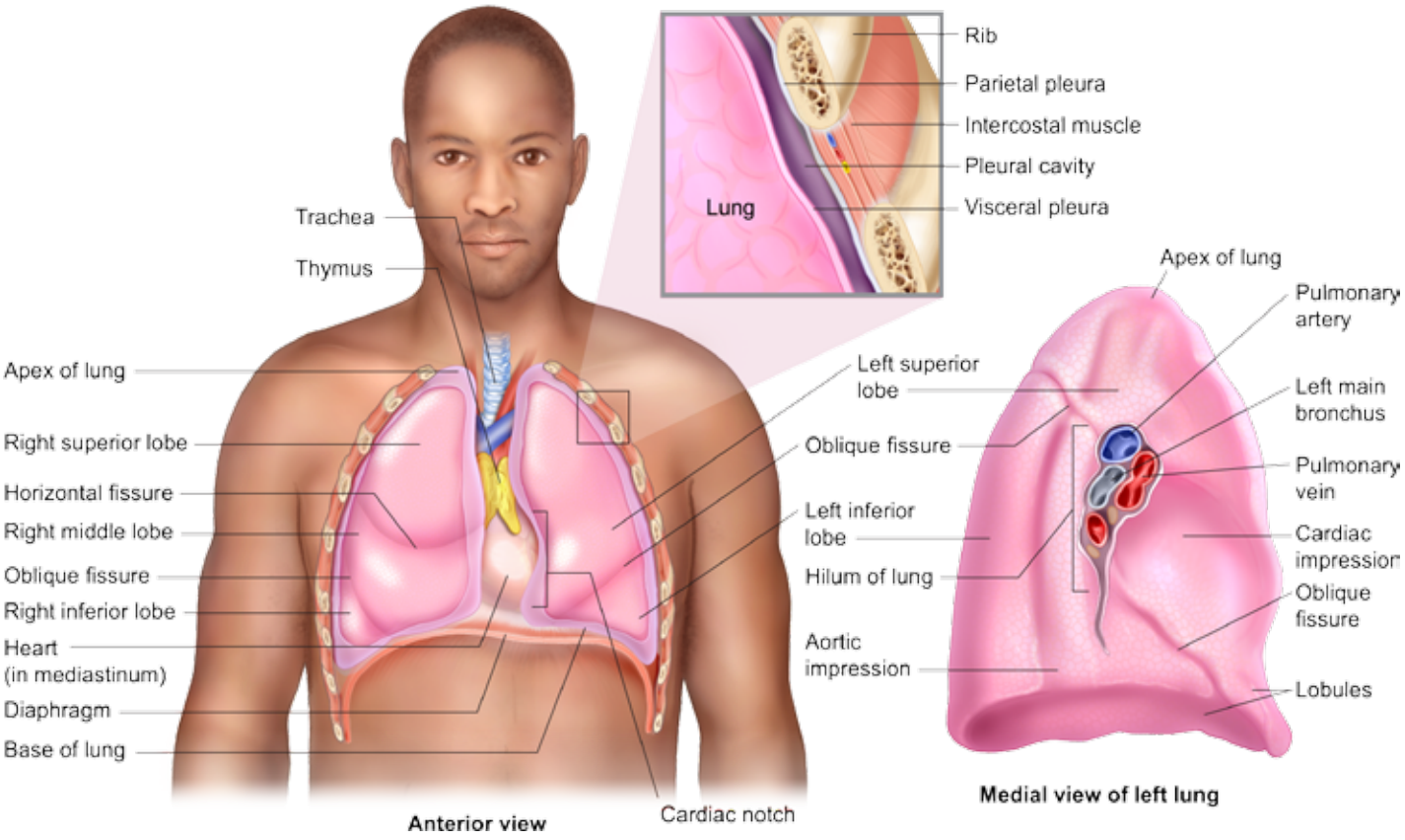
Note that, when discussing anatomy, the structures of the body are referred to in the orientation they are in the body.[10] That is to say, the right lung refers to the patient's right side. Thus, in illustrations looking at the front of the body, the right lung appears on the left side of the illustration, while the left lung appears on the right side of the illustration.

1.1.1 The Lungs

The lungs are 2 sponge-like organs located in the chest which are surrounded by the rib cage **(Figure 1.1B).** Each lung consists of lobes: 3 on the right (superior, middle, and inferior) and 2 on the left (superior and inferior).[11] The left lung is slightly smaller because the heart takes up some room on that side of the chest.

A bronchopulmonary segment is a division of the lobe; each lobe houses multiple bronchopulmonary segments. Each of these segments receives air from its own tertiary bronchus **(see Figure 1.1B)** and is supplied by blood from its own artery.[11]

Figure 1.1B. ***The lungs***[10]



Adapted from Marieb et al, 2014.

Did You Know?

One way in which lung cancer may be managed surgically is by removing 1 or more diseased segments of the lung, a procedure known as segmentectomy.[47] Other types of surgery performed on the lung include pneumonectomy, or removal of an entire lung, and lobectomy, or removal of just one lobe of the lung.

1.1.2 Structures Surrounding the Lungs

- The diaphragm is a muscle underlying the lungs that contracts and relaxes. When it contracts, it moves downward and flattens out. As a result, the dimension of the thoracic cavity increases, causing the lungs to expand.[10] The diaphragm works together with the intercostal muscles, which lift the rib cage[10]
- The pleurae are thin, double-layered tissues; the layer called the parietal pleura covers the wall of the thorax and the face of the diaphragm facing the lungs. It continues around the heart and between the lungs. From there, it extends as the layer called the visceral pleura to cover the external lung surface.[10] The pleurae produce pleural fluid, which fills the pleural cavity and provides lubrication, allowing the lungs to glide over the wall of the thorax during breathing[10]
- The heart is located between the lungs, inside the mediastinum. The heart is surrounded by a sac known as the pericardium; major vessels of the heart include the aorta, the vena cavae, and pulmonary arteries[11]
- The spine, and its components, the vertebrae, are in close proximity to the lungs

1.1.3 The Lower Respiratory System: The Conducting and Respiratory Zones

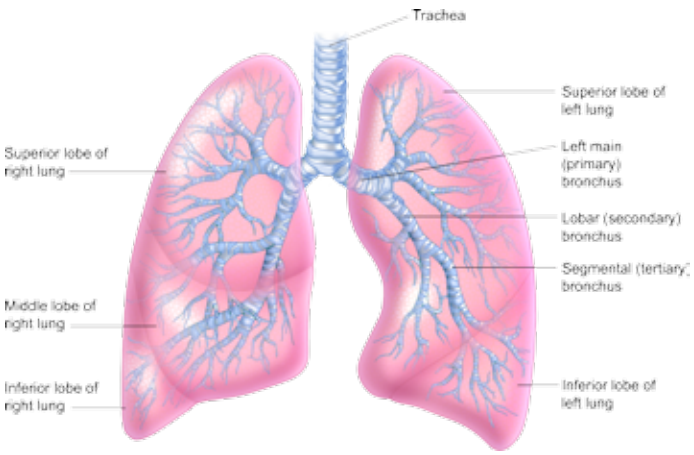
The lower respiratory system is divided into 2 sets of structures: a conducting zone, which simply provides for the passage of air, and the respiratory zone, where gas exchange actually occurs.[10]

- The conducting zone of the lower respiratory system consists of the trachea and the bronchi
- The respiratory zone consists of the respiratory bronchioles, alveolar ducts, and alveoli. All of these structures are microscopic

The Conducting Zone

The trachea, also known as the "windpipe," is part of the air-conducting zone of the lower respiratory system **(Figure 1.1C).** It descends from the larynx through the neck and into the mediastinum.[10] It ends by dividing into 2 bronchi, at approximately mid-chest level. In humans, it is about 4 in (10-12 cm) in length and about 3.4 inches (1.9 cm) in diameter, and it is very flexible.[10] The trachea is surrounded and supported by rings made of cartilage that prevent it from collapsing, despite pressure changes during normal breathing.

Figure 1.1C. *The conducting zone passages*[10]



Adapted from Marieb et al, 2014.

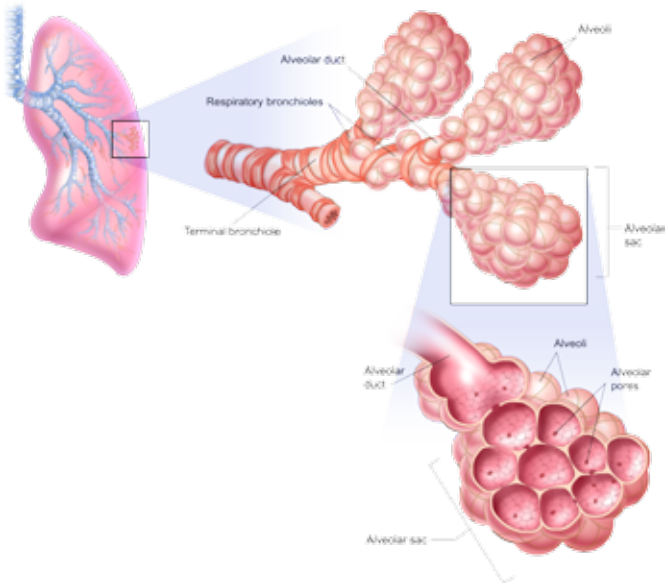
The bronchi are air passages that extend from the trachea, which divides to form the right and left main (pulmonary) bronchi.[10] The bronchi branch multiple times, forming a structure sometimes referred to as the bronchial tree, because it resembles an inverted tree.[10] Each bronchus enters the lung through the medial depression (hilum) of the lung.[10]

Once the bronchi enter the lungs, they first divide into the lobar (or secondary) bronchi. There are 3 lobar bronchi in the right lung and 2 in the left lung, each supplying 1 lung lobe. The lobar bronchi, in turn, branch again into the segmental (tertiary) bronchi, which divide repeatedly into smaller and smaller bronchi. Passages smaller than 1 mm in diameter are known as bronchioles; the smallest of these are called terminal bronchioles, which are less than 0.5 mm in diameter.[10]

The Respiratory Zone

The respiratory zone, which is defined by the presence of alveoli or thin-walled air sacs, begins where the terminal bronchioles feed into respiratory bronchioles within the lung **(Figure 1.1D)**.[10] The terminal bronchioles lead to the alveolar ducts, which consist of rings of smooth muscle, connective tissues, and outpocketing alveoli. The alveolar ducts terminate in clusters of alveoli known as alveolar sacs. Gas exchange occurs in the alveoli.[10]

Figure 1.1D. *The structures of the respiratory zone: Respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. Note that the alveoli are very thin-walled.*[10]



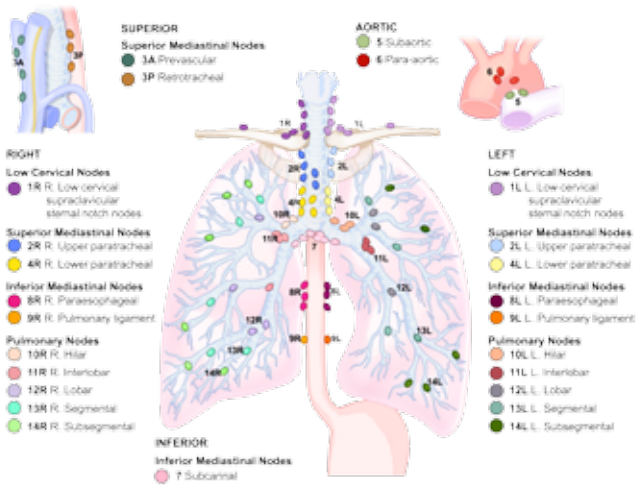
Adapted from Marieb et al, 2014.

1.1.4 The Lymphatic System of the Lungs

Understanding the lymphatic structures surrounding the lungs is important because lung cancer metastasizes through the lymph and blood. The lymphatic system consists of interstitial fluid called lymph, which is transported by lymphatic vessels through lymph nodes and other organs of the lymphatic system.[11] The primary role of the lymphatic system is to circulate lymph and thereby help defend against disease.[11] As will be discussed later, if allowed to continue to grow, most lung cancers will eventually invade the lymphatic vessels. In fact, metastasis to the regional lymph nodes often precedes distant metastasis.

In the lungs, the lymphatic drainage parallels the arteries, with lymph nodes located adjacent to the bronchi. The lymph nodes that are most important in staging lung cancer are shown in **Figure 1.1E**.[12]

Figure 1.1E. *Lymphatic system of the lungs (map developed by the International Association for the Study of Lung Cancer [IASLC])*[12]



Adapted from El-Sherief et al, 2014

Did You Know?

Knowing the location and names of the lymph nodes is important because NSCLC is staged using the "tumor, (lymph) **node**, metastasis" (TNM) system, which is driven in part by the location of lymph nodes involved in disease. The TNM system facilitates treatment planning and prognostication.[12]

1.2 The Circulatory System

Lung cancer can invade the circulatory system and use it to spread to distant sites. Further, because of the proximity of the heart to the lungs, lung cancer may directly invade the heart and great vessels.[13]

1.2.1 Overview of the Cardiovascular System

The cardiovascular system consists of the heart, blood vessels, and blood. The cardiovascular system has 2 key functions[11]:

- It provides oxygen and nutrients to every cell and tissue in the body
- It removes waste products produced by cell metabolism

The specific functions of blood also include defense of the body from infection, distribution of heat, and maintenance of balance within the body (homeostasis).

The cardiovascular system consists of the following components[14]:

- The heart: Pump that propels blood through the system
- The vessels: Carry blood throughout the body
  - Arteries are vessels that carry blood from the heart to the tissues
  - Capillaries, the smallest vessels, are the sites where oxygen (O2), carbon dioxide (CO2), and nutrient/waste-product exchange occur between the blood and the tissues
  - Veins carry blood back to the heart to be pumped again

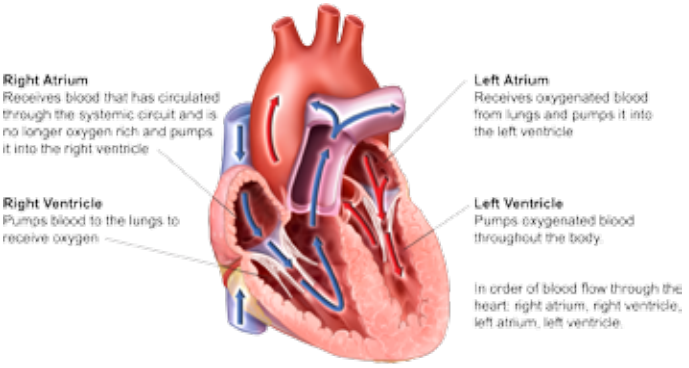
The circulation of blood can be thought of as 2 separate but interconnected circuits: pulmonary and systemic. The pulmonary circuit carries deoxygenated blood from the heart to the lungs and then carries oxygenated blood from the lungs back to the heart. The systemic circuit carries oxygenated blood to the body tissues and returns deoxygenated blood back to the heart.[14]

1.2.2 The Heart

The heart is a 4-chambered, hollow, muscular organ located slightly to the left of the middle of the chest between the lungs. Each of the 4 chambers of the heart contracts and relaxes regularly to pump blood continuously through the arteries and veins.[11] The chambers of the heart are illustrated in **Figure 1.2A**.

- The two upper chambers of the heart are the right and left atria (singular: atrium). These small chambers collect blood from the veins of the pulmonary and systemic circuits of the cardiovascular system.[15] The right atrium receives deoxygenated venous blood from the systemic circulation and pumps it into the right ventricle.[15] The left atrium receives oxygenated blood from the pulmonary circulation and pumps it into the left ventricle[15]
- The 2 lower chambers of the heart are the right and left ventricles. These chambers are muscular structures that pump blood into the arteries of the pulmonary and systemic circulation with each contraction. The right ventricle receives blood from the right atrium and is responsible for pumping blood into the pulmonary circulation.[15] The left ventricle receives oxygenated blood from the left atrium and pumps blood into the systemic circulation[15]

Figure 1.2A. *The chambers of the heart*[11]



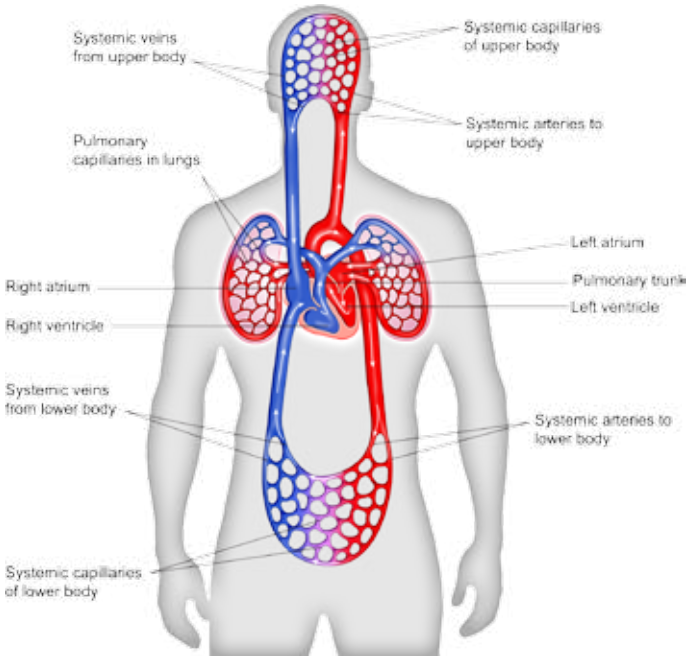
Adapted from Betts, 2014.

1.2.3 The Circulation

In **Figure 1.2B**, oxygenated blood is shown in red, and deoxygenated blood is shown in blue.[11] Blood flows from the right atrium to the right ventricle, where it is pumped into the pulmonary circuit. The blood in the pulmonary artery branches is low in oxygen but relatively high in carbon dioxide. Gas exchange occurs in the pulmonary capillaries, meaning that oxygen is loaded into red blood cells and carbon dioxide is dropped off.[11]

Blood high in oxygen and low in carbon dioxide returns to the left atrium and is then expelled into the left ventricle, which pumps it into the systemic arteries of the upper and lower body. The blood then flows into the systemic capillaries of the upper and lower body. Exchange occurs in the systemic capillaries, where oxygen and nutrients are delivered and carbon dioxide and wastes are loaded on. Blood then returns to the right atrium via the systemic veins. The cycle is then repeated.[11]

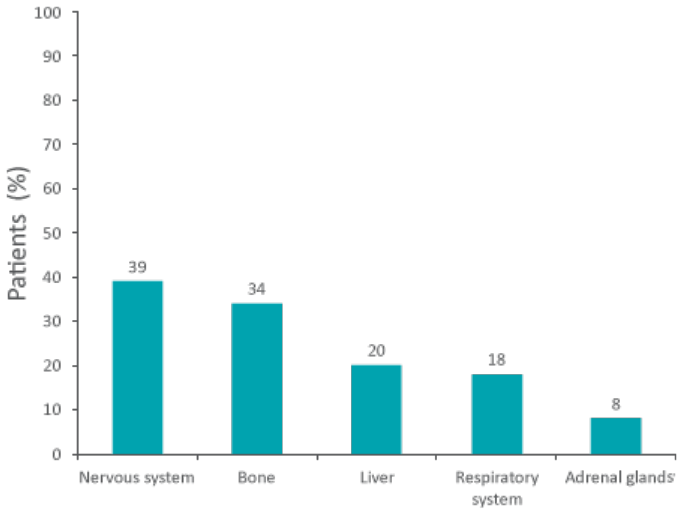
Figure 1.2B. *The circulation*[11]



1.3 Additional Relevant Anatomy

In many patients, lung cancer is not just a disease of the lungs. In fact, metastatic spread of cancer to distant organs is the primary reason for most cancer deaths.[16] The most common sites of metastasis among patients with lung cancer are the nervous system, bone, liver, respiratory system and adrenal glands **(Figure 1.3A)**. Sites of metastasis have a strong influence on patients survival in with lung cancer.[16]

Figure 1.3A. *Most common sites of metastasis in lung cancer*[16]

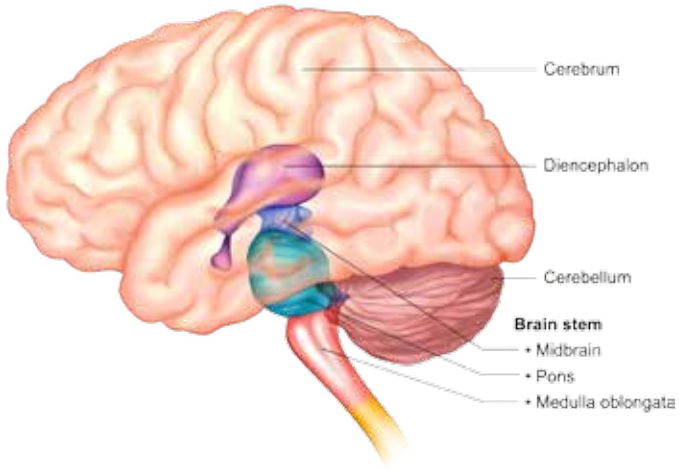


A detailed discussion of the anatomy and physiology of each of these sites of metastases is beyond the scope of this backgrounder; however, a brief introduction to each relevant organ is provided here.

Brain and Spinal Cord

About 39% of patients with metastatic lung cancer have metastases of the nervous system.[16] The brain and spinal cord represent the main organs of the nervous system. The spinal cord is a single structure, whereas the adult brain has 4 major regions: the cerebrum, diencephalon, brain stem, and cerebellum **(Figure 1.3B).** The brain is responsible for consciousness, maintaining homeostasis, and coordination of reflexes.[11]

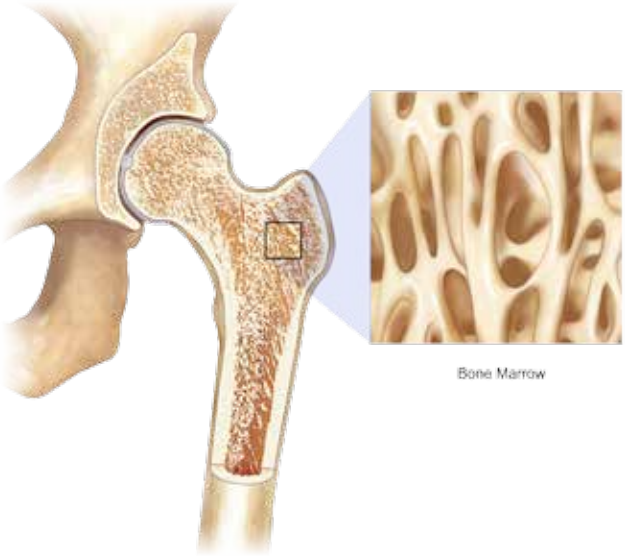
Figure 1.3B. *Major regions of the brain*



Bone

Bone, or osseous tissue, is a hard, dense connective tissue that forms most of the adult skeleton. Bone has multiple functions including supporting the body, facilitating movement, protecting internal organs, production of red blood cells, and storage and release of minerals and fat.[11] The interior of the cancellous (spongy) bone contains the red marrow, the site of most hematopoiesis in adults **(Figure 1.3C).**[11]

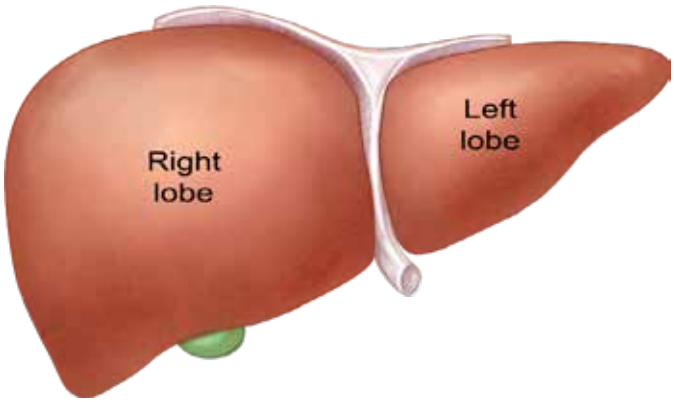
Figure 1.3C. *Cancellous bone contains the red marrow—the site of hematopoiesis*[11]



Liver

The liver is the largest gland in the body, weighing about 3 pounds in an adult. It plays an important role as an accessory digestive organ, but also plays a number of roles in metabolism and regulation, including detoxification of toxic substances and metabolizing drugs.[11] The liver is divided into 2 primary lobes: a large right lobe and a much smaller left lobe **(Figure 1.3D**).[11] After processing nutrients and toxins, the liver releases nutrients needed by other cells back into the blood; toxins are excreted.[11]

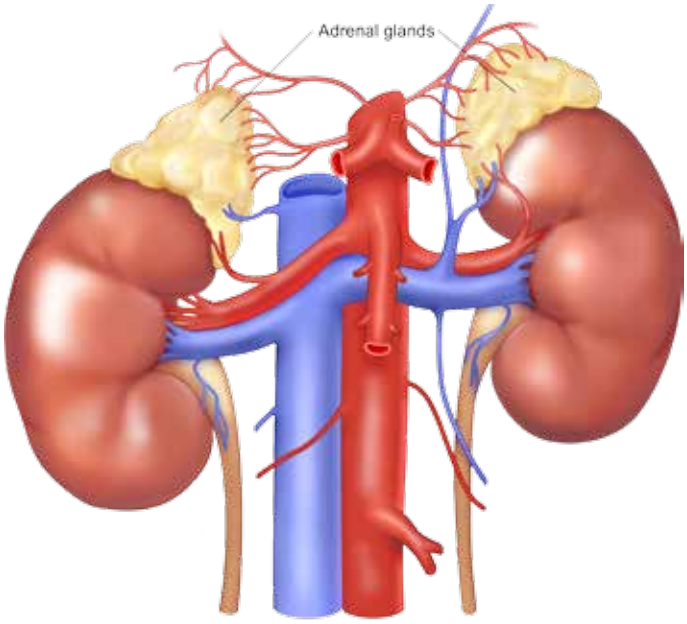
Figure 1.3D. *The liver*[11]



Adrenal Glands

The adrenal glands are wedges of glandular and endocrine tissue adhering to the top of the kidneys **(Figure 1.3E)**.[11] The adrenal glands have a rich blood supply and have one of the highest rates of blood flow in the body. The adrenal cortex secretes steroid hormones important for the regulation of the long-term stress response, blood pressure and blood volume, nutrient uptake and storage, fluid and electrolyte balance, and inflammation. The adrenal medulla helps regulate homeostasis in the body.[11]

Figure 1.3E. *The adrenal glands*



1.4 Summary: Anatomy and Physiology: The Lung and Other Relevant Organ Systems

The Lung and Associated Structures

- The lung consists of 5 lobes: 3 on the right (superior, middle, inferior) and 2 on the left (superior and inferior)
- Each lobe consists of multiple bronchopulmonary segments; each segment receives its own tertiary bronchus
- Structures surrounding the lung include:
  - The diaphragm: A muscle that contracts and relaxes to facilitate breathing
  - The pleurae: Thin, double-layered tissues that produce a fluid that fills the pleural cavity, providing lubrication
  - The heart: Located between the lungs
  - The spine and vertebrae
  - Associated lymph nodes
- The lower respiratory system consists of:
  - The conducting zone: Trachea and bronchi
  - The respiratory zone: Respiratory bronchioles, alveolar ducts, alveoli
- The bronchi extend from the trachea and branch multiple times, forming a structure that appears like an inverted tree
  - Passages smaller than 1 mm in diameter are known as bronchioles
  - Passages smaller than 0.5 mm in diameter are known as terminal bronchioles
- The terminal bronchioles lead to the alveolar ducts, which consist of rings of smooth muscle, connective tissues, and outpocketing alveoli
  - Gas exchange occurs in the alveoli

The Circulatory System

- The primary purpose of the heart is to distribute blood throughout the circulation
- Blood flows from the right atrium to the right ventricle, where it is pumped into the pulmonary circuit
- Gas exchange occurs in the pulmonary capillaries
- Blood high in oxygen and low in carbon dioxide returns to the left atrium and is then expelled into the left ventricle, which pumps it into the systemic arteries of the upper and lower body
- Following exchange in the systemic capillaries, blood returns to the right atrium via the systemic veins

Additional Relevant Anatomy

- Lung cancer is most commonly metastatic to the nervous system, bone, liver, respiratory system, and adrenal glands
  - The brain is responsible for consciousness, maintaining homeostasis, and coordination of reflexes
  - The bone is responsible for supporting the body, facilitating movement, protecting internal organs, production of red blood cells, and storage and release of minerals and fat
  - The liver is an accessory digestive organ that also plays roles in metabolism and regulation
  - The adrenal glands secrete steroid hormones and regulate homeostasis

CHAPTER 2

Tumor Growth, Angiogenesis, and Signaling

2.0 Introduction

Cancers are caused by the uncontrolled proliferation of the body's own cells and start with a mutation in a single cell that may, for example, render inactive the cell's normal mechanisms for monitoring the fidelity of DNA replication. Additional changes accumulate in these cells, allowing them to outgrow, out-divide, and outlive their neighboring cells. This competitive advantage ultimately results in a tumor.[17] However, solid tumors cannot grow indefinitely in the absence of support mechanisms. For this reason, cancers have mechanisms that can commandeer supplies of oxygen and nutrients from the host body.

Further, all cells, including those of tumors, must be able to coordinate their growth and activities through some means of intercellular communication.[17] An important part of this communication process involves signal transduction, in which specific molecules from outside the cell initiate a pathway of biochemical reactions within the cell, leading to a change in the cell's behavior. Signals and their effects in the cell are finely balanced, with many positive and negative feedback loops to control growth.[17]

This chapter will first review 2 key concepts related to tumor growth: doubling time and angiogenesis. Next, it will explore signaling pathways, both in general, and those that are thought to be involved in oncogenesis.

Objectives

After completing this chapter, you will be able to:

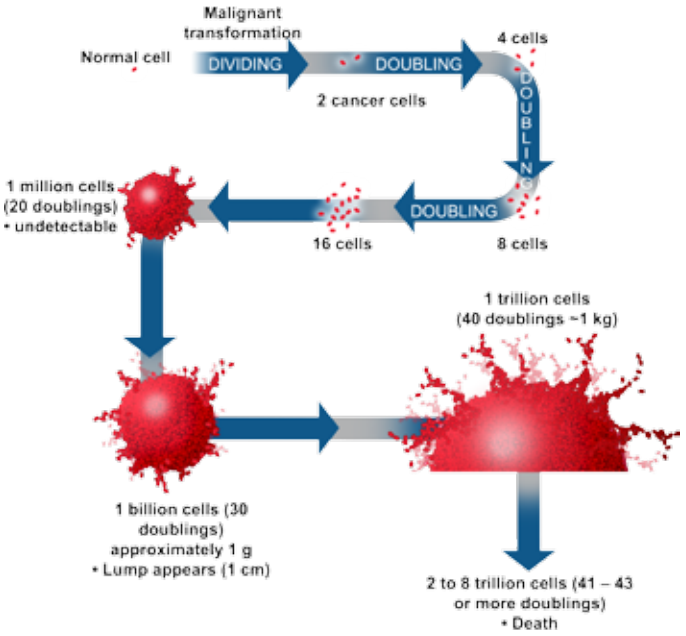
- Discuss tumor growth in terms of doubling time
- Describe the process of angiogenesis and its importance in tumor growth and metastasis
- Define signal transduction
- Relate the structure of receptor tyrosine kinases (RTKs) to the transmission of extracellular signals within the cell
- Recognize signal transduction pathways involved in cancer

2.1 Doubling Time of Tumor Cells

As shown in **Figure 2.1A**, the formation of a tumor begins with a single cell doubling, or dividing into 2. Each of those 2 cells divides to make 4 cells, then 8, and so on. This doubling time is called exponential growth, and it is expressed using factors of 10 (eg, 10, 100, 1000, 10,000). When a cancer is microscopic and nonpalpable, growth is exponential.[18]

Tumors may reach 10^9 (1 billion) cells (which represents about 30 doublings), before they are clinically detectable. At this point, the tumor may be about 1 cm in diameter and can grow undetected for a long time to a relatively large size, which means that there may be a relatively short amount of time between detectability and lethality of the tumor mass.[19] Patients most often present with tumors that consist of 10^{10} cells; only 10 more doublings can result in more than 1 trillion cells, which is lethal in many cases.[19] In general, tumor cells do not grow faster than normal cells; instead, they have more cells in the active phases of replication and are unable to undergo programmed cell death. In contrast, normal tissues have a greater number of cells in quiescent (resting) phase.[18]

Figure 2.1A. *In many cases, only 41 to 43 doublings is required for a tumor to become lethal*[18]

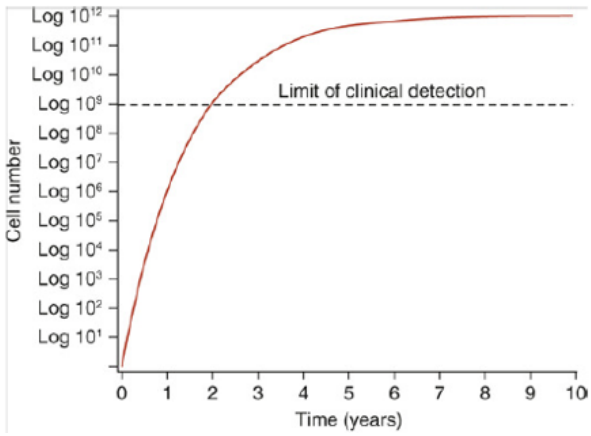


Did You Know?

Cancer chemotherapies (as opposed to targeted agents or biologics) are nonselective; they will kill a broad range of cells. However, in order for most cancer chemotherapies to kill cells, they require the cell to be actively dividing. This principle underlies the selectivity of chemotherapy: While most normal cells are in the resting phase of the cell cycle, cancer cells are actively dividing. Thus, cancer chemotherapy more or less selectively targets tumor cells, while relatively sparing normal tissue.[18]

Tumors are characterized by what is known as a gompertzian growth curve **(Figure 2.1B).**[18] Tumor masses require progressively longer times to double in size as they enlarge, because as the tumor grows, limitations in the supply of oxygen and nutrients hamper its growth. In order for a tumor to grow beyond a certain size, it must encourage the formation of its own blood supply, which we will discuss in the next section.[18]

Figure 2.1B. *The gompertzian growth curve.* During the early stages of solid tumor expansion, growth is exponential. As the tumor grows, growth slows. Most tumors complete the exponential phase of their growth before detection, which may have implications for the efficacy of conventional chemotherapy.[18]



2.2 Angiogenesis and Tumor Growth

To survive and grow, cells require oxygen and a constant supply of nutrients from blood vessels. Metabolic wastes must be carried away by the same mechanism. For this reason, cells are usually located in close proximity to blood vessels. When a tumor is small (<1-2 mm), simple diffusion permits exchange of these substances through existing vasculature. For a tumor to grow larger than about 1 to 2 mm in diameter, it must establish its own blood supply through a process called angiogenesis.[20] Angiogenesis, and the consequent production of new blood vessels, has a dual effect on tumor growth: it supplies needed nutrients and oxygen, and newly formed blood vessel cells secrete growth factors that stimulate the growth of adjacent tumor cells. The blood vessels that are formed in this process are haphazard and "leaky." Thus, beyond their role in nourishing the tumor, access of tumor cells to these abnormally formed blood vessels contributes to metastasis.[20]

Angiogenesis, along with many other normal and abnormal cellular functions, requires coordinated signaling among cells. The next section will discuss signal transduction: the way in which normal cells and tumor cells "talk" to each other.

Did You Know?

In order to capitalize on the selectivity of cancer chemotherapies for actively growing cells, patients may undergo surgery to remove the primary tumor and debulk large masses. This leaves only microscopic residual disease. The remaining tumor cells are pushed into active replication, and are thus more sensitive to conventional chemotherapies.[18]

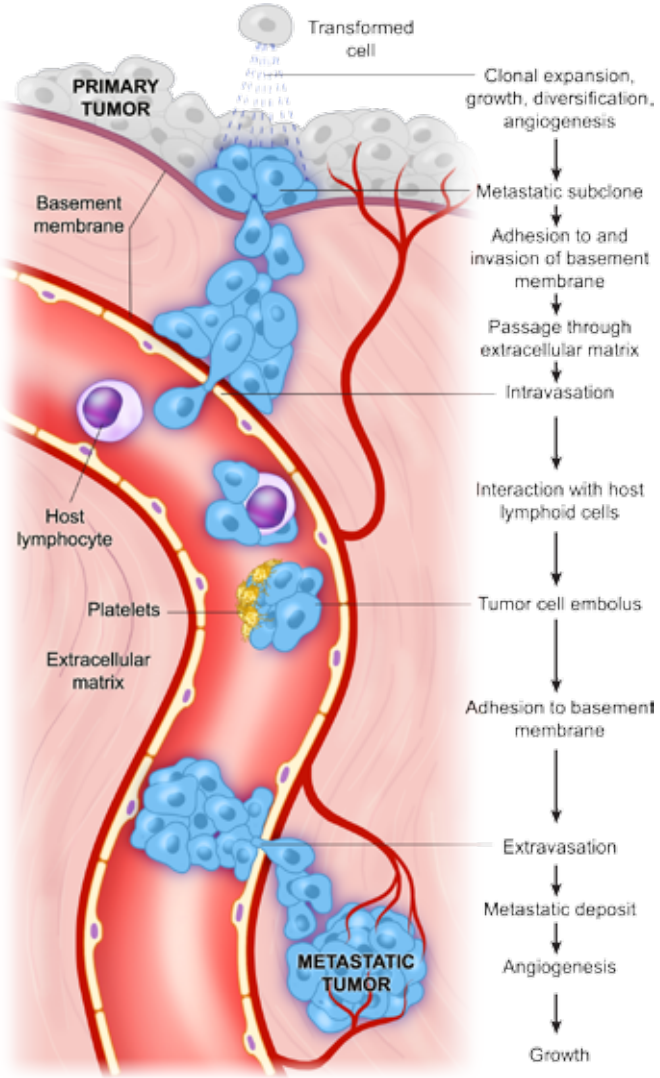
2.3 Invasion and Metastasis

Invasion of cancer cells and metastasis, the development of secondary malignant growths at a distance from the primary site of cancer, are major causes of cancer-related morbidity and mortality.[20] Each day, millions of cells are released from the primary tumor; however, only a few metastases are produced. In fact, tumor cells can be detected in the blood of many patients with cancer who do not ever develop metastatic disease.[20] The metastatic process is inefficient: In order to form a metastasis, the tumor must go through a series of steps **(Figure 2.3A)**.

There are 2 phases of the metastatic cascade:[20]

1. Invasion of the extracellular matrix
2. Dissemination of tumor cells through the vasculature, homing of tumor cells, and colonization

Figure 2.3A. *The metastatic cascade[20] (See text for details)[20]*



2.3.1 Invasion of the Extracellular Matrix

The extracellular matrix is composed of the basement membrane and connective tissue. In order for a metastasis to occur, the tumor must first breach the basement membrane of the surrounding tissue, transit through the connective tissue, and then breach the basement membrane of the surrounding vasculature.[20]

This process must be repeated in reverse when the tumor cell attaches to the distant site. There are a number of steps that are required in order for the tumor cell to breach the basement membrane. First, tumor cells detach from one another because of reduced adhesiveness. Inflammatory cells attracted to the site of the tumor, as well as the tumor cells themselves, release proteases that degrade the basement membrane. This process leads to migration and invasion of tumor cells.[20]

2.3.2 Dissemination and Colonization

Once in the circulation, the tumor cells aggregate in clumps.[20] These clumps adhere to the endothelium, the inner layer of the blood vessel, and leave the blood vessel through degradation of the blood vessel's basement membrane.[20] The site at which the circulating tumor cells leave the blood vessels to form metastases is related to the anatomic location and vascular supply of the primary tumor, as well as the tropism of particular tumors for specific tissues.[20] For example, as we have already discussed, lung cancer metastases have a preference for nervous tissue, the liver, bone, and the adrenal glands (along with other respiratory tissue).[16]

2.4 Signal Transduction—Communication Among Cells

For the human body to function, its cells must be able to coordinate their growth and activities through intercellular communication.[17] An important part of this communication process involves signal transduction, in which specific molecules from outside the cell initiate a pathway of biochemical reactions within the cell, leading to a change in the cell's behavior.[21] Signals and their effects in the cell are finely balanced, with many positive and negative feedback loops to control growth.

2.4.1 Overview of Signal Transduction

Signal transduction is not only a mechanism for intercellular communication, but is also the way in which signals are propagated and integrated intracellularly to modulate the cell's behaviors. A broad range of stimuli can initiate signaling cascades, including proteins, short peptides, amino acids, nucleotides, steroids, retinoids, fatty acids, and dissolved gases.[21] However, the primary examples of signaling molecules—also known as ligands—are growth factors and cytokines. Ligands bind to receptor proteins, activating signal transduction pathways.[17]

Signal transduction pathways can be classified into 2 broad categories **(Figure 2.4A):**[21]

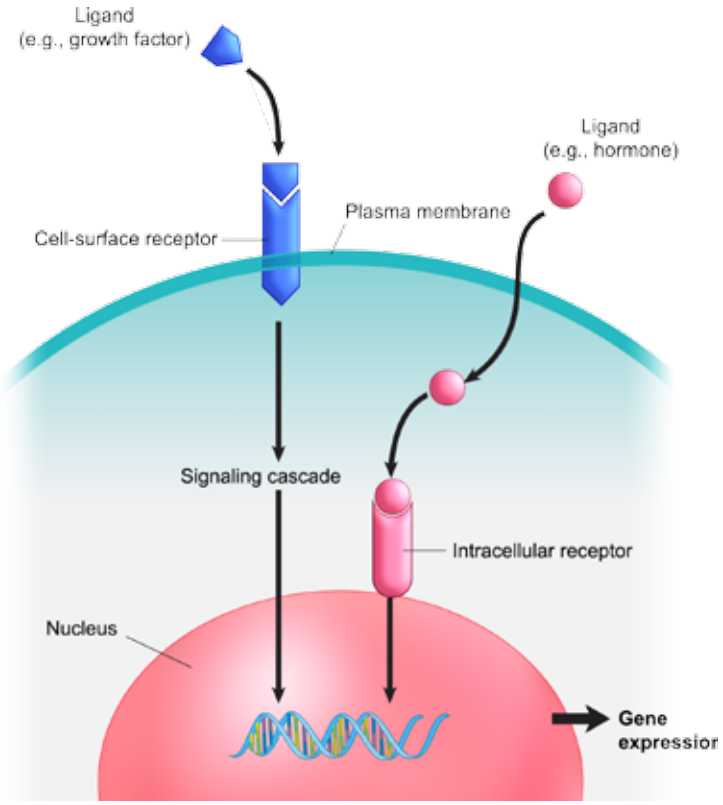
- Those that transmit signals from outside the cell to the inside of the cell (cell-surface receptor pathways). Most signaling molecules cannot pass through the cell membrane. These molecules must bind to a cell-surface receptor, which transduces the signal from outside the cell to the inside
- In some cases, signaling molecules can pass through the cell membrane. In these cases, the signaling molecule binds to an intracellular receptor in the cytoplasm of the cell or the nucleus

In both cases, the ultimate effect is a signal that enters the nucleus of the cell and controls gene expression.[21]

When the receptor is activated by the ligand, a signal is transduced to appropriate parts of the cell through a cascade of downstream intracellular signaling proteins.[17] Depending on the signal, the end result of the pathway could be to alter gene expression, a metabolic pathway, survival, growth or proliferation.[17]

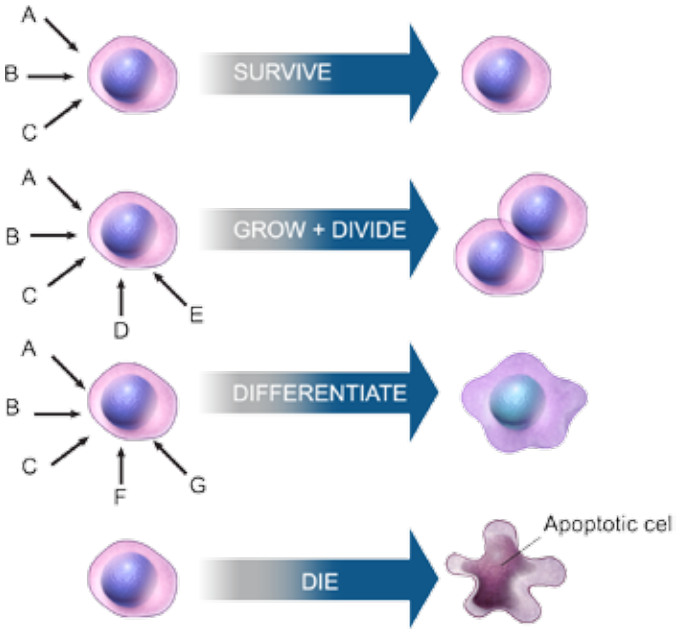
A typical cell is exposed to multiple, and often hundreds, of signals from different signaling molecules in its environment. The cell responds to this mixture of signals selectively, according to its own specific characteristics.[17] For example, a cell may respond to one combination of signals by differentiating into another type of cell; another set of signals may cause it to perform a specialized function, such as contraction or secretion; a third set of signals may cause it to undergo programmed cell death.

Figure 2.4A. *Cell surface and intracellular receptor pathways*[21]



Cells integrate and balance this information, resulting in a net effect that allows them to make crucial decisions, such as whether to divide, move, or differentiate **(Figure 2.4B).** Further, most cells are dependent on different combinations of signals in order to continue to survive. In the absence of these signals, they die by apoptosis, a process of programmed cell death.[17]

Figure 2.4B. *Cells depend on multiple extracellular signals to drive their behavior*[17]



Adapted from Alberts, 2008.

2.4.2 Signaling Through Tyrosine Kinases

Although there are many kinds of ligands, receptors, and intracellular signaling proteins, we will focus on growth factors, their receptors, and their downstream intracellular pathways. Growth factors are extracellular signaling proteins that promote the growth, proliferation, or survival of cells.[17] In most, but not all, cases, the receptors to which these growth factors bind are kinases, and their signals are transduced by a cascade of additional kinases that ultimately alter the behavior of the cell. Let's take a closer look at what kinases are and how they function.

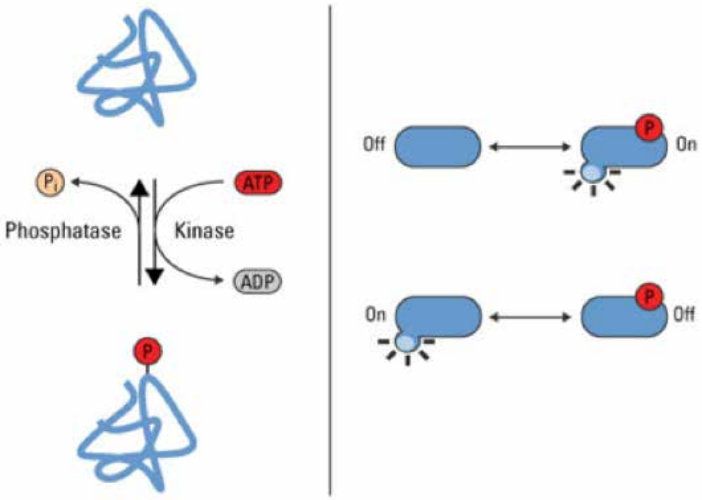
2.4.3 Phosphorylation and Dephosphorylation: The Fundamental Processes Underlying Signaling Cascades

Kinases are proteins that can pass on a signal by mediating a chemical reaction at a specific site on another protein.[17] The specific chemical reaction involves attaching a phosphate group, donated by adenosine triphosphate (ATP), to a protein. Phosphatases are proteins that remove phosphates from another protein. Once a protein is phosphorylated, it becomes activated, thus participating in a cascade of molecular interactions known as a signaling cascade. Phosphorylation can alter the function of a protein in 2 general ways.[17]

1. Changing the shape of the protein, and thus conferring new activities
2. Creating binding sites for other proteins that specifically recognize the phosphorylated protein

Conversely, dephosphorylation by a protein phosphatase "shuts off" the protein, halting its participation in a signaling cascade **(Figure 2.4C)**.[17]

Figure 2.4C. *Phosphorylation and dephosphorylation;* "P" represents a phosphate group.[17] (Left) Kinases, using ATP as a source, add phosphates to proteins. Phosphatases, conversely, remove phosphates. (Right) Addition of a phosphate can turn on a protein (top) or turn the protein "off" (bottom)[17]



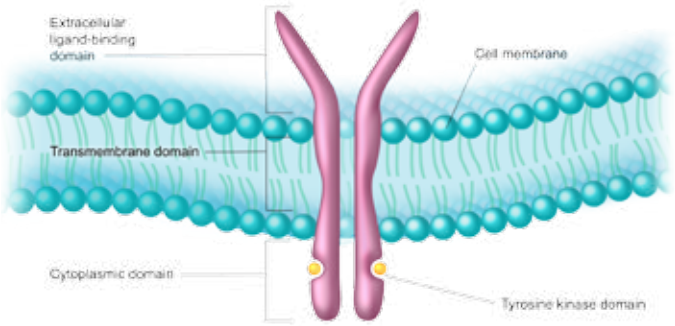
Kinases that phosphorylate tyrosine sites on other proteins are among the oncogenes that play a key role in cancer development and progression.[20] Tyrosine kinases (RTKs) are further categorized as transmembrane receptor tyrosine kinases and nonreceptor tyrosine kinases (NRTKs), also known as intracellular or cytoplasmic kinases.[20] There are at least 60 genes encoding human receptor tyrosine kinases; these receptors can be divided into 16 subfamilies.[17]

2.4.4 Receptor Tyrosine Kinases

Receptor tyrosine kinases are common receptors for growth factors and are the major focus of this section.[17] As shown in **Figure 2.4D**, the structure of each receptor tyrosine kinase includes: an extracellular domain, which carries the ligand-binding site; a transmembrane domain, spanning the cell membrane; and a cytoplasmic domain, which contains the tyrosine kinase catalytic sites.[17,20] Each subfamily of receptor tyrosine kinases is dedicated to binding with its complementary family of ligands.[17] For example, epidermal growth factor (EGF) binds to the epidermal growth factor receptor (EGFR). Similarly, insulin-like growth factor (IGF) ligands bind to the insulin-like growth factor receptor (IGFR).[17]

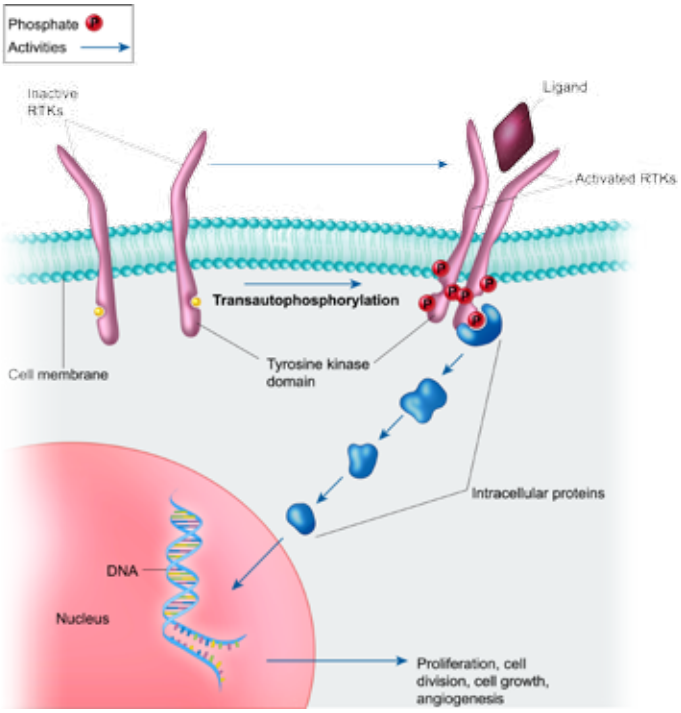
When a ligand binds to the receptors of 2 receptor tyrosine kinase molecules, they come together to form a dimer **(Figure 2.4E)**. Most tyrosine kinases are activated when they are cross-linked by a ligand, which brings the tyrosine kinase domains into close proximity.[17] The cytoplasmic tails of the receptor tyrosine kinase have kinase activity. When these cytoplasmic tails are brought together, they cross-phosphorylate each other. Cross-phosphorylation triggers the assembly of an intracellular signaling complex, which then broadcasts the signal through the cell, often along several different downstream pathways.[17] Different receptor tyrosine kinases bind to different sets and subsets of intracellular signaling molecules; thus, different signals are produced depending on the specific ligand-receptor pairing, and different responses are activated within the cell.[17]

Figure 2.4D. *Typical receptor tyrosine kinase structure*[17,20]



Ligand binding promotes dimerization of receptor tyrosine kinases, promoting cross-phosphorylation. This induces a downstream signaling cascade that alters the behavior of the target cell.[17]

Figure 2.4E. *Activation of receptor tyrosine kinase signaling pathways*[17]



2.5 Signal Transduction and Cancer

Mutations in signal transduction pathways can allow a cell to escape usual controls and replicate abnormally, a characteristic of cancer. For example, tyrosine kinase activity is normally short lived and reversed by phosphatases. However, in many human cancers, tyrosine kinases or components of their downstream pathways are constitutively activated by mutation, amplification, or chromosomal changes.[20]

Specifically, alterations in signal transduction pathways can lead to:[19]

- Deregulation of cell proliferation: Allowing cells to grow out of control
- Loss of normal apoptosis: Abnormal cells no longer die in response to specific signals
- Failure to differentiate: Allowing stem cells to maintain a “primitive” stem-cell phenotype
- Genetic instability: Allows the cells to accumulate mutations that would normally result in programmed cell death
- Loss of replicative senescence: Normal cells stop dividing after 25 to 50 divisions; malignant cells may continue dividing past this point
- Increased angiogenesis: Allowing the growth of blood vessels to supply the interior of the tumor with oxygen and nutrients
- Invasion: Allowing the cells to ignore normal signals that halt growth when they contact another cell
- Metastasis: Allowing the cell to spread to lymph nodes or distant sites
- Evasion of the immune system: Allowing the malignant cell to escape detection and destruction by the immune system

Now that you understand the function of tyrosine kinases and the potential ways in which altered signaling from these critical pathways can influence the development of cancer, the next chapter will provide a top-line overview of the key pathways involved in lung cancer.

2.6 Summary:

Tumor growth, Angiogenesis, and Signaling

- Tumors usually reach 10^9 (1 billion) cells before they are clinically detectable
- Tumors grow exponentially when small; as they grow, nutrient and oxygen limitations slow their growth resulting in a gompertzian growth curve

Angiogenesis

- To survive and grow, tumor cells require oxygen and nutrients
- Tumor cells elicit the growth of their own blood supply in a process called angiogenesis
- The resulting blood vessels are haphazard and "leaky," promoting metastases

Metastasis

- Metastasis is a multistep, inefficient process that requires invasion of the extracellular matrix, dissemination of the tumor cells through the vasculature, homing of tumor cells, and colonization

Signal Transduction

- The cells of multicellular organisms, such as humans, must communicate to coordinate growth and behavior
- Signal transduction involves sending and receiving signals that permit communication at the cellular level
- Depending on the signal, the end result of the pathway could be to alter gene expression, a metabolic pathway, survival, growth, proliferation, or parts of the cytoskeleton, among other effects
- Many signals rely on kinases (tyrosine or serine/threonine)
- Proteins are turned "on" and "off" through phosphorylation and dephosphorylation
- Kinases can be receptors or strictly intracellular
- Tyrosine kinase receptors, in general, require cross-linking by a ligand; when the intracellular tails are brought together, they phosphorylate each other, initiating an intracellular signaling cascade
- Mutations of any of the proteins in a signal transduction pathway can allow a cell to escape usual controls and replicate abnormally, a characteristic of cancer. Such alterations may result in:
  - Deregulation of cell proliferation: Allowing cells to grow out of control
  - Failure to differentiate: Allowing stem cells to maintain a "primitive" stem-cell phenotype
  - Loss of normal apoptosis: Abnormal cells no longer die in response to specific signals
  - Genetic instability: Allows the cells to accumulate mutations that would normally result in programmed cell death
  - Loss of replicative senescence: Normal cells stop dividing after 25 to 50 divisions; malignant cells may continue dividing past this point
  - Increased angiogenesis: Allowing the growth of blood vessels to supply the interior of the tumor with oxygen and nutrients
  - Invasion: Allowing the cells to ignore normal signals that halt growth when they contact another cell
  - Metastasis: Allowing the cell to spread to lymph nodes or distant sites
  - Evasion of the immune system: Allowing the malignant cell to escape detection and destruction by the immune system

CHAPTER 3

Introduction to Lung Cancer

3.0 Introduction

Although lung cancer is often thought of outside the medical community as a homogeneous disease, it is actually a complex set of diseases that are commonly divided into 2 histologies: small-cell lung cancer (SCLC) and non–small-cell lung cancer (NSCLC). Within each histology, there are multiple driver mutations and predictive biomarkers. Here, we will briefly review SCLC and then turn our focus to a more detailed discussion of NSCLC.

Please note that this is a top-line introduction to lung cancer. You will receive detailed training on relevant pathways in the Brigatinib Learning System.

Objectives

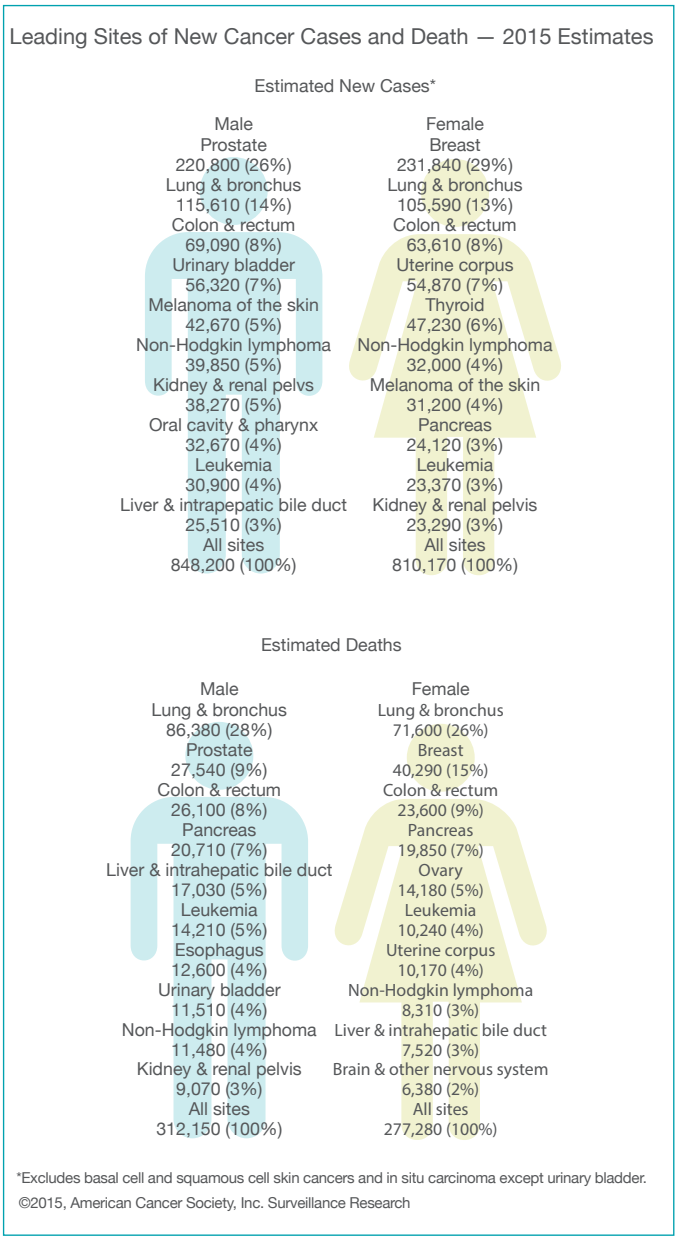
After completing this chapter, you will be able to:

- Recognize the key statistics about lung cancer
- Differentiate between SCLC and NSCLC
- Identify the subtypes of NSCLC and their key features
- Describe risk factors for NSCLC

3.1 Lung Cancer Statistics—Overall

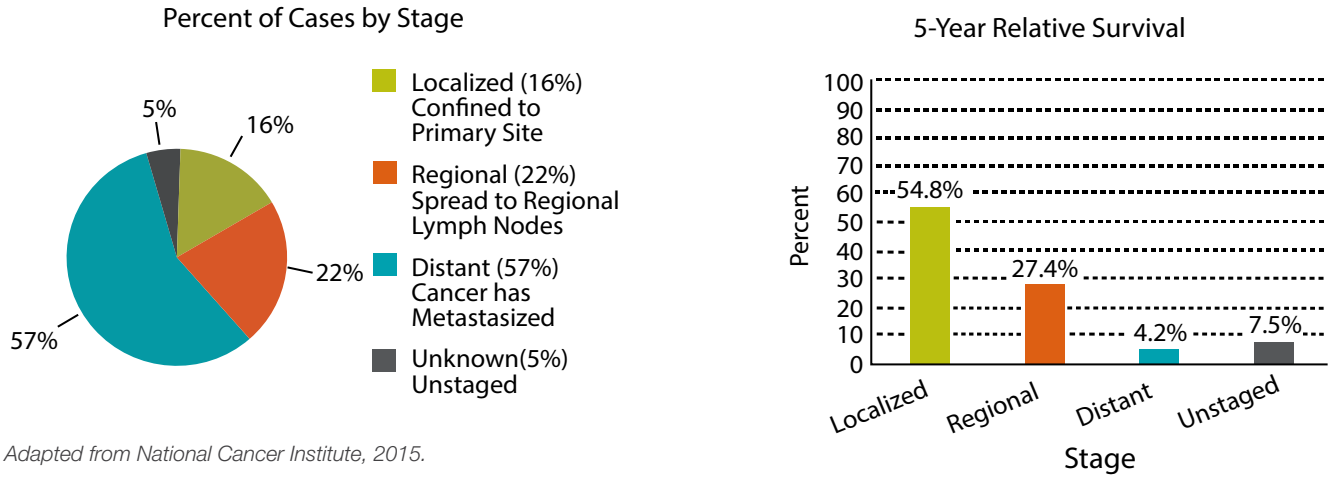
In the United States, lung and bronchus cancer was estimated to be the leading cause of cancer deaths in both men and women in 2015. In fact, it is expected to cause more deaths than the next 3 most common cancers combined (prostate, colon/rectum, and pancreatic cancers in men and breast, colon/rectum, and pancreatic cancers in women). In total, lung and bronchus cancer is estimated to cause 158,000 deaths every year, or about 27% of all cancer deaths in the United States. Globally, there were 1.8 million new cases of lung cancer and 1.6 million deaths attributable to lung cancer in 2012.[1,2] Notably, the rate of new lung cancer cases over the past 37 years has dropped by 28% among men, but has risen 98% among women. The age-adjusted death rate from lung cancer is higher among men (56.1 per 100,000 persons) than among women (36.4 per 100,000 persons). It is also higher among black people (48.3 per 100,000 persons) than among white people (45.6 per 100,000 persons).[2] Lung cancer is primarily a disease of the elderly; in 2011, 82% of people with lung cancer were aged 60 years or older.[2]

Figure 3.1A. *Leading cancer types and cancer deaths in the United States (2015 estimates)*[1]



Adapted from American Cancer Society, 2014.

Figure 3.1B. *Percentage of cases and 5-year relative survival by stage at diagnosis for lung cancer from 2005 - 2011*[23]



Adapted from National Cancer Institute, 2015.

Lung cancer is a deadly disease. In fact, the 5-year relative survival rate of 17.8% is lower than for many other leading cancer sites, such as colon cancer (65.4%) and prostate cancer (99.6%).[2] While the 5-year relative survival rate for lung cancer that is detected while the disease is still localized is relatively high (55%), 5-year survival among patients with metastases is only 4%.[2] Unfortunately only 15% of lung cancer cases are diagnosed at an early stage. In fact, the earlier a patient is diagnosed, the better the chance for survival, as shown in **Figure 3.1B.**[23]

Now that we've covered the statistics for lung cancer overall, let's take a closer look at how the different subtypes of lung cancer are distinguished and the statistics for each.

3.2 Small-Cell Lung Cancer

SCLC accounts for only about 15% of lung cancers.[24] SCLC is usually clinically aggressive, and centrally located.[25] Virtually all patients with SCLC are cigarette smokers, usually heavy smokers.[26] SCLCs are associated with early metastases. Patients with SCLC often present with advanced disease, and they therefore have a poor prognosis.[25]

Although SCLC is highly responsive to chemotherapy, 5-year relative survival is less than 10%.[5]

3.3 Non–Small-Cell Lung Cancer

NSCLC accounts for the majority of lung cancers in the United States. Unlike SCLC, it is not a homogenous disease state; in fact, NSCLC is any type of epithelial lung cancer other than SCLC.[4] The most common types of NSCLC are squamous cell carcinoma, large cell carcinoma, and adenocarcinoma; however, there are several other types that occur less frequently.[4] Although NSCLCs are commonly associated with cigarette smoking, adenocarcinomas can be found in patients who have never smoked. As a class, NSCLC is relatively insensitive to chemotherapy and radiation therapy, especially as compared with SCLC.[4] The five-year relative survival rate is better than that seen in SCLC, but remains poor at only about 22.3%.[27] Survival by stage of diagnosis is summarized in **Table 3.3A**. Note that 5-year relative survival among patients with metastases is only 4.7% (data for patients diagnosed between 2005 and 2011), strongly supporting a need for better therapies for patients; note also that the majority of patients are diagnosed at this stage.[27]

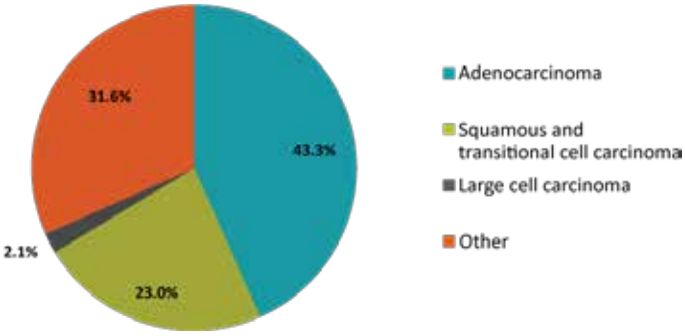
Table 3.3A. *NSCLC relative survival by stage for patients diagnosed between 2005 and 2011*[27]

Stage	Stage Distribution (%)	Survival (%)
All stages	100	21.2
Localized	19	58.7
Regional	24	30.6
Distant	55	4.7
Unstaged	3	12.8

3.3.1 NSCLC Histology

The 3 main NSCLC histologies are: adenocarcinoma, which accounts for about 43.3% of cases; squamous and transitional cell carcinoma (SCC), which accounts for about 23% of NSCLC cases; and large-cell carcinoma, which accounts for only 2.1% of NSCLC cases. Other histologies account for the remaining 31.6% **(Figure 3.3C).**[22]

Figure 3.3C. *Distribution of histologic subtypes in NSCLC for patients with confirmed histologies between 2008 and 2012*[22]



Historically, the different subtypes of NSCLC have been grouped together because the approach to treatment, response to therapy, and prognoses were similar.[4] However, as you will see later in this backgrounder and in the full Brigatinib Learning System, advances in the molecular characterization of NSCLC have led to more tailored therapies that are used in response to specific tumor characteristics.

3.3.2 Adenocarcinoma

Adenocarcinomas are the most common histologic subtype of NSCLC, and are almost twice as common as the next most frequent subtype, squamous and transitional cell carcinomas.[22] They are histologically heterogeneous and metastasize early. They often occur in patients with underlying lung disease.[25] As with other histologies, they occur primarily in smokers, although nonsmokers are more likely to develop adenocarcinomas than other lung cancer types.[3] While adenocarcinomas can occur anywhere in the lung, they most often tend to occur peripherally.[3]

Did You Know?

ALK translocations are most frequently seen in adenocarcinomas.[32]

3.3.3 Squamous and Transitional Cell Carcinomas

Squamous cell carcinomas are typically centrally located and often present with hemoptysis, pneumonia, or outright collapse of the affected lobe.[25] Unlike adenocarcinomas, squamous cell carcinomas typically do not metastasize until late in the disease. Squamous cell carcinomas are highly associated with smoking history.[3]

3.3.4. Large Cell Carcinoma

Large-cell NSCLC is the least common of the major subtypes.[22] Large-cell NSCLC tends to occur peripherally, and is defined as poorly differentiated carcinomas of the lung that are composed of larger malignant cells without evidence of squamous or glandular differentiation or features of SCLC by light microscopy.[3]

3.4 Risk Factors for NSCLC

Compared with those who have never smoked, people who smoke have at least a 10-fold increased risk for developing lung cancer.[28] In fact, one study showed that smokers accumulate at least 1 genetic mutation for every 15 cigarettes smoked. Former male smokers have a 9-fold increased risk of developing lung cancer as compared with men who have never smoked. The size of the risk reduction increases progressively with length of time since the person has quit smoking, although risk remains higher among former smokers than among those who have never smoked.[28]

Second-hand smoke has also been shown to increase risk for lung cancer, although the risk conferred is much lower than from active smoking, ranging from 20% to 30%, as compared to a 2000% increase among active smokers.[28]

Cigarette smoking is the cause of the majority of lung cancers; however, other risk factors have been identified, particularly occupational exposures to asbestos, arsenic, bischloromethyl ether, hexavalent chromium, mustard gas, nickel, and polycyclic aromatic hydrocarbons.[28] Risk of lung cancer appears higher among individuals with low fruit and vegetable intake during adulthood. Ionizing radiation is also a lung carcinogen, as demonstrated in studies showing increased rates of lung cancer among survivors of the Hiroshima and Nagasaki bombs. Prolonged exposure to low-level radon in homes may be associated with a risk for lung cancer equivalent to that conferred by environmental tobacco smoke. Prior lung diseases (eg, chronic bronchitis, emphysema, and tuberculosis) may also increase the risk for lung cancer.[28]

While smoking is considered the primary risk factor for lung cancer, it is clear that nonsmokers and light smokers are at risk for lung cancer, particularly adenocarcinomas. As discussed previously, many of these patients have cancers that are characterized by ALK fusions.

3.5 Summary: Introduction to Lung Cancer

Lung Cancer Statistics

- Lung cancer is the leading cause of cancer deaths in both men and women in the United States
- Lung cancer is more common among men than women and among black people than white people
- Lung cancer is primarily a disease of elderly people (83% of people with lung cancer are aged 60 or older)
- Lung cancer is associated with a 5-year relative survival rate of 17.8%, in part because it is usually only detected after metastasis
- Smoking is the primary risk factor for lung cancer; however, exposure to a number of different chemicals has also been shown to increase the risk for lung cancer

Small-Cell Lung Cancer

- Accounts for only about 15% of lung cancers
- Most patients with SCLC are heavy smokers
- Associated with early metastasis and poor prognosis

Non–Small-Cell Lung Cancer

- Accounts for majority of lung cancers in the United States
- Composed of multiple histologies
  - Adenocarcinoma (most common)
  - Squamous and transitional cell
  - Large-cell carcinoma
- Five-year relative survival is better than in SCLC, but is still only about 22.3%; 5-year survival among patients with metastases is only 4.7%

CHAPTER 4

Selected Signaling Pathways Involved in Lung Cancer

4.0 Introduction

Fundamentally, cancer is a genetic disease.[17] A single cell acquires 1 or more heritable changes that influence its ability to divide, differentiate, or survive allowing it to outcompete its neighbors, eventually forming a tumor.[17] Although there are a broad range of signaling pathways that can influence these parameters, some pathways can be targeted specifically by current treatments.[29] In this chapter, we cover the normal function of some of the key pathways that are altered in lung cancer and that can be addressed by currently available treatments or agents in development. We will also briefly discuss how these pathways may be altered in lung cancer.

Please note that this is a topline introduction to the function of specific pathways that are involved in lung cancer and are targeted by current treatments. You will receive detailed training on relevant pathways in the Brigatinib Learning System.

Objectives

After completing this chapter, you will be able to:

- Identify the normal function of key targetable pathways in lung cancer
- Describe, on a topline level, where these pathways may be altered in lung cancers

Did You Know?

Unlike Ph+ chronic myeloid leukemia, which is nearly always the result of a translocation that fuses Bcr with Abl, lung cancers are highly heterogeneous, both histologically and genetically. Even within a single subtype of NSCLC, there is substantial variability on the molecular level. Rearrangements in ALK, which are discussed below, are one example of a mutation that may be present in some lung cancers, but not in others.

4.1 Anaplastic Lymphoma Receptor Tyrosine Kinase

In this section, we will take a topline look at the anaplastic lymphoma receptor tyrosine kinase (ALK) in health and disease. *Please note that you will receive detailed training on this critical pathway in the Brigatinib Learning System.*

Anaplastic lymphoma kinase (ALK) was originally described in anaplastic large cell lymphoma. Since that time it has been identified as an important mutation in certain subsets of patients with NSCLC.[30,31] ALK is a transmembrane receptor tyrosine kinase, with the typical features of this family including an extracellular ligand-binding domain, a transmembrane-spanning domain, and an intracellular tyrosine kinase domain **(Figure 4.1A)**.[30]

Under normal conditions, pleiotrophin (PTN), osteoblast-specific factor-1 (OSF-1), heparin affinitiy regulatory peptide (HARP), heparin-binding neurotrophic factor (HBNF), and midkine (MK) are thought to be the ligands for ALK.[30] Both MK and PTN are implicated in a broad range of cellular processes including neural development, cell migration, and angiogenesis.[30]

Chromosomal rearrangement of the ALK gene and its activation generally takes place through the position of one of several different 5 fusion partners and their associated promoter region upstream of the kinase domain of ALK, inducing its transcription and protein expression **(Figure 4.1A)**.[32]

Figure 4.1A. *The EML4-ALK fusion protein arises from an inversion in chromosome 2*

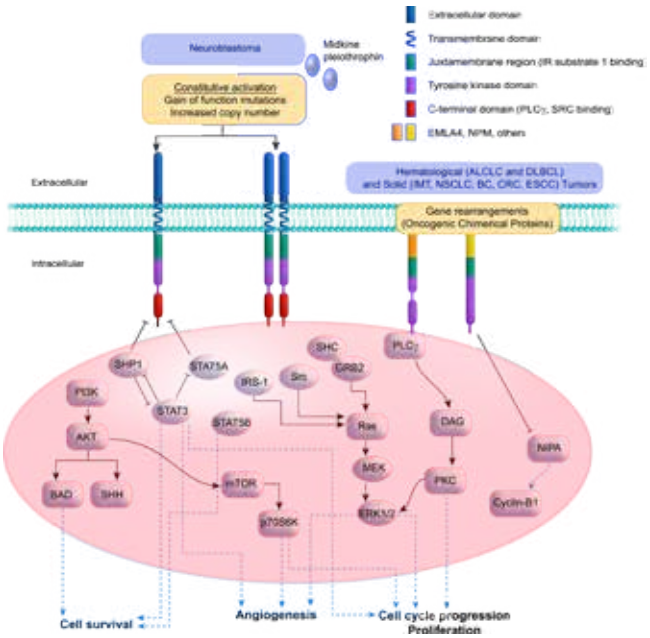
Approximately 2% to 5% of patients with NSCLC have tumors with an inversion in chromosome 2, which results in the fusion of ALK with the echinoderm microtubule-associated protein-like 4 (EML4) gene, leading to the production of an EML4-ALK fusion tyrosine kinase that is involved in cell proliferation, differentiation, and survival.[32] It is important to note that a number of other ALK fusions and point mutations have been observed, but all are far less frequent than the EML4-ALK fusion. ALK translocation-positive tumors often have a distinct histology and often occur in younger patients who are never or former/light smokers.[33] Although ALK mutations can coexist with other mutations in NSCLC, patients with multiple mutations are rare.[33]

The signaling pathways that are activated by ALK remain incompletely characterized in lung cancer **(Figure 4.1B)**. In anaplastic large cell lymphoma, a number of intracellular signaling cascades have been implicated in the oncogenic transformation induced by aberrant ALK. ALK fusions have been shown to activate 3 key signaling pathways[31]:

- The Janus kinase 3-STAT3 pathway. This pathway is likely to be a key player in promoting survival of tumor cells, and is likely important in the pathogenesis of EML-4 tumors
- The phosphoinositide 3-kinase (PI3K) pathway
- The RAS-extracellular signal regulated kinase (ERK) pathway

Together, these pathways play a role in cell survival, angiogenesis, cell cycle progression, and cell proliferation.

Figure 4.1B. *Signaling through ALK4,*[30,31]

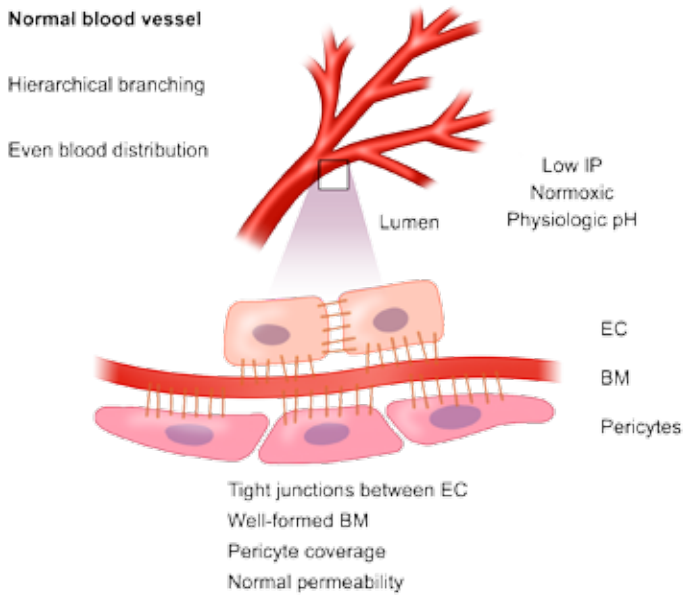


4.2 Vascular Endothelial Growth Factor

For a tumor to grow larger than about 1 to 2 mm in diameter, it must establish its own blood supply through a process called angiogenesis.[20]

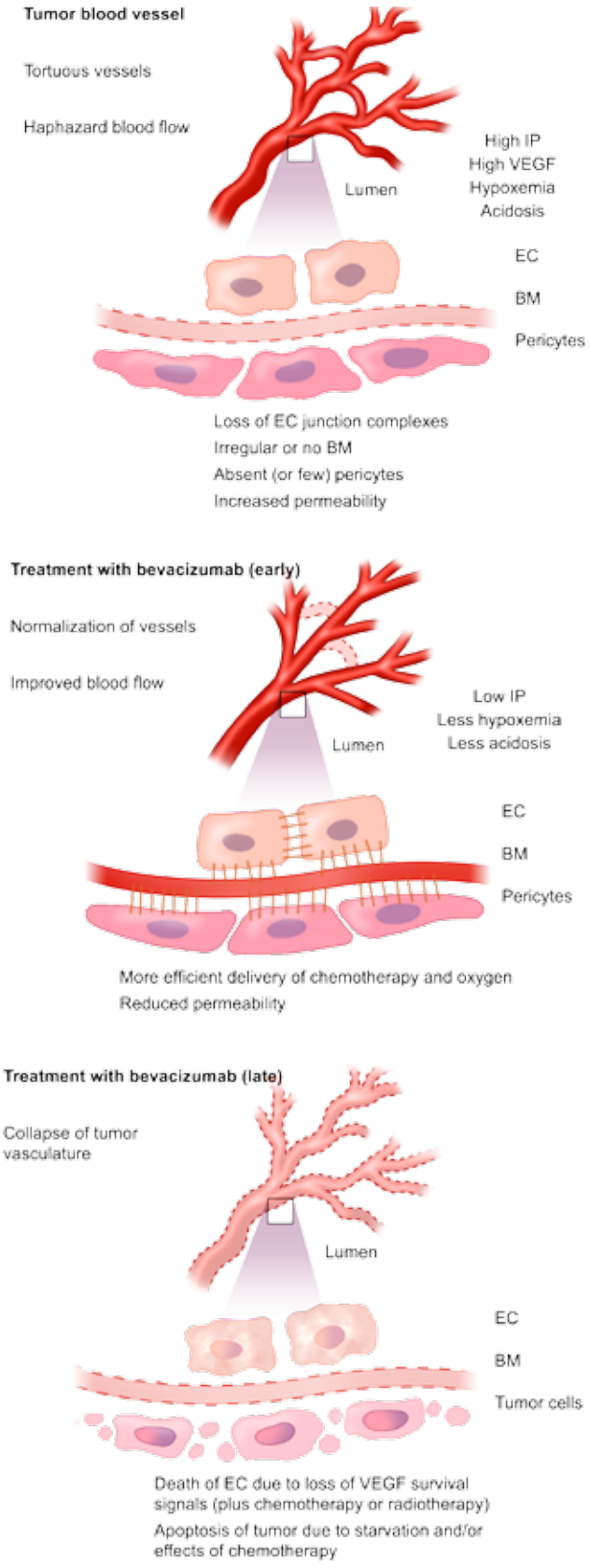
Angiogenesis is induced by hypoxemia, which in turn induces the expression of vascular endothelial growth factor (VEGF).[34] VEGF and its receptors are normally required for blood vessel development in the embryo and during wound healing. In normal tissues, blood vessels exhibit a regular, branching pattern that delivers blood to the tissues in an efficient manner **(Figure 4.2A)**.[34] Tumors, on the other hand, have tortuous vascular branching and dilated, irregular interconnecting branches, causing uneven blood flow. In experimental systems, treatment with a VEGF inhibitor, like bevacizumab, results initially in normalization of blood vessels. Continued treatment with VEGF inhibitors results in death of the endothelial cells that line blood vessels, ultimately leading to tumor cell death.[34]

Figure 4.2A. *Normal blood vessels, tumor blood vessels, and impact of treatment with an anti-VEGF agent such as bevacizumab*[34]



Adapted from Clark 2015.

Figure 4.2A. *Normal blood vessels, tumor blood vessels, and impact of treatment with an anti-VEGF agent such as bevacizumab*[34]



Adapted from Clark 2015.

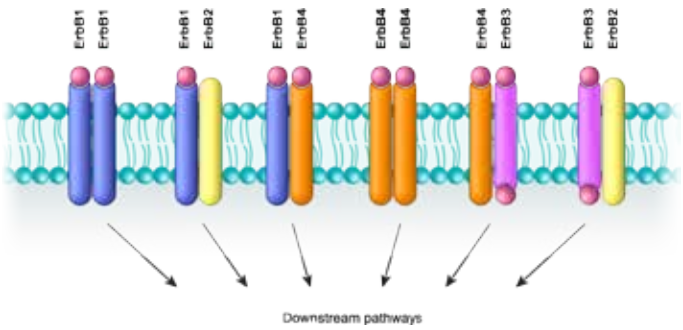
4.3 The ErbB (HER) Family

The ErbB (or HER) family includes 4 receptors that are overexpressed in many cancers including NSCLC.[35] The Erb family includes:[35]

- ErbB1 (EGFR)
- ErbB2, which is most commonly referred to as HER2
- ErbB3
- ErbB4

A set of at least 11 different ligands can activate ErbB receptors.[35] When these ligands bind to ErbB receptors, the receptors pair to form a number of homo- or heterodimers. As already discussed, this pairing results in activation of their tyrosine kinase domains, cross-phosphorylation, and downstream signaling **(Figure 4.3A)**.[35] The ErbB family of receptors are transmembrane proteins; all except ErbB3 have kinase activity in their cytoplasmic tail.[35] Instead, ErbB3 binds to ligands, but only transmits signals when paired with an active family member.[35]

Figure 4.3A. *The ErbB family of receptor tyrosine kinases*[35]



Adapted from Tannock 2013.

The primary intracellular pathways implicated in EGFR signaling include:[36]

- The phosphoinositol 3-kinase (PI3K) pathway, which is involved in survival signaling
- The RAS/mitogen-activated protein kinase (MAPK) pathway, which is involved in cell cycle progression and cell proliferation

4.3.1 ErbB Pathways in Cancer

ErbB receptors are associated with many types of cancer including lung cancer. Some of the alterations in ErbB receptors that are believed to play a role in human tumors include[35,36]:

- Amplification of the EGFR gene, leading to overexpression of the receptor
- Point mutations in the kinase domain, which promote constitutive activation even in the absence of ligand-induced dimerization (especially seen in NSCLC)
- Deletions within the extracellular domain of EGFR, which leave the receptors in a permanently activated conformation
- Mutations that prevent feedback mechanisms. Normally, EGF signaling is reduced by a feedback mechanism that involves removal of the receptors from the cell membrane. Some point mutations disrupt this mechanism, resulting in hyperactivation of the EGF pathway

Activating point mutations in the cytoplasmic tail of the receptor results in an intermediate phosphorylation status between that of unstimulated and growth-factor–stimulated normal receptors. An important consequence of this "partial" activation is a shift in the downstream signaling from these mutant receptors. Whereas stimulation of normal EGFRs activates a range of downstream pathways, the mutant receptors tend to more prominently activate the MAP kinase and PI3 kinase signaling pathways at the expense of other pathways.[35]

Mutations in EGFR, most commonly deletions and point mutations, are present in 10% of Caucasian patients and up to 50% of Asian patients.[6] These EGFR mutations are associated with high response rates to EGFR tyrosine kinase inhibitor therapy. In patients with squamous cell carcinoma, the observed incidence of EGFR mutations is about 3.6%.[6]

According to the National Comprehensive Cancer Network (NCCN) guidelines, first-line targeted treatments for EGFR mutation-positive NSCLC include erlotinib, afatinib, and gefitinib.[6] Osimertinib is a later-line option in patients with sensitizing EGFR mutations.[6] HER2 has been found to be overexpressed in between 13% and 20% of NSCLC cases, although strong overexpression is only found in 2% to 6%.[83] According to current NCCN guidelines, these

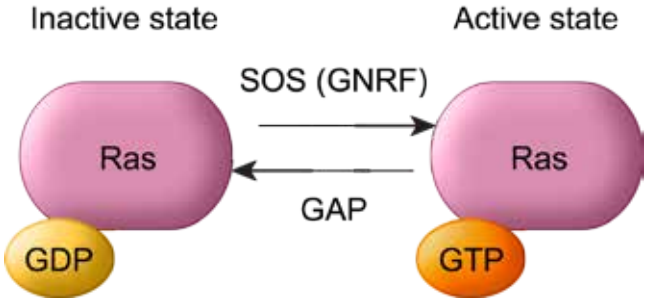
tumors can be treated with HER2-specific agents, such as trastuzumab, or the multi-kinase inhibitor afatinib. Only afatinib has a specific indication for NSCLC (although it should be noted that afatinib is indicated for patients with specific EGFR mutations and not for HER2-mutated NSCLC).[6,83,84]

4.4 KRAS and BRAF

Point mutations in RAS family genes are the most common type of abnormality in human tumors.[20] There are 3 RAS genes in humans, known as HRAS, KRAS, and NRAS. About 15% to 20% of all human tumors express mutated RAS proteins, but in some types of cancers, the frequency of RAS mutations is much higher; in fact, about 30% of lung adenocarcinomas contain a RAS point mutation.[20]

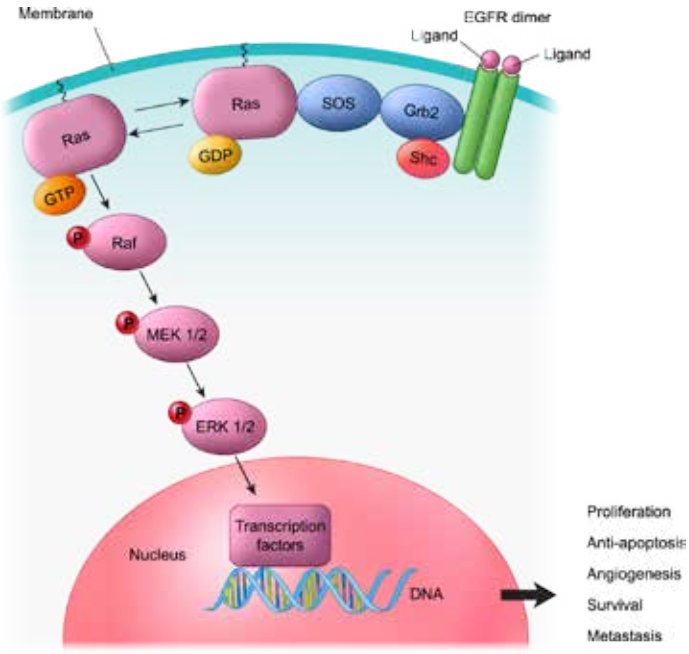
KRAS is involved in transducing the signal from EGFR into the nucleus of the cell **(Figure 4.4A)**.[37]

Figure 4.4A. *Activation of KRAS.*[37] Here, SOS exchanges GDP for GTP, resulting in activation of KRAS. GTPase-activating proteins (GAPs), on the other hand, activate and amplify the GTPase activate of KRAS, encouraging the conversion of GTP into GDP, inactivating KRAS[37]



Activation of KRAS initiates a signaling cascade through Raf, MEK1/2, and ERK1/2. Ultimately this cascade results in activation of genes involved in cell proliferation, angiogenesis, survival, and metastasis **(Figure 4.4B).**[37]

Figure 4.4B. *The KRAS signaling cascade*[37]



Mutations in KRAS inactivate the inherent GTPase activity of KRAS, resulting in a protein that is unresponsive to GAP proteins and remains constitutively active, promoting proliferation, angiogenesis, survival, and metastasis.[37] Targeted therapy is not currently available for patients with KRAS mutations.

BRAF is a member of the RAF family, and acts downstream of KRAS.[38] BRAF is a serine/threonine protein kinase. As shown in the figure, RAS-family proteins stimulate and recruit RAF proteins, such as BRAF, to the cell membrane, where they are activated. Active BRAF then activates a second protein kinase called MEK, which in turn activates a third protein kinase called ERK. Once activated, ERK can translocate to the nucleus and phosphorylate transcription factors, which can activate genes involved in cancer.

BRAF V600E is the most common BRAF mutation.[38] It increases the activity of BRAF by about 500-fold compared with wild type protein, resulting in constitutive signaling and activation of genes that can potentially be involved in cancer.[38]

4.5 RET

RET is a receptor tyrosine kinase.[39] RET fusions, which are well-known mutations in thyroid cancer, have also been identified in approximately 1% to 2% of NSCLCs. Such mutations appear more common among young, never-smoker, and light-smoker patients.[40] In lung cancer, an inversion in chromosome 13 results in the fusion between a gene known as KIF5B and RET, resulting in constitutively active signaling.[40]

According to current guidelines, cabozantinib, a multi-tyrosine kinase inhibitor with activity on RET, may have some benefit in patients with RET rearrangements.[41,6] Note that cabozantinib is not currently indicated for NSCLC.[41]

4.6 ROS1

ROS1 is a receptor tyrosine kinase that is closely related to ALK.[42] In normal situations, expression of ROS1 is highest in the kidney, but is also present in the brain, peripheral neural tissue, stomach, small intestine, and colon.[42] Notably, ROS1 protein expression is absent in normal human lung tissue. Little is known about the normal function of ROS1, and its ligand is unknown.[42]

Gene fusions involving the ROS1 kinase domain have the potential to cause cancer. These fusions often involve a fusion with a gene known as FIG, although other fusions have been identified.[42] In cancer, the ROS1 fusions have been shown to activate growth and survival signaling pathways common to other receptor tyrosine kinase pathways.[42] ROS1 rearrangements tend to occur in younger patients and patients who have never smoked.[42] In lung cancer, studies suggest that between 0.9% and 5% of NSCLC samples have ROS1 rearrangements, with the majority of cases having an adenocarcinoma histology.[42]

According to current NCCN guidelines, there may be some benefit associated with crizotinib in patients with ROS1 mutations.[6]

4.7 Summary: Selected Signaling Pathways Involved in Lung Cancer

ALK

- Receptor tyrosine kinase activated in cancer by fusion with EML4, forming the EML4-ALK fusion protein
- Activates at least the JAK/STAT3, RAS/MAPK, and PLC-gamma pathways, which are involved in cellular proliferation, differentiation, and survival by suppressing programmed cell death
- ALK fusions are present in between 2% and 5% of patients with NSCLC
- ALK mutations are most common in younger patients who are never smokers or light smokers

VEGF

- Involved in angiogenesis
- Treatment with VEGF inhibitors results in suppression of new blood vessel growth in tumors, starving them of oxygen and nutrients

HER

- A family of receptors that includes HER2 and EGFR
- Signal through the PI3K pathway (involved in survival) and the RAS/MAPK pathway (involved in cell cycle progression and cell proliferation)
- May be amplified, have point mutations, deletions, or mutations that prevent feedback mechanisms, all of which lead to overactivity

KRAS and BRAF

- Intracellular signaling molecules
- KRAS is activated by GTP and deactivated by degradation of GTP to GDP
- KRAS activates downstream signaling pathways involved in cell proliferation, angiogenesis, survival, and metastasis
- BRAF is an intracellular serine/threonine kinase that acts downstream of KRAS

RET

- A receptor tyrosine kinase that is activated by fusion with another gene
- Normally involved in the development of the nervous system of the gut
- Constitutive signaling by RET fusions activates pathways involved in oncogenesis
- More common among young, never-smoker, and light-smoker patients

ROS1

- Receptor tyrosine kinase closely related to ALK; activated by gene fusion in NSCLC
- Normally absent in the lung
- Most common in younger patients and those who have never smoked

CHAPTER *5*

Diagnosis of NSCLC

5.0 Introduction

As discussed earlier, data suggests that the majority of lung cancers are not found until they are at an advanced stage. In fact, more than half of NSCLC cases are diagnosed only after distant metastases have occurred.[23] Patients suspected of having NSCLC usually undergo a diagnostic workup such as that recommended by the National Comprehensive Cancer Network (NCCN)[6]:

- Pathology review
- History and physical examination
- A computerized tomography (CT) scan of the chest and upper abdomen
- A complete blood count, including platelet count
- Blood chemistry

In this chapter, we will examine screening, signs and symptoms, and diagnostic procedures used in patients with NSCLC.

Please note that this is a topline introduction to the diagnosis of lung cancer. You will receive detailed training on the NSCLC diagnostic paradigm in the Brigatinib Learning System.

Objectives

After completing this chapter, you will be able to:

- Discuss the importance of screening for early detection of NSCLC
- List the signs and symptoms of NSCLC
- Describe the procedures used to diagnose NSCLC

5.1 Screening for Lung Cancer

As discussed earlier, the majority of lung cancers are diagnosed late, when disseminated disease has occurred.[23] These patients have a very low 5-year survival rate of around 4.7%; however, earlier diagnosis, particularly when the cancer remains localized, is associated with relatively good 5-year survival rates in excess of 50%.[23]

The potential role of screening is illustrated by the results of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). This study, in which investigators enrolled nearly 155,000 people aged 55 to 74, found that there was almost no difference between the receipt of an annual x-ray screening and no screening in terms of lung cancer mortality or the stage or histology of lung cancers.The PLCO study included both women and non-smokers.[28]

The National Lung Screen Trial (NLST) was a randomized study conducted by investigators to determine whether routine low-dose, non-contrast, thin-splice spiral chest computed tomography (LDCT) has the potential to reduce mortality from lung cancer in high-risk populations as compared with a standard x-ray.[28] In this study, “high risk” was defined as people aged 55 to 74, who had smoked cigarettes for 30 years or more; former smokers must have quit within the previous 15 years. The study showed that, compared with the standard x-ray group, the rate of death in the LDCT group was reduced by 6.7%, and the number needed to screen to prevent 1 lung cancer death was 320.[28] The benefits and harms of LDCT versus standard x-ray are summarized in **Table 5.1A.**

Table 5.1A. *Benefit and harm of CT scanning for screening as compared with standard x-ray.*[28]

Benefits: How did CT scans compare with standard x-ray?		
	LDCT	**Standard X-Ray**
4 in 1000 fewer deaths from lung cancer	13 in 1000	17 in 1000
5 in 1000 fewer died from all causes	70 in 1000	75 in 1000
Harms: What problems did CT scans cause compared with standard x-ray?		
223 in 1000 had at least 1 false alarm	365 in 1000	142 in 1000
18 in 1000 had a false alarm leading to an invasive procedure	25 in 1000	7 in 1000
2 in 1000 had a major complication from an invasive procedure	3 in 1000	1 in 1000

Adapted Horn 2015.

Despite limited efficacy for the early detection of lung cancer, the American Cancer Society recommends that certain patients who meet all the following criteria undergo routine lung cancer screening[43]:

- 55 to 74 years old
- In fairly good health (eg, those who do not already have symptoms consistent with lung cancer)
- Have at least a 30 pack-year smoking history
- Are either still smoking or have quit smoking within the last 15 years

5.2 Signs and Symptoms of Lung Cancer

Only about 10% of patients are diagnosed before they have symptoms of lung cancer; in fact, most tumors are discovered during a chest x-ray for an unrelated medical condition.[25] The remaining 90% are not diagnosed until the patient is symptomatic, generally when the cancer has spread too far to be cured. Symptoms of lung cancer can be divided into those that are associated with nonspecific systemic symptoms, symptoms associated with the intrathoracic or extrathoracic spread of the primary tumor, and **paraneoplastic syndromes**. The most common symptoms of NSCLC at diagnosis are summarized in **Table 5.2A**.

Table 5.2A. *Symptoms at diagnosis of NSCLC*[44]

Symptoms	Present at Diagnosis (%)
Cough	45-75
Dyspnea	40-60
Weight loss	20-70
Chest pain	30-45
Hemoptysis	25-35
Bone pain	6-25
Fatigue	0-20
Dysphagia	0-2
Wheezing and/or stridor	0-2
None	2-5

5.3 Diagnosis of Lung Cancer: Imaging and Biopsy Procedures

In general, patients suspected of having NSCLC undergo the following initial diagnostic workup[6]:

- Pathology review
- History and physical examination
- CT scan of the chest and upper abdomen
- Complete blood count, including platelet count
- Blood chemistry

Table 5.3A summarizes available imaging tests.

The results of these tests provide information to guide staging, which will be covered in the next chapter.

5.3.1 Imaging Tests

After the physical examination, the next step in the diagnostic workup is a chest x-ray and CT scans of the chest and upper abdomen. Chest x-rays will reveal large peripheral lung cancers or central obstructing lesions; however, it is not particularly sensitive and a normal chest x-ray does not exclude lung cancer.[45] In fact, conventional chest x-rays fail to detect up to 80% of histologically proven CT-detected lung cancers of 2 cm or more in diameter. CT scans are preferred; their accuracy can be enhanced by using super high-resolution scanning techniques. Further, patients with a newly diagnosed lesion suspicious for lung cancer may undergo positron emission tomography (PET) scanning.

Table 5.3A. *Imaging tests for NSCLC*[43,45]

Chest x-ray	• Plain x-ray • Can be conducted at imaging centers, hospitals, and some physician's offices • Poor ability to detect lung cancers; CT is preferred
Computed tomography (CT) scan	• Utilizes x-rays to provide a series of detailed cross-sections of the body; able to provide detailed images of soft tissues • Requires administration of oral or IV contrast solution • Conventional CT scans have a sensitivity of 60% and specificity of 80% for the detection of lung cancer • Super high-resolution techniques have a sensitivity of 85% and specificity of 100%
Magnetic resonance imaging (MRI) scan	• Provide detailed images of soft tissues in the body • Use radiofrequency energy rather than x-rays • Gadolinium contrast may be used
Positron emission tomography (PET) scan	• Use radioactive sugar (fluorodeoxyglucose [FDG]) as a tracer; rapidly growing cancer cells absorb this sugar and are labeled • PET scans identify areas of rapid metabolism • Useful for diagnosis of metastases • May be combined with a CT scan in a single machine
Bone scan	• Used to detect metastasis to the bone • Radioactive material is injected intravenously; areas of active bone changes show up as "hot spots" on the bone scan • Usually not necessary if a PET scan is conducted
Endobronchial ultrasound	• Endoscope passed into bronchi; uses ultrasound to create an image of the surrounding areas
Endoscopic esophageal ultrasound	• Endoscope passed into esophagus; uses ultrasound to create a picture of the areas adjacent to the esophagus

5.3.2 Methods to Establish Tissue Diagnosis

Confirmation of the masses identified during imaging is conducted by sampling the tissue. Tissue diagnosis also aids in determining the histology, molecular features, and stage of lung cancer. Tissue samples can be acquired from a variety of methods.[43] The method used depends on the location of the tumor, as well as the amount of tissue needed for the pathologic examination.[45] Note that some guidelines suggest that, among patients with a strong clinical suspicion of stage I or II lung cancer (based on risk factors and radiologic appearance), a biopsy is not required before surgery.[6] Biopsies may add time, costs, and procedural risks without providing important additional information on which to base treatment decisions in early-stage lung cancer. **Table 5.3B** reviews the modalities used for tissue sampling.

Table 5.3B. *Tissue sampling modalities*[25,43]

Sputum cytology	• Noninvasive • Most helpful in diagnosing central tumors and hemoptysis • 3 early morning samples are usually taken • 99% sensitivity • Further testing needed after a negative result
Thoracentesis	• Invasive; used to obtain a sample of fluid around the lungs (pleural effusion) • Needle is inserted between ribs to drain fluid; fluid is checked microscopically • 80% sensitivity
Needle biopsy	• Uses a hollow needle to obtain a small sample from a mass • Fine-needle aspiration (FNA) uses a thin, hollow needle to aspirate cells and small fragments of tissue • Core biopsies use a larger needle to remove cores (often preferred because a larger sample is obtained) • May be guided by fluoroscopy or CT • May also be conducted to evaluate spread through lymph nodes via the trachea (transtracheal FNA) or bronchi (transbracheal FNA)
Bronchoscopy	• Involves passage of a lighted, fiber-optic tube (bronchoscope) through the mouth and into the bronchi • Small instruments can be passed down the bronchoscope to take biopsies and sample tissues, including brushes; tissue may also be washed to obtain cells for analysis • 88% sensitive for central tumors; 60% to 70% sensitive for peripheral tumors • May be guided fluoroscopically or by CT
Mediastinoscopy and mediastinotomy	• Mediastinoscopy: Conducted under general anesthesia by creating a small cut in the neck; a thin, hollow tube (mediastinoscope) is inserted behind the sternum to visualize the area; instruments can be passed through a tube to take samples from lymph nodes along the trachea and major bronchi • Mediatinotomy: A slightly larger incision is made between left second and third ribs; allows surgeon to reach lymph nodes that cannot be reached by mediastinoscopy
Thorascopy	• Conducted under general anesthesia; thorascope is inserted to visualize space between the lungs and chest wall
Open surgical biopsy	• Conducted by creating a larger incision in the chest wall as part of a full surgical procedure

Samples obtained from biopsies are used for extensive histologic and molecular testing. We will review the modalities used to assess tumor tissue in the next chapter.

5.4 Summary: Diagnosis of NSCLC

Screening for Lung Cancer

- Lung cancer screening, while not very sensitive or specific, is recommended in certain at-risk populations
- Signs and symptoms of lung cancer include, but are not limited to:
  - Cough
  - Dyspnea
  - Weight loss
  - Chest pain
  - Hemoptysis
  - Bone pain
  - Fatigue
  - Dysphagia
  - Wheezing
- Symptoms of the primary tumor, intrathoracic spread, and extrathoracic spread can be differentiated but often overlap
  - Primary tumor symptoms include the signs and symptoms listed in this chapter
  - Symptoms of intrathoracic spread may include hoarseness, Pancoast syndrome, pleuritic pain, dysphagia, facial swelling
  - Symptoms of extrathoracic spread are varied and depend on the involved organs; may include weakness, weight loss, bone pain and fracture, headaches, nausea, vomiting, neurologic deficits, seizures, confusion and personality changes
  - Paraneoplastic syndromes are caused by bioactive substances that affect other organ systems
- Imaging tests commonly used in lung cancer include plain chest x-rays; CT, MRI, and PET scans; bone scans; endobrachial ultrasound; and endoscopic esophageal ultrasound
- A number of modalities are used for obtaining tissue samples; these range from truly minimally invasive, such as sputum cytology, to moderately invasive, such as needle biopsies and bronchoscopies, to open surgical biopsies

CHAPTER 6

Histologic and Molecular Analysis of Lung Cancer Tissue

6.0 Introduction

Histology is the study of the tissues of the body and how these tissues are arranged to constitute organs. This subject involves all aspects of tissue biology, with the focus on how cells' structure and arrangement optimize functions specific to each organ. Molecular analysis has become an increasingly important component of the analysis of lung cancer tissues as more agents are developed that specifically target mutations commonly identified in cancers, including NSCLC.

There are a broad range of different tests used to evaluate the specific characteristics of NSCLC, many of which are beyond the scope of this backgrounder. Here, we will focus on the principles underlying histologic analysis of NSCLC and the most commonly used techniques for evaluating the genetics of this disease.

Objectives

After completing this chapter, you will be able to:

- Describe how tissue is processed after a biopsy or surgery
- Identify the components of a pathology report
- Describe key molecular tests used in the diagnosis and assessment for treatment of NSCLC
- Describe topline guideline recommendations on molecular testing for NSCLC

6.1 How Is Tissue Processed After a Biopsy or Surgery?

The tissue removed during a biopsy or surgery must be cut into thin sections, placed on slides, and stained with dyes before it can be examined under a microscope. Two methods are used to make the tissue firm enough to cut into thin sections: frozen sections and paraffin-embedded (permanent) sections. All tissue samples are prepared as permanent sections, but sometimes frozen sections are also prepared.[48]

Permanent sections are prepared by placing the tissue in fixative (usually formalin) to preserve the tissue, processing it through additional solutions, and then placing it in paraffin wax. After the wax has hardened, the tissue is cut into very thin slices, which are placed on slides and stained. The process normally takes several days. A permanent section provides the best quality for examination by the pathologist and produces more accurate results than a frozen section.[48]

6.2 What Is a Pathology Report?

A pathology report is simply a document that contains the diagnosis determined by the microscopic examination of cells and tissues.[48] The report may also contain information about the size, shape, and appearance of a specimen as it looks to the naked eye. This information is known as the gross description. In addition, pathology reports include cytogenetic information to evaluate the tumor for specific genetic alterations that may influence prognosis or treatment selection.

6.3 What Information Does a Pathology Report Usually Include?

Basic information contained in all pathology reports includes[48]:

- Patient information: Name, birth date and biopsy date
- Gross description: Color, weight, and size of tissue as seen by the naked eye
- Microscopic description: How the sample looks under the microscope and how it compares with normal cells
- Diagnosis: Type of tumor/cancer and grade (how abnormal the cells look under the microscope and how quickly the tumor is likely to grow and spread)
- Tumor size: Measured in centimeters
- Tumor margins: There are 3 possible findings when the biopsy sample is for the entire tumor:
  - Positive margins mean that cancer cells are found at the edge of the material removed
  - Negative, not involved, clear, or free margins mean that no cancer cells are found at the outer edge
  - Close margins are neither negative nor positive
- Other information: Usually notes about samples that have been sent for other tests or a second opinion
- Pathologist's signature and name and address of the laboratory

In addition to this basic information, the pathology report often includes data on the physical, chemical, and genetic characteristics of the tissue. After identifying the tissue as cancerous, the pathologist may perform additional tests

to get more information about the tumor that cannot be determined by looking at the tissue with routine stains, such as hematoxylin and eosin (also known as H&E), under a microscope. The pathology report will include the results of these tests. For example, the pathology report may include information obtained from immunochemical stains that involve a method known as immunohistochemistry (IHC). Scientists utilize IHC through antibodies to identify specific antigens on the surface of cancer cells. IHC is frequently used to determine the site of the primary cancer (in the case of metastatic disease) and to distinguish different cancer types.[48]

The pathology report may include the results of molecular diagnostic and cytogenetic studies. Such studies investigate the presence or absence of malignant cells, and genetic or molecular abnormalities in specimens.[48] Techniques used in these analyses are discussed in the next section.

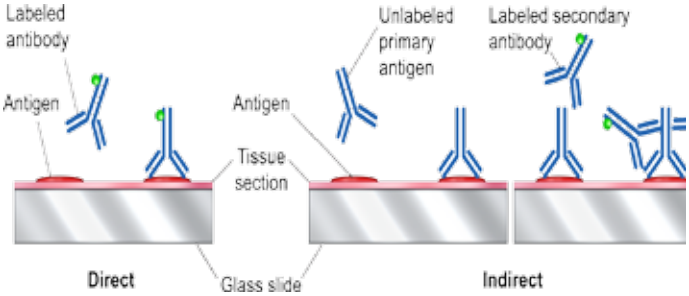
6.4 Molecular Genetics Techniques

Recurrent mutations in oncogenes comprise a large proportion of the therapeutically targetable alterations in lung cancer. For example, EGFR mutations in NSCLC, which were first discovered in 2004, are present in 10% to 15% of Caucasian patients with advanced disease. 49 ALK rearrangements occur in 3% to 5% of lung adenocarcinomas and are associated with response rates of 60% to 80% with ALK inhibitors.[49] In this section, we will discuss the methods by which these fundamental alterations in cancer cell genetics and physiology are evaluated.

6.4.1 Immunohistochemistry

IHC uses antibodies to test for certain antigens in a sample of tissue. The antibody is usually linked to a radioactive substance or dye that causes the antigens to be detectable under a microscope. In IHC, a tissue section that may contain the protein of interest is incubated with a solution of antibody against the protein. The antibody binds specifically to the protein and, after a rinse, the protein's location in the tissues or cells can be seen with a microscope by visualizing the antibody. Antibodies are commonly tagged with fluorescent compounds or with enzymes that convert substances to colored deposits that are visible under a microscope **(Figure 6.4A)**.[14]

Figure 6.4A. *IHC techniques*[14] (A) The direct method uses an antibody made against the tissue protein of interest and is tagged with a fluorescent compound or an enzyme that converts substances into colored deposits. (B) In the indirect method, a primary antibody is bound to the antigen of interest, and a labeled secondary antibody is introduced that binds to the first antibody. Because more than 1 labeled secondary antibody can bind to each primary antibody, labeling of the protein of interest is amplified in this method.



In lung cancer, IHC has been used to distinguish between different EGFR mutations.[50] However, its primary use is in the differential diagnosis of lung carcinomas, including differentiating among squamous cell carcinoma, large cell carcinoma, metastatic carcinoma, and malignant mesothelioma.[50]

6.4.2 Fluorescent In-Situ Hybridization

Fluorescent in-situ hybridization (FISH) has multiple uses, but in NSCLC it is often used to detect ALK rearrangements.[51] Although other techniques, such as polymerase chain reaction (PCR) and IHC staining are under investigation, FISH is currently the most commonly used method for detecting ALK rearrangements in NSCLC.[6]

In the United States, prescription of an ALK inhibitor is dependent upon the use of the Vysis Break Apart FISH Probe Kit (Abbott Molecular, Inc).[51]

FISH utilizes specific probes that bind to sequences on DNA. ALK and EML4 are located on chromosome 2 **(Figure 6.4B)**.[51] EML is normally on the opposite strand from ALK, and both of the 2 probes in the Vysis Break Apart FISH Kit (red-orange and green) are normally closely juxtaposed. As we have already learned, the EML4-ALK fusion gene is the result of an inversion of EML4 fused to ALK. This inversion leads to an increased distance between the red-orange and green probes. A deletion of the proximal part combined with the inversion explains a single red-orange signal. Cells are considered ALK FISH positive when there is: (1) ≥1 set of red and green signals that are ≥2 signal diameters apart, or (2) a single red signal without a corresponding green signal in addition to fused (normal) signals. A sample is considered negative if <5 cells (<10%) are positive and positive if >25 cells (>50%) are positive. A sample is considered equivocal if 5 to 25 cells (10%-50%) are positive.

Figure 6.4B. *EML/ALK Translocation by FISH*

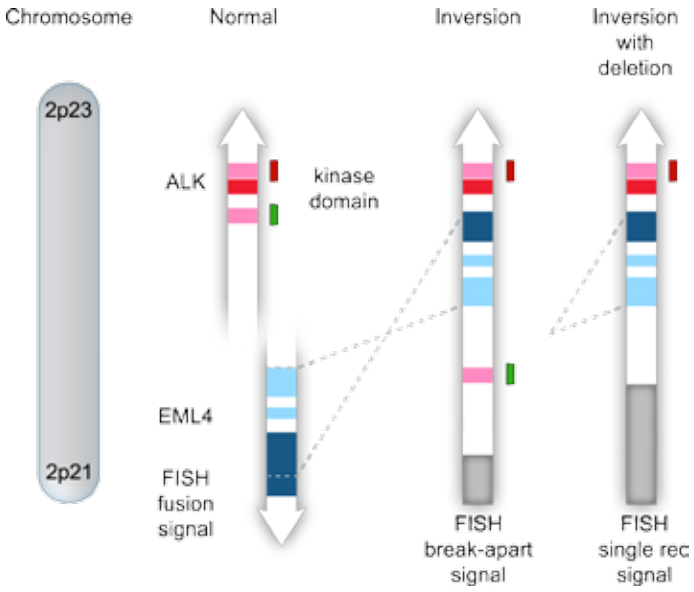
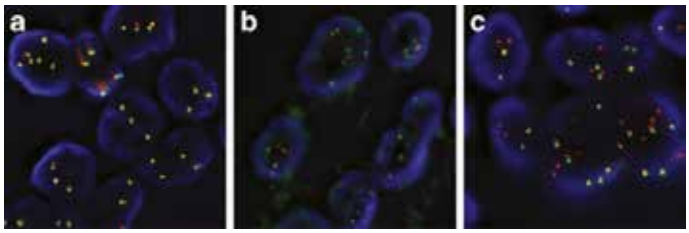


Figure 6.4C shows what these findings actually look like under a fluorescence microscope.

Figure 6.4C. *Representative examples of ALK FISH findings in pulmonary adenocarcinomas*[51]



(a) Normal signals, no rearrangement. Note that some of the signals are fused and produce a yellow signal, while others have green and red signals in close proximity. (b) One or 2 break-apart signals per nucleus, indicative of inversion. (c) Single red signals, indicative of inversion and deletion. Note that the cancer cells in (b) and (c) contain both rearranged and normal ALK signals.

6.4.3 Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a method that allows for rapid amplification of DNA segments.[52] The method used to detect ALK rearrangements relies on reverse transcribing the RNA generated by the EML4-ALK or KIF5B-ALK fusion, creating a DNA product. This DNA is amplified using PCR, and the size of the PCR product is evaluated to determine if a fusion is present. This approach allows for the analysis of non-fixed tissues.

While reverse transcriptase (RT)-PCR-based assays are highly sensitive and specific, the technique carries inherent disadvantages that may limit its utility as a primary clinical screening test for the detection of ALK rearrangements:[51]

- Multiplex RT-PCR systems are required because of the wide variation in fusion types
- Since specific primer sets are required to detect each ALK variant, unknown variants are undetectable (ie, only known alterations can be assayed)
- Proper cryopreservation of tumor samples to ensure intact nucleic acid substrate may be lacking in routine practice

To date, there are no approved PCR-based companion diagnostic assays.

6.4.4 Next Generation Sequencing

Next generation sequencing (or NGS) refers to various massively parallel, high-throughput sequencing platforms that offer many advantages over traditional sequencing (eg, full sequencing of a large number of genes in a single test, simultaneous detection of deletions, insertions, copy number alterations, rearrangements, and exome-wide base substitutions). The advent of NGS technologies is expanding the possibilities for researchers and clinicians to query genomic data. NGS currently represents an emerging methodology for detection of oncogene fusions and mutations in tumor samples.[79]

6.5 Example Guidelines for the Molecular Analysis of NSCLC

In 2013, the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology released guidelines on molecular testing for the selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors.[53] Although a detailed discussion of these recommendations are beyond the scope of this backgrounder, the major recommendations were: 1) to use testing for EGFR mutations and ALK fusions to guide patient selection for therapy with EGFR and ALK inhibitors, respectively, in all patients with advanced-stage adenocarcinoma, regardless of sex, race, smoking history, or other clinical risk factors; and 2) to prioritize EGFR and ALK testing over other molecular predictive tests.[53]

The NCCN guidelines also recommend molecular diagnostic studies focused on EGFR, KRAS, and ALK. ALK testing should be conducted as part of the broad molecular profiling at diagnosis, before selection of first-line therapy. Patients with the following characteristics should be tested for ALK rearrangements[6]:

- Adenocarcinoma histology
- Those with large cell carcinoma or NSCLC not otherwise specified
- Those with small biopsy specimens or mixed squamous cell histology
- Never/light smokers

The current standard method for detecting ALK NSCLC is FISH, although other methods are currently being evaluated, such as PCR and IHC. As of June 2016, the VENTANA ALK Assay was the first and only FDA-approved, fully automated IHC companion diagnostic used to test for ALK+ NSCLC.[85] The appropriate antibody and detection method for ALK protein expression can be used for rapid prescreening of ALK-rearranged lung adenocarcinomas and selection of cases that will be confirmed by FISH testing.[6]

6.6 Summary: Histologic and Molecular Analysis of Lung Cancer Tissue

How Is Tissue Processed After Biopsy or Surgery?

- Tissue removed during biopsies is processed by freezing or embedding into paraffin and then sectioned

What Information Does a Pathology Report Usually Include?

- Samples are assessed by pathologists, who provide a gross and microscopic description, a preliminary diagnosis based on the microscopic appearance of the cells, and an evaluation of the margins

Molecular Genetics Techniques

- In addition to visual inspection, a number of molecular tests may be conducted to provide more information on the diagnosis and/or to identify patients who qualify for targeted therapy:
  - IHC: Provides information on tumor type; can also be used to identify specific mutations
  - FISH: Helps to identify chromosomal translocations such as the EML4/ALK fusion
  - PCR: Detects ALK rearrangements through the analysis of non-fixed tissues
  - NGS: High-throughput sequencing technology enabling the detection and characterization of many genetic variants

Example Guidelines for the Molecular Analysis of NSCLC

- The College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology Guidelines suggest:
  - Using testing for EGFR mutations and ALK fusions to guide patient selection for therapy with EGFR and ALK inhibitors, respectively, in all patients with advanced-stage adenocarcinoma, regardless of sex, race, smoking history, or other clinical risk factors
  - Prioritizing EGFR and ALK testing over other molecular predictive test
- NCCN guidelines suggest:
  - ALK testing be conducted at diagnosis before selection of first-line therapy for those with: adenocarcinoma histology (including large cell carcinoma or NSCLC not otherwise specified), small biopsy specimens or mixed squamous cell histology and never/light smokers
  - The current standard method for detecting ALK NSCLC is FISH, PCR and IHC are currently being evaluated

CHAPTER 7

Staging and Prognosis of NSCLC

7.0 Introduction

As in most cancers, staging is important in lung cancer to help the treating physician identify appropriate treatment options. Staging also provides prognostic information that can be combined with other prognostic factors to help predict short- and long-term outcomes. This section discusses the staging and prognosis of both NSCLC and SCLC.

Objectives

Upon completion of this chapter you will be able to:

- Distinguish between the stages of NSCLC
- List the prognostic factors in NSCLC
- Describe the staging and prognosis of SCLC

7.1 Staging of NSCLC

TNM Staging

NSCLC is staged using the American Joint Committee on Cancer (AJCC) tumor, node, metastasis (TNM) staging system; the most recent version is the seventh edition, which was published in 2010.[13] As shown in **Table 7.1A**, this system stages the tumor according to:

- Primary tumor (T): Size of the tumor, location, and involvement of local tissues
- Lymph node involvement (N): Presence and location of lymph node involvement
- Metastasis (M): Presence or absence of metastasis

Table 7.1A. *TNM staging*[13]

Primary Tumor (T)	
TX	Primary tumor cannot be assessed, or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 3 cm or less in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus (ie, not in the main bronchus)*
T1a	Tumor 2 cm or less in greatest dimension
T1b	Tumor more than 2 cm but 3 cm or less in greatest dimension
T2	Tumor more than 3 cm but 7 cm or less, or tumor with any of the following features (T2 tumors with these features are classified T2a if 5 cm or less): • Involves main bronchus, 2 cm or more distal to the carina • Invades visceral pleura (PL1 or PL2) • Associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung
T2a	Tumor more than 3 cm but 5 cm or less in greatest dimension
T2b	Tumor more than 5 cm but 7 cm or less in greatest dimension

Table 7.1A. *TNM staging*[13]

Primary Tumor (T)	
T3	Tumor more than 7 cm or one that directly invades any of the following: • Parietal pleural (PL3) chest wall (including superior sulcus tumors) • Diaphragm • Phrenic nerve • Mediastinal pleura • Parietal pericardium OR • Tumor in the main bronchus (less than 2 cm distal to the carina* but without involvement of the carina) • Associated atelectasis or obstructive pneumonitis of the entire lung or separate tumor nodule(s) in the same lobe
T4	Tumor of any size that invades any of the following: • Mediastinum • Heart • Great vessels • Trachea • Recurrent laryngeal nerve • Esophagus • Vertebral body • Carina • Separate tumor nodule(s) in a different ipsilateral lobe
Regional Lymph Nodes (N)	
NX	• Regional lymph nodes cannot be assessed
N0	• No regional lymph node involvement
N1	• Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2	• Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
N3	• Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)
Distant Metastasis (M)	
M0	• No distant metastasis
M1	• Distant metastasis
M1a	• Separate tumor nodule(s) in a contralateral lobe OR • Tumor with pleural nodules OR • Malignant pleural or pericardial effusion
M1b	• Distant metastasis

*The uncommon superficial spreading tumor of any size with its invasive component limited to the bronchial wall, which may extend proximally to the main bronchus, is also classified as T1a.

Based on the T, N, and M stages of the patient's disease, the cancer is determined to be 1 of 4 stages (from I to IV, some with subcategories), as shown in **Table 7.1B.**[13] For example, a T2a tumor with metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s) (N2) would be considered a stage IIIA tumor (highlighted in the table).

Table 7.1B. *TNM stage groupings*[13]

T/M	N0	N1	N2	N3
T1a	IA	IIA	IIIA	IIIB
T1b	IA	IIA	IIIA	IIIB
T2a	IB	IIA	IIIA	IIIB
T3	IIB	IIIA	IIIA	IIIB
T4	IIIA	IIIA	IIIB	IIIB
M1a	IV	IV	IV	IV
M1b	IV	IV	IV	IV

Did You Know?

You may also hear physicians use the following terms to describe a patient's lung cancer[6]:

- Early stage, which most often refers to stage I disease
- Locally advanced disease, which refers to stage II-III disease
- Advanced/metastatic disease, which refers to stage IV disease

Clinical and Pathologic Staging

There are 2 major types of staging in NSCLC: clinical and pathologic.[13] Clinical stages are denoted by a "c" before the stage (for example, cT3), whereas pathologic stages are denoted by a "p" (for example, pT2). **Table 7.1C** outlines the key features of both clinical and pathologic staging.

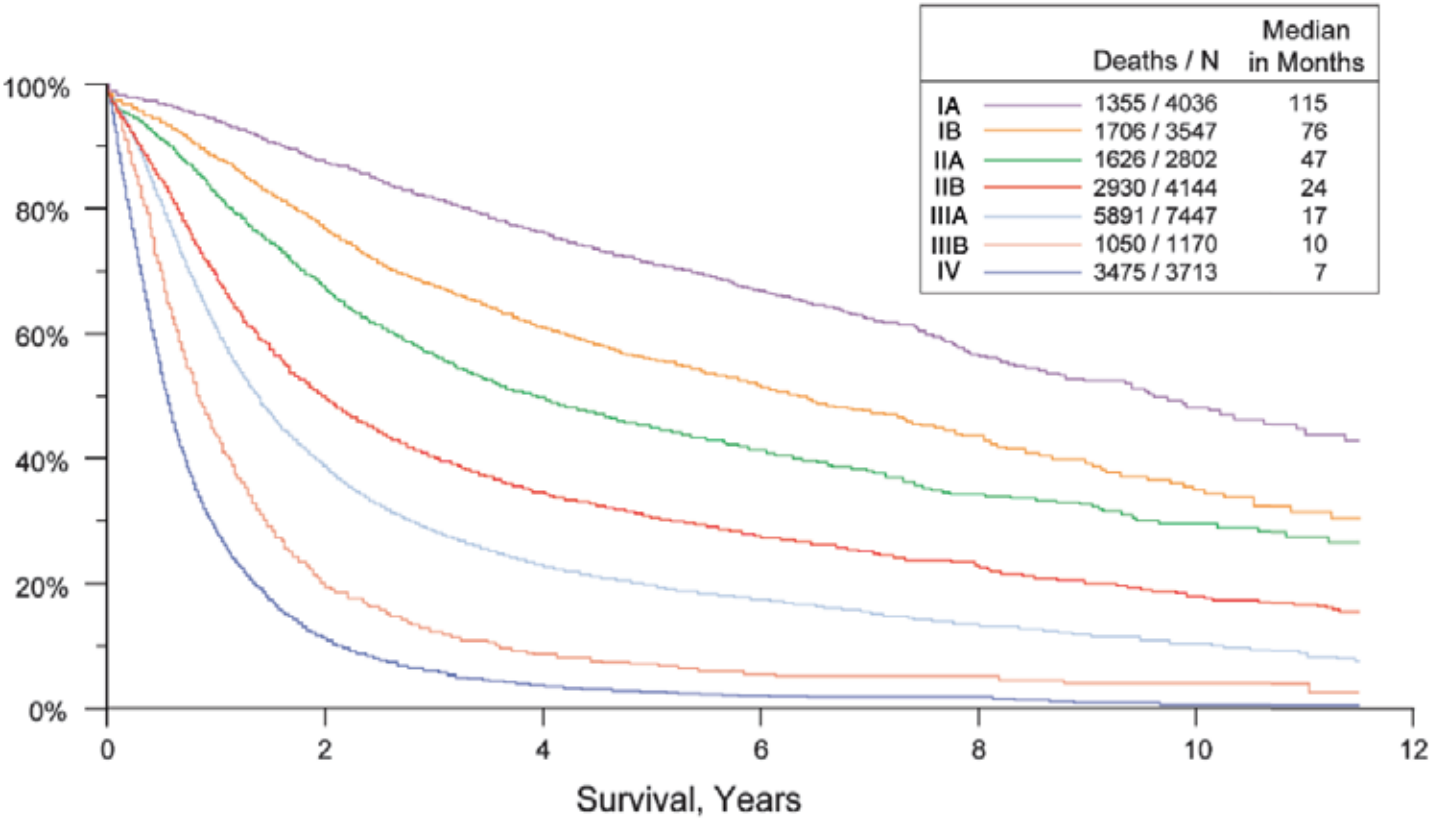
Table 7.1C. *Clinical and pathologic staging of NSCLC, according to the AJCC*[13]

Clinical Staging[13]	Pathologic Staging[13]
Based on evidence acquired before treatment: • Physical exam • Imaging • Laboratory tests • Staging procedures (eg, bronchoscopy or esophagoscopy with ultrasound-directed biopsies, mediastinoscopy, mediastinotomy, thoracentesis, thorascopy, exploratory thoracotomy) • May also be called radiologic staging	• Staging procedures (eg, bronchoscopy or esophagoscopy with ultrasound-directed biopsies, mediastinoscopy, mediastinotomy, thoracentesis, thorascopy, exploratory thoracotomy) • May also be called radiologic staging • Depends on the proven anatomic extent of disease • Uses evidence acquired before treatment, supplemented or modified by the additional evidence acquired during and after surgery, particularly from pathologic examination • Provides additional precise data used for estimating prognosis and calculating end results • Pathologic assessment of the primary tumor requires resection of the primary tumor sufficient to evaluate the highest pT category • Complete pathologic assessment of the regional lymph nodes ideally entails removal of a sufficient number of lymph nodes to evaluate the highest pN category • Pathologic assessment of metastases may be either clinical or pathologic when the T and/or N categories meet the criteria for pathologic staging (pT, pN, and cM or pM)

7.2 Prognosis of NSCLC

There are differences in survival in NSCLC among the TNM stage groupings **(Figure 7.2A)**.

Figure 7.2A. Overall survival in NSCLC by TNM stage[13]



Prognostic factors that predict the survival of patients with lung cancer have been identified over and above stage and histologic classification. Additional prognostic factors include patient's sex, performance status, various laboratory values, and molecular markers.[13] Favorable prognostic factors include early stage disease at diagnosis, a good performance status (Eastern Cooperative Oncology Group [ECOG] PS of 0, 1, or 2), no significant weight loss (≤5%), and female gender.[6]

7.3 Summary: Staging and Prognosis of NSCLC

Staging of NSCLC

- NSCLC is staged using the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control's (IUCC) tumor, node, metastasis (TNM) staging system
  - Primary tumor (T): Depends on size, site, and local involvement
  - Lymph node involvement (N): Presence and location of lymph node involvement
  - Metastasis (M): Presence or absence of metastasis
- Stages range from IA (early, localized disease) to IV (advanced, metastatic disease)
  - Survival varies widely; among patients diagnosed with stage IA disease, median survival is 115 months; among patients with stage IV disease, median survival is only 7 months

Prognosis of NSCLC

- Prognostic factors can modify expected survival; positive prognostic factors include early stage disease at diagnosis, a good performance status (Eastern Cooperative Oncology Group [ECOG] PS of 0, 1, or 2), no significant weight loss (≤5%), and female gender

CHAPTER 8

A Topline Overview of NSCLC Treatment

8.0 Introduction

Treatment for NSCLC has advanced considerably over the past decade, yet it remains one of the most deadly cancers with the poorest prognosis among cancers. The advent of targeted therapies has improved the outlook for many cancer patients, but there is a clear need for additional treatment options.

Here, we will discuss the management of NSCLC in broad terms; **please note that this discussion is not intended as a substitute for the detailed content contained within the Brigatinib Learning System**. Also note that we will cover the ALK-specific therapies in greater detail in the final chapter of this module.

Objectives

After completing this chapter, you will be able to:

- Describe the major management modalities for lung cancer: surgery, radiation, chemotherapy, and targeted therapy
- Describe the treatments used for systemic therapy for metastatic disease
- Describe treatment pathways for patients with NSCLC

8.1 Overview: Treatment Modalities for Localized NSCLC

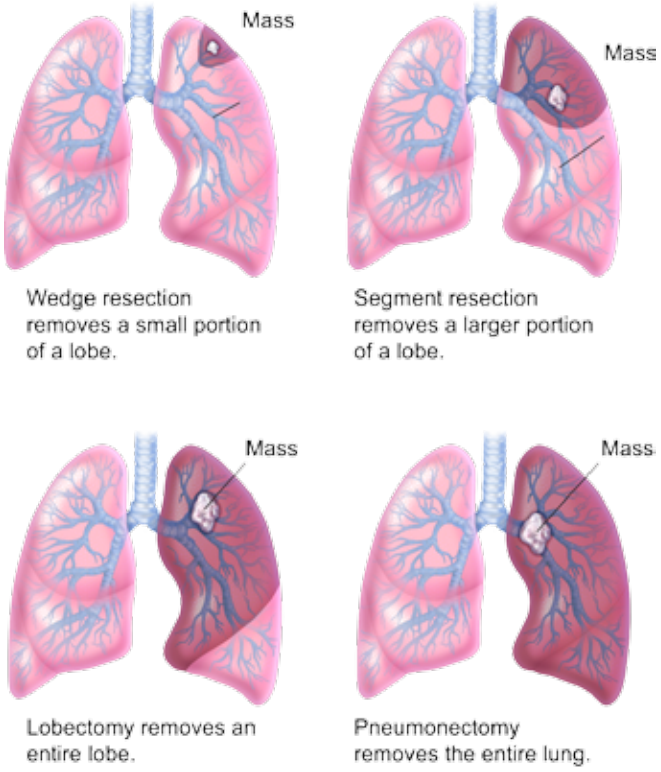
Three treatment modalities are commonly used to treat patients with NSCLC: surgery, radiation therapy, and systemic therapy. Depending on the disease status and other patient characteristics, they can be used either alone or in combination. The primary factor driving choice of therapy is the stage of the patient's cancer; other factors include the molecular characteristics of the tumor, prior treatments, and comorbidities. In general, treatment recommendations for a patient are made after consultation with a multidisciplinary team including thoracic surgeons, thoracic radiologists, and pulmonologists.[6]

8.2 Surgery for Localized NSCLC

Surgical resection of NSCLC offers a chance for cure and prolonged survival, particularly if the patient is diagnosed with early stage disease.[54] In general, patients with Stage IIIB and IV are not resectable.

The surgical procedure used to resect NSCLC depends on both the extent of disease and the pulmonary reserve of the patient. Surgical options for NSCLC include wedge resection, segmentectomy, lobectomy, and pneumonectomy **(Figure 8.2A)**.[6] The choice of procedure is highly patient-specific and depends on the patient's underlying cardiovascular health and extent of disease.[6]

Figure 8.2A. *Surgical procedures used for NSCLC*



8.3 Radiation Therapy for Localized NSCLC

Radiation therapy has a potential role in all stages of NSCLC as definitive therapy (the primary treatment) or palliative treatment.[6] The amount of radiation given, or the dosage, is usually measured in Grays (Gy), a unit that is defined as the absorption of 1 joule of radiation energy per 1 kilogram of matter.[46]

Conventional radiotherapy relies on small doses of radiation (also known as fractions) given daily for an extended period, often over as long as several weeks. In contrast, stereotactic ablative radiotherapy (SABR) uses focused high-dose radiation and is given in fewer (usually 1 to 5) treatments. Several beams are aimed at the tumor from different angles. Patients are placed in a body frame for each treatment to reduce movement of the tumor during breathing and enhance the precision of treatment.[55]

The dose of radiation given to a patient depends on[6]: **(Table 8.3A)**

- The treatment setting (ie, preoperative, postoperative, or definitive)
- Tumor features (ie, location of metastasis)
- Whether it is given in combination with chemotherapy

Adverse effects of radiation therapy include[45,55]:

- Fatigue
- Nausea and vomiting
- Loss of appetite
- Weight loss
- Skin changes in the area being treated (ranging from mild redness to blistering and peeling)
- Hair loss on the skin over targeted sites
- Pulmonary toxicity
- Sore throat and trouble swallowing (if the esophagus is targeted)
- Cardiac toxicity (infrequent)

Table 8.3A. *Principles of Radiation Therapy*[6]

	Total Dose	Fraction Size	Treatment Duration
Definitive with or without chemotherapy	60-70 Gy	2 Gy	6-7 weeks
Preoperative	45-54 Gy	1.8-2 Gy	5 weeks
Postoperative			
Negative margins	50-54 Gy	1.8-2 Gy	5-6 weeks
Extracapsular nodal extension or microscopic positive margins	54-60 Gy	1.8-2 Gy	6 weeks
Gross residual tumor	60-70 Gy	2 Gy	6-7 weeks
Palliative			
Obstructive disease	30-45 Gy	3 Gy	2-3 weeks
Bone metastases with soft tissue mass	20-30 Gy	4-3 Gy	1-2 weeks
Bone metastases without soft tissue mass	8-30 GY	8-3 Gy	1 day-2 weeks
Brain metastases	*	*	*
Symptomatic chest disease in patients with poor PS	17 Gy	8.5 Gy	1-2 weeks
Any metastasis in patients with poor PS	8-20 Gy	8-4 Gy	1 day-1 week

PS=performance status.
*Recommendations provided in Central Nervous System Cancers guidelines

8.4 Systemic Therapy

Systemic therapy for cancer can be divided into 2 broad categories: chemotherapy and targeted therapies[56]:

- Chemotherapies act on all rapidly dividing normal and cancerous cells; often cytotoxic (kill cancer cells)
- Targeted therapies act on specific molecular targets associated with cancer; often cytostatic (block proliferation of tumor cells, but may not kill them)

Chemotherapy

Chemotherapies are formally not considered targeted agents; instead, they act on all rapidly dividing normal and cancerous cells. However, this mechanism allows for a degree of targeting because tumor cells are generally proliferating at a higher rate than most other tissues.

Chemotherapies generally induce acute toxicities in rapidly dividing normal tissues (eg, bone marrow, GI tract, gonads, hair follicles). Side effects such as stomatitis, alopecia, myelosuppression, nausea, vomiting, anorexia, and diarrhea are common, depending on the specific agent administered. These expected side effects are generally manageable and usually do not necessitate treatment cessation. Because virtually every organ is affected by systemic chemotherapy, the toxicities of the drug will commonly determine the maximum amount that can be administered safely. Cumulative and potentially irreversible damage to vital organs limits total chemotherapy exposure.[80]

Multiple classes of chemotherapies are available. **Table 8.4A** reviews the key characteristics of agents used in the management of NSCLC.[6]

Table 8.4A. *Classes of chemotherapy used in the treatment of NSCLC*

Agent	Mechanism of Action
Platinums	
Cisplatin[57]	Bind to DNA and form crosslinks, preventing DNA replication; may also inhibit protein synthesis, change transport across the cell membrane, and suppress the function of the mitochondria[80]
Carboplatin[58]	
Taxanes	
Docetaxel[59]	Bind to microtubules (the cytoskeleton) and inhibit disassembly, which is necessary for normal functioning of the cytoskeleton[80]
Paclitaxel[60]	
Topoisomerase Inhibitors	
Etoposide[61]	Cause drug-induced DNA strand breaks; inhibit reunion of broken DNA strands[80]
Irinotecan[62]	
Antimetabolites	
Gemcitabine[63]	Inhibits synthesis of nucleotides, the building blocks of DNA[80]
Pemetrexed[64]	
Vinca Alkaloids	
Vinorelbine[65]	Interact with microtubules (the cytoskeleton of the cell) and interfere with cell division[80]
Vinblastine[66]	
Antitumor Antibiotic	
Mitomycin[67]	Binds tightly to DNA, inhibiting DNA synthesis[80]
Alkylating Agent	
Ifosfamide[68]	Binds to DNA, causing DNA breakage and other damage[80]

Targeted Therapy

Targeted therapies act on specific molecular targets associated with cancer.[56] In general, they were specifically developed for their ability to interact with their target.

Targeted therapies are often cytostatic, meaning they block tumor cell proliferation, whereas standard chemotherapies are cytotoxic. A number of different targeted therapies, including both small molecules and monoclonal antibodies, are available for the management of NSCLC. Here, we will provide a brief overview of these agents; the Brigatinib Learning System will cover relevant targeted therapies in detail. **Table 8.4B** summarizes agents that are currently widely used for the treatment of NSCLC.

Table 8.4B. *Targeted therapies for NSCLC*[6] Refer to Chapter 3 for details on the pathways targeted by these agents; Chapter 9 will cover current and pipeline ALK- directed therapies in greater detail.

Agent	Target/MOA	Indication in NSCLC
TARGET: VEGF		
Bevacizumab (Avastin®)	Binds VEGF and prevents the interaction of VEGF with its receptors on endothelial cells, inhibiting angiogenesis[69]	Nonsquamous NSCLC, with carboplatin and paclitaxel for first- line treatment of unresectable, locally advanced, recurrent or metastatic disease[69]
Ramucirumab (Cyramza®)	Binds VEGF 2; inhibits angiogenesis[70]	In combination with docetaxel for the treatment of metastatic NSCLC with disease progression on or after platinum-based chemotherapy (patients with EGFR or ALK genomic tumor aberrations should have disease progression on treatment for these aberrations prior to receiving ramucirumab)[70]

Agent	Target/MOA	Indication in NSCLC
TARGET: EGFR		
Erlotinib (Tarceva®)	Tyrosine kinase inhibitor; reversibly inhibits kinase activity of EGFR[71]	First-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test[71] Also indicated as maintenance treatment for patients with locally advanced or metastatic NSCLC whose disease has not progressed after 4 cycles of platinum-based first-line chemotherapy and for treatment of locally advanced or metastatic NSCLC after failure of at least 1 prior chemotherapy regimen
Gefitinib (Iressa®)	Tyrosine kinase inhibitor; inhibits EGFR and some activating mutations of this receptor[72]	First-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test[72]
Afatinib (Gilotrif®)	Tyrosine kinase inhibitor; inhibits EGFR, HER2, HER4[73]	First-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test[73]
Osimertinib (Tagrisso®)	Tyrosine kinase inhibitor targeting EGFR[82]	Treatment of patients with metastatic EGFR T790M mutation-positive NSCLC, as detected by an FDA-approved test, who have progressed on or after EGFR TKI therapy[82]

Agent	Target/MOA	Indication in NSCLC
TARGET: ALK		
Crizotinib (Xalkori®)	Tyrosine kinase inhibitor; targets ALK, insulin-like growth factor, insulin receptor, and ROS1[74]	Treatment of patients with metastatic NSCLC whose tumors are anaplastic lymphoma kinase (ALK)-positive as detected by an FDA-approved test; metastatic NSCLC whose tumors are ROS1-positive[74]
Ceritinib (Zykadia®)	Tyrosine kinase inhibitor; targets ALK, insulin-like growth factor, insulin receptor, and ROS1[76]	Treatment of ALK-positive metastatic NSCLC patients who have progressed on or are intolerant to crizotinib[76]
Alectinib (Alecensa®)	Tyrosine kinase inhibitor; targets ALK and RET[75]	Treatment of ALK-positive metastatic NSCLC patients who have progressed on or who are intolerant to crizotinib[75]
TARGET: PD-1		
Nivolumab (Opdivo®)	Inhibits PD-1 receptor; releases inhibition of the immune response, allowing the immune system to attack the tumor[77]	Metastatic non-small cell lung cancer and progression on or after platinum-based chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving nivolumab[77]
Pembrolizumab (Keytruda®)	Inhibits PD-1 receptor; releases inhibition of the immune response, allowing the immune system to attack the tumor[78]	Patients with metastatic NSCLC whose tumors express PD-L1 as determined by an FDA-approved test and who have disease progression on or after platinum-containing chemotherapy (patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab)[78]

8.5 Treatment of Advanced and Metastatic NSCLC

Recurrent and metastatic NSCLC have historically been regarded as incurable, with a shift in focus of care to palliative therapy.[6] In the metastatic setting for patients with a known oncogenic driver mutation, first-line treatment options generally include targeted agents and chemotherapy. Therapy is generally chosen based on histologic subtype, molecular testing (ie, mutational testing), and performance status of the patient. Many patients with advanced NSCLC receive supportive care to treat complications of the disease and/or their treatment. Moreover, many patients with advanced disease eventually reach a point at which the focus of care shifts from anticancer treatment to palliative care.[81] Please note that a comprehensive discussion of the management of patients with metastatic disease, with a focus on patients with ALK rearrangements, can be found in the full Brigatinib Learning System.[81]

8.5.1 Local Therapies

Surgery plays a limited role in the treatment of advanced NSCLC.[6] Surgical removal of a solitary brain metastasis may extend survival in selected patients; patients with adrenal metastases may benefit from removal of the adrenal glands.[6] Radiation therapy may be used after surgical resection in patients with brain metastases and in patients with adrenal metastases.[6]

8.5.2 Systemic Therapies

In general, the drug regimen with the highest likelihood of benefit with a level of toxicity that is deemed acceptable to the physician and patient is given as initial therapy for patients with advanced NSCLC.[6]

Many pivotal trials have been conducted over the past several decades that have shaped how advanced NSCLC is treated today; for example, results from these trials have led to NCCN recommendations such as:

- Platinum-based chemotherapy is more effective than best supportive care; it prolongs survival, improves symptom control, and yields a superior quality of life[6]
- There is superior efficacy and reduced toxicity of cisplatin/pemetrexed in patients with nonsquamous histology compared with cisplatin/gemcitabine; the opposite is true of patients with squamous histology[6]
- Two-drug regimens are preferred; a third cytotoxic drug increases response rate but not survival. Single-agent chemotherapy may be appropriate in select patients[6]
- With a few exceptions, (such as patients with specific mutations, including ALK and EGFR) systemic chemotherapy should not be given to patients with a performance status of 3 or 4[6]

Some patients with advanced or metastatic disease may receive continuation maintenance therapy, in which at least 1 of the agents given in the first line is used beyond 4 to 6 cycles in the absence of disease progression. Others may receive “switch maintenance,” which refers to the initiation of a different agent, not included as part of the first-line regimen, in the absence of disease progression after 5 to 6 cycles of chemotherapy.[6]

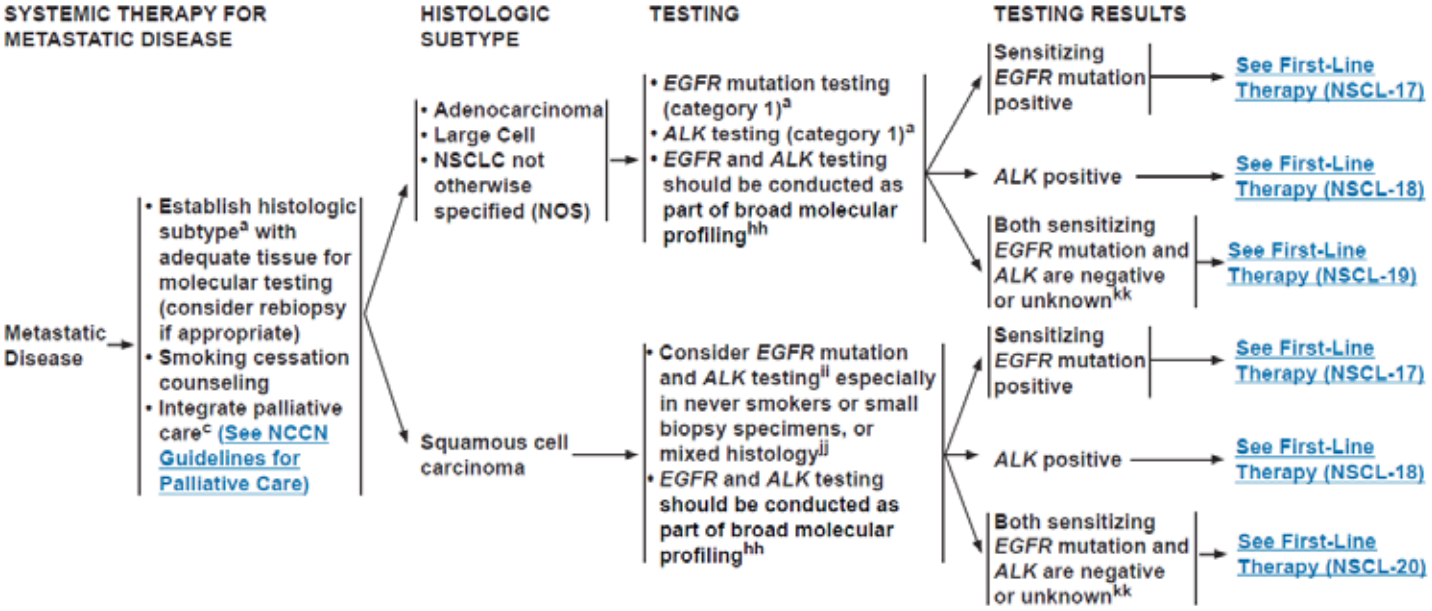
Patients who progress during or after first-line therapy have the option of receiving second-line therapy. In these patients[6]:

- Nivolumab improves survival when compared with docetaxel
- Pembrolizumab improves overall survival in PD-L1 positive tumors when compared with docetaxel
- Docetaxel is superior to vinorelbine or ifosfamide
- Pemetrexed is considered equivalent to docetaxel with less toxicity in patients with adenocarcinoma and large cell carcinoma
- Ramucirumab + docetaxel improves survival when compared to docetaxel alone
- Erlotinib is superior to best supportive care

Impact of Driver Mutations

As noted throughout this backgrounder, some NSCLCs are characterized by specific mutations that can be addressed therapeutically through the use of targeted agents. At present, the 2 main categories in NSCLC that can be managed with targeted therapies are specific EGFR and ALK. The NCCN guidelines recommend testing of all patients with metastatic disease for EGFR mutations and ALK rearrangements **(Figure 8.5A)**.[6]

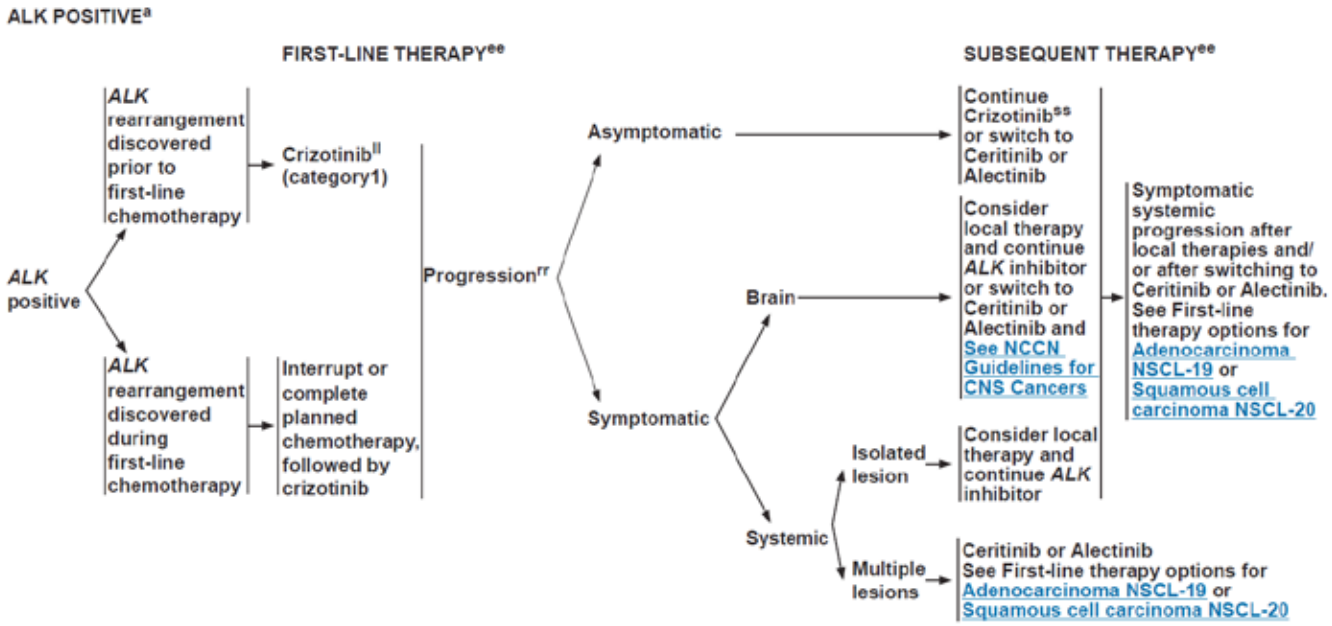
Figure 8.5A. *Recommendations for mutation testing for patients with metastatic NSCLC*[6]



[a] See Principles of Pathologic Review (NSCL-A).
[c] Temel JS, Greer JA, Muzikansky A, et al. Early palliative care for patients with metastatic non-small-cell lung cancer. N Engl J Med 2010;363:733-742.
[hh] The NCCN NSCLC Guidelines Panel strongly endorses broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC. See Emerging Targeted Agents for Patients With Genetic Alterations (NSCL-H).
[ii] In patients with squamous cell carcinoma, the observed incidence of *EGFR* mutations is 2.7% with a confidence that the true incidence of mutations is less than 3.6%. This frequency of *EGFR* mutations does not justify routine testing of all tumor specimens. Forbes SA, Bharma G, Bamford S, et al. The catalogue of somatic mutations in cancer (COSMIS). Curr Protoc Hum Genet 2008;chapter 10:unit 10.11.
[ii] Paik PK, Varghese AM, Sima CS, et al. Response to erlotinib in patients with *EGFR* mutant advanced non-small cell lung cancers with a squamous or squamous-like component. Mol Cancer Ther 2012;11:2535-2540.
[kk] Consider ROS1 testing; if positive, may treat with crizotinib. Shaw AT, Ou S-HI, Bang Y-J, et al. Crizotinib in ROS1-rearranged non-small cell lung cancer. N Engl J Med 2014;371:1963-1971.

As an example, crizotinib is recommended as first-line therapy in patients with metastatic disease with ALK rearrangements **(Figure 8.5B).**[74] In fact, the evidence for crizotinib is strong enough that the NCCN guidelines suggest considering interrupting chemotherapy and starting patients on crizotinib, for those whom an ALK rearrangement is discovered during first-line chemotherapy. Later-line options among patients who progress on crizotinib include the alternative ALK inhibitors ceritinib and alectinib. A similar paradigm applies to patients with specific EGFR mutations.

Figure 8.5B. *Pathway for patients with metastatic NSCLC with an ALK rearrangement*[6]



[a]See Principles of Pathologic Review (NSCL-A).
[ee]See Systemic Therapy for Advanced or Metastatic Disease (NSCL-F).
[ll]For performance status 0-4.
[rr]Patients who are intolerant to crizotinib may be switched to ceritinib or alectinib.
[ss]For rapid radiologic progression or threatened organ function, alternate therapy should be instituted.

8.6 Summary: A Topline Overview of NSCLC Treatment

Treatment Modalities for Localized NSCLC

- Three major modalities are used in the management of NSCLC:
  - Surgery
  - Radiation therapy
  - Systemic therapy
- These therapies may be used alone, simultaneously, or sequentially

Surgery for Localized NSCLC

- Surgery is often curative when NSCLC is diagnosed early; surgeries may be:
  - Wedge resections (removes small portion of lobe)
  - Segment resections (removes larger portion of lobe)
  - Lobectomy (removes an entire lobe of the lung)
  - Pneumonectomy (removes entire lung)

Radiation Therapy for Localized NSCLC

- Radiation therapy is a key option in patients who refuse surgery or who are medically inoperable
  - SABR is commonly used in these patients

Systemic Therapy

- Systemic therapies may be divided into 2 broad categories:
  - Chemotherapies, which rely on rapid cell division to semi-selectively target tumor tissues
  - Targeted therapies, which rely on unique molecular characteristics of the tumor to affect these cells while sparing normal tissues to varying degrees
- Targets for currently available therapies for NSCLC include:
  - VEGF
  - EGFR
  - ALK
  - PD-1

Management of NSCLC

- The presence of driver mutations strongly influences the treatment pathway of patients with metastatic NSCLC
  - Patients with EGFR or ALK mutations are preferentially treated with agents directed against these targets, even if the mutation is discovered after initiation of chemotherapy

CHAPTER 9

ALK Inhibitors

9.0 Introduction

Several protein kinases have emerged as attractive therapeutic targets for the treatment of various cancers. One of these, ALK, has attracted a great deal of attention due to its oncogenic potential and essential role in the pathogenesis of a wide variety of human cancers such as NSCLC. At present, multiple ALK inhibitors have entered clinical investigation for the treatment of cancers, and 3 have been approved in various countries worldwide as of 2016, including crizotinib, ceritinib (in the United States and European Union), and alectinib (in the United States and Japan).

The first ALK inhibitor, crizotinib, is currently established, where approved, as first-line therapy in patients with metastatic NSCLC with ALK rearrangements. Two "second generation" agents, alectinib and ceritinib, were recently approved by several regulatory authorities worldwide as second-line treatment options among patients with ALK rearrangements who have progressed on crizotinib. Here, we will focus on the product characteristics of the 3 marketed agents. Please note that you will receive detailed training on each of these agents as well as brigatinib in the Brigatinib Learning System.

Objectives

After completing this chapter, you will be able to:

- Describe the key product characteristics of currently available ALK inhibitors

9.1 Crizotinib (Xalkori®)

9.1.1 Introduction

Crizotinib was approved by the US FDA in 2011, and was the first ALK inhibitor approved for NSCLC.[74] Crizotinib has activity on ALK, but also has activity on a number of other receptor tyrosine kinases, including hepatocyte growth factor receptor (HGFR, c-MET), ROS1, and Recepteur d'Origine Nantais (RON).[74]

9.1.2 Dosing

Crizotinib is administered orally at a dosage of 250 mg twice daily; treatment is continued until disease progression slows or until it is no longer tolerated by the patient.[74] The dosage is reduced in patients with severe renal impairment to once daily. The dose can be modified or treatment discontinued for the management of adverse events, including hematologic toxicities, liver enzyme elevations, interstitial lung disease/pneumonitis, QTc prolongation and bradycardia.[74]

9.1.3 Warnings and Precautions and Adverse Events[74]

- Hepatotoxicity: Fatal hepatotoxicity occurred in 0.1% of patients. Monitor with periodic liver testing. Temporarily suspend, dose reduce, or permanently discontinue crizotinib. (section 5.1)
- Interstitial Lung Disease (ILD)/Pneumonitis: Occurred in 2.9% of patients. Permanently discontinue in patientswith ILD/pneumonitis. (section 5.2)
- QT Interval Prolongation: Occurred in 2.1% of patients. Monitor with electrocardiograms and electrolytes in patients who have a history of or predisposition for QTc prolongation, or who are taking medications that prolong QT. Temporarily suspend, dose reduce, or permanently discontinue crizotinib. (section 5.3)
- Bradycardia: Crizotinib can cause bradycardia. Monitor heart rate and blood pressure regularly. Temporarily suspend, dose reduce, or permanently discontinue crizotinib. (section 5.4)
- Severe Visual Loss: Reported in 0.2% of patients. Discontinue crizotinib in patients with severe visual loss. Perform an ophthalmological evaluation. (section 5.5)
- Embryofetal Toxicity: Can cause fetal harm. Advise females of reproductive potential of the potential risk to a fetus and use of effective contraception. (sections 5.6, 8.1, 8.3)
- The most common adverse reactions (≥25%) are vision disorders, nausea, diarrhea, vomiting, edema, constipation, elevated transaminases, fatigue, decreased appetite, upper respiratory infection, dizziness, and neuropathy. (section 6)

9.1.4 Clinical Studies

The efficacy and safety of crizotinib in patients with ALK- positive metastatic NSCLC, who had not received previous systemic treatment for advanced disease, was evaluated in a randomized, multicenter, open-label, active-controlled study.[74] Patients were randomized to receive crizotinib (n=172) or chemotherapy (n=171). Patients received crizotinib 250 mg orally twice daily until documented disease progression, intolerance to therapy, or the investigator determined that the patient was no longer experiencing clinical benefit. Chemotherapy consisted of pemetrexed 500 mg/m2 with cisplatin 75 mg/m2 or carboplatin AUC of 5 or 6 mg·min/mL by intravenous infusion every 3 weeks for up to 6 cycles. Patients in the chemotherapy arm were not permitted to receive maintenance chemotherapy.[74]

This study demonstrated a statistically significant improvement in PFS in the patients treated with crizotinib; OS analysis conducted at the time of the PFS analysis did not suggest a difference in survival between arms.[74] Efficacy results are summarized in **Table 9.1A** and **Figure 9.1A**.

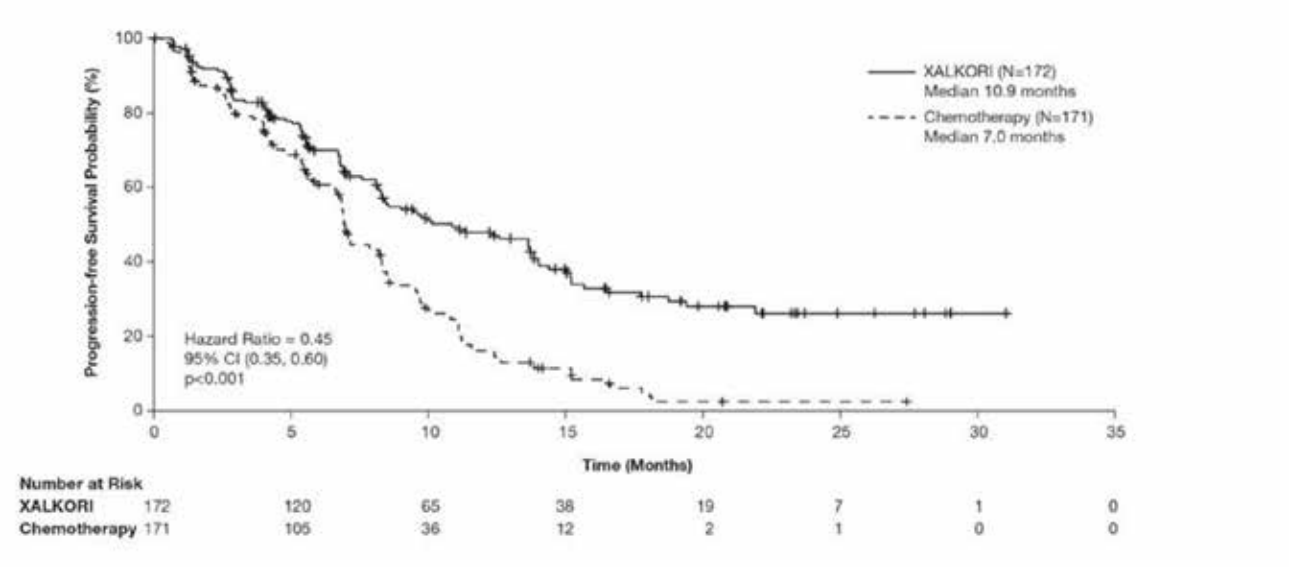
Table 9.1A. Efficacy results for crizotinib in ALK-positive metastatic NSCLC patients who had not received previous systemic treatment for advanced disease[74]

	XALKORI (N=172)	Chemotherapy (N=171)
Progression-Free Survival (Based on IRR)		
Number of Events (%)	100 (58%)	137 (80%)
Progressive Disease	89 (52%)	132 (77%)
Death	11 (6%)	5 (3%)
Median, Months (95% CI)	10.9 (8.3, 13.9)	7.0 (6.8, 8.2)
HR (95% CI)*	0.45 (0.35, 0.60)	
p-value†	<0.001	
Overall Survival‡		
Number of Events (%)	44 (26%)	46 (27%)
Median, Months (95% CI)	NR	NR
HR (95% CI)*	0.82 (0.54, 1.26)	
p-value†	0.36	
Tumor Responses (Based on IRR)		
Objective Response Rate % (95% CI)	74% (67, 81)	45% (37, 53)
CR, n (%)	3 (1.7%)	2 (1.2%)
PR, n (%)	125 (73%)	75 (44%)
p-value§	<0.001	
Duration of Response		
Median, Months¶ (95% CI)	11.3 (8.1, 13.8)	5.3 (4.1, 5.8)

HR=hazard ratio; CI=confidence interval; IRR=independent radiology review; NR=not reached; CR=complete response; PR=partial response.

* Based on the Cox proportional hazards stratified analysis.
† Based on the stratified log-rank test.
‡ OS analysis was not adjusted for the potentially confounding effects of cross over.
§ Based on the stratified Cochran-Mantel-Haenszel test.
¶ Estimated using the Kaplan Meier method.

Figure 9.1A. PFS as assessed by IRR (independent radiology review) in a study of crizotinib in ALK-positive metastatic NSCLC patients who had not received previous systemic treatment for advanced disease[74]



In a second randomized, multicenter, open-label, active-controlled study, crizotinib monotherapy was evaluated in 347 patients with ALK-positive metastatic NSCLC that were previously treated with 1 platinum-based chemotherapy regimen. The major efficacy outcome was PFS as assessed by IRR. Additional efficacy outcomes included objective response rate (ORR) as assessed by IRR, duration of response, and OS.[74]

Patients were randomly allocated to treatment with crizotinib 250 mg orally twice daily (n=173) or chemotherapy (n=174). Chemotherapy consisted of pemetrexed 500 mg/m (if pemetrexed naïve; n=99) or docetaxel 75 mg/m (n=72) intravenously (IV) every 21 days. Patients in both treatment arms continued treatment until documented disease progression, intolerance to therapy, or the investigator determined that the patient was no longer experiencing clinical benefit.[74]

This study demonstrated a statistically significant improvement in PFS in the patients treated with crizotinib. Efficacy results are summarized in **Table 9.1B** and **Figure 9.1B.**

Table 9.1B. Efficacy results for crizotinib in previously treated ALK-positive metastatic NSCLC patients[74]

	XALKORI (N=173)	Chemotherapy (N=174)
Progression-Free Survival (Based on IRR)		
Number of Events (%)	100 (58%)	127 (73%)
Progressive Disease	84 (49%)	119 (68%)
Death	16 (9%)	8 (5%)
Median, Months (95% CI)	7.7 (6.0, 8.8)	3.0* (2.6, 4.3)
HR (95% CI)†	0.49 (0.37, 0.64)	
p-value‡	<0.001	
Overall Survival§		
Number of Events (%)	49 (28%)	47 (27%)
Median, Months (95% CI)	20.3 (18.1, NR)	22.8 (18.6, NR)
HR (95% CI)†	1.02 (0.68, 1.54)	
p-value‡	0.92	
Tumor Responses (Based on IRR)		
Objective Response Rate % (95% CI)	65% (58, 72)	20% (14, 26)
CR, n (%)	1 (0.6%)	0
PR, n (%)	112 (65%)	34 (20%)
p-value¶	<0.001	
Duration of Response		
Median, Months (95% CI)	7.4 (6.1, 9.7)	5.6 (3.4, 8.3)

HR=hazard ratio; CI=confidence interval; IRR=independent radiology review; NR=not reached; CR=complete response; PR=partial response.

* For pemetrexed, the median PFS was 4.2 months. For docetaxel, the median PFS was 2.6 months.

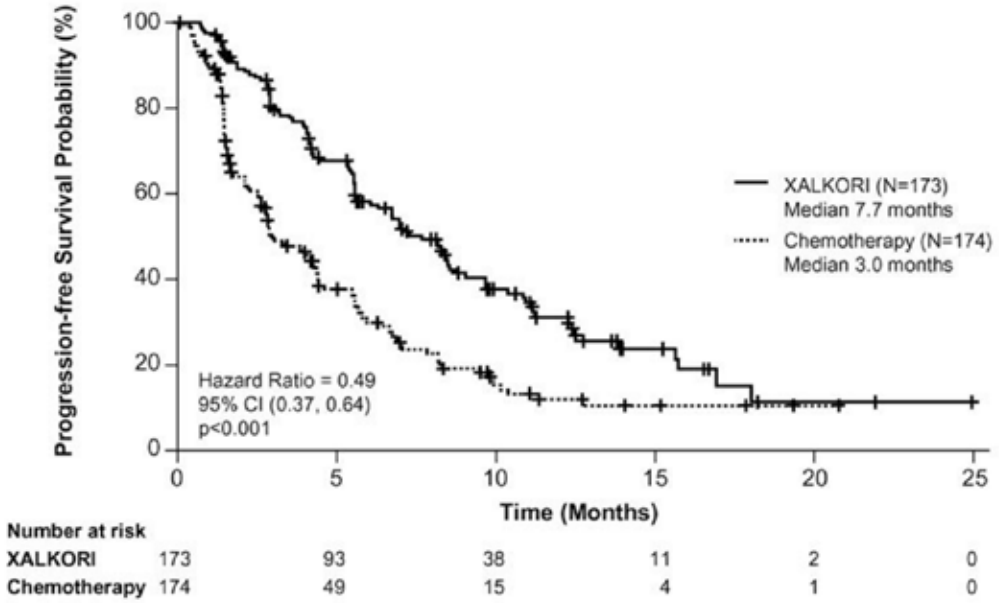
† Based on the Cox proportional hazards stratified analysis.

‡ Based on the stratified log-rank test.

§ Interim OS analysis conducted at 40% of total events required for final analysis.

¶ Based on the stratified Cochran-Mantel-Haenszel test.

Figure 9.1B. PFS results for crizotinib in previously treated ALK-positive metastatic NSCLC patients[74]



9.2 Ceritinib (Zykadia®)

9.2.1 Introduction

Ceritinib was approved by the US FDA in 2014.[76] At present, it is indicated for treatment of patients with ALK-positive metastatic NSCLC who have progressed on or are intolerant to crizotinib.[80] As with other agents in this class, ceritinib is a tyrosine kinase inhibitor; data suggests that it targets ALK, insulin-like growth factor 1 receptor, insulin receptor, and ROS1.[76]

9.2.2 Dosing

Ceritinib is administered once daily at a dosage of 750 mg until disease progression or unacceptable toxicity. Ceritinib must be administered on an empty stomach.[76] The dose may be reduced or treatment discontinued for the management of adverse events including liver enzyme elevations, interstitial lung disease, QTc prolongation, bradycardia, elevated blood sugar, severe gastrointestinal adverse events, and lipase or amylase elevations.[76] The dose may also require modification in patients who are taking strong CYP3A4 inhibitors.[76]

9.2.3 Warnings and Precautions and Adverse Events[76]

- Severe or Persistent Gastrointestinal Toxicity: Dose modification due to diarrhea, nausea, vomiting or abdominal pain occurred in 38% of patients. Withhold if not responsive to anti-emetics or anti-diarrheals, then dose reduce ceritinib. (sections 2.2, 5.1)
- Hepatotoxicity: Ceritinib can cause hepatotoxicity. Monitor liver laboratory tests at least monthly. Withhold then dose reduce, or permanently discontinue ceritinib. (sections 2.2, 5.2)
- Interstitial Lung Disease (ILD)/Pneumonitis: Occurred in 4% of patients. Permanently discontinue ceritinib in patients diagnosed with treatment-related ILD/ pneumonitis. (sections 2.2, 5.3)
- QT Interval Prolongation: Ceritinib can cause QTc interval prolongation. Monitor electrocardiograms and electrolytes in patients with congestive heart failure, bradyarrhythmias, electrolyte abnormalities, or those who are taking medications that are known to prolong the QTc interval. Withhold then dose reduce, or permanently discontinue ceritinib. (sections 2.2, 5.4)
- Hyperglycemia: Ceritinib can cause hyperglycemia. Monitor fasting glucose prior to treatment and periodically thereafter as clinically indicated. Initiate or optimize anti-hyperglycemic medications as indicated. Withhold then dose reduce, or permanently discontinue ceritinib. (sectons 2.2, 5.5)
- Bradycardia: Ceritinib can cause bradycardia. Monitor heart rate and blood pressure regularly. Withhold then dose reduce, or permanently discontinue ceritinib. (sections 2.2, 5.6)
- Pancreatitis: Elevations of lipase and/or amylase and pancreatitis can occur. Monitor lipase and amylase prior to treatment and periodically thereafter as clinically indicated. (sections 2.2, 5.7)
- Embryofetal Toxicity: Ceritinib may cause fetal harm. Advise females of reproductive potential of the potential risk to a fetus. (sections 5.8, 8.1, 8.7)
- The most common adverse reactions (incidence of at least 25%) are diarrhea, nausea, elevated transaminases, vomiting, abdominal pain, fatigue, decreased appetite, and constipation. (section 6)

9.2.4 Clinical Study

The efficacy of ceritinib was established in a multicenter, single-arm, open-label clinical trial. A total of 163 patients with metastatic ALK-positive NSCLC who progressed while receiving or were intolerant to crizotinib were enrolled. All patients received ceritinib at a dose of 750 mg once daily. Efficacy results are summarized in **Table 9.2A.**[76]

Table 9.2A. Efficacy of ceritinib in a clinical trial of ALK- positive NSCLC who received prior crizotinib[76]

Efficacy Parameter	Investigator Assessment (N=163)	BIRC Assessment (N=163)
Overall Response Rate (95% CI)	54.6% (47, 62)	43.6% (36, 52)
CR	1.2%	2.5%
PR	53.4%	41.1%
Duration of Response, median (months) (95% CI)	7.4 (5.4, 10.1)	7.1 (5.6, NE)
[1]Overall Response Rate and Duration of Response determined by RECIST v1.0 BIRC, blinded independent review committee; CR, complete response; NE, not estimable; PR, partial response.		

9.3 Alectinib®

9.3.1 Introduction

Alectinib was approved by the US FDA in 2015, and was the third ALK inhibitor approved in NSCLC.[75] It is approved for the treatment of ALK-positive metastatic NSCLC in patients who have progressed on or who are intolerant to crizotinib.[75] As with other agents in this class, alectinib targets ALK; it also appears to have activity on RET.[75]

9.3.2 Dosing

Alectinib 600 mg is administered twice daily with food; treatment is continued until disease progression or unacceptable toxicity.[75] The dosage may be reduced or treatment discontinued for the management of adverse events, including liver enzyme elevations, bilirubin elevations, interstitial lung disease, bradycardia, or creatinine phosphokinase elevations.[75]

9.3.3 Warnings and Precautions and Adverse Events[75]

- Hepatotoxicity: Monitor liver laboratory tests every 2 weeks during the first 2 months of treatment, and then periodically during treatment. In case of severe ALT, AST, or bilirubin elevations, withhold, then reduce dose, or permanently discontinue alectinib. (sections 2.2, 5.1)
- Interstitial Lung Disease (ILD)/Pneumonitis: Occurred in 0.4% of patients. Immediately withhold alectinib in patients diagnosed with ILD/pneumonitis and permanently discontinue if no other potential causes of ILD/pneumonitis have been identified. (sections 2.2, 5.2)
- Bradycardia: Monitor heart rate and blood pressure regularly. If symptomatic, withhold alectinib then reduce dose, or permanently discontinue. (sections 2.2, 5.3)
- Severe Myalgia and Creatine Phosphokinase (CPK) Elevation: Occurred in 1.2% and 4.6% of patients, respectively. Assess CPK every 2 weeks during the first month of treatment and in patients reporting unexplained muscle pain, tenderness, or weakness. In case of severe CPK elevations, withhold, then resume or reduce dose. (sections 2.2, 5.4)
- Embryo-Fetal Toxicity: Alectinib can cause fetal harm. Advise females of reproductive potential of the potential risk to a fetus and to use effective contraception. (sections 5.5, 8.1, 8.3)
- The most common adverse reactions (incidence ≥20%) were fatigue, constipation, edema and myalgia. (section 6)

Clinical Studies

The safety and efficacy of alectinib were established in 2 single-arm, multicenter clinical trials. Patients with locally advanced or metastatic ALK-positive NSCLC, who had progressed on crizotinib, with documented ALK-positive NSCLC based on an FDA-approved test, and ECOG PS of 0-2 were enrolled in both studies.[75] The major efficacy outcome measure in both studies was ORR. Efficacy results for these studies are summarized in **Table 9.3A.**

Table 9.3A. Efficacy results for alectinib in patients with locally advanced or metastatic ALK-positive NSCLC who had progressed on crizotinib[75]

Efficacy Results in Studies 1 and 2

Efficacy Parameter	Study 1 (N=87)		Study 2 (N=138)	
	IRC* Assessment	Investigator Assessment	IRC* Assessment	Investigator Assessment
Objective Response Rate (95% CI)	38% (28; 49)	46% (35; 57)	44% (36; 53)	48% (39; 57)
Number of Responders	33	40	61	66
Duration of Response, median in months (95% CI)	7.5 (4.9, Not Estimable)	NE (4.9, Not Estimable)	11.2 (9.6, Not Estimable)	7.8 (7.4, 9.2)

The package insert for alectinib is unique among current ALK inhibitors in that it includes specific data on patients with brain metastases.[75] An assessment of ORR and duration of response for CNS metastases in the subgroup of 51 patients with baseline measurable CNS lesions is summarized in **Table 9.3B**. Thirty-five (69%) patients with measurable CNS lesions according to RECIST v 1.1 had received prior brain radiation, including 25 (49%) who completed radiation treatment at least 6 months before starting treatment with alectinib.

Responses were observed irrespective of prior brain radiation status.[75]

Table 9.3B. CNS objective response in patients with measurable CNS lesions in patients who had previously progressed on crizotinib[75]

Efficacy Parameter	N=51
CNS Objective Response Rate (95% CI)	61% (46,74)
Complete Response	18%
Partial Response	43%
CNS Duration of Response, median in months (95% CI)	9.1 (5.8, not evaluable)

9.4 Summary: ALK Inhibitors

- At present, 3 ALK inhibitors are available:
  - Crizotinib
  - Ceritinib
  - Alectinib
- Only crizotinib has an indication as first-line treatment in patients with metastatic disease who are ALK-positive
- Only ceritinib is dosed once daily; both crizotinib and alectinib require twice daily dosing
- Agents in this class have several Warnings and Precautions in common: hepatotoxicity, effects on heart rate, risk for interstitial lung disease, and embryofetal toxicity
- All agents have demonstrated significant, durable responses in patients selected on the basis of ALK rearrangement

GLOSSARY

GLOSSARY

Adjuvant: Chemotherapy given in addition to surgical therapy, in order to reduce the risk of local or systemic relapse.[46]

Atelectasis: Complete or partial collapse of a lung or lobe of a lung. [46]

Basement membrane: A thin, delicate layer of protein fibers and other substances separating epithelial tissue from underlying tissue.[46]

Bradycardia: Slow heart rate.[46]

Chromatin: The genetic material of the nucleus. [46]

Cushing syndrome: A disorder resulting from increased secretion of cortisol; characterized by obesity, moon face, acne, hypertension, osteoporosis, and other symptoms.[46]

Dysphagia: Difficulty or discomfort in swallowing as a symptom of disease.[46]

Dyspnea: Shortness of breath.[46]

Endothelium: A layer of flat cells lining the blood, lymphatic vessels, and the heart.[46]

Hemoptysis: Coughing up of blood.[46]

Mediastinum: A partition in the thoracic cavity that contains all of the organs except the lungs.[46]

Metastasis: The spread of a disease process from one part of the body to another, such as in the appearance of tumors in parts of the body remote from the site of the primary tumor. Results from dissemination of tumor cells by the lymphatics or blood vessels or by direct extension of the tumor.[46]

Mucositis: Inflammation of a mucous membrane, such as the lining of the mouth.[46]

Necrosis: Pathologic death of cells or a portion of a tissue or organ resulting from irreversible damage.[46]

Nucleoli (sing, nucleolus): A small, rounded mass within the cell nucleus.[46]

Protease: An enzyme that breaks down certain tissues.[46]

Pulmonary reserve: The additional volume of air that the lungs can inhale and exhale when breathing to the limit of capacity.[46]

Segmentectomy: Surgery to remove part of an organ or a gland. In lung cancer surgery, segmentectomy refers to removing a section of a lobe of the lung. Also known as a segmental resection.[46]

REFERENCES

REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2014. American Cancer Society. Available at http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151.pdf. Accessed February 10, 2015. .
2. American Lung Association. Lung cancer fact sheet. Available at: http://www.lung.org/lung-disease/lung-cancer/resources/facts-figures/lung-cancer-fact-sheet.html. Accessed January 30, 2016.
3. Abeloff MD. Abeloff's Clinical Oncology. 4th ed. Philadelphia, PA: Elsevier Churchill Livingstone 2011.
4. National Cancer Institute. Non-small cell lung cancer treatment--for professionals (PDQ). Available at: http://www.cancer.gov/types/lung/hp/non-small-cell-lung-treatment-pdq. Accessed January 30, 2016.
5. National Cancer Institute. SEER Statistics: Small Cell Cancer of the Bronchus and Lung. Available at: http://seer.cancer.gov/csr/1975_2012/browse_csr.php?sectionSEL=15&pageSEL=sect_15_table.13.html. Accessed January 30, 2016.
6. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Non-small Cell Lung Cancer. Version 4.2016. Available at: NCCN.org. Accessed February 1, 2016.
7. Oxnard GR, Binder A, Janne PA. New targetable oncogenes in non-small-cell lung cancer. J Clin Oncol. 2013;31:1097-1104.
8. Scagliotti GV, Bironzo P, Vansteenkiste JF. Addressing the unmet need in lung cancer: The potential of immuno-oncology. Cancer Treat Rev. 2015;41:465-475.
9. National Cancer Institute. Metastatic cancer. Available at: http://www.cancer.gov/about-cancer/what-is-cancer/metastatic-fact-sheet. Accessed January 12.
10. Marieb EN, Hoehn K. Human Anatomy and Physiology. Boston. Pearson Inc. 2015.
11. Betts JG, Desaix P, Johnson E. Anatomy and Physiology. OpenStax College. Rice University. 2013 Edition.
12. El-Sherief AH, Lau CT, Wu CC, Drake RL, Abbott GF, Rice TW. International association for the study of lung cancer (IASLC) lymph node map: radiologic review with CT illustration. Radiographics. 2014;34:1680-1691.
13. Edge SB, Byrd DR, Compton CC, et al. AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer; 2010.
14. Mescher AL. Histology & Its Methods of Study. In: Mescher AL. eds. Junqueira's Basic Histology, 14e. New York, NY: McGraw-Hill; 2016.
15. Heart. In: Morton DA, Foreman K, Albertine KH. eds. The Big Picture: Gross Anatomy. New York: McGraw-Hill; 2011. http://accessmedicine.mhmedical.com/content.aspx?bookid=381&Sectionid=40140010. Accessed February 15, 2014.
16. Riihimaki M, Hemminki A, Fallah M, et al. Metastatic sites and survival in cancer. Lung Cancer. 2014;86:78-84.
17. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Water P. Molecular Biology of the Cell. 5th ed. New York, NY: Garland Science/Taylor & Francis Group; 2008.
18. Hoffman BL, Schorge JO, Schaffer JI, et al. Principles of chemotherapy. In: Hoffman BL, Schorge JO, Schaffer JI, et.al eds. Williams Gynecology. 2nd ed. New York, NY: McGraw-Hill; 2012:1-31.
19. Longo DL. Chapter 84. Cancer Cell Biology and Angiogenesis. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson J, Loscalzo J. eds. Harrison's Principles of Internal Medicine, 18e. New York, NY: McGraw-Hill; 2012. http://accessmedicine.mhmedical.com/content.aspx?bookid=331&Sectionid=40726820. Accessed January 02, 2015.
20. Kumar V, Abbas AK, Aster JC. Neoplasia. In: Kumar V, Abbas AK, Aster JC, eds. Robbins and Cotran Pathologic Basis of Disease. Philadelphia, PA: Elsevier/Saunders; 2015:265-340.
21. Feng X, Lin X, Brunicardi F. Molecular and genomic surgery. In: Brunicardi F, Andersen DK, Billiar TR, et al, eds. Schwartz's Principles of Surgery. 10th ed. New York, NY: McGraw-Hill; 2015:1-43.

22. National Cancer Institute. Cancer of the Lung and Bronchus (Invasive). Percent Distribution and Counts by Histology among Histologically Confirmed Cases, 2008-2012 Both Sexes by Race. Available at: www.cancer.gov. Accessed January 19, 2016.
23. National Cancer Institute. SEER Stat Fact Sheets: Lung and Bronchus Cancer. Available at: http://seer.cancer.gov/statfacts/html/lungb.html. Accessed April 12, 2016.
24. National Cancer Institute. Small cell lung cancer treatment--for professionals (PDQ). Available at: http://www.cancer.gov/types/lung/hp/small-cell-lung-treatment-pdq. Accessed January 30, 2016.
25. Collins LG, Haines C, Perkel R, Enck RE. Lung cancer: diagnosis and management. Am Fam Physician. 2007;75:56-63.
26. Travis WD. Update on small cell carcinoma and its differentiation from squamous cell carcinoma and other non-small cell carcinomas. Mod Pathol. 2012;25 Suppl 1:S18-30.
27. National Cancer Institute. Non-small cell cancer of the lung and bronchus (invasive). 5-year relative and period survival (percent) by race, sex, diagnosis year, stage, and age. Available at: www.cancer.gov. Accessed February 20, 2016.
28. Horn L, Lovly CM, Johnson DH. Neoplasms of the Lung. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. eds. Harrison's Principles of Internal Medicine, 19e. New York, NY: McGraw-Hill; 2015. .
29. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology. Non-small cell lung cancer. Version I.2015. Available at: http://www.nccn.org/professionals/physician_gls/PDF/nscl.pdf. Accessed November 21, 2014.
30. Palmer RH, Vernersson E, Grabbe C, Hallberg B. Anaplastic lymphoma kinase: signalling in development and disease. Biochem J. 2009;420:345-361.
31. Grande E, Bolos MV, Arriola E. Targeting oncogenic ALK: a promising strategy for cancer treatment. Mol Cancer Ther. 2011;10:569-579.
32. Rossi A, Maione P, Sacco PC, et al. ALK inhibitors and advanced non-small cell lung cancer (review). Int J Oncol. 2014;45:499-508.
33. Shackelford RE, Vora M, Mayhall K, Cotelingam J. ALK-rearrangements and testing methods in non-small cell lung cancer: a review. Genes Cancer. 2014;5:1-14.
34. Clark JW, Longo DL. Cancer Cell Biology. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. eds. Harrison's Principles of Internal Medicine, 19e. New York, NY: McGraw-Hill; 2015. http://accessmedicine.mhmedical.com/content.aspx?bookid=1130&Sectionid=71748230. Accessed February 02, 2016.
35. Tannock IF, Bristow RG, Hill RP, Harrington L. The Basic Science of Oncology. 5th ed. New York, NY: McGraw Hill; 2013.
36. Yoon HH, Sinicrope FA. Personalized medicine and targeted therapy of esophageal cancer. In: Tan, D Lynch HT, eds: Principles of Molecular DIagnostics and Personalized Cancer Medicine. Lippincott Williams & Wilkins. 2013: Chap 55.
37. Jancik S, Drabek J, Radzioch D, Hajduch M. Clinical relevance of KRAS in human cancers. J Biomed Biotechnol. 2010;2010:150960.
38. Sanchez-Torres JM, Viteri S, Molina MA, Rosell R. BRAF mutant non-small cell lung cancer and treatment with BRAF inhibitors. Transl Lung Cancer Res. 2013;2:244-250.
39. Ibanez CF. Structure and physiology of the RET receptor tyrosine kinase. Cold Spring Harb Perspect Biol. 2013;5.
40. Kohno T, Tsuta K, Tsuchihara K, Nakaoku T, Yoh K, Goto K. RET fusion gene: translation to personalized lung cancer therapy. Cancer Sci. 2013;104:1396-1400.
41. Cometriq (cabozantinib) [package insert]. Exelexis, Inc. South San Francisco, CA. 2012.

42. Davies KD, Doebele RC. Molecular pathways: ROS1 fusion proteins in cancer. Clin Cancer Res. 2013;19:4040-4045.
43. American Cancer Society. Lung Cancer (non-small cell). Available at: http://www.cancer.org/acs/groups/cid/documents/webcontent/003115-pdf.pdf. Accessed November 25, 2014.
44. Pass HI, Carbone DP, Johnson DH, et al. Principles and Practice of Lung Cancer: The Official Reference Text of the IASLC. Philadelphia, PA: Lippincott Williams & Wilkins; 2010.
45. DeVita VT, Lawrence TS, Rosenberg SA. DeVita, Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology. 8th ed. Vol 1. Philadelphia, PA: Lippincott Williams & Wilkins; 2008.
46. Stedman T. Stedman's Online Medical Dictionary. 2015;Available at: http://www.stedmansonline.com. Accessed February 20, 2015.
47. National Institute of Health. Segmentectomy (definition). Available at: http://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=538164. Accessed January 15, 2016.
48. National Cancer Institute. Pathology reports. Available at: http://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis/pathology-reports-fact-sheet. Accessed February 12, 2016.
49. Naidoo J, Drilon A. Molecular diagnostic testing in non-small cell lung cancer. Am J Hematol/Oncol. September 2014.
50. Walsh K, Wallace WA. Molecular pathology in lung cancer: a guide to the techniques used in clinical practice. Histopathology. 2014;65:731-741.
51. Thunnissen E, Bubendorf L, Dietel M, et al. EML4-ALK testing in non-small cell carcinomas of the lung: a review with recommendations. Virchows Arch. 2012;461:245-257.
52. Soda M, Isobe K, Inoue A, et al. A prospective PCR-based screening for the EML4-ALK oncogene in non-small cell lung cancer. Clin Cancer Res. 2012;18:5682-5689.
53. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Arch Pathol Lab Med. 2013;137:828-860.
54. Abeloff MD. Abeloff's Clinical Oncology. 4th ed. Philadelphia, PA: Elsevier Churchill Livingstone 2008.
55. American Cancer Society. Radiation therapy for non-small cell lung cancer. Available at: http://www.cancer.org/cancer/lungcancer-non-smallcell/detailedguide/non-small-cell-lung-cancer-treating-radiation-therapy. Accessed November 25, 2014.
56. National Cancer Institute. Targeted cancer therapies. Available at: http://www.cancer.gov/about-cancer/treatment/types/targeted-therapies/targeted-therapies-fact-sheet. Accessed February 15, 2016.
57. Cisplatin [package insert]. Bristol-Myers Squibb Company. Princeton, NJ. 2010.
58. Carboplatin [package insert]. Bristol-Meyers Squibb Company. Princeton, NJ. 2010.
59. Taxotere (docetaxel) [package insert]. Sanofi-Aventis US LLC. Bridgewater, NJ. 2010.
60. Taxol (paclitaxel) injection [package insert]. Bristol-Meyers Squibb Company. Princeton, NJ. 2011.
61. Etopophos (etoposide for injection) [package insert]. Bristol-Meyers Squibb Company. Princeton, NJ. 2013.
62. Camptosar (irinotecan) [package insert]. Pfizer Inc. New York, NY. 2012.
63. Gemzar (gemcitabine for injection) [package insert]. Lilly USA, LLC. Indianapolis, IN. 2014.
64. Alimta (pemetrexed for injection) [package insert]. Lilly USA, LLC. Indianapolis, IN. 2013.

65. Navelbine (vinorelbine tartrate) injection [package insert]. GlaxoSmithKline. Research Triangle Park, NC. 2002.
66. Vinblastine [package insert]. APP Pharmaceuticals. Schaumburg, IL. 2011.
67. Mitomycin [package insert]. Bedford Laboratories. Bedford, OH. 2013.
68. Ifosfamide [package insert]. Baxter Healthcare Corporation. Deerfield, IL. 2012.
69. Avastin (bevacizumab) Prescribing Information. Genentech Inc. South San Francisco, CA. 2015.
70. Cyramza (ramucirumab) [package insert]. Eli Lilly and Company. Indianapolis, IN. 2015.
71. Tarceva (erolotinib) [package insert]. Genentech Inc. South San Francisco, CA. 2015.
72. Iressa (gefitinib) [package insert]. AstraZeneca Pharmaceuticals LP. Wilmington, DE. 2015.
73. Gilotrif (afatinib) [prescribing information]. Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, CT. 2015.
74. Xalkori (crizotinib) Prescribing Information. Pfizer Inc. New York, NY. 2016.
75. Alecensa (alectinib) capsules [package insert]. Genentech Inc. South San Francisco, CA. 2015.
76. Zykadia (certinib) [package insert]. Novartis Inc. East Hanover, NJ. 2015.
77. Opdivo (nivolumab) [package insert]. Bristol-Meyers Squibb Company. Princeton, NJ. 2016.
78. Keytruda (pembrolizumab) [package insert]. Merck & Co. Whitehouse Station, NJ. 2015.
79. Tsao MS, Hirsch FR, Tatabe Y. IASLC Atlas of ALK Testing in Lung Cancer. IASLC Press. Aurora, CO, USA. 2013.
80. Yarbro CH, Wujcik D, Gobel BH. Cancer Nursing: Principles and Practice. Jones and Bartlett Publishers. Sudbury, Mass. 2011.
81. Sausville EA, Longo DL. Principles of cancer treatment. In: Kasper D, Fauci A, Hauser. S, Longo D, Jameson J, Loscalzo J, eds. Harrison's Principles of Internal Medicine. 19th ed. New York, NY: McGraw-Hill; 2015.
82. Tagrisso (osimertinib) tablet [package insert]. AstraZeneca Pharmaceuticals LP. Wilmington, DE. 2015.
83. Peters S, Zimmermann S. Targeted therapy in NSCLC driven by HER2 insertions. Transl Lung Cancer Res. 2014;3:84-88.
84. Herceptin (trastuzumab) [package insert]. Genentech, Inc. South San Francisco, CA. 2015.
85. Ventana receives FDA approval for the first fully automated, IHC companion diagnostic to identify lung cancer patients eligible for XALKORI® (crizotinib). Ventana Medical Systems, Inc. Tucson, AZ. 2015.