Lung Cancer

SJMC Molecular Diagnostics Laboratory

CME Prepared 2021-2022

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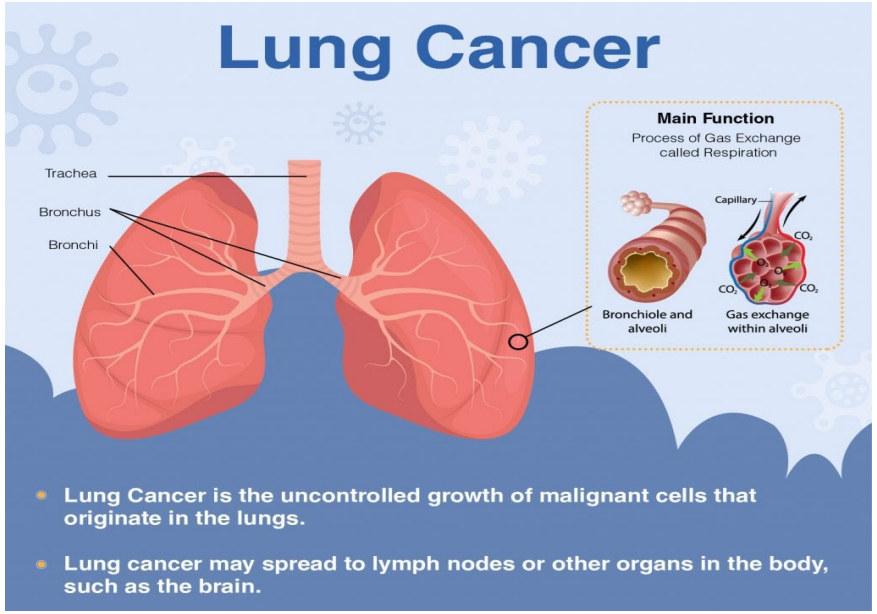
Background

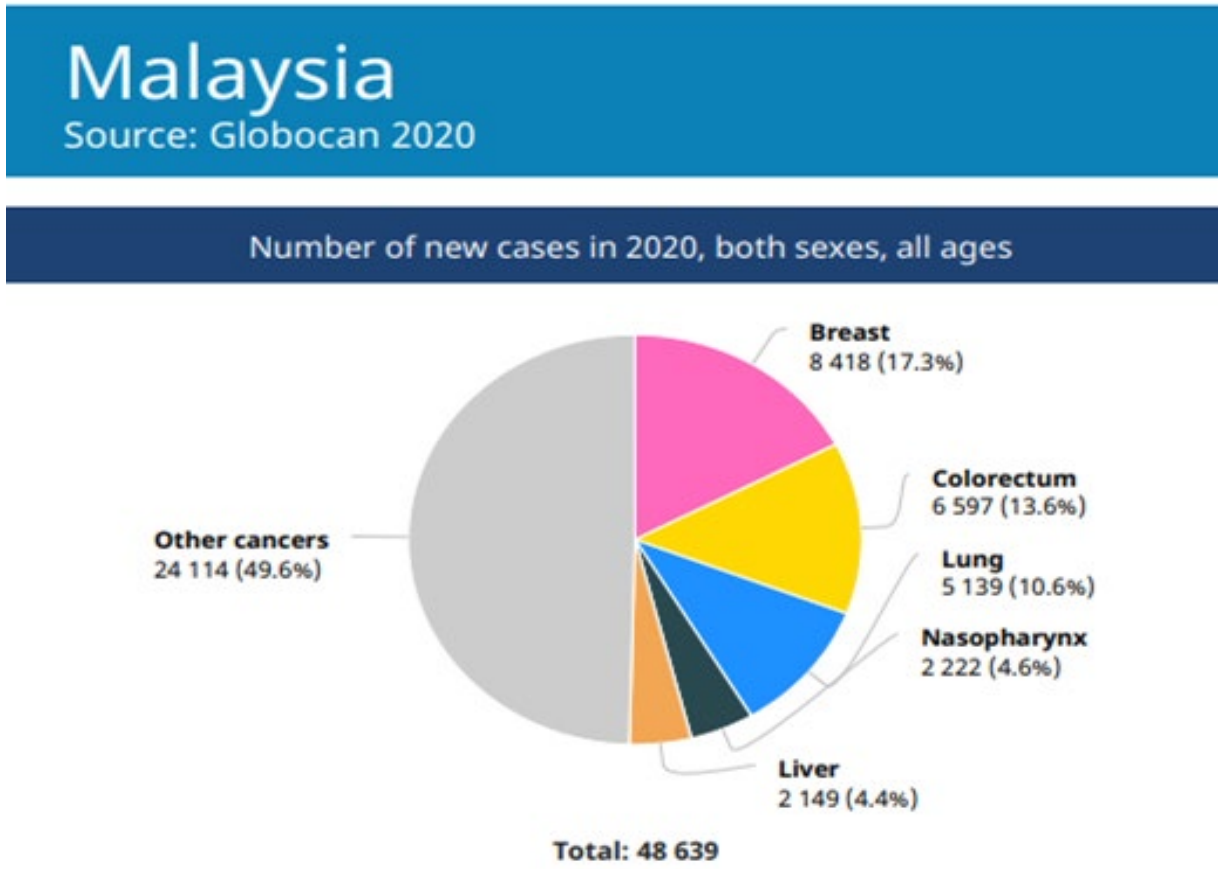
- **Definition**: Lung cancer = uncontrolled growth of tumour cells that begin in the lungs.
- Cancerous cells may spread to surrounding healthy tissues, such as the lining of the lungs and nearby lobes. This is known as local metastasis.
- However, if the cancerous cells invade the lymph nodes and travel through the lymphatic system to other organs, such as the brain and liver, this is known as distant metastasis(1).
- The median age of lung cancer diagnosis for men and women is 70 years(2).



Anatomy of the lung (3)

Background

- Lung cancer is the leading cause of cancer-related deaths making up almost 20% of all cancer deaths.
- Number of new cases for lung cancer is third highest in Malaysia (10.6%) after breast and colorectal cancer.
- The lifetime risk is approximately 1 in 55 for Malaysian males: risk is highest in Chinese males (1 in 43), followed by Malays (1 in 62) and Indians (1 in 103).
- For women, the risk is approximately 1 in 135[4].
- Most common type of lung cancer is non-small cell lung cancer (NSCLC) adenocarcinoma[5].



Number of new cancer cases in Malaysia in 2020 for males and females of all ages[6]

Types of Lung Cancer

There are 2 main types of lung cancer. Treatment and prognosis differ depending on the type of lung cancer.

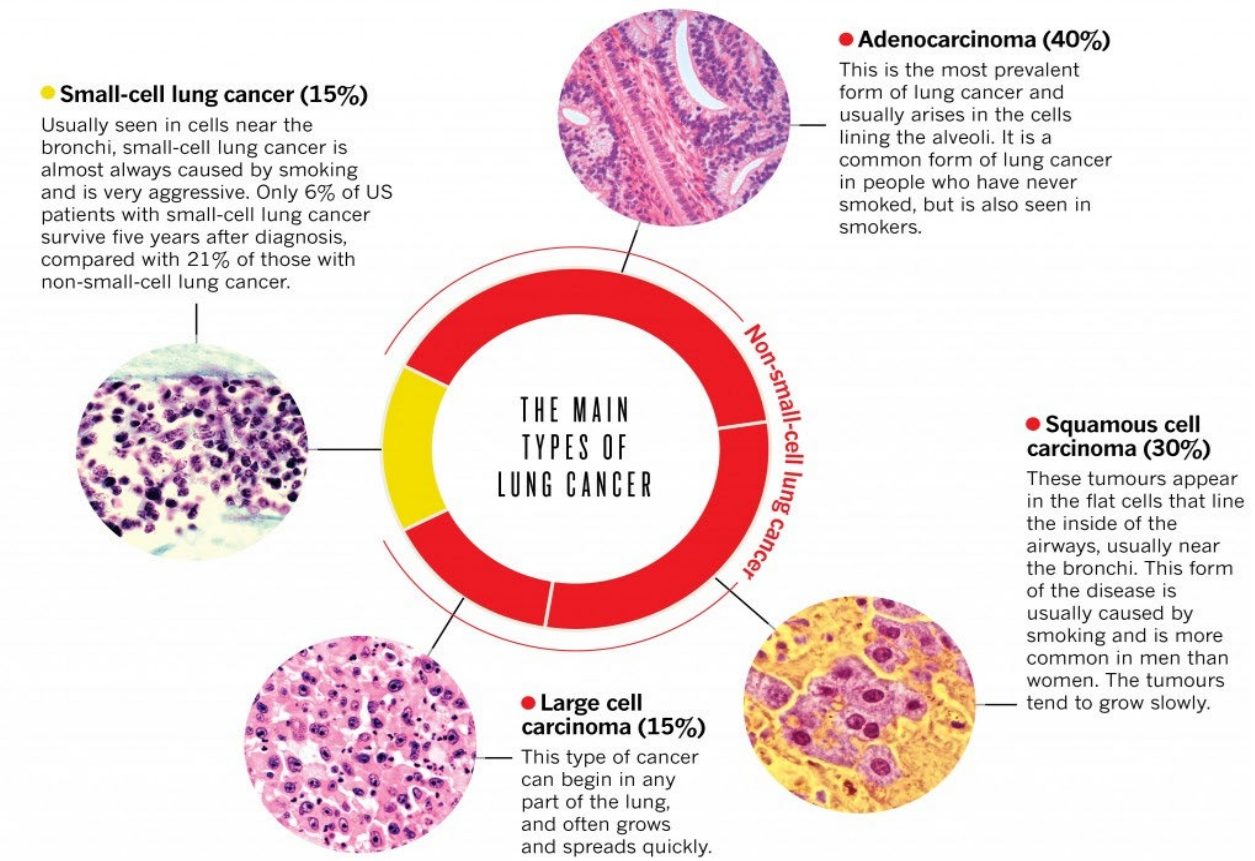
Non-small cell lung cancer (NSCLC)

About 80% to 85% of lung cancers are NSCLC. Most common sub types of NSCLC are:

- Adenocarcinoma (30-40%)
- Squamous cell carcinoma (30%)
- Large cell carcinoma (15%)
- Adenosquamous carcinoma (ASC) (4%)
  a relatively rare subtype- a malignancy containing components of lung adenocarcinoma (ADC) and lung squamous cell carcinoma (SCC).

Small cell lung cancer (SCLC)

About 15% of all lung cancers are SCLC, sometimes called oat cell cancer.



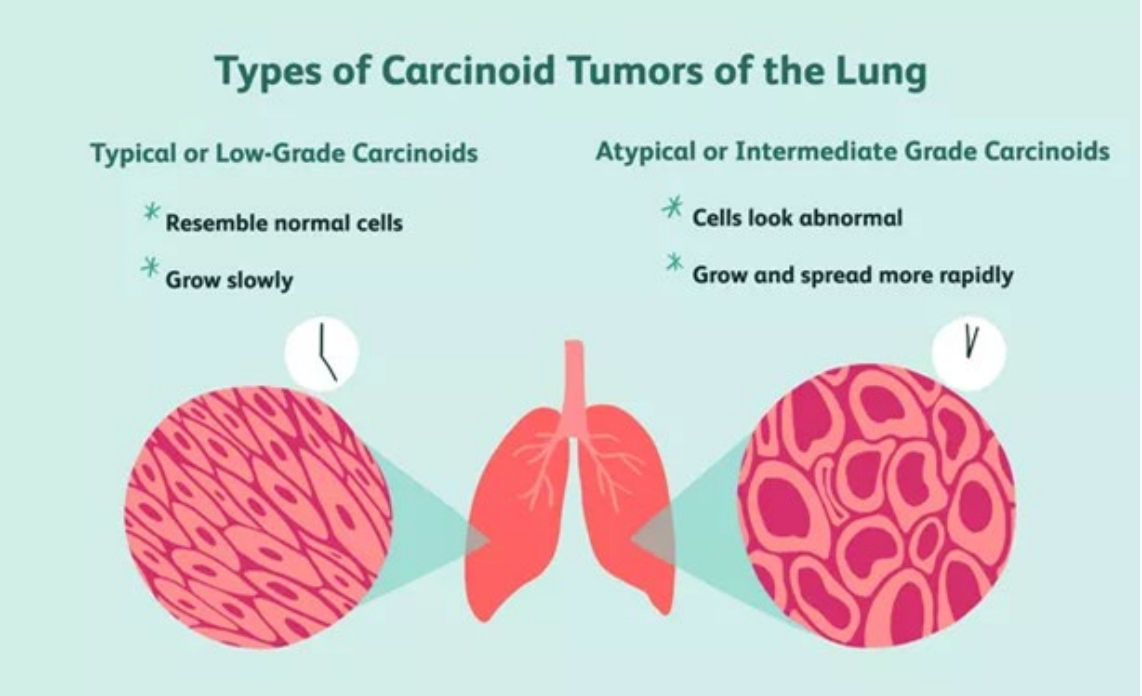
The different types of lung cancer, including the histology of each type[7]

Types of Lung Cancer

There are other types of lung tumours

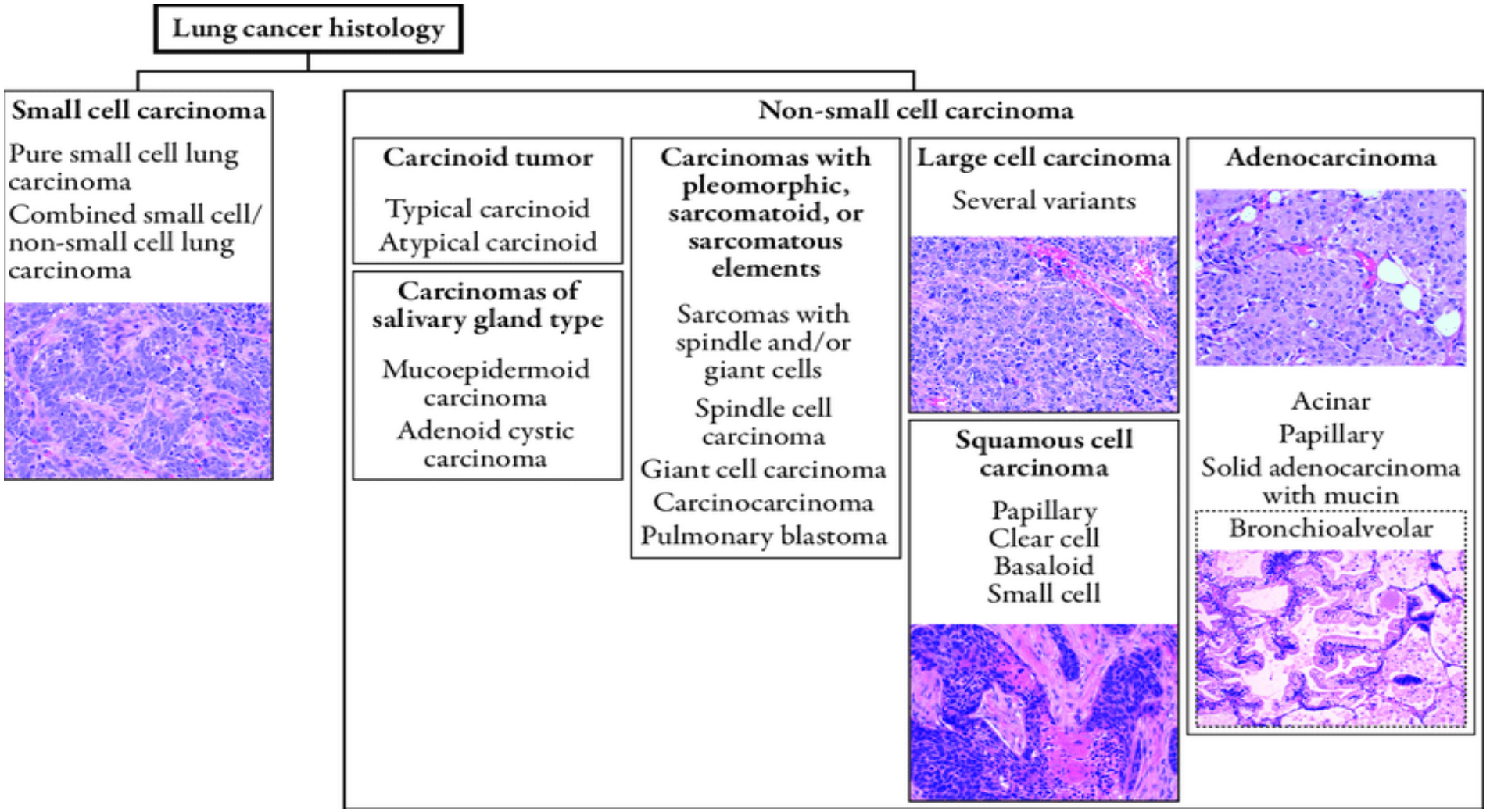
Lung carcinoid tumours account for fewer than 5% of lung tumours, most of these grow slowly.

Other types of lung cancer such as adenoid cystic carcinomas, lymphomas, and sarcomas are rare.



Two types of lung carcinoid tumours: typical and atypical. The differences in histology and growth rate between typical and atypical tumours are shown above[8]

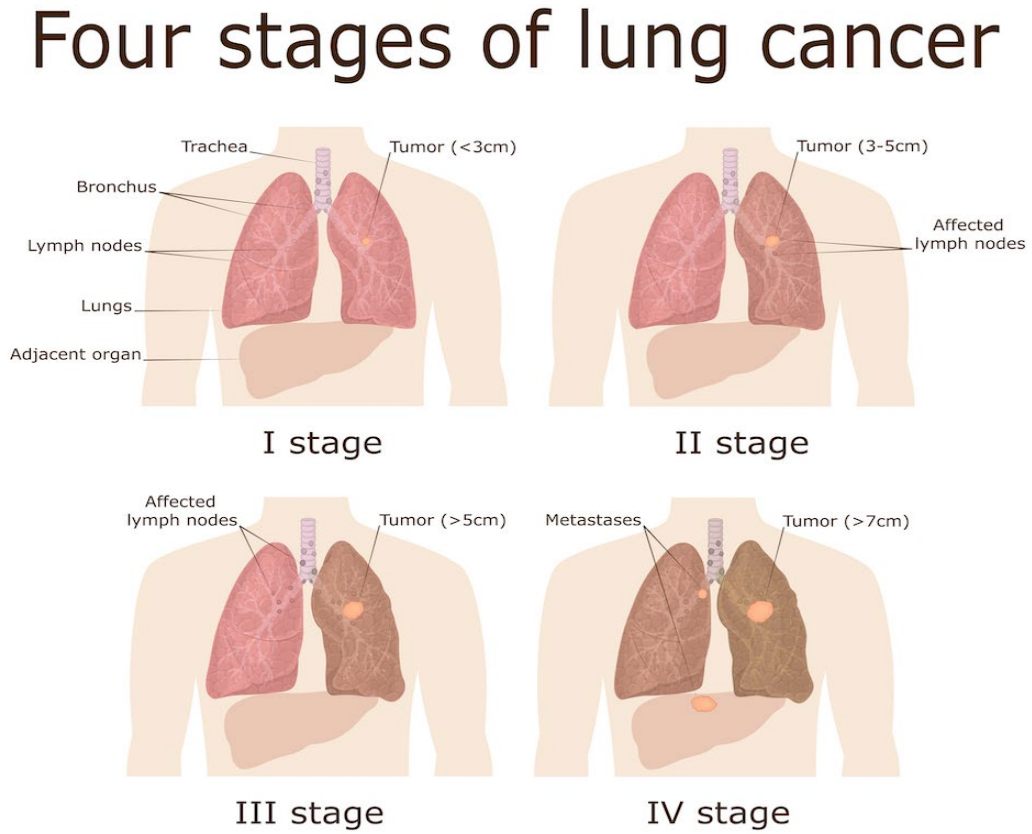
Types of Lung Cancer (Histology)



Histology of the different types of lung cancers - Small Cell Carcinoma (SCLC) and Non-Small Cell Carcinomas (NSCLC)[9]

Stages of Lung Cancer for NSCLC

Stage	What it means
Stage I	The cancer is relatively small and contained within the organ which it started
Stage II	The cancer has not spread into surrounding tissue but the tumour is larger than in stage I. Sometimes stage II means that cancer cells have spread into lymph nodes close to the tumour
Stage III	The cancer is large and may have spread into surrounding tissues and lymph nodes in the area
Stage IV	The cancer has spread to distant parts of the body

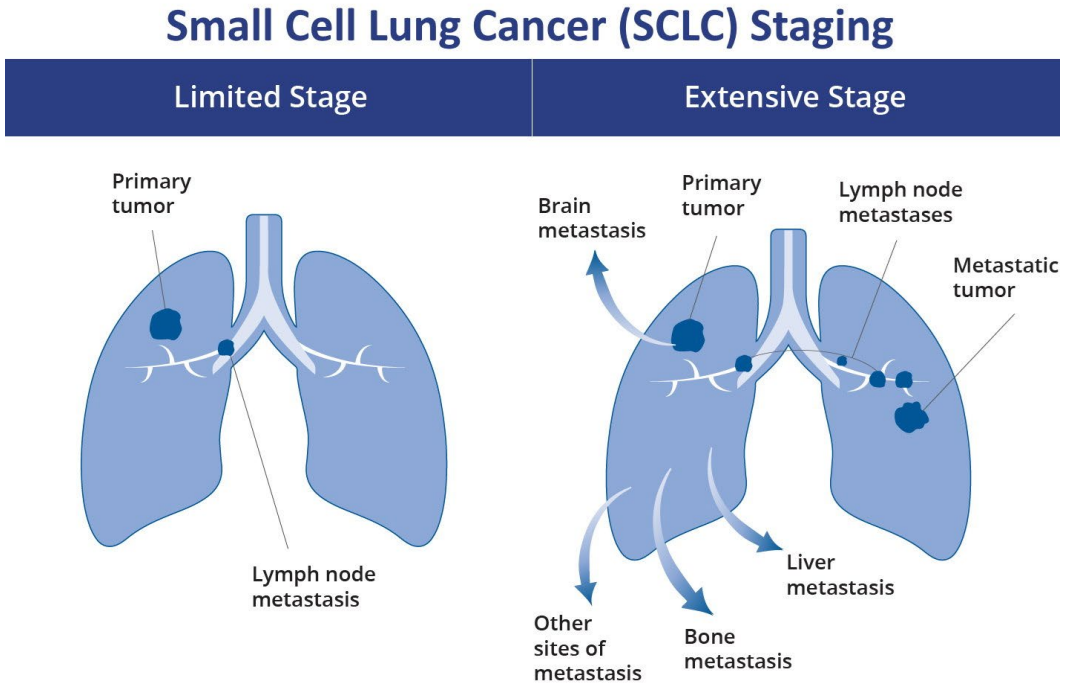


The staging system for any cancer was created to put cancers into groups that correlate with the prognosis and into categories that determine treatment. A simplified description of the 4 stages of lung cancer is shown above[10]

Stages of Lung Cancer for SCLC

1. **Limited** stage - cancer cells are **only on one side of the lung and can be treated with radiotherapy to that one area**. This includes cancer cells that might have spread to nearby lymph nodes on the same side of the chest[11].

1. **Extensive** stage – Cancer cells that have **spread throughout the lung**. For instance, to the other lung, to lymph nodes on the other side of the chest, or to other parts of the body, such as bone, brain and bone marrow. Patients in this stage cannot be treated with radiotherapy to just one area[12].



Staging of SCLC. Staging is required to determine course of treatment[13]

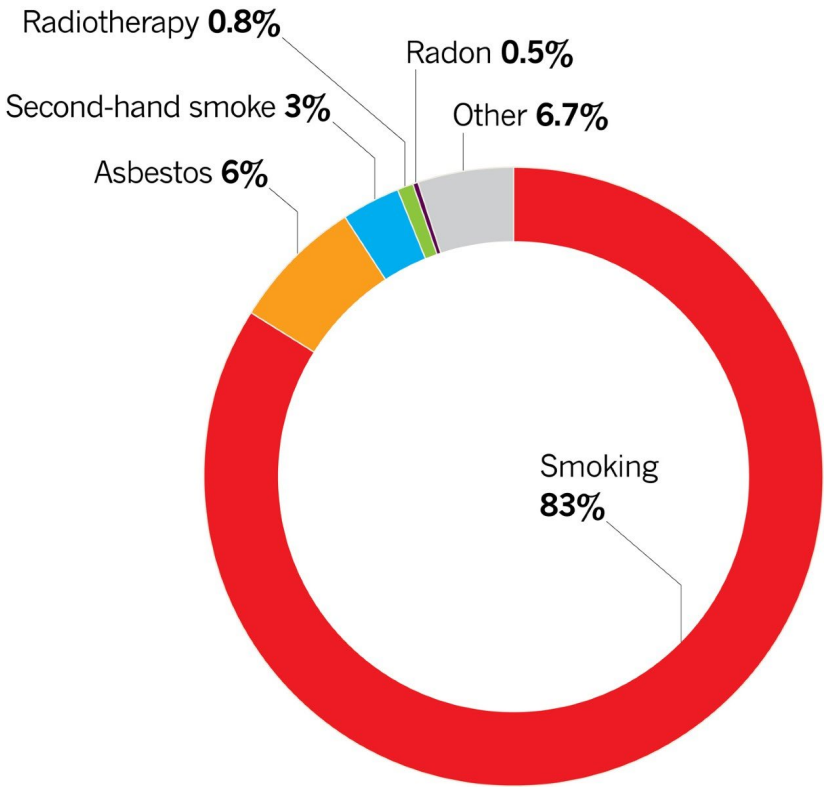
Risk Factors, Symptoms & Treatment

Risk factors:
- Smoking (tobacco)
- Secondhand smoke
- Exposure to radon, asbestos

Symptoms: Usually appears during advanced stages

- Persistent coughing
- Coughing up blood
- Chest pain
- Unexplained weight loss

Treatment:
- Surgery
- Radiation therapy
- Chemotherapy
- Targeted drug therapy
- Immunotherapy
- Palliative care



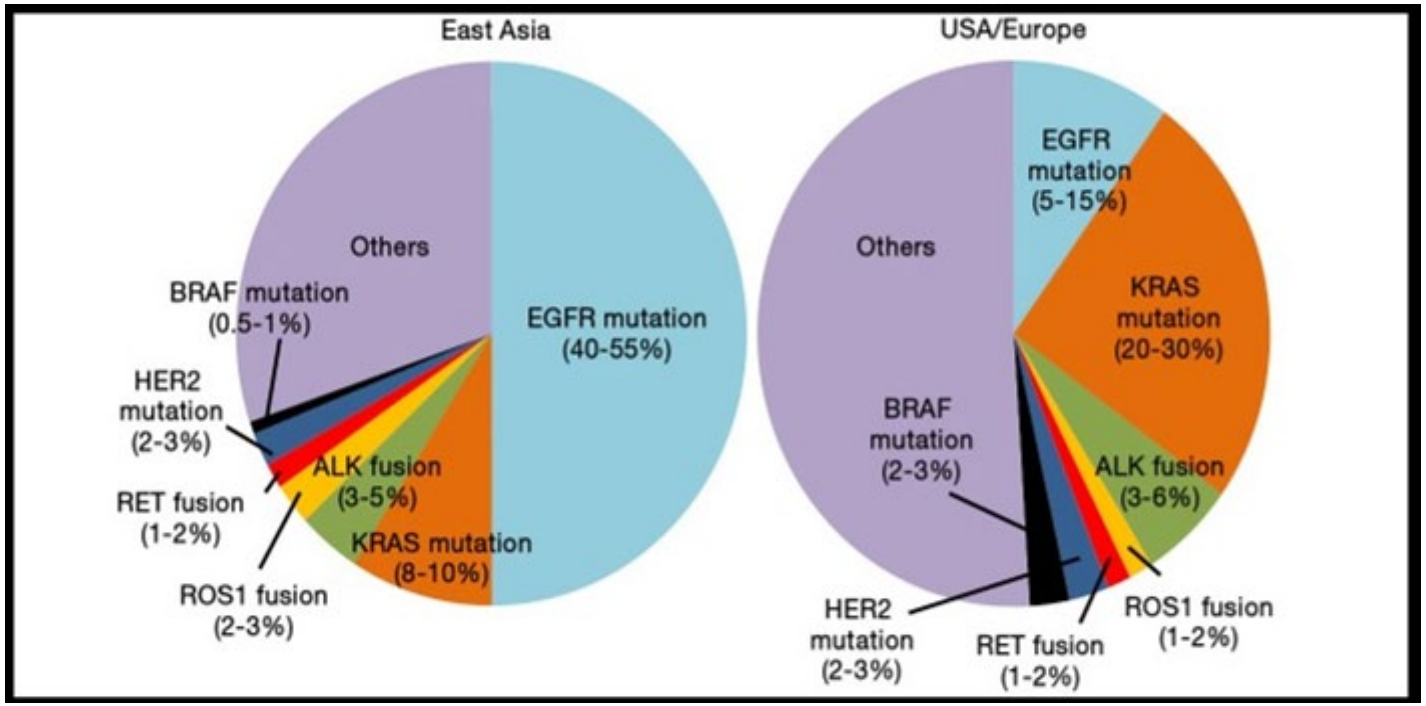
Most cases of lung cancer are attributable to smoking[7]

Driver oncogenes

Driver oncogenes include *EGFR*, *KRAS*, *BRAF*, and *HER2*/*ERBB2*, which are activated by missense and/or insertion/deletion mutations.

The *ALK* gene, which is activated by fusion to other genes also called partner genes. Aberrations of these genes are mutually exclusively, therefore they are believed to drive lung adenocarcinoma development.

EGFR mutation account for 40-55% of lung adenocarcinoma in East Asia population vs Europe population which is approximately 5-15%[14].



Pie charts showing the proportion of lung adenocarcinoma harboring aberrations in driver oncogenes. Data from patients in East Asia (Japan, Korea, and China) and from those of European descent[14]

EGFR Mutation Frequency

- One study has shown that EGFR mutations were present in 39.5% of patients in Malaysia.
- Most common mutations: exon 19 deletion (23.5%) & exon 21 substitution (14.9%).
- Mutations were more frequent in women (52.5%) than men (27.8%).
- EGFR mutations were more common among Chinese (40.8%), followed by Malay (37.2%) and Indian (33.3%). The difference was not statistically significant.
- Patients with smoking record: 211 patients.
- Mutation rate of never-smokers (54.8%) higher than never-smokers (20.7%)[15].

Clinical Characteristics	*n*	(%)
Age, mean + SD	812	58.9 + 12.0
Sex, *n* (%)		
Women	398	(49)
Men	414	(51)
Ethnicity, *n* (%)		
Chinese	517	(63.7)
Malay	239	(29.4)
Indian	39	(4.8)
Other	17	(2.1)
Smoking status*, n (%)	211	
Never-smoker	124	(58.8)
Former smoker	38	(18.0)
Current smoker	49	(23.2)
EGFR mutation detection method, *n* (%)		
Direct sequencing	416	(51.2)
Real-time PCR	396	(48.8)
EGFR gene mutation status, *n* (%)		
Negative (wild-type)	491	(60.5)
Positive	321	(39.5)
Exon 18	5	(0.6)
Exon 19 deletion	191	(23.5)
Exon 20	16	(2.0)
Exon 21 substitution	121	(14.9)
No. of mutations		
Single	310	(38.2)
Two	10	(1.2)
Three	1	(0.1)

* Smoking status known in only 211 patients.
EGFR, epidermal growth factor receptor; SD, standard deviation; PCR, polymerase chain reaction

Clinical characteristics of patients and EGFR mutation results[15]

Molecular Testing and Targeted Therapy in NSCLC

- Molecular testing has become a mandatory component of the non-small cell lung cancer (NSCLC) management.
- The detection of EGFR, BRAF and MET mutations as well as the analysis of ALK, ROS1, RET and NTRK translocations have already been incorporated in the NSCLC diagnostic standards, and the inhibitors of these kinases are in routine clinical use.
- Comprehensive NSCLC testing for multiple predictive markers requires the analysis of distinct biological molecules (DNA, RNA, proteins) and, therefore the involvement of different analytical platforms:

- Real-time polymerase chain reaction (real-time PCR)
- Droplet digital PCR (ddPCR)
- DNA Sequencing
- Immunohistochemistry (IHC)
- Fluorescence In Situ Hybridization (FISH)
- Next Generation Sequencing (NGS) - an ongoing effort aimed at the integration of multiple NSCLC molecular assays into a single diagnostic pipeline.

Diagnostic Methods for Somatic Mutation Testing in NSCLC

1. Real-time PCR

- Technique based on the polymerase chain reaction which monitors the amplification of a targeted DNA molecule in real time. Two types of detection methods:
  - Fluorescence dye/DNA binding dye
  -DNA probe

- Tests available in SJMC

-EGFR (cobas® EGFR Mutation Test v2 identifies 42 mutations in exons 18, 19, 20 and 21 of the epidermal growth factor receptor (EGFR) gene, including the T790M resistance mutation.

-KRAS/NRAS (AmoyDx KRAS/NRAS Detection of 19 KRAS mutations (exons 2, 3 and 4) and 13 NRAS mutations (exons 2, 3 and 4)

Basic Principles of Real-time PCR

Three main steps in Real-time PCR:

1. Denaturation

Occurs at 95°C

Double-stranded DNA is denatured and forms two single-stranded DNA templates.

2. Annealing

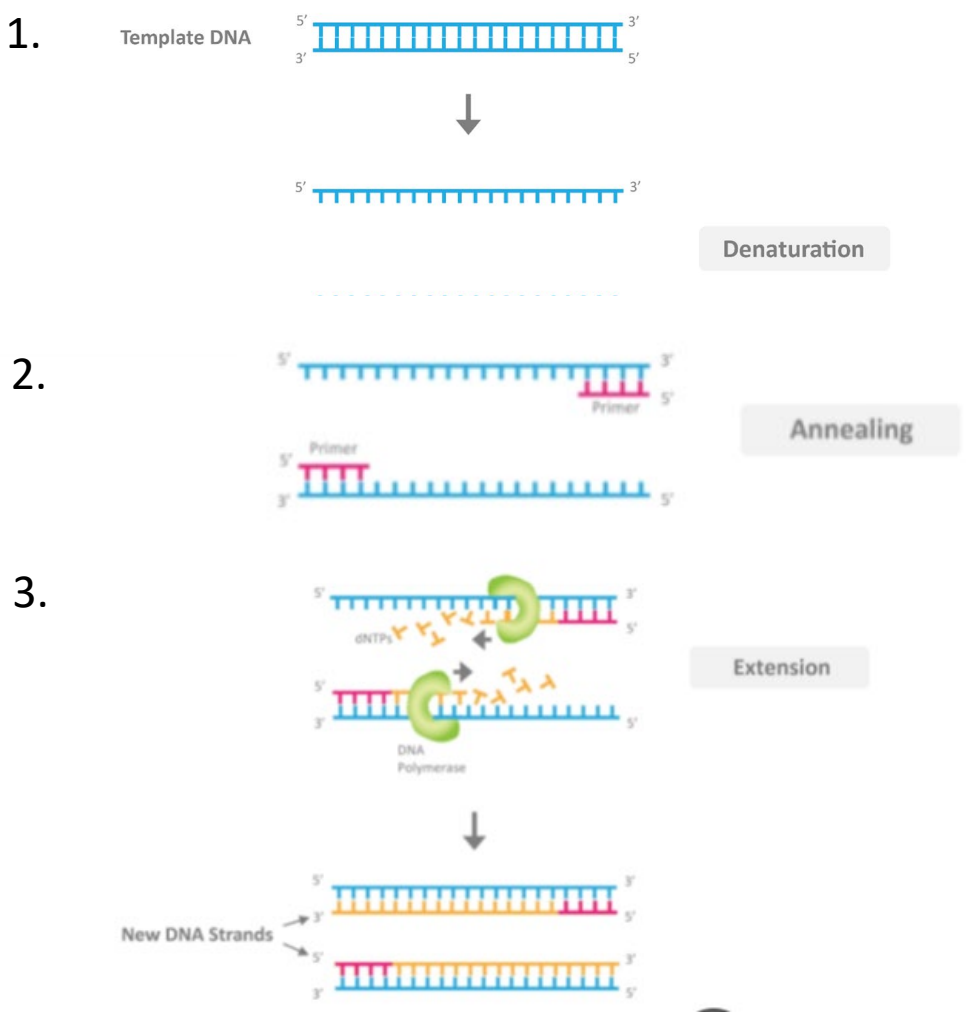
Occurs at 50-56°C

Sequence-specific primers bind to regions on the target DNA along with fluorescent dye / DNA probe.

3. Extension

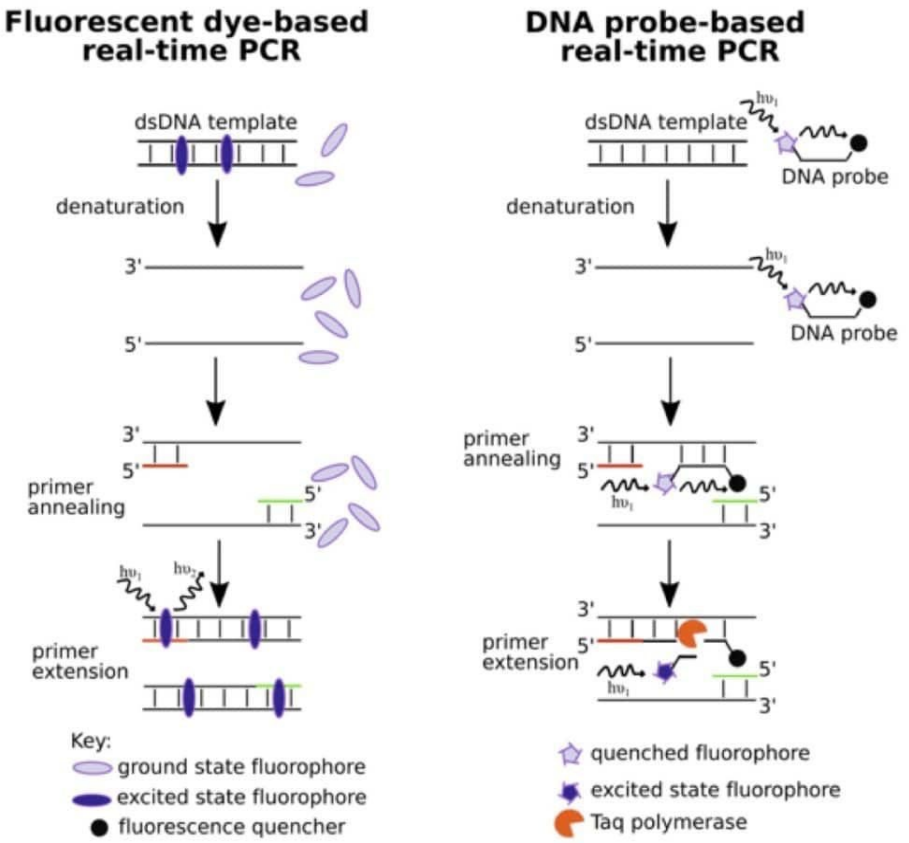
Occurs at 72°C

Taq DNA polymerase adds nucleotides to template strands.



The PCR process(16)

Two types chemistry in Real-time PCR



The real-time PCR process highlighting the two types of chemistry (fluorescence-based and DNA probe-based)[17]

Fluorescent dye-based: *eg:* SYBR Green which binds to the minor groove of DNA double helix. The intensity of fluorescence increases as the dye binds to DNA, which increases after each amplification cycle[18].

DNA-probe based: *eg:* TaqMan probes are labeled with a reporter fluorophore at 5' end and quencher fluorophore at 3' end. The probe, which is complementary to the target sequence is added to PCR mixture. During the PCR extension process, the polymerase cleaves the probe and releases the reporter fluorophore and hence, increases fluorescence intensity[19].

EGFR Real-time PCR

The cobas® EGFR Mutation Test v2 – orthogonal method for EGFR

- A real-time polymerase chain reaction (PCR) test that identifies 42 mutations in exons 18, 19, 20 and 21 of the epidermal growth factor receptor (EGFR) gene, including the T790M resistance mutation[20].
- Clinically validated in multiple clinical trials as a companion diagnostic (CDx) for both 1st and 2nd line EGFR TKI therapy in patients with advanced NSCLC in cfDNA and FFPET
- The sensitivity of real-time PCR is as low as **5% mutation** in wild type background of DNA.

The difference in duration and workflow process of cobas EGFR real-time PCR with plasma and tissue samples[20]

2.Droplet digital PCR (ddPCR) Bio-Rad

- A digital PCR method based on water-oil emulsion droplet technology. The ddPCR System partitions nucleic acid samples into thousands of nanolitre-sized droplets, and PCR amplification is carried out within each droplet[21]. The sensitivity of ddPCR is as low as **0.1% mutation** in wild type background of DNA.

- Test available in SJMC
  -Liquid biopsy : EGFR (exon 19 Deletion/exon 21 L858R/ exon 20 T790M/ exon 20 C797S)
  -Solid tumour: BRAF

Workflow of Bio-Rad ddPCR test[22]

Consists of two instruments: the QX200 Droplet Generator and the QX200 Droplet Reader.

- QX200 Droplet Generator produces thousands of nanolitre-sized droplets from the ddPCR reaction mix.
- QX200 Droplet reader analyzes each sample after PCR on a thermal cycler.
- Target DNA is quantified by counting number of PCR-positive and negative droplets.
- This method can process up to 96 samples per run[22].

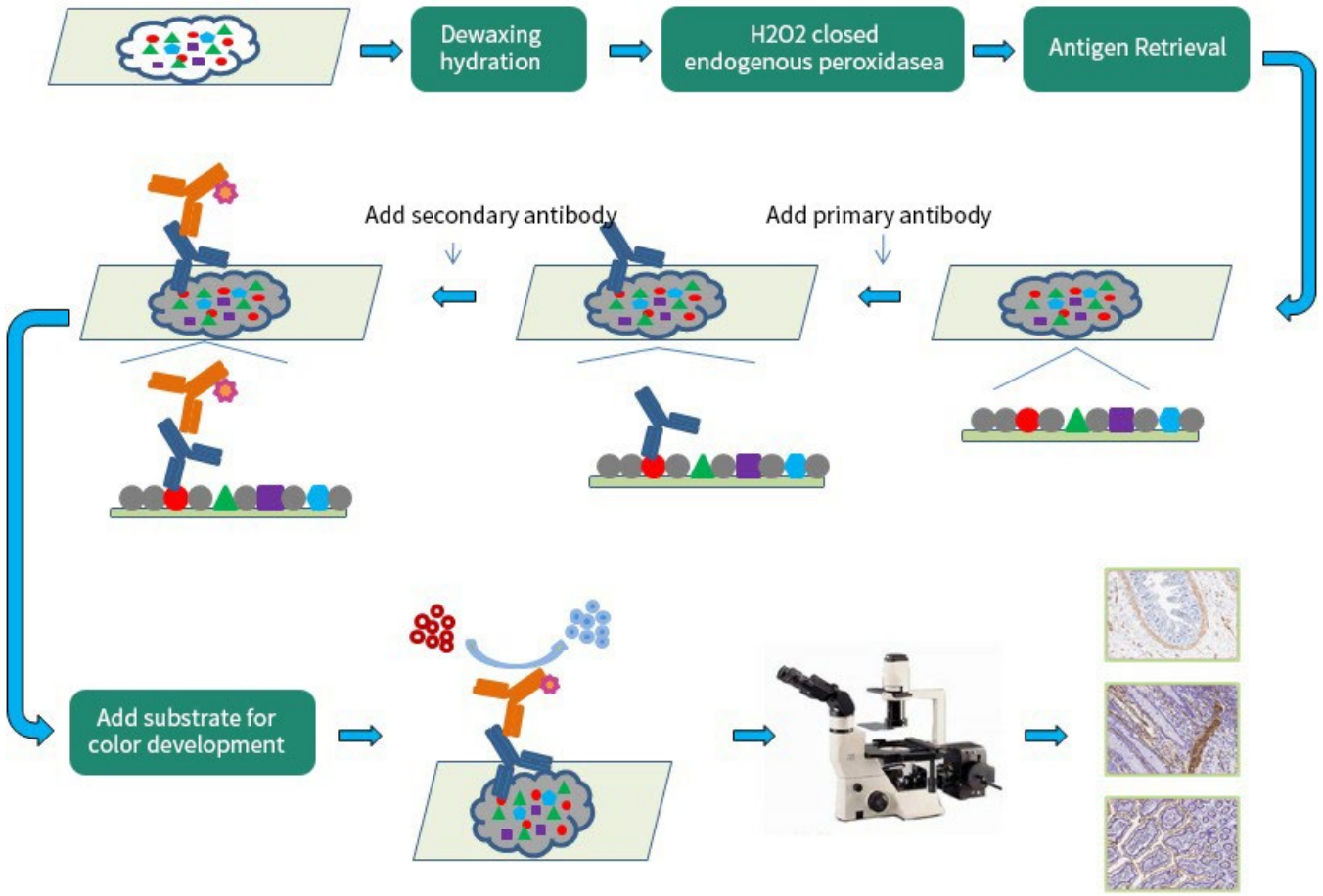
3. Immunohistochemistry (IHC)

- Immunohistochemistry (IHC) is based on the binding of antibodies tagged with a visible label to the specific antigens in tissue sections.

- Recently, the FDA has approved an immunohistochemical assay utilizing ALK antibody clone D5F3 as a companion diagnostic assay for patients that have positive ALK-rearrangement NSCLC. This is because recent studies have demonstrated that ALK antibody clones D5F3 and 5A4 exhibit the highest sensitivity and specificity for ALK rearrangements, as compared to other available anti-ALK antibodies. Hence, NSCLC patients with positive ALK mutation (verified by immunostaining using clone D5F3), may benefit from ALK inhibitor treatment[23,24].

- Test available in SJMC
  - -ALK rearrangement
  - -Microsatellite instability (MSI)
  - -PD-L1

Immunohistochemistry (IHC) Workflow



IHC process including tissue fixation, blocking, antigen retrieval, antibody labeling and visualization[25]

Tissue Fixation - using the snap-frozen and acetone-fixed or the formalin-fixed and paraffin-embedded (FFPE) method.

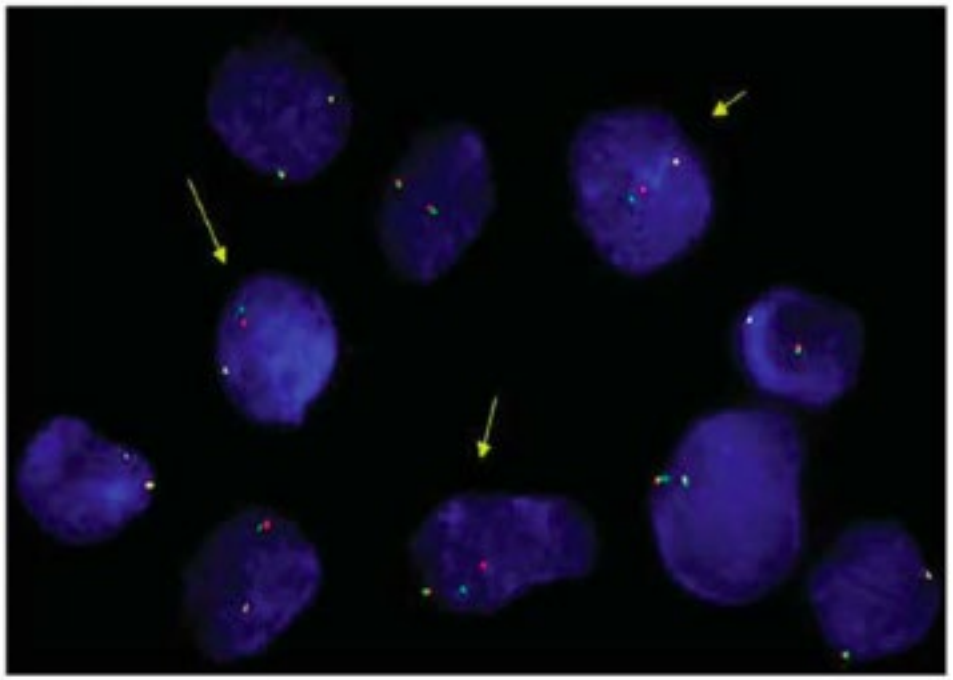
Blocking - to block endogenous enzymes that could activate substrates used for visualization. Peroxidase or alkaline phosphatase may be used for blocking.

Antigen Retrieval - pre-treatment with antigen retrieval agents can improve antigen expression of samples.

Antibody Labeling and Visualization – by indirect or direct detection.

4. Fluorescence in situ hybridization (FISH)

- FISH is a molecular cytogenetic analysis technique that can detect and locate a specific DNA sequence on a chromosome.
- Principle of FISH: Chromosomes are exposed to a fluorescently-labeled DNA probe which complementary binds to the region of interest on the chromosome. Binding of probe to DNA sequence can be visualized using a fluorescence microscope[26]. Test offered in SJMC : ERBB2 amplification, MET amplification, ROS1 fusion, ALK Fusion



FISH test for RET gene rearrangement from tissue. Three positive cells with split signal pattern are observed and labeled with arrows (1 red, 1 green, 1 fusion)[27]

5. Sanger Sequencing

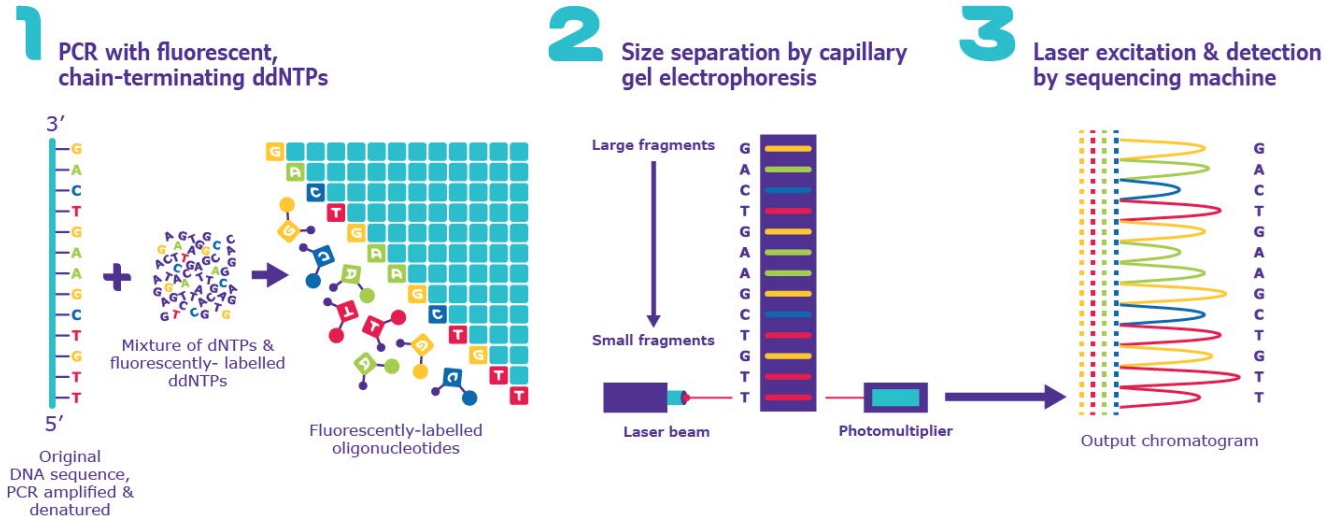
- Also known as “chain termination method”.
- Developed in 1977 by Frederick Sanger and his colleagues.
- A DNA sequencing method - used to determine the nucleotide sequence of DNA.
- Can be performed manually or by a sequencing machine (automated).
- However, next-generation sequencing technologies are becoming increasingly popular, supplanting the conventional Sanger Sequencing method.

SJMC no longer offers sanger sequencing due to major limitations of this method; high cost, labor intensiveness, and low sensitivity. The sensitivity of this method is **10%–20% mutations in the wild-type background.** Thus, low frequent mutations (< 10%) in tumor samples cannot be determined using Sanger sequencing.

Ion Torrent PGM Semiconductor instrument for Sanger sequencing[28]

Basic Principles of Sanger Sequencing

Sanger sequencing involves chain-termination PCR, gel electrophoresis, laser excitation and detection either manually or by sequencing machine (automated).



The basic steps of Sanger Sequencing[29]

1. Chain-termination PCR uses dideoxyribonucleotides (ddNTPs), which lack the 3'-OH group needed for phosphodiester bond formation between nucleotides. When ddNTPs are added, during the extension step of PCR, extension ceases as DNA polymerase incorporates a ddNTP at random. Oligonucleotide copies of the DNA sequence of interest, terminated at random lengths are created.
2. Gel electrophoresis is carried out to separate the oligonucleotides by size.
3. The gel is read either by a sequencing machine or manually to determine the DNA sequence[29].

6. Next-Generation Sequencing

- Also known as massive parallel or deep sequencing which offers ultra-high throughput, scalability, and speed.
- The entire human genome can be sequenced in a day, as compared to the Sanger sequencing method which took over 10 years.
- NGS technology enables the detection of relevant SNVs, CNVs, gene fusions and indels from unique genes using multiplex PCR to help inform drug discovery research and clinical trial research programs.
- Enables data analysis from as little as 10ng of nucleic acid.

- Test available in SJMC

  -Oncomine Comprehensive Assay v3M (161 unique genes including fusion), report within 3 weeks.

  -Oncomine Precision Assay GX specifically for lung/colon cancer (50 unique genes, including key targets within EGFR, BRAF, KRAS, ALK, ROS1, NTRK, RET, and others), both solid tissue and liquid biopsy samples. Report as early as 2-3 working days.

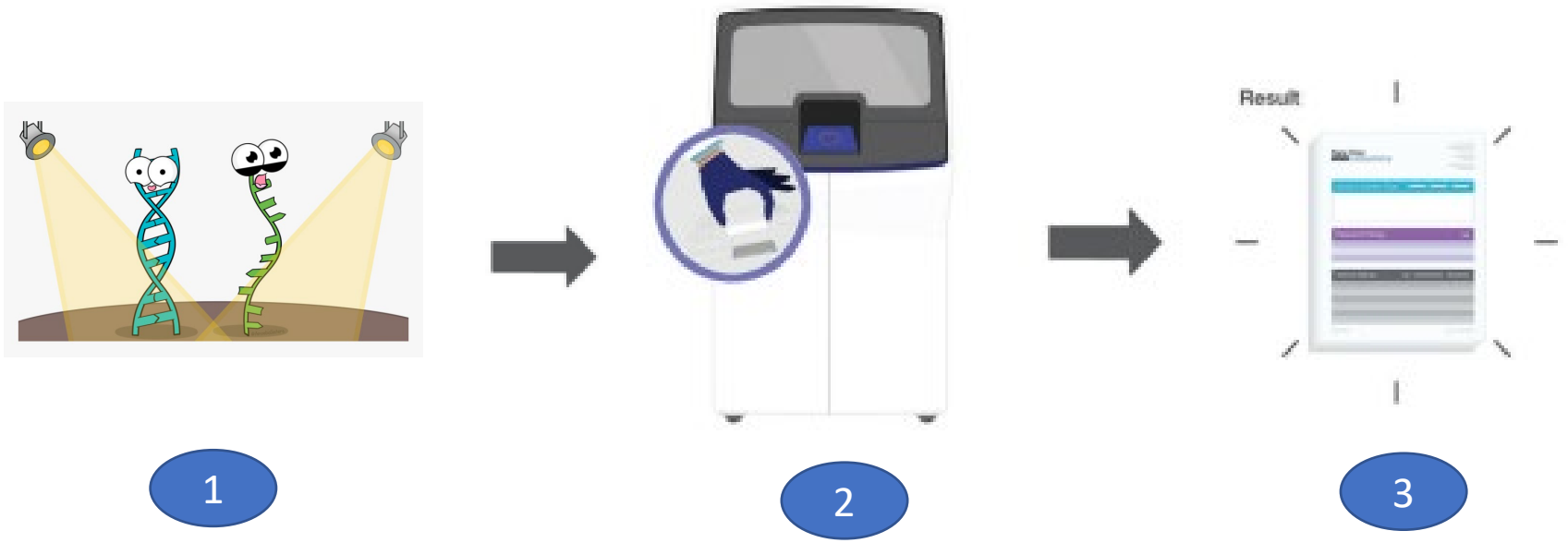
Oncomine Precision Assay GX for Ion-Torrent NGS[30]

Next-Generation Sequencing Ion GeneStudioS5 (*eg*: 161 unique genes)

NGS workflow using Ion GeneStudio S5 system[31].

1) Tumour sample: Nucleic acid extraction from FFPET samples, nucleic acid quantification.
2) Library Preparation: Library construction as little as 10ng of DNA/RNA.
3) NGS Templating and sequencing.
4) Post-sequencing analysis: Identification of variants and generating custom reports.

Next-Generation Sequencing Ion Torrent Genexus System / NGS Express (*eg*: Oncomine Precision Assay 50 genes delivers result wthin 2-3 working days)



NGS workflow using Ion Torrent Genexus instrument[32].

1. Nucleic acid (DNA/RNA) extraction and quantification, sequencing sample plate preparation.
2. Library preparation, templating and sequencing.
3. Analysis.

Targeted Therapy for Advanced/Metastatic NSCLC

Recent Targeted therapy for NSCLC

KRAS G12C Mutation

Targeted therapy : Sotorasib

In May 2021, FDA approves Lumakras (sotorasib) targeted therapy for adult patients with NSCLC whose tumours have KRAS G12C, and who have received at least one prior systemic therapy[33].

KRAS mutation was previously considered resistant to drug therapy and with this accelerated approval of sotorasib, the KRAS G12C is likely to predict clinical benefit to patients.

KRAS mutation accounts for approximately 25% of mutations in NSCLC. KRAS G12C mutations represent about 13% of mutations in NSCLC[34].

Recent Targeted therapy for NSCLC

EGFR Exon 20 Insertion

Targeted therapy: Amivantamab-vmjw

Within the same year 2021, FDA approves Rybrevant (amivantamab-vmjw) for adults with NSCLC with EGFR exon 20 insertion mutations[35]. Amivantamab-vmjw is a bispecific antibody targeting mutations in the *EGFR* and *MET* pathways

Approximately 2% to 3% of patients with NSCLC will have EGFR exon 20 insertion mutations[35].

EGFR resistant variants T790M & C797S

T790M

Lung cancer patients with an activating mutation in the EGFR (epidermal growth factor receptor) can develop resistance to EGFR tyrosine kinase inhibitors (TKI), which is often mediated by the T790M resistance mutation in exon 20 of the EGFR gene.

Patients with acquired resistance to first- or second-generation EGFR-TKIs, osimertinib is approved in the presence of the T790M resistance mutation. Osimertinib is a third-generation EGFR TKI that is selective for both EGFR-TKI-sensitizing and T790M.

C797S

EGFR mutation C797S in exon 20 is a resistance mutation to third generation EGFR TKI Osimertinib. There is no major breakthroughs that have been achieved so far to target C797S variant.

However, in some studies, patients harboring EGFR C797S in trans with T790M are sensitive to a combination of first- and third-generation EGFR TKI. However, patients harboring EGFR C797S in cis with T790M are resistant to combination therapy or each single reagent [37]

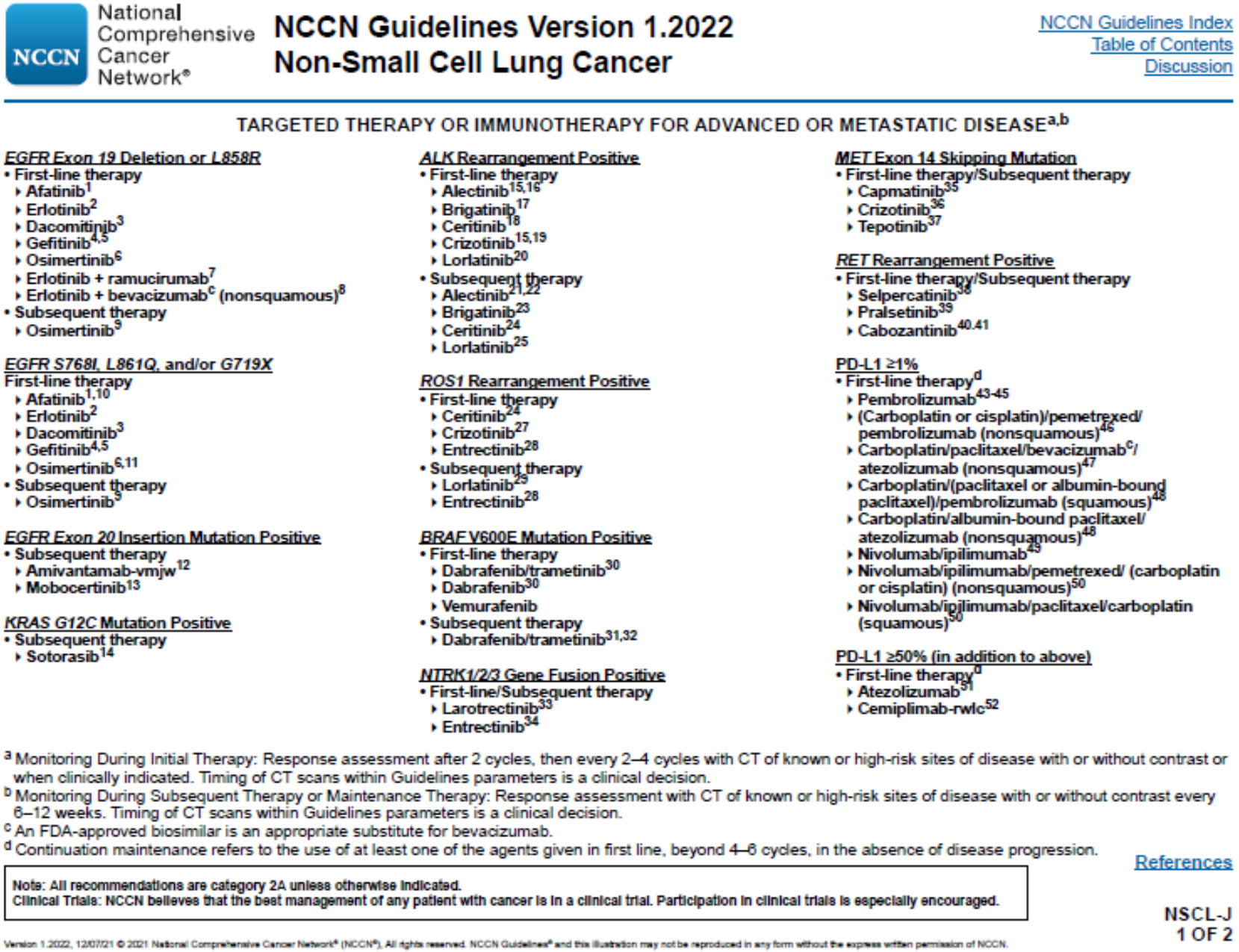
Summary of Targeted Therapy for Advanced/Metastatic NSCLC

EGFR Exon 19 Deletion or L858R	First line therapy
	Afatinib, Erlotinib, Dacomitinib, Gefitinib, Osimertinib, Erlotinib + Ramucirumab, Erlotinib + Bevacizumab (non squamous)
	Subsequent therapy
	Osimertinib
EGFR S768I, L861Q, and/or G719X	First line therapy
	Afatinib, Erlotinib, Dacomitinib, Gefitinib, Osimertinib
	Subsequent therapy
	Osimertinib
EGFR Exon 20 insertion	Subsequent therapy
	Amivantamab-vmjw
	Mobocertinib
KRAS G12C	Subsequent therapy
	Sotorasib
ALK fusion	First line therapy
	Alectinib, Brigatinib, Ceritinib, Crizotinib, Lorlatinib
	Subsequent therapy
	Alectinib, Brigatinib, Ceritinib, Lorlatinib

Summary of Targeted Therapy for Advanced/Metastatic NSCLC

ROS fusion	First line therapy
	Ceritinib, Crizotinib, Entrectinib
	Subsequent therapy
	Lorlatinib, Entrectinib
BRAF V600E Mutation Positive	First line therapy
	Dabrafenib/trametinib, Dabrafenib, Vemurafenib
	Subsequent therapy
	Dabrafenib/trametinib.
NRTK1/2/3 Gene fusion	First line therapy/Subsequent therapy
	Larotrectinib, Entrectinib
MET Exon 14 Skipping Mutation	First line therapy/Subsequent therapy
	Capmatinib, crizotinib, Tepotinib
RET Fusion	First line therapy/Subsequent therapy
	Selpercatinib, Pralsetinib, Cabozantinib

Adapted from NCCN guideline version 1. 2022 Non-Small Cell Lung Cancer[36]



National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2022
Non-Small Cell Lung Cancer

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  - Erlotinib[2]
  - Dacomitinib[3]
  - Gefitinib[4,5]
  - Osimertinib[6]
  - Erlotinib + ramucirumab[7]
  - Erlotinib + bevacizumab[c] (nonsquamous)[8]
- Subsequent therapy
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EGFR S768I, L861Q,* and/or *G719X
First-line therapy
- Afatinib[1,10]
- Erlotinib[2]
- Dacomitinib[3]
- Gefitinib[4,5]
- Osimertinib[6,11]
- Subsequent therapy
  - Osimertinib[9]

***EGFR Exon 20* Insertion Mutation Positive**
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  - Amivantamab-vmjw[12]
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***KRAS G12C* Mutation Positive**
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- First-line therapy
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  - Brigatinib[17]
  - Ceritinib[18]
  - Crizotinib[15,19]
  - Lorlatinib[20]
- Subsequent therapy
  - Alectinib[21,22]
  - Brigatinib[23]
  - Ceritinib[24]
  - Lorlatinib[25]

***ROS1* Rearrangement Positive**
- First-line therapy
  - Ceritinib[24]
  - Crizotinib[27]
  - Entrectinib[28]
- Subsequent therapy
  - Lorlatinib[25]
  - Entrectinib[28]

***BRAF* V600E Mutation Positive**
- First-line therapy
  - Dabrafenib/trametinib[30]
  - Dabrafenib[30]
  - Vemurafenib
- Subsequent therapy
  - Dabrafenib/trametinib[31,32]

***NTRK1/2/3* Gene Fusion Positive**
- First-line/Subsequent therapy
  - Larotrectinib[33]
  - Entrectinib[34]

***MET* Exon 14 Skipping Mutation**
- First-line therapy/Subsequent therapy
  - Capmatinib[35]
  - Crizotinib[36]
  - Tepotinib[37]

***RET* Rearrangement Positive**
- First-line therapy/Subsequent therapy
  - Selpercatinib[38]
  - Pralsetinib[39]
  - Cabozantinib[40,41]

PD-L1 ≥1%
- First-line therapy[d]
  - Pembrolizumab[43-45]
  - (Carboplatin or cisplatin)/pemetrexed/ pembrolizumab (nonsquamous)[46]
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  - Carboplatin/(paclitaxel or albumin-bound paclitaxel)/pembrolizumab (squamous)[48]
  - Carboplatin/albumin-bound paclitaxel/ atezolizumab (nonsquamous)[48]
  - Nivolumab/ipilimumab[49]
  - Nivolumab/ipilimumab/pemetrexed/ (carboplatin or cisplatin) (nonsquamous)[50]
  - Nivolumab/ipilimumab/paclitaxel/carboplatin (squamous)[50]

PD-L1 ≥50% (in addition to above)
- First-line therapy[d]
  - Atezolizumab[51]
  - Cemiplimab-rwlc[52]

[a] Monitoring During Initial Therapy: Response assessment after 2 cycles, then every 2–4 cycles with CT of known or high-risk sites of disease with or without contrast or when clinically indicated. Timing of CT scans within Guidelines parameters is a clinical decision.
[b] Monitoring During Subsequent Therapy or Maintenance Therapy: Response assessment with CT of known or high-risk sites of disease with or without contrast every 6–12 weeks. Timing of CT scans within Guidelines parameters is a clinical decision.
[c] An FDA-approved biosimilar is an appropriate substitute for bevacizumab.
[d] Continuation maintenance refers to the use of at least one of the agents given in first line, beyond 4–6 cycles, in the absence of disease progression.

References

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

Targeted therapies for various mutations for NSCLC[(36)].

EMERGING BIOMARKERS TO IDENTIFY NOVEL THERAPIES FOR PATIENTS WITH METASTATIC NSCLC

Genetic Alteration (ie, Driver event)	Available Targeted Agents with Activity Against Driver Event in Lung Cancer
High-level *MET* amplification*	Crizotinib[1-2] Capmatinib[3] Tepotinib[4]
ERBB2 (HER2) mutations**	Ado-trastuzumab emtansine[5] Fam-trastuzumab deruxtecan-nxki[6]

* The definition of high-level *MET* amplification is evolving and may differ according to the assay used for testing. For NGS-based results, a copy number greater than 10 is consistent with high-level *MET* amplification.

** For oncogenic or likely oncogenic *HER2* mutations, refer to definitions at oncokb.org.

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