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SUSTAINABLE ADVANCED CANCER CARE THROUGH WAQF

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Community-Driven University For a Sustainable World

Molecular Profiling in Cancer: Towards a Switch to Next-Generation Sequencing (NGS)Testing

Sustainable Advanced Cancer Care Through Waqf

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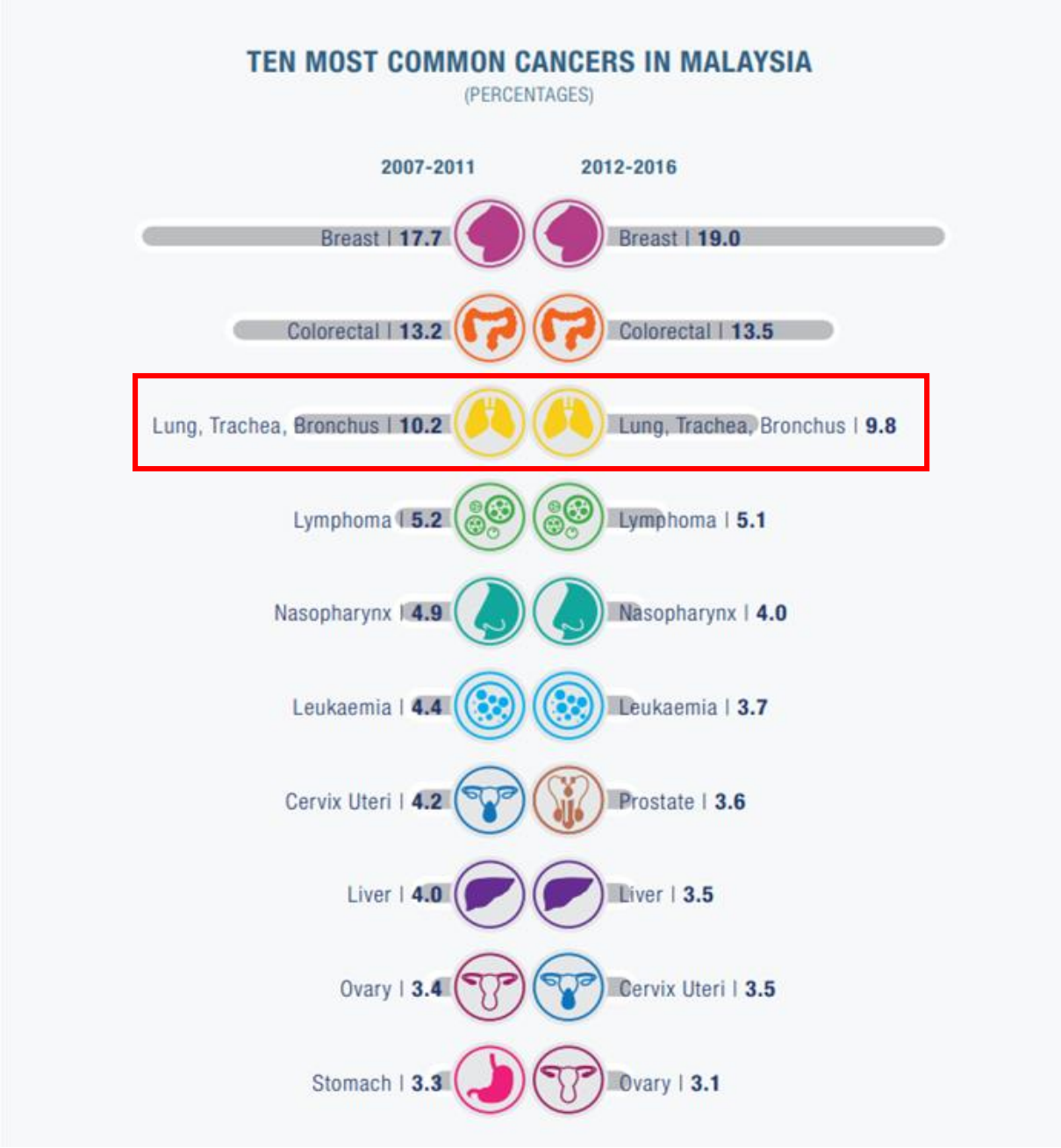
17th MARCH 2023

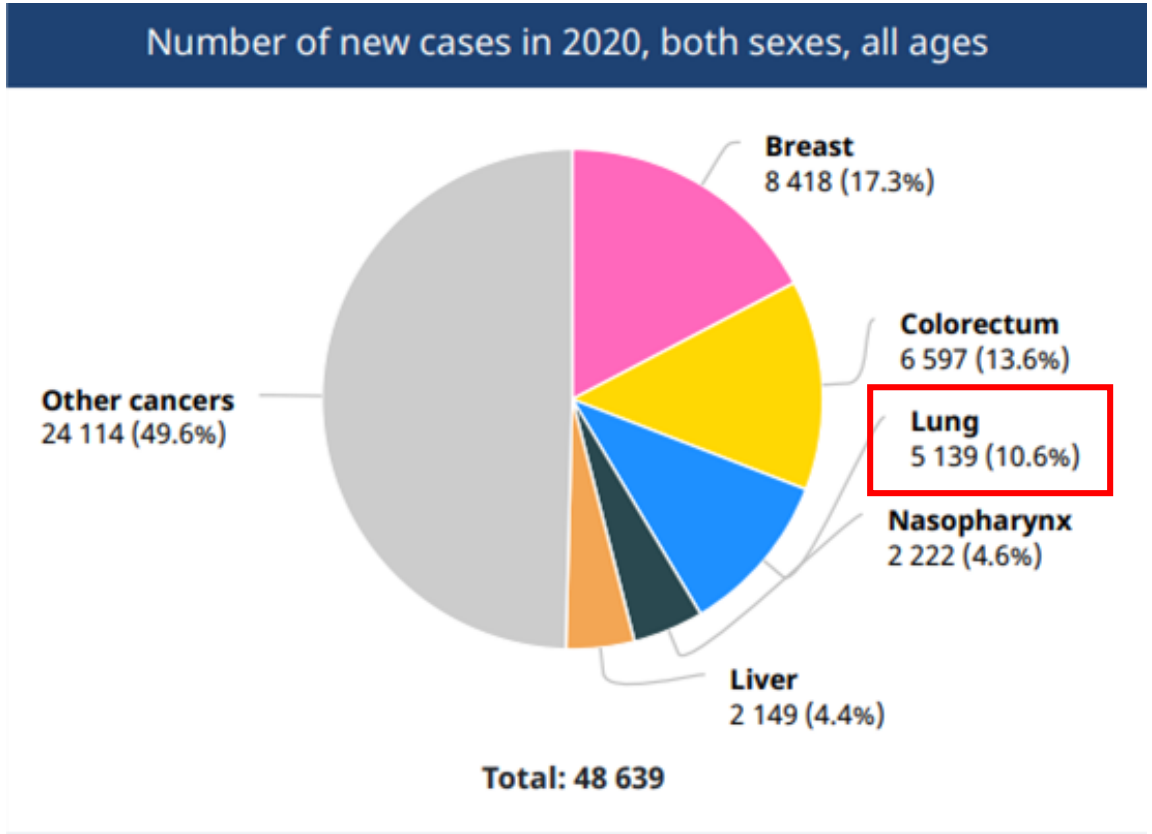
WATERFRONT HOTEL, KUCHING, SARAWAK

OVERVIEW

1. Molecular Testing Focusing on Lung and Colorectal Cancer In Sarawak
2. Polymerase Chain Reaction (PCR)
3. Next Generation Sequencing (NGS)

Prevalence of Lung Cancer in Malaysia





Source:

1. Summary of Malaysia National Cancer Registry Report 2012-2016
2. Globocan 2020

Prevalence of Lung Cancer in Malaysia

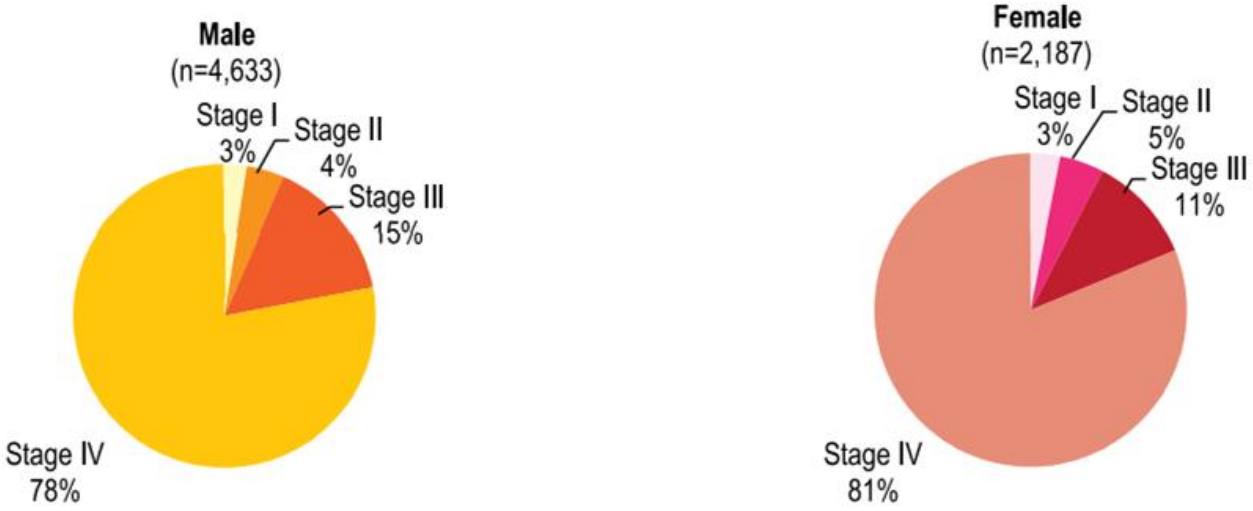


Figure 35. Trachea, bronchus and lung: Staging percentage by sex, Malaysia, 2012-2016

Lung cancer is the third most common cancer after breast and colorectal cancers.

Most patients present at late stage (stages 3-4), contributing to poor survival outcomes.

Non-small cell lung cancer (NSCLC) is a predominant type of lung cancer in Malaysia (72-90% of diagnosed cases).

Testing recommendations from international guidelines

a

Approved biomarkers	ESMO Guidelines (2020)	NCCN Guidelines® (2022)[a]	CAP/IASLC/AMP Guidelines (2018)	ASCO Guidelines (2014)	Pan-Asian Guidelines (2019)
EGFR	●	●	●	●	●
ALK	●	●	●	●	●
ROS1	●	●	●	●	●
BRAF	●	●	●	●	●
NTRK	●	●	●	●	●
PD-L1	●	●	●	●	●

b

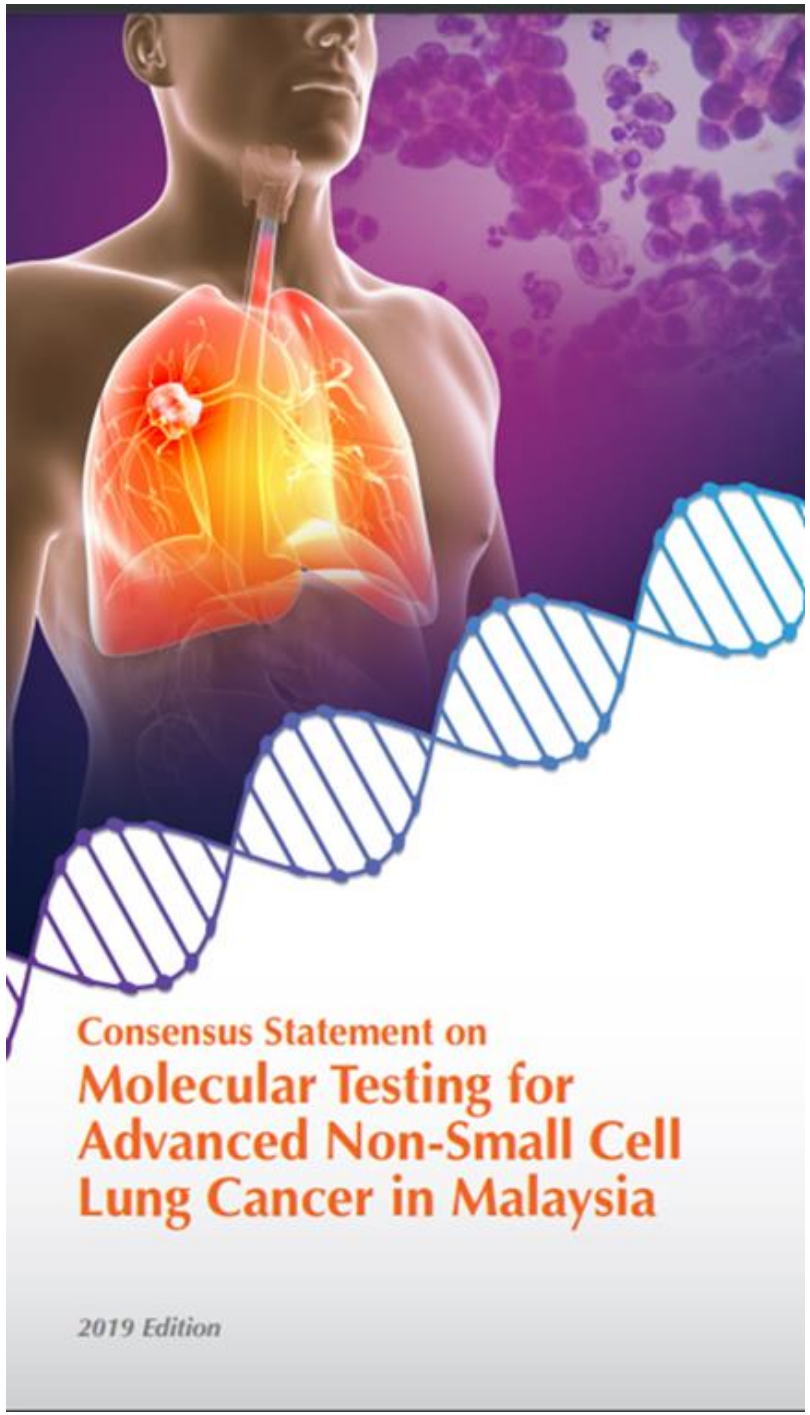
Emerging biomarkers	ESMO Guidelines (2020)	NCCN Guidelines® (2022)[a]	CAP/IASLC/AMP Guidelines (2018)	ASCO Guidelines (2014)	Pan-Asian Guidelines (2019)
KRAS[b]	●	●	▨	●	●
MET	●	●	●	●	●
RET[b]	●	●	●	●	●
ERBB2/HER2	●	●	●	●	●
TMB[c]	●	○	●	●	●

● Testing recommended ● Expanded panel testing recommended ▨ Single gene or expanded panel testing recommended ● IHC testing recommended ● No guideline recommendations to date ○ Testing not recommended

Source: Penault-Llorca, F., Kerr, K.M., Garrido, P. et al. Expert opinion on NSCLC small specimen biomarker testing — Part 2: Analysis, reporting, and quality assessment. Virchows Arch 481, 351–366 (2022). https://doi.org/10.1007/s00428-022-03344-1

Testing recommendations from international guidelines

- Most guidelines recommend expanded panel testing, in order to detect all clinically relevant biomarkers in a single test.


Consensus Statement on
Molecular Testing for Advanced Non-Small Cell Lung Cancer in Malaysia
2019 Edition

Consensus Statement on Molecular Testing for Advanced NSCLC in Malaysia, 2019 Edition

- Each approved therapy is accompanied by molecular testing to detect the most suitable targeted therapy for patients.

Consensus Statement on Molecular Testing for Advanced NSCLC in Malaysia, 2019 Edition

- The Malaysian consensus statement on molecular testing has recommended molecular testing for advanced NSCLC:
  - Patients with non-squamous cell NSCLC and patients with squamous cell carcinoma who are light smokers are recommended *EGFR, ALK* and *ROS1* testing.
  - Extended testing may be considered if approved therapies are available.
- Patient who received a first- or second-generation EGFR TKI: Upon disease progression after first-line treatment, *EGFR* T790M mutation and an expanded panel testing are recommended for patients
- Patient who received a third-generation EGFR TKI: An expanded panel testing is recommended for patients

Testing algorithm for first-line molecular testing

FIGURE 1. Algorithm for mutational testing of non-squamous cell NSCLC in the first line setting.

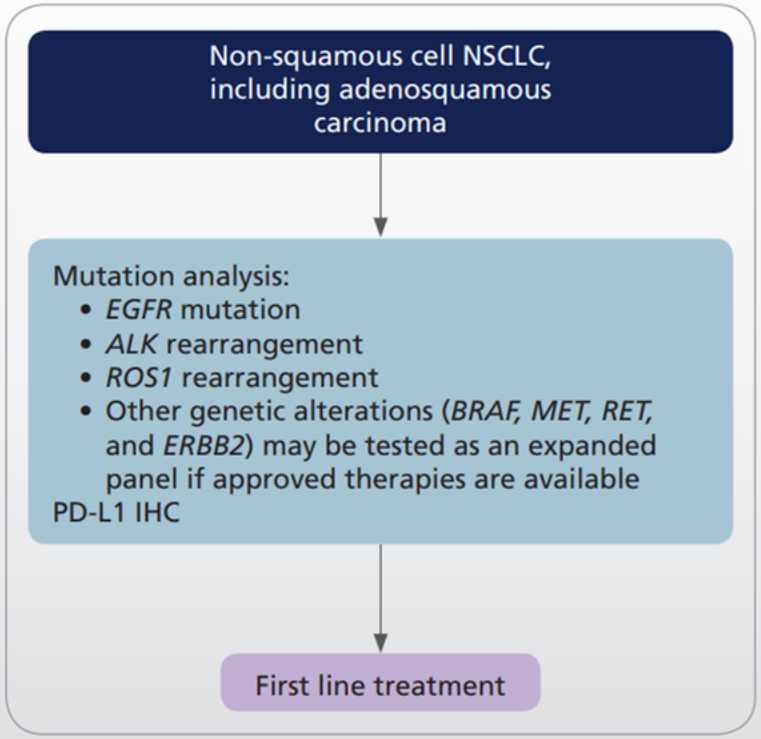
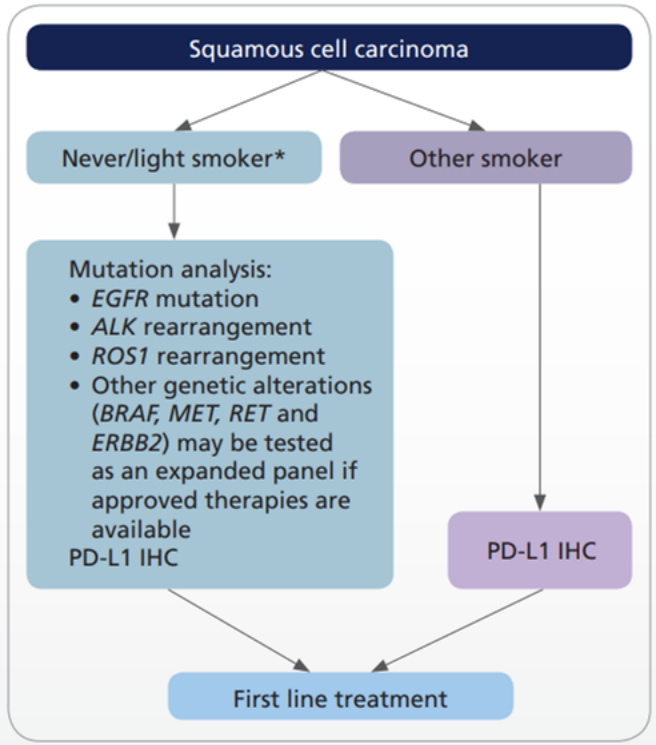
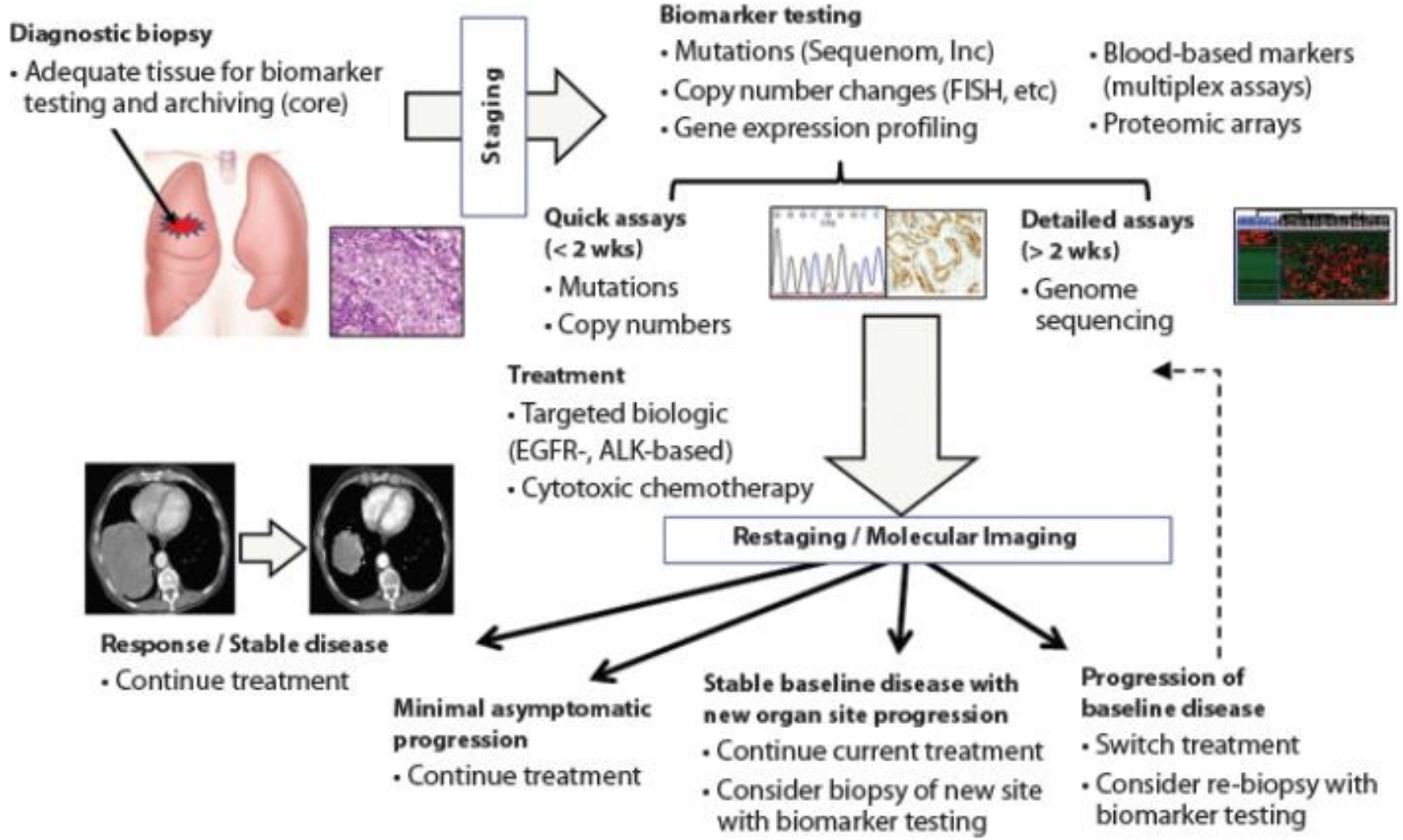


FIGURE 2. Algorithm for mutational testing of squamous cell carcinoma in the first line setting.



*Light smoker is defined as < 15 pack-years.[25]

Source: Consensus Statement on Molecular Testing for Advanced NSCLC in Malaysia, 2019 Edition.



ALGORITHM FOR PERSONALIZED SYSTEMATIC APPROACH TO THE DIAGNOSIS, EVALUATION AND TREATMENT OF LUNG CANCER PATIENTS

Liquid biopsy

- A laboratory test done on a sample of blood, urine, or other body fluid to look for cancer cells from a tumor or small pieces of DNA, RNA, or other molecules released by tumor cells into a person's body fluids.

- may help doctors understand what kind of genetic or molecular changes are taking place in a tumor

- may help find cancer at an early stage

- help plan treatment or to find out how well treatment is working or if cancer has come back.

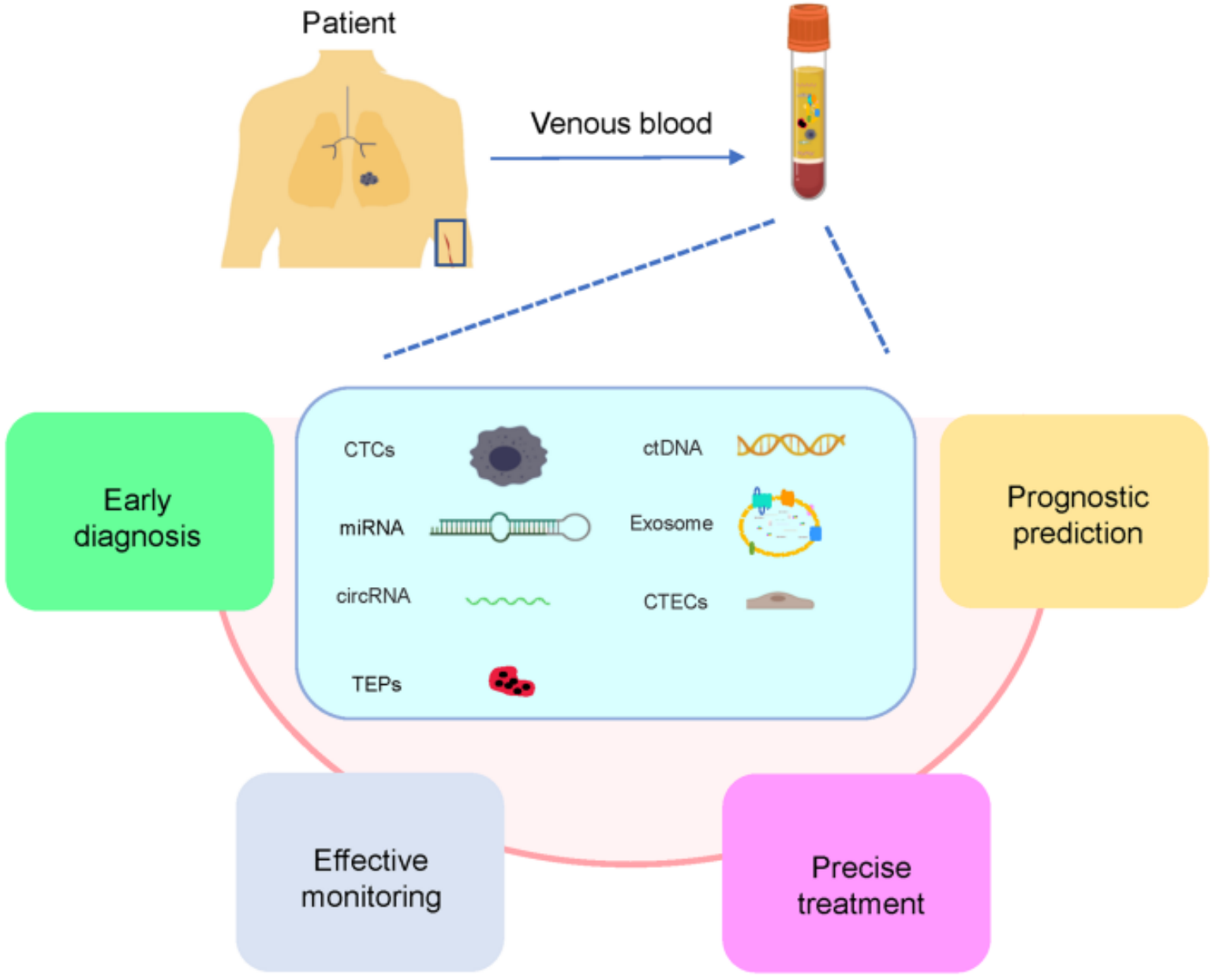
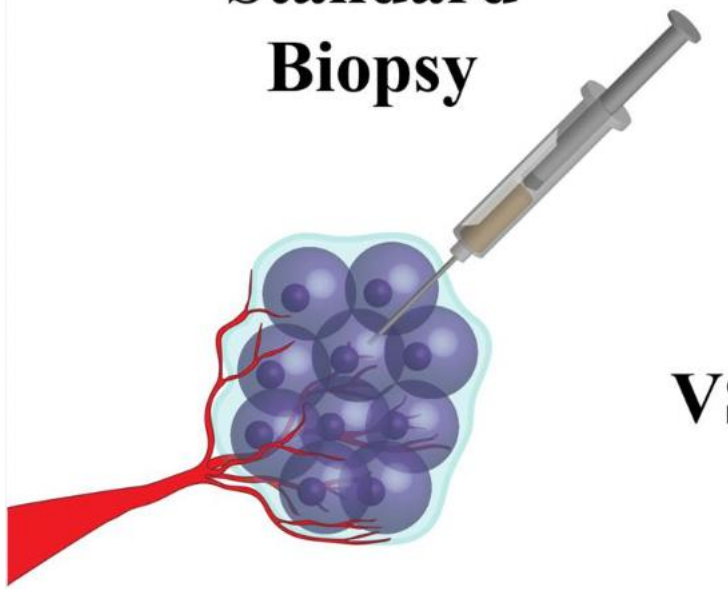
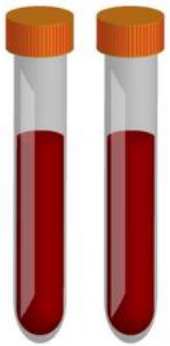


Fig. 2 Clinical application of liquid biopsy in lung cancer. Circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, microRNAs (miRNAs), circular RNAs (circRNAs), tumor-educated platelets (TEPs), and circulating tumor vascular endothelial cells (CTECs) in the venous blood from lung cancer patients(e.g., liquid biopsy) have the potential to be used clinically to provide unique opportunities for the real-time monitoring of disease progression and treatment response, in addition to studying tumor heterogeneity

Standard Biopsy		Liquid Biopsy
	VS.	
Time-Intensive Procedure		Quick
Localized Sampling of Tissue		Comprehensive Tissue Profile
Not Easily Obtained		Easily Obtained
Some Pain/Risk		Minimal Pain/Risk
Invasive		Minimally Invasive

Liquid biopsy

- If liquid biopsy is negative → rebiopsy is recommended, due to the lower sensitivity of liquid biopsy (50-80% of tumour testing sensitivity).
- Studies have shown that site of metastasis and stage of disease can affect sensitivity of liquid biopsy.

Available testing modalities

- Immunohistochemistry [IHC]: PD-L1
- PCR ; polymerase chain reaction
- NGS; next generation sequencing

- Factors to consider when selecting a molecular method include the sample type, type of variant to be detected, turnaround time, availability or sufficiency of tissue, sensitivity, and cost.

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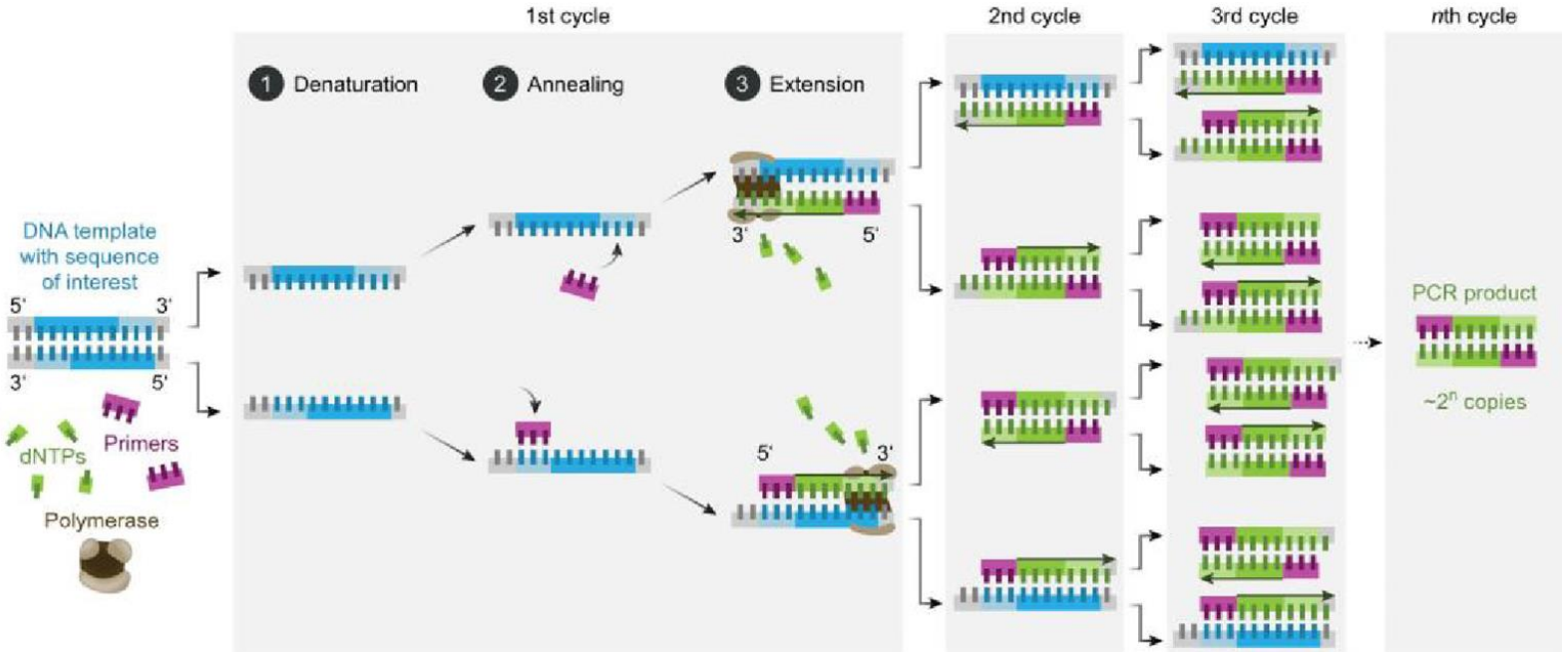
Polymerase chain reaction (PCR)



- Allow us to obtain genetic information through the specific amplification of nucleic acid sequences starting with a very low number of target copies.
- These reactions are characterized by a logarithmic amplification of the target sequences, i.e. an increase of PCR copies, followed by a plateau phase showing a rapid decrease to zero of copy number increment per cycle.

- Gene profiling opens new possibilities to classify the disease into subtypes and guide a differentiated treatment.
- High sensitivity, excellent precision and large dynamic range, and has become the method of choice for quantitative gene expression measurements.

Current molecular service in SGH Kuching

- In SGH, molecular service was started in January 2022. Previously send over to HTA, KL
- Facing logistic issue and delay TAT; especially challenging COVID-19 pandemic era, long festival public holidays and suspended courier service
- Test is limited to RT-PCR
  - *EGFR* mutations, KRAS (for lung cancer): January 2023
  - KRAS, NRAS, BRAF (for colorectal cancer); July 2023
  - BRAF (for melanoma) ; July 2023
- *ALK* (D5F3) IHC are performed on ULTRA Ventana platform and PD-L1 22C3 clone IHC are performed on Dako's Autostainer Link 48 platform; 2018

PCR analyzer

- The test is intended to be used to identify patients with advanced NSCLC whose tumours harbour mutations in exons 18, 19, 20 and 21 of the EGFR gene.
- Able to detect 42 exons; CE IVD
- To select patients for treatment with small molecule tyrosine kinase inhibitors (TKIs) that target EGFR.

PCR Advantages

- RT-PCR produces quick and reliable result.
- RT-PCR is easy to use and is more tolerant of variable DNA quality compared to NGS, but has limited multiplex capability.

The Role of PCR in EGFR with exon 20 Insertion

- In SGH, solid molecular tumour testing is new; commencing January 2022
- The service has been extended to Sabah as well, as Sabah also faced logistic issues with sending specimens to the Peninsular Malaysia.
- From January to June 2022 in SGH, e.g. six out of 284 samples have been detected with ex20ins mutation (2.11%)
- The DNA extractions were sent to HTA, KL for confirmation,
  - Three (3) samples were confirmed as ex20ins by Sanger sequencing
  - One (1) sample was confirmed through the Amoy (NGS) platform
  - One (1) sample was confirmed using Idylla (PCR) method
  - One (1) sample false positive by Sanger sequencing; initially performed on COBAS 4800z

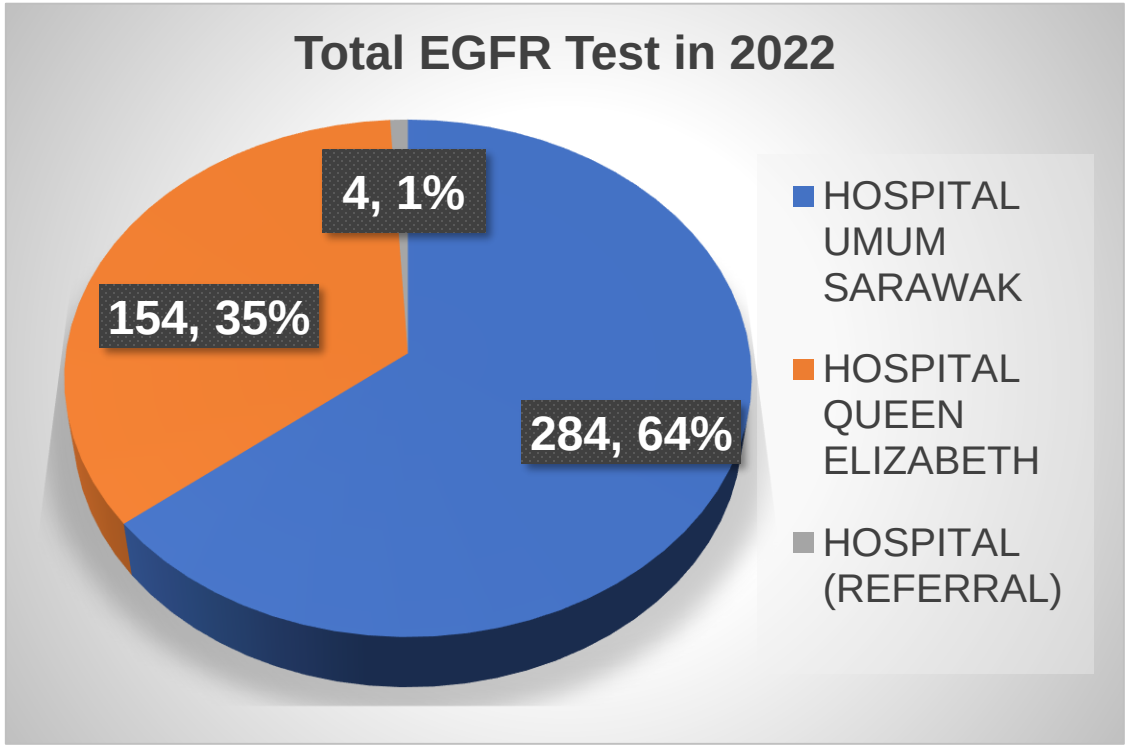
MOLECULAR TESTS IN ANATOMICAL PATHOLOGY UNIT, SARAWAK GENERAL HOSPITAL

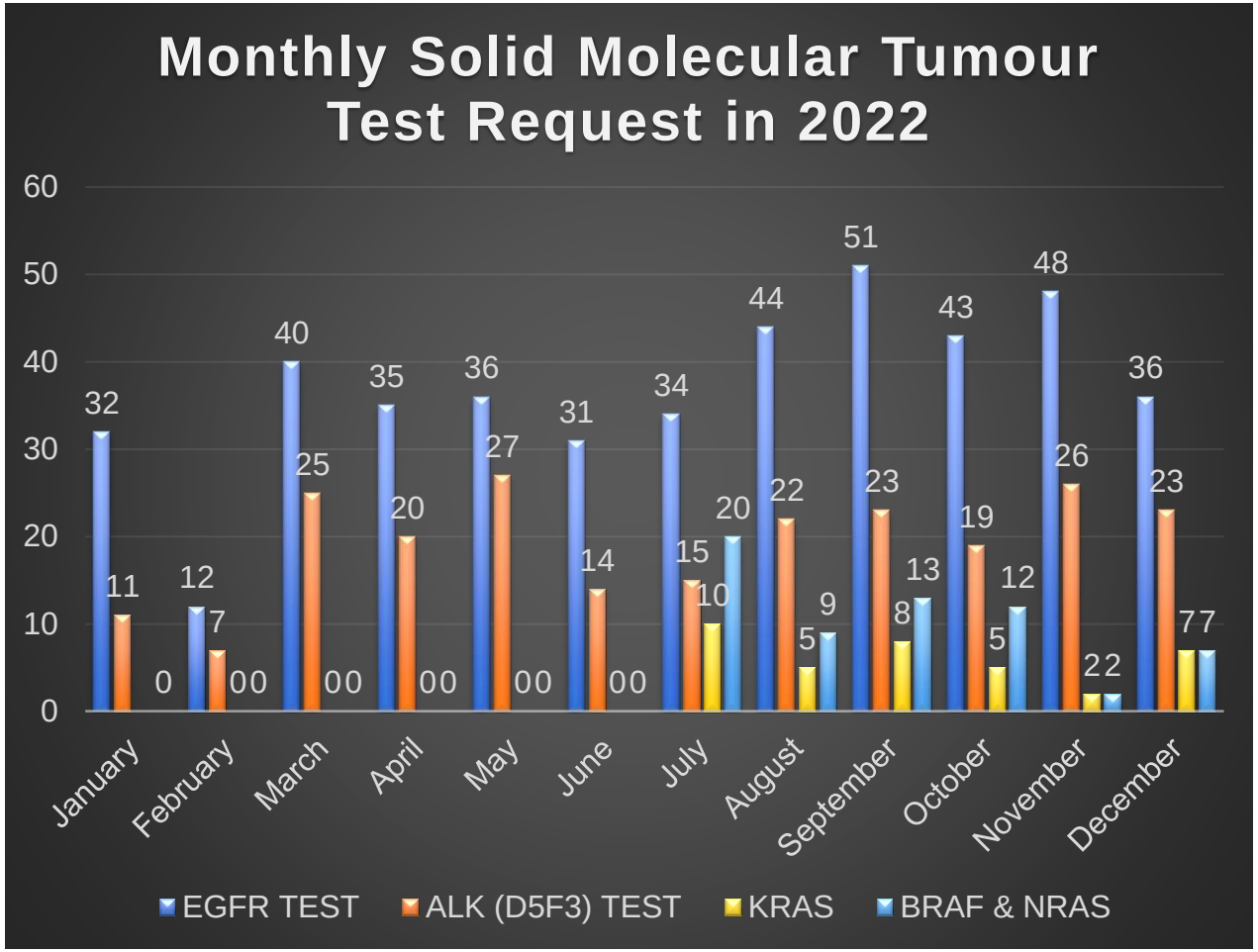
EGFR, NRAS, KRAS, BRAF

JANUARY TO DECEMBER 2022

REPORT ON MOLECULAR TESTS IN SGH 2022; PCR METHOD

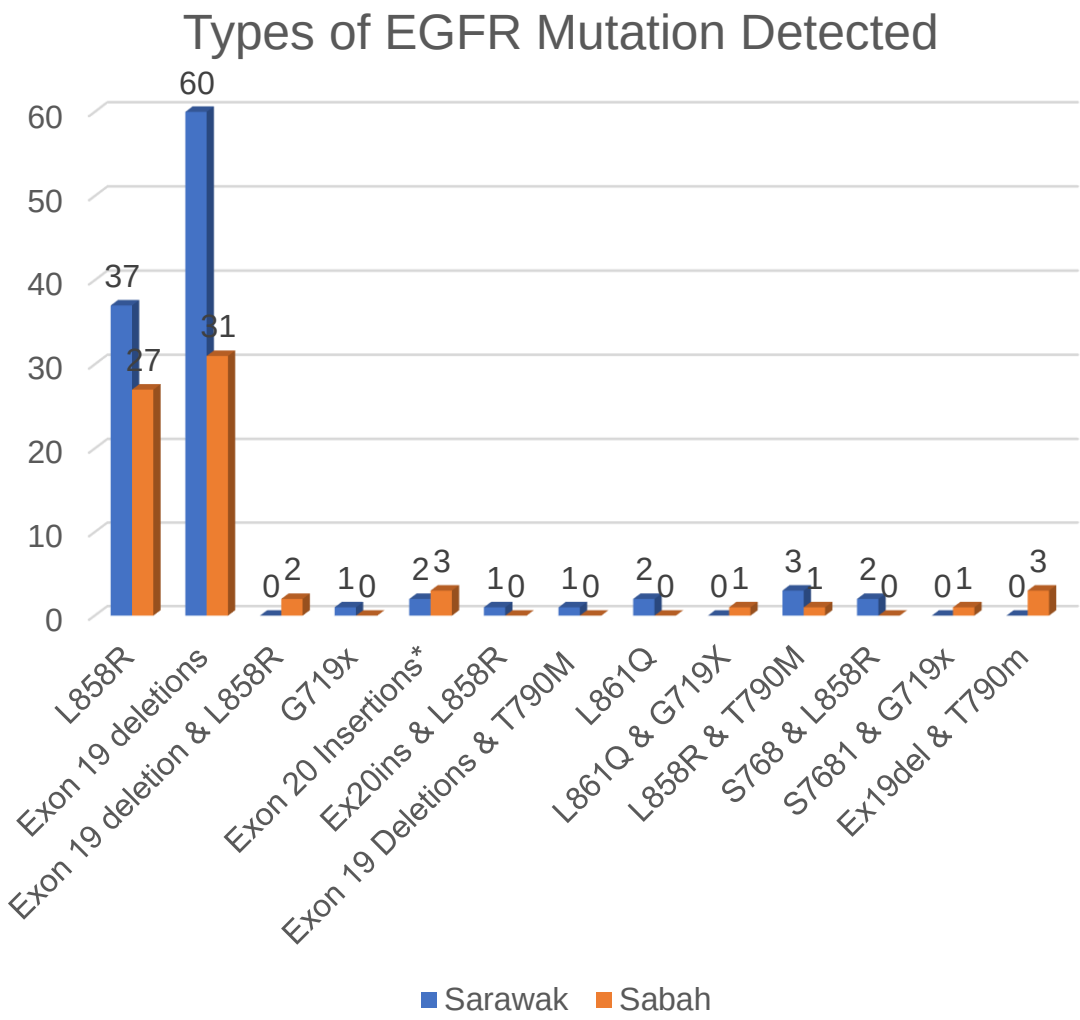
TYPE OF TEST	EGFR			KRAS	BRAF & NRAS
Source	HOSPITAL UMUM SARAWAK	HOSPITAL QUEEN ELIZABETH	OTHER HOSPITALS (REFERRALS)	HOSPITAL UMUM SARAWAK	HOSPITAL UMUM SARAWAK
January	24	7	1	0	0
February	9	3	0	0	0
March	27	12	1	0	0
April	23	11	1	0	0
May	24	11	1	0	0
June	25	6	0	0	0
July	22	12	0	10	20
August	26	18	0	5	9
September	29	22	0	8	13
October	23	20	0	5	12
November	29	19	0	2	2
December	23	13	0	7	7
Total According To Hospitals	284	154	4	37	63
Grand Total:	442			100	


Total EGFR Test in 2022
4, 1%
154, 35%
284, 64%
HOSPITAL UMUM SARAWAK
HOSPITAL QUEEN ELIZABETH
HOSPITAL (REFERRAL)

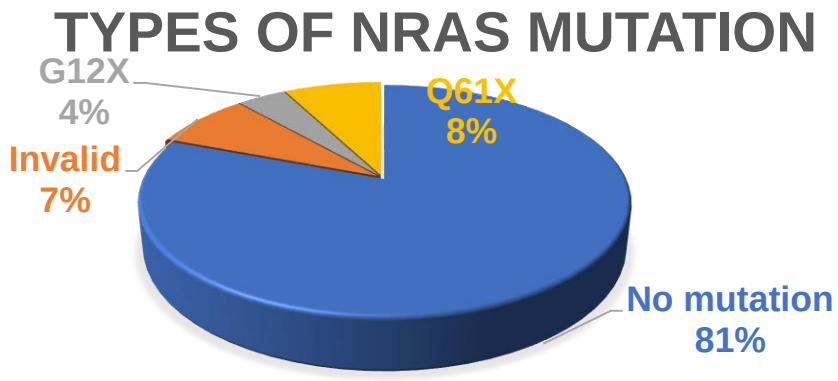

Monthly Solid Molecular Tumour Test Request in 2022
60
50
40
30
20
10
0
January 32 11 0 0
February 12 7 0 0
March 40 25 0 0
April 35 20 0 0
May 36 27 0 0
June 31 14 0 0
July 34 15 10 20
August 44 22 5 9
September 51 23 8 13
October 43 19 5 12
November 48 26 2 2
December 36 23 7 7
EGFR TEST
ALK (D5F3) TEST
KRAS
BRAF & NRAS

Types of EGFR Mutation Detected

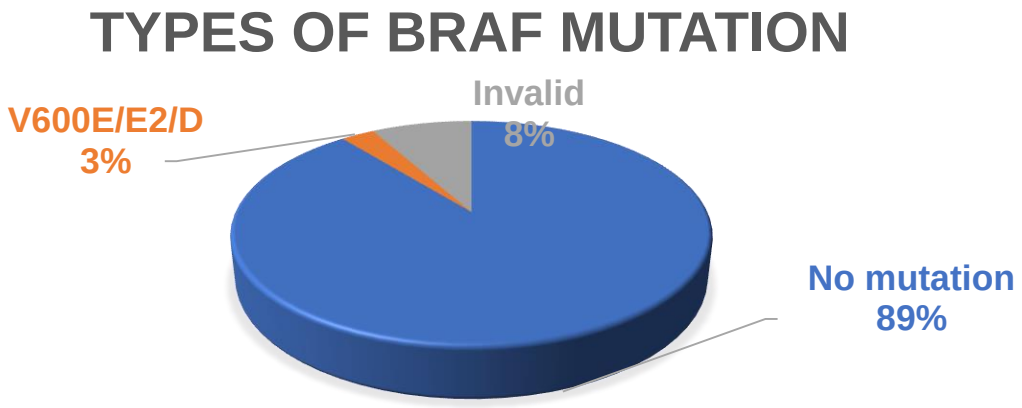
Types of EGFR Mutation Detected	State		TOTAL
Genotyping	Sarawak	Sabah	
L858R	37	27	64
Exon 19 deletions	60	31	91
Exon 19 deletion & L858R	0	2	2
G719x	1	0	1
Exon 20 Insertions	2	3	5
Ex20ins & L858R	1	0	1
Exon 19 Deletions & T790M	1	0	1
L861Q	2	0	2
L861Q & G719X	0	1	1
L858R & T790M	3	1	4
S768 & L858R	2	0	2
S7681 & G719x	0	1	1
Ex19del & T790m	0	3	3
Total Mutations Detected	**109**	**69**	**178**



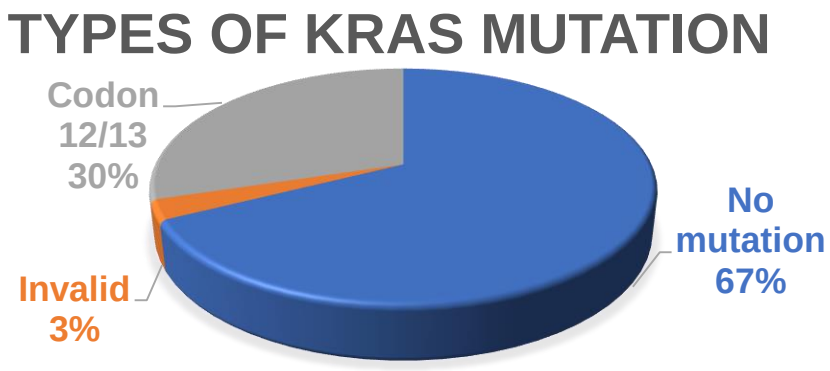
Types of NRAS Mutation Detected	
No mutation	21
Invalid	2
G12X	1
Q61X	2



Types of BRAF Mutation Detected	
No mutation	33
V600E/E2/D	1
Invalid	3

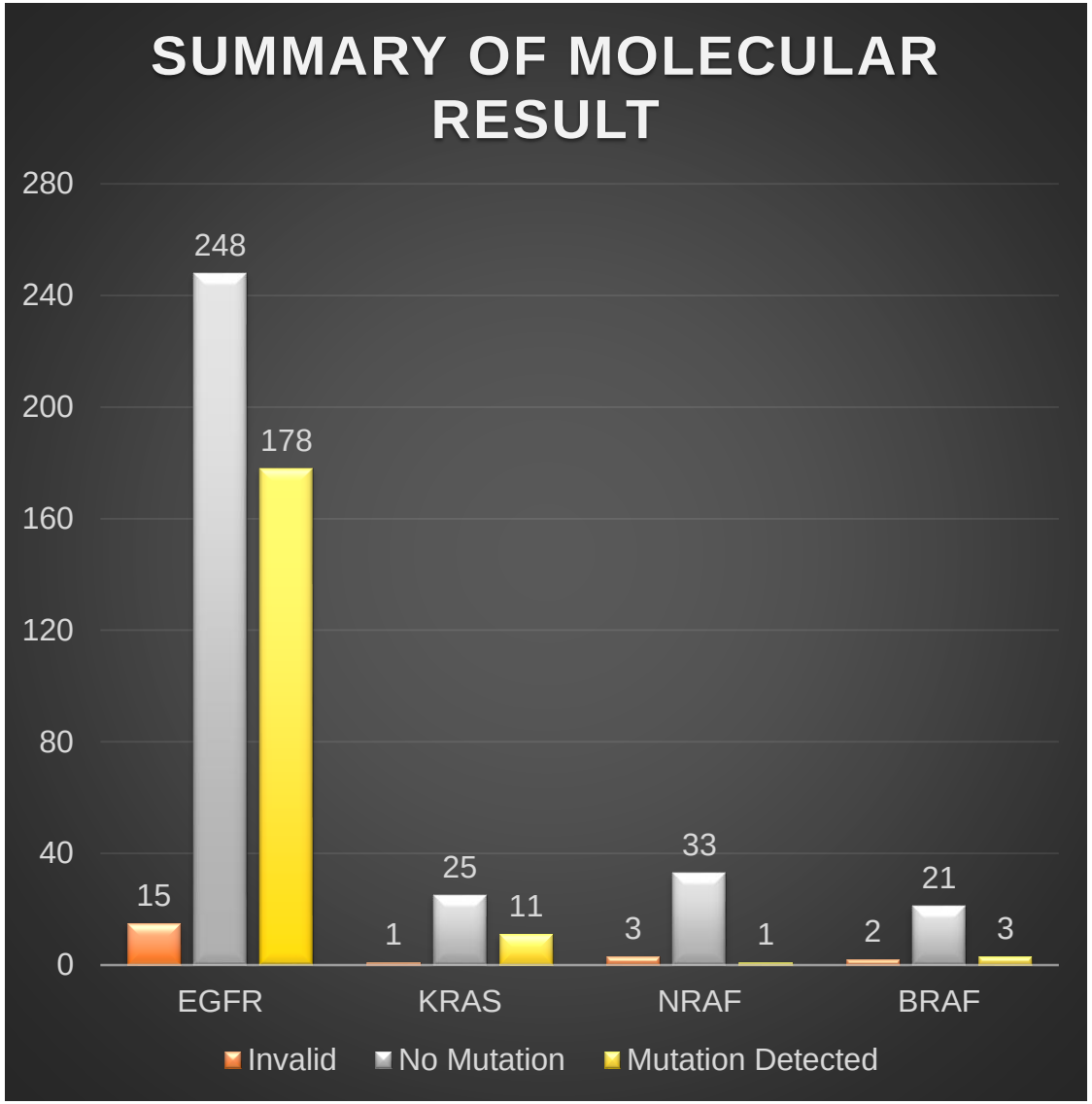


Types of KRAS Mutation Detected	
No mutation	25
Invalid	1
Codon 12/13	11



Summary of Molecular Tests Results

RESULT	EGFR	KRAS	NRAF	BRAF
Invalid	15	1	3	2
No Mutation	248	25	33	21
Mutation Detected	178	11	1	3
Total sample	441	37	37	26



OVERVIEW

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NGS as a Modality for Molecular Testing

- The therapies employed depend on the availability of the therapies and the underlying mutation.
- Proportion of patients with e.g. EGFR mutation is different between Western (< 20%) and Asian (39-50%) populations.
- Therefore, lung cancer differs not only molecularly but also geographically.

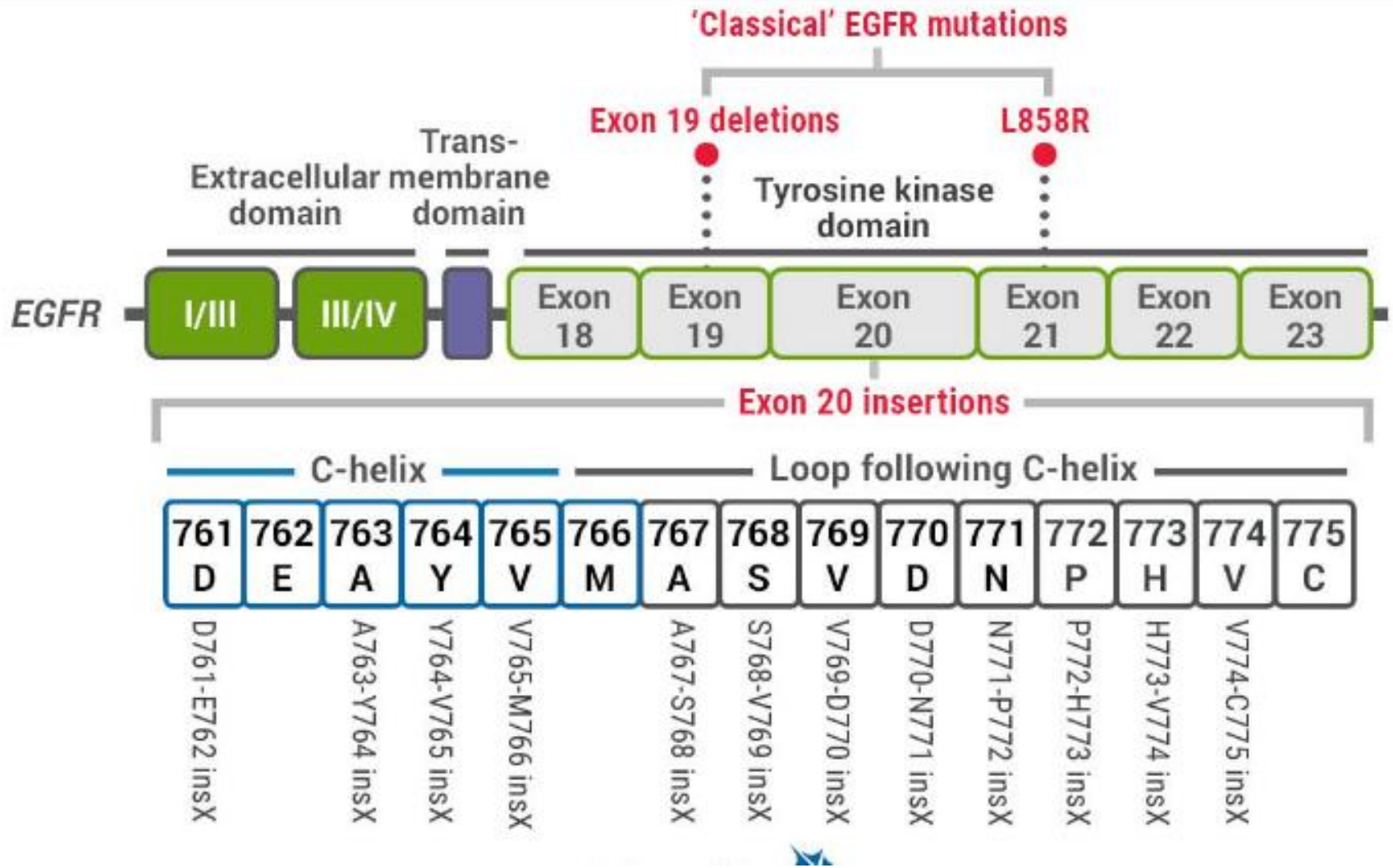
EGFR Mutations in NSCLC

- The most common mutations are ex19del and L858R in exon 21.
- Exon 20 mutation comprised up to 6% of *EGFR* mutations (third most common *EGFR* mutation subtype). Among all NSCLC, the incidence is similar to *ROS1* and *RET* at 1%.
- Exon 20 mutations are inherently resistant to approved TKIs, and patients' prognosis is generally poorer.

EGFR ex20ins Mutation in NSCLC

- A C-helix region is present in exon 20.
- Mutations in the C-helix region is amenable to first-, second- and third-generation TKIs.
- However, most ex20 mutations occur in the loop following the C-helix. Mutations in this region are generally resistant to first-generation TKIs.
- It is difficult for routine RT-PCR and Sanger sequencing to detect molecular events in the loop following C-helix.
- Among unique *EGFR* mutations, approximately 60% are targetable.

EGFR exon 20 mutations in Non-Small Cell Lung Cancer



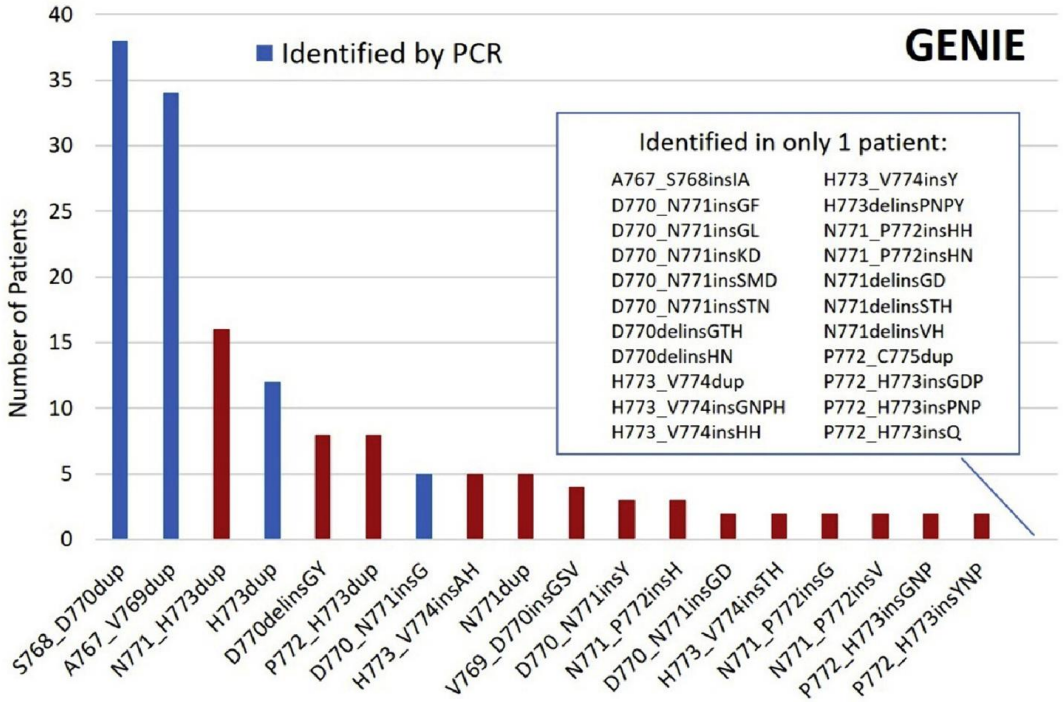
IASLC 2020 World Conference on Lung Cancer Singapore
wclc2020.IASLC.com | #WCLC20
CONQUERING THORACIC CANCERS WORLDWIDE

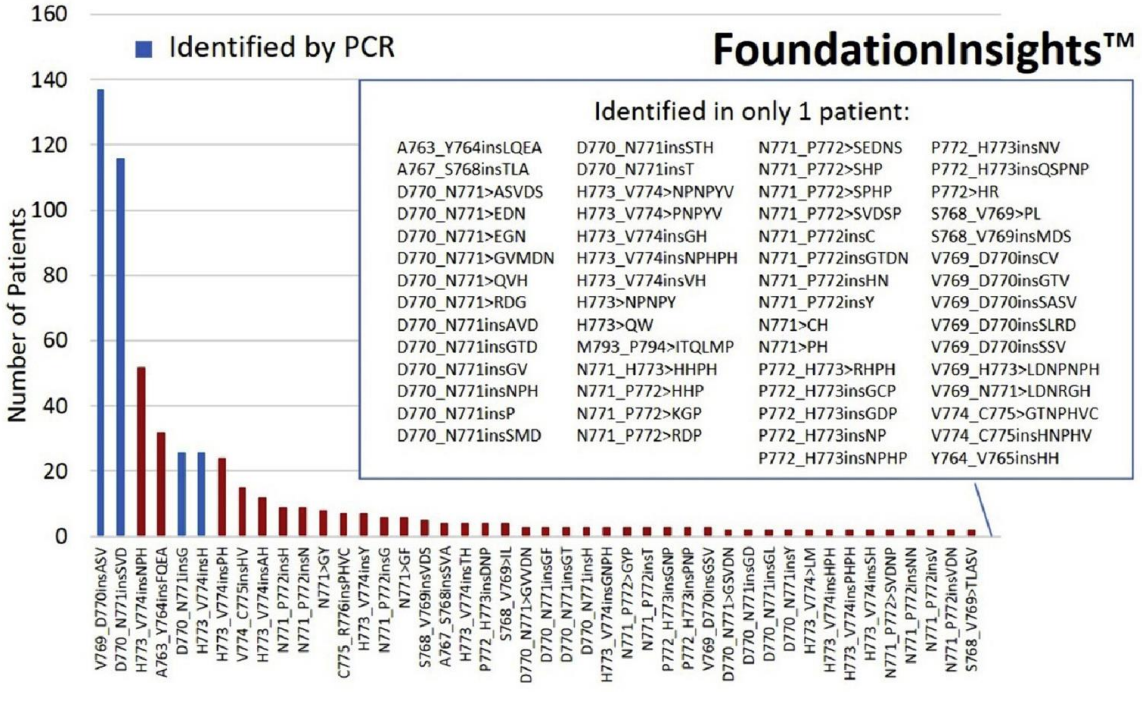
Underdiagnosis of EGFR Exon 20 Insertion Mutation Variants: Estimates from NGS-based Real-World Datasets

Joshua M. Bauml[1], Santiago Viteri[2], Anna Minchom[3], Lyudmila Bazhenova[4], Sai-Hong Ignatius Ou[5], Michael Schaffer[6], Nicholas LeCroy[6], Ralph Riley[6], Parthiv Mahadevia[6], Nicolas Girard[7]

[1]Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA; [2]Instituto Oncológico Dr Rosell, Centro Médico Teknon, Grupo QuironSalud, Barcelona, Spain; [3]Drug Development Unit, Royal Marsden/Institute of Cancer Research, Sutton, UK; [4]University of California San Diego, San Diego, CA, USA; [5]University of California Irvine, Orange, CA, USA; [6]Janssen R&D, Spring House, PA, USA; [7]Institut Curie, Paris, France

PCR can miss ~50% of Exon20ins variant cases





Exon20ins variants identified by GENIE
PCR misses 49.1% of cases identified by NGS

Exon20ins variants identified by FMI
PCR misses 51.4% of cases identified by NGS

Source: Bauml, J.M. & Viteri, Santiago & Minchom, A. & Bazhenova, Lyudmila & Ou, S. & Schaffer, M. & Croy, N. & Riley, R. & Mahadevia, P. & Girard, N.. (2021). FP07.12 Underdiagnosis of EGFR Exon 20 Insertion Mutation Variants: Estimates from NGS-based Real-World Datasets. Journal of Thoracic Oncology. 16. S208-S209. 10.1016/j.jtho.2021.01.112.

PCR can miss ~50% of Exon20ins variant cases

- Two large databases (GENIE and FOUNDATION) were used to compare NGS vs. PCR.
- GENIE is genomic data from 13 US institutes, and FOUNDATIONS is a database on 150 cancers, in which data on ex20ins was compared using PCR and NGS.
- In both databases, 77% of patients were white (therefore data may differ form the Asian population).
- The study found that PCR misses ~50% of exon20ins variant cases.
- The study strongly supports using NGS to detect mutations.

PCR vs NGS

- Several other subsequent studies also reached a similar conclusion that NGS outperforms PCR in e.g. ex20ins detection.
- NGS provides a wide map of the possible mutations that can occur, unlike RT-PCR which only detects mutations within a particular gene (e.g. *EGFR*).
- Information on concomitant mutations is important, e.g. exon 19 or 21 molecular events with concomitant TP53 mutation do not respond well to TKIs.
- A similar case is seen with MET exon 14 skipping; this information changes how patients are managed.

In conclusion..

- **Mutation specific targeted PCR based tests**
- Identify common EGFR mutations, might missed Exon 20 insertions

- **NGS**
- Routine testing of entire exons 18 to 21
- Increased detection of a broad array of EGFR mutations, including Exon 20 insertions
- Current IASLC recommendation is that NGS genotyping of tumour tissue should be the standard of molecular diagnosis for lung cancer

- **Liquid biopsy**
- Early diagnosis, prognostic prediction, effective monitoring and precise treatment.

Conclusion (2)

- To have our very own Sarawak Cancer Centre/ Institute and able to perform advanced molecular testing for ALL type of cancer in the future

- Way to go forward. Test expensive and high expertise and technical ability. Equal patient opportunity for testing. Offer broad range option of personalized/ targeted treatment

- Sustainable Advanced Cancer Care Through Waqf; for sustainability of advanced molecular testing and treatment/ management