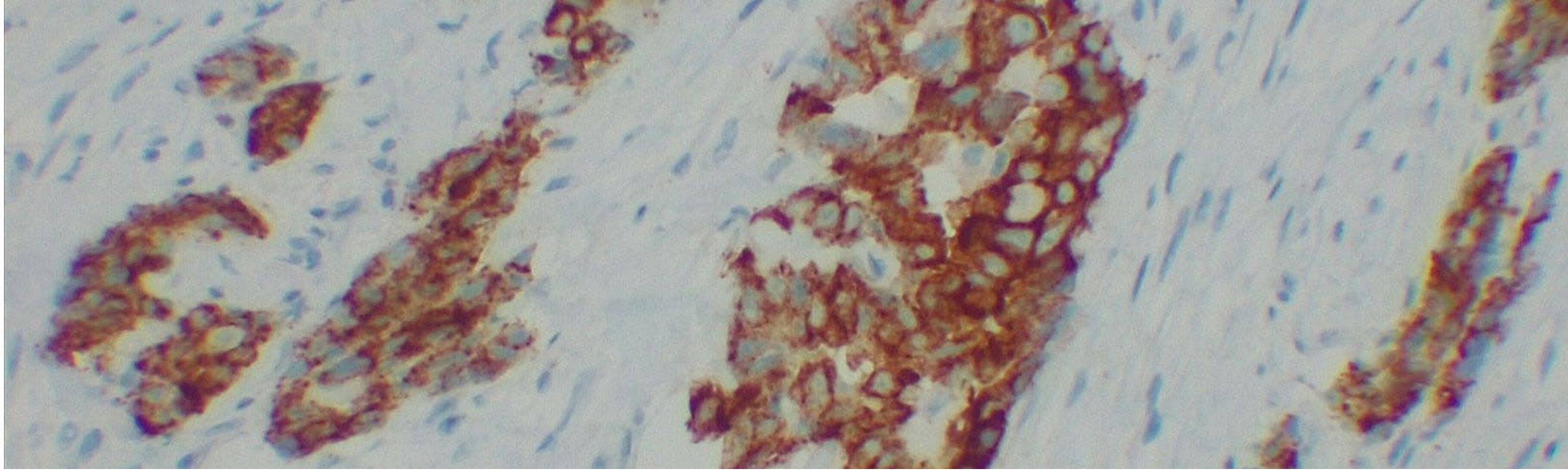


EFGR testing in non- small cell lung cancer

Prof.dr Živka Eri, patolog
Univerzitetski Klinički Centar Srbije

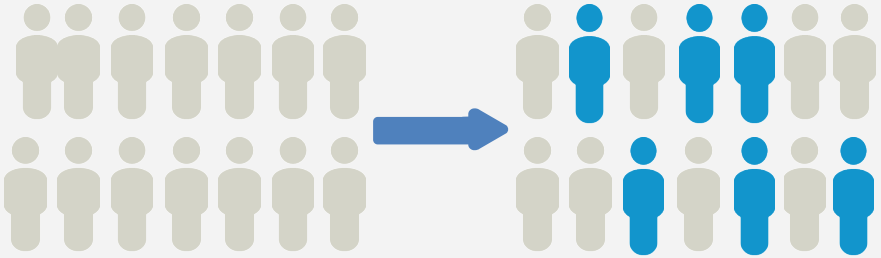
The meeting is initiated, organized and funded by Takeda

Only for Healthcare Professionals

ONCOLOGY

Personalized/precision medicine

right treatment
to the
right patient
at the
right time

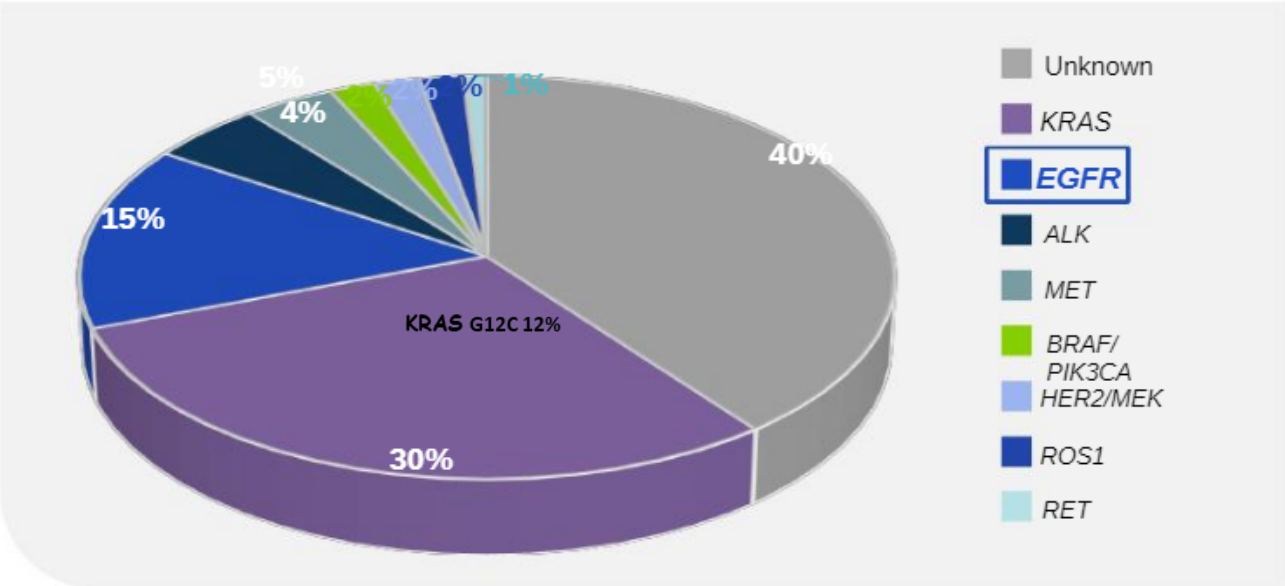


✓ therapeutic decisions are based on the specific histologic and genetic characteristics of the patient's tumor

✓ knowledge of EGFR mutation status is crucial to therapeutic decision-making for patients with advanced-stage disease

NSCLC Is Associated With Several Oncogenic Driver Mutations, With *EGFR* Driver Mutations Occurring in ≈15% of NSCLC Adenocarcinomas[1]

Adenocarcinomas are the most common subtype of NSCLC, occurring in 40% to 50% of cases[1,2]



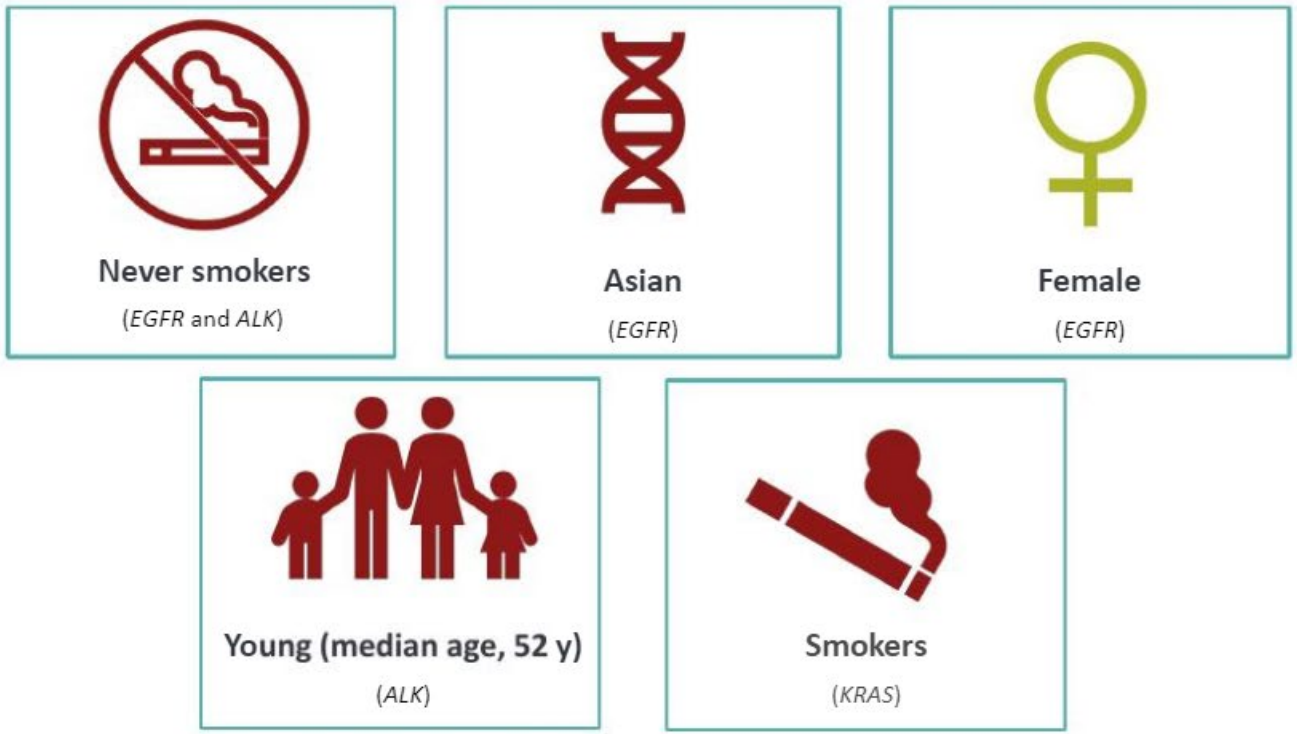
- Oncogenic driver mutations in genes such as EGFR are responsible for both the initiation and the progression of cancer[3]
- **≈11,000 to 15,000 patients** are diagnosed with *EGFR* mutations each year in the US[1,2,4]

ALK, anaplastic lymphoma kinase; BRAF, proto-oncogene B-Raf; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; KRAS, Kirsten rat sarcoma virus oncogene; MEK, mitogen-activated protein kinase kinase; MET, mesenchymal epithelial transition factor; NSCLC, non-small cell lung cancer; PIK3CA, phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit α; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase.

1. Chan B, Hughes B. *Transl Lung Cancer Res.* 2015;4:36-54; 2. American Cancer Society. Accessed October 26, 2022. https://www.cancer.org/cancer/lung-cancer/about/what-is.html; 3. Luo SY, Lam DC. *Transl Respir Med.* 2013;1:6; 4. Surveillance, Epidemiology, and End Results Program. Accessed October 26, 2022. http://seer.cancer.gov/statfacts/html/lungb.html

Clinical Features of Patients With NSCLC With Subsets of Driver Mutations

A higher prevalence of oncogenic driver mutations is found in patients who are[1-10]:



ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; NSCLC, non-small cell lung cancer.

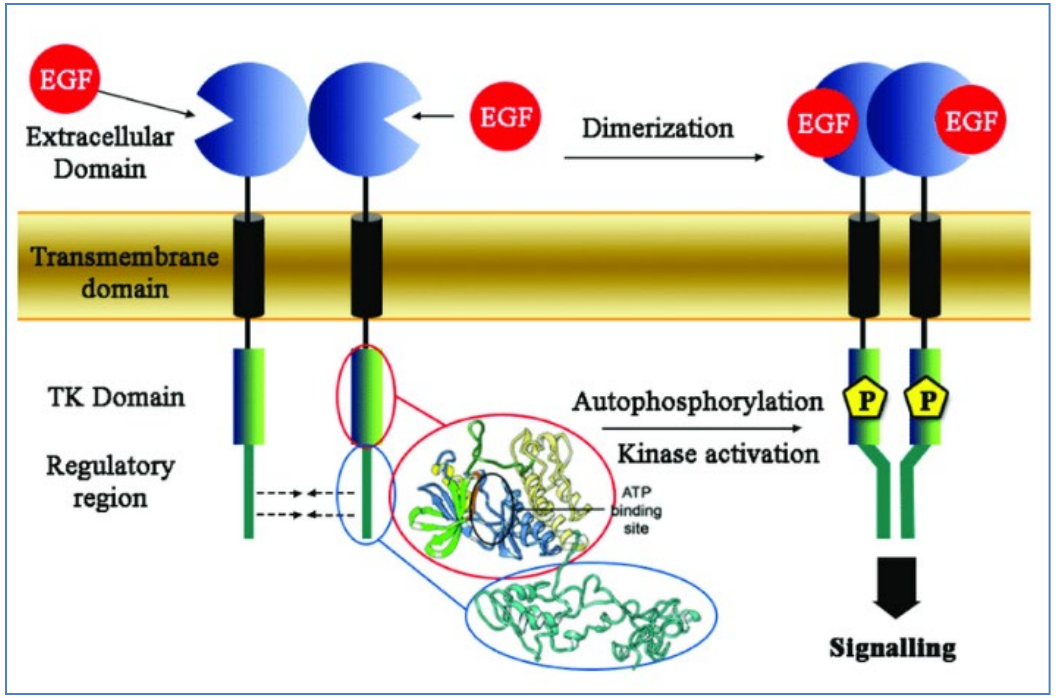
1. Shigematsu H. *J Natl Cancer Inst.* 2005;97:339-346; 2. Reck M, Rabe K. *N Engl J Med.* 2017;377:849-861; 3. Chan BA, Hughes BGM. *Transl Lung Cancer Res.* 2015;4:36-54; 4. O'Kane G, et al. *Lung Cancer.* 2017;109:137-144; 5. Midha A, et al. *Am J Cancer Res.* 2015;5:2892-2911; 6. Hirsch V. *Ther Adv Med Oncol.* 2018;10:1-12; 7. Shi YS, et al. *J Thorac Oncol.* 2014;9:154-162; 8. Chapman AM, et al. *Lung Cancer.* 2016;102:122-134; 9. Sacher AG, et al. *JAMA Oncol.* 2016;2:313-320; 10. Chia PL, et al. *Clin Epidemiol.* 2014;6:423-432.

Structure and normal function EGFR

- **E**pidermal **G**rowth **F**actor **R**eceptor is essential for normal cellular functions such as:
  growth,proliferation,differentiation, migration and survival.

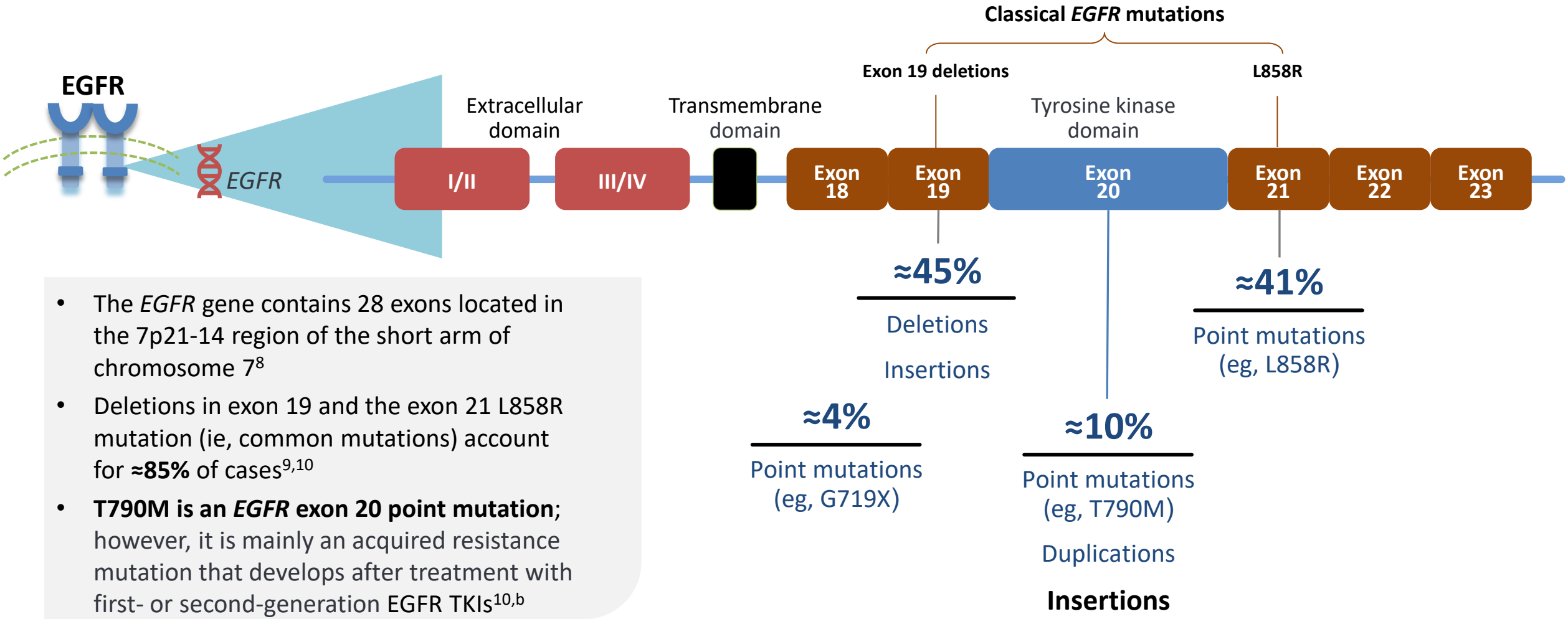
- EGFR gene-short arm of chromosome 7

- Overexpression, gene amplification, mutations of EGFR's kinase domain can cause dysregulation leading to non-small cell lung cancer (NSCLC)



Voldborg B.R.Epidermal growth factor receptor (EGFR) and EGFR mutations, function and possible role in clinical trials Ann Oncol. 1997 Dec;8(12):1197-206.

EGFR Oncogenic Driver Mutations Are Predominantly Found Within Exons 18 to 21 Encoding the Tyrosine Kinase Domain

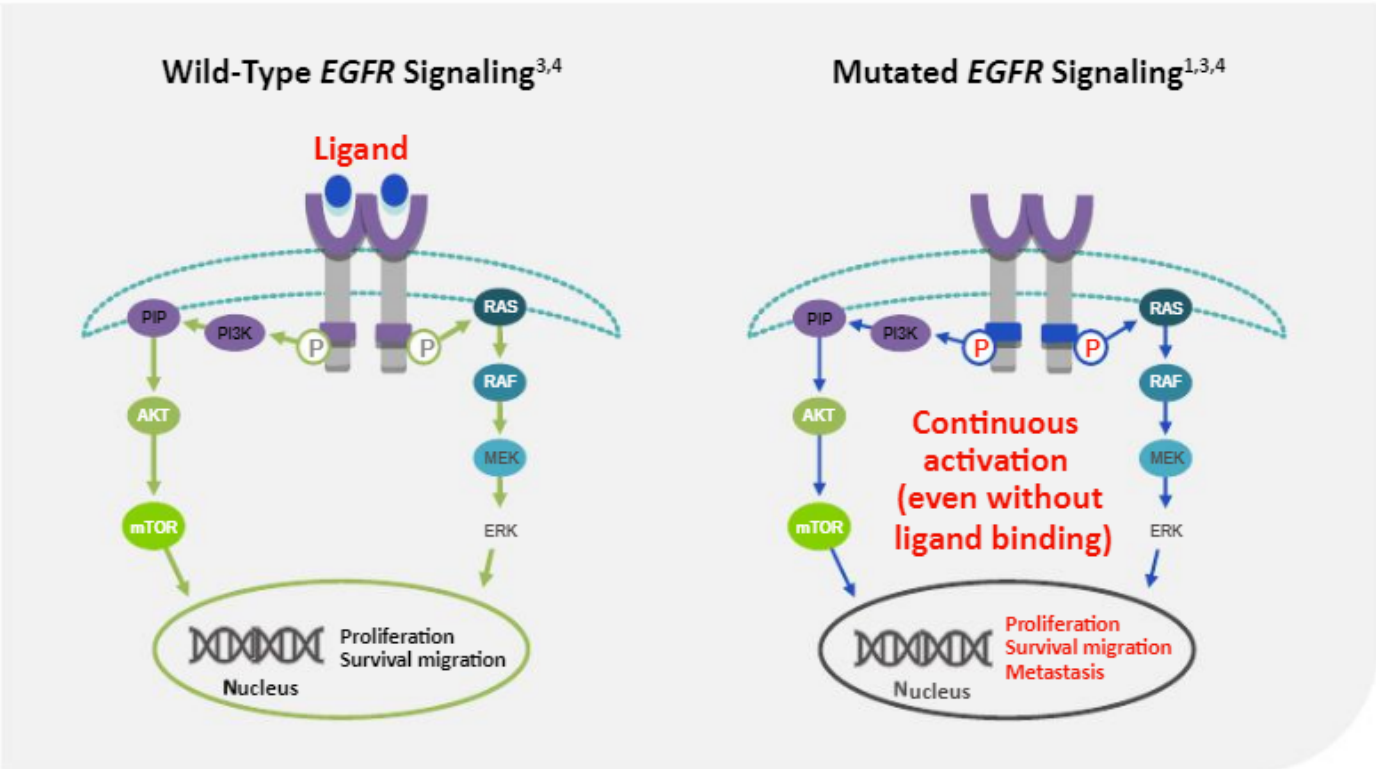


- The *EGFR* gene contains 28 exons located in the 7p21-14 region of the short arm of chromosome 7[8]
- Deletions in exon 19 and the exon 21 L858R mutation (ie, common mutations) account for **≈85%** of cases[9,10]
- **T790M is an *EGFR* exon 20 point mutation**; however, it is mainly an acquired resistance mutation that develops after treatment with first- or second-generation EGFR TKIs[10,b]

[a] The incidence of mutations may vary due to the range of techniques used; [b] EGFR TKIs not designed to target *EGFR* exon 20 insertions.
EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

1. Chong C, Jänne P. *Nat Med.* 2013;19:1389-1400; 2. Crossland V, et al. *J Thorac Oncol.* 2018;13(10 suppl):S612-S613; 3. Gazdar A, Minna J. *PLoS Med.* 2005;2:e377; 4. Gazdar A. *Oncogene.* 2009;28(suppl 1):S24-S31; 5. Jorge S, et al. *Braz J Med Biol Res.* 2014;47:929-939; 6. Kobayashi Y, Mitsudomi T. *Cancer Sci.* 2016;107:1179-1186; 7. Lee J, et al. *Ann Oncol.* 2013;24:2080-2087; 8. Wang F, et al. *Transl Cancer Res*. 2020;9:2982-2991; 9. O'Kane G, et al. *Lung Cancer.* 2017;109:137-144; 10. Nagano T, et al. *Cells.* 2018;7:212.

EGFR Oncogenic Driver Mutations Constitutively Activate the Receptor

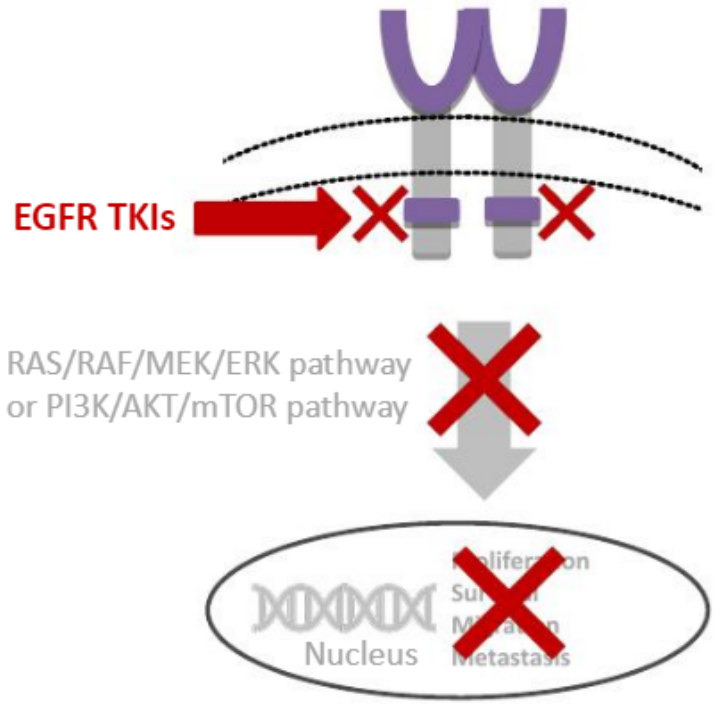


- The most common *EGFR* oncogenic driver mutations are found within the genes encoding the tyrosine kinase domain[1]
- These mutations cause increased and sustained phosphorylation of the receptor (without ligand stimulation)[1-3]
- This results in continuous cell survival, proliferation, invasion, and metastasis[1,3]

AKT, protein kinase B; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; P, phosphorylation; PI3K, phosphatidylinositol-3-kinase; PIP, phosphatidylinositol-4,5-bisphosphate; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma.

1. Gazdar A. *Oncogene*. 2009;28(suppl 1):S24-S31; 2. Kobayashi Y, Mitsudomi T. *Cancer Sci*. 2016;107:1179-1186; 3. Siegelin M, Borczuk A. *Lab Invest*. 2014;94:129-137; 4. Wee P, Wang Z. *Cancers (Basel)*. 2017;9:52.

EGFR Oncogenic Driver Mutations Can Be Blocked by EGFR TKIs[1,2]



EGFR TKIs:

- Bind to the ATP pocket of the tyrosine kinase domain
- Inhibit intracellular signaling pathways
- Impede cell survival, proliferation, invasion, and metastasis

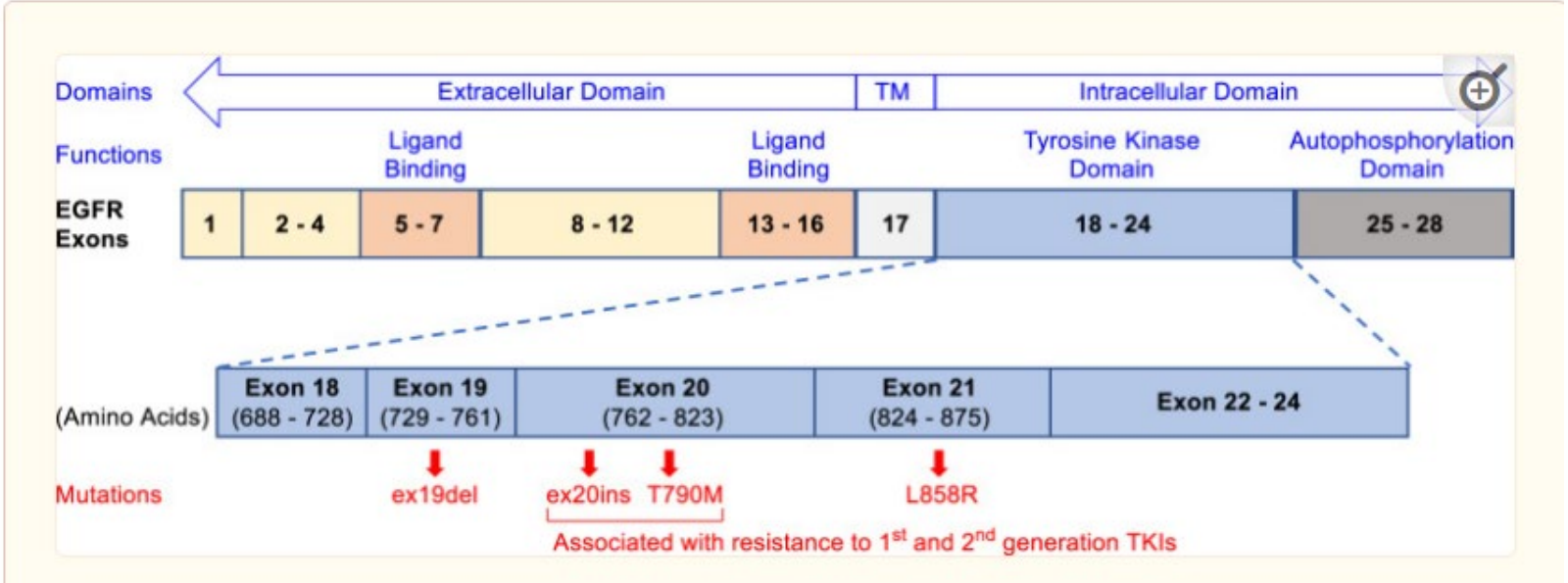
EGFR TKIs bind to the ATP-binding site within the EGFR tyrosine kinase domain, inhibiting its kinase activity. However, not all *EGFR* mutations are sensitive to current TKIs

ATP, adenosine triphosphate; AKT, protein kinase B; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol-3-kinase; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; TKI, tyrosine kinase inhibitor.

1. Kobayashi Y, Mitsudomi T. *Cancer Sci.* 2016;107:1179-1186; 2. Du Z, Lovly C. *Mol Cancer.* 2018;17:58.

EGFR tyrosine kinase inhibitors

- First-generation EGFR tyrosine kinase inhibitors (TKIs), erlotinib and gefitinib and second-generation TKIs, afatinib and dacomitinib are effective for these common mutations but they lose their effectiveness with the occurrence of EGFR T790M mutation, an acquired mutation that confers drug resistance.
- **EGFR exon 20 insertions (ex20ins) are the third most frequent mutations are resistant to both first and second-generation TKIs.**
- Third-generation irreversible TKI, osimertinib, has shown activity against ex20ins in some studies but was only approved for EGFR T790M-positive NSCLC



Med Chem Res. 2022; 31(10): 1647–1662.
Published online 2022 Sep 1. doi: 10.1007/s00044-022-02952-5
PMCID: PMC9433531
PMID: 36065226

Discovery of mobocertinib, a new irreversible tyrosine kinase inhibitor indicated for the treatment of non-small-cell lung cancer harboring EGFR exon 20 insertion mutations

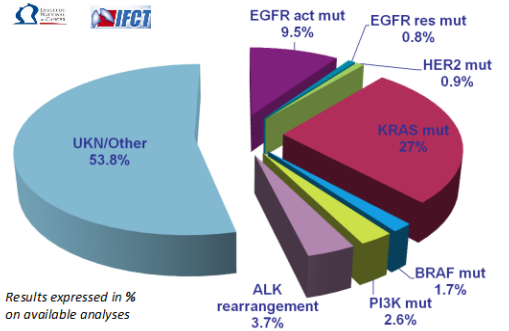
Jun Wang,[1] Daniel Lam,[2] Jeffrey Yang,[1] and Longqin Hu[1,3]

- On September 15, 2021, mobocertinib received accelerated FDA approval for use in adults with locally advanced or metastatic NSCLC patients with EGFR ex20ins mutations, as detected by an FDA-approved test, who are on or have had platinum therapy.

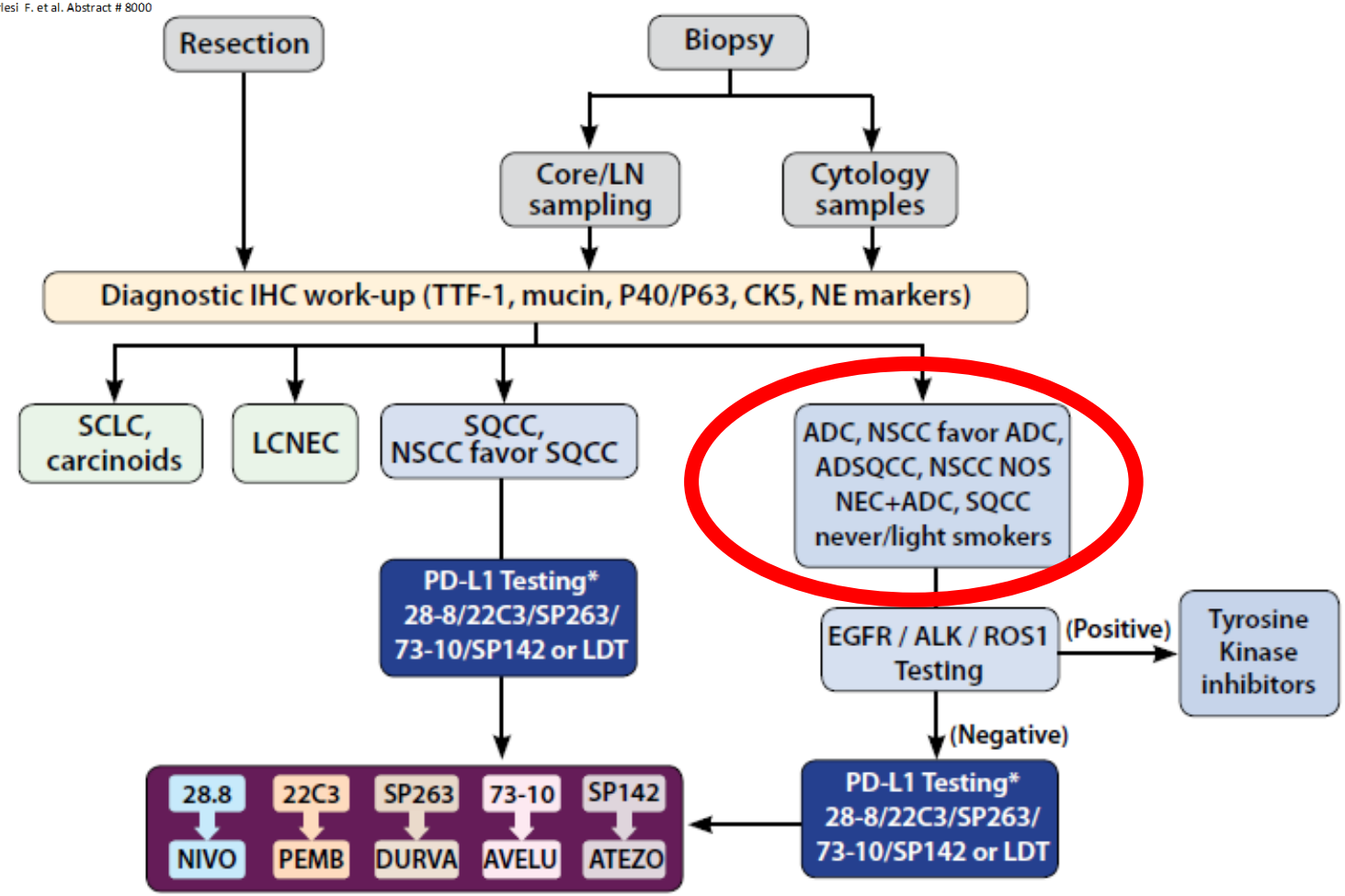
Timing of EGFR testing

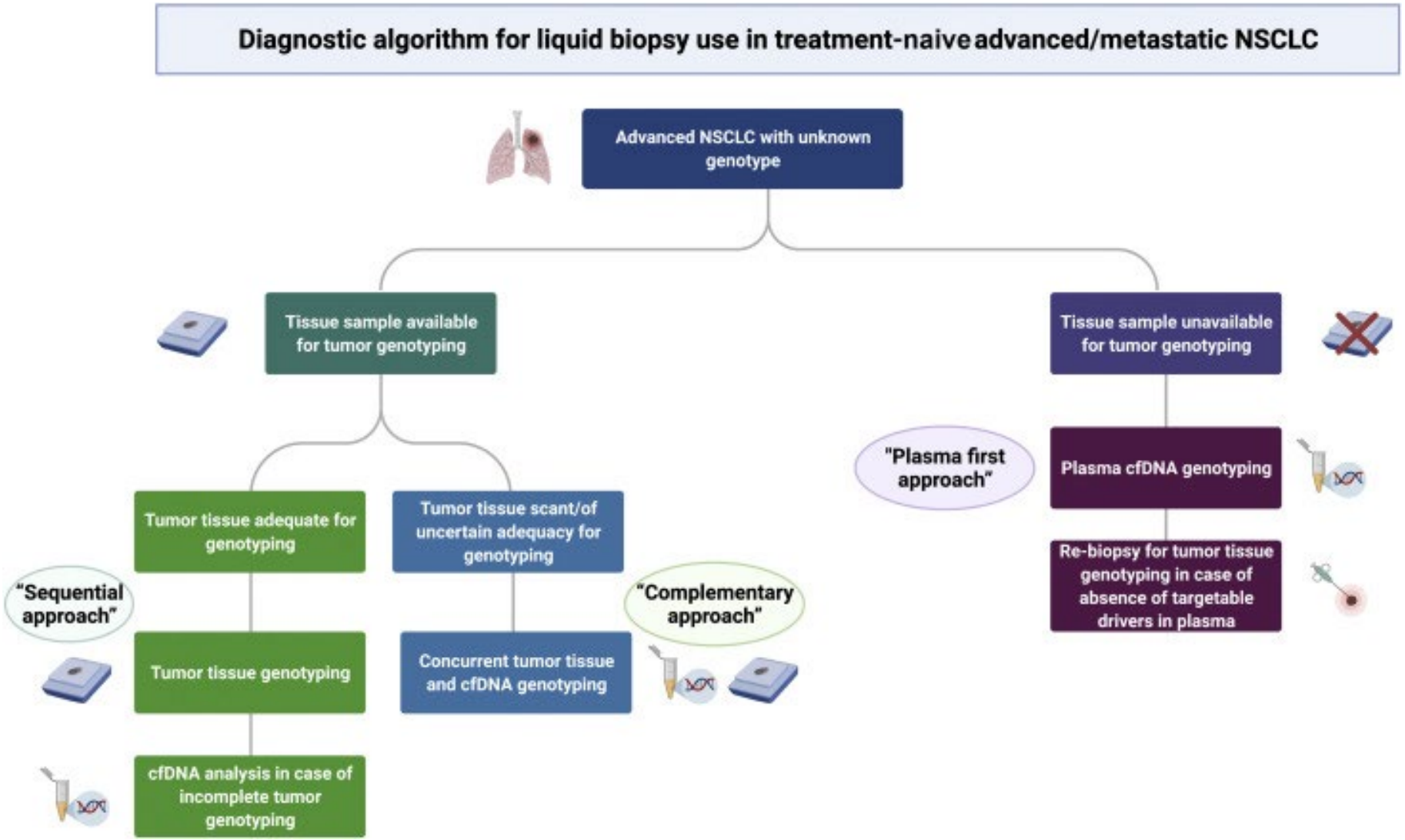
- ✓ ***Primary diagnosis***
- ✓ ***Dynamic monitoring-*** EGFR driver and resistance mutation status during treatment
- ✓ ***Disease progression***

Biomarkers assessment (n=9911)



Barlesi F. et al. Abstract # 8000





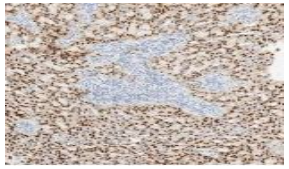
- Rolfo C.et al.Liquid Biopsy for Advanced NSCLC: A Consensus Statement From the International Association for the Study of Lung Cancer. Journal of Thoracic Oncology Vol. 16 No. 10, October 2021, Pages 1647-1662

When to test: reflex or on demand?

- More expensive
- Shorter turnaround times
- Tissue saving
- Results of testing are include into initial report

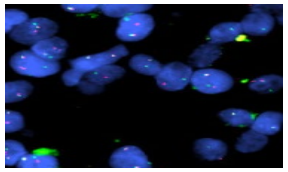
- Less costly
-Takes more time
- Addition sectioning of specimens
- Result of testing separately reported

Which methods ?



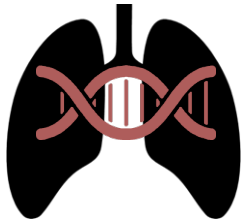
IMMUNOHISTOCHEMISTRY

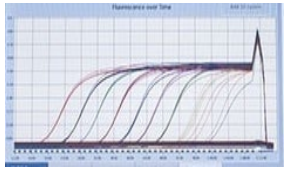
- Short turnaround time, cheap
- Validated detection method for ALK and PD-L1
- Screening method : ROS1,BRAF,NTRK



FISH *(fluorescence in situ hybridisation)*

- Historical gold standard for detection of gene fusions (ALK, ROS1
- Expert pathologist !!
- False- negative results can be above 30%





PCR *(polymerase chain reaction)*

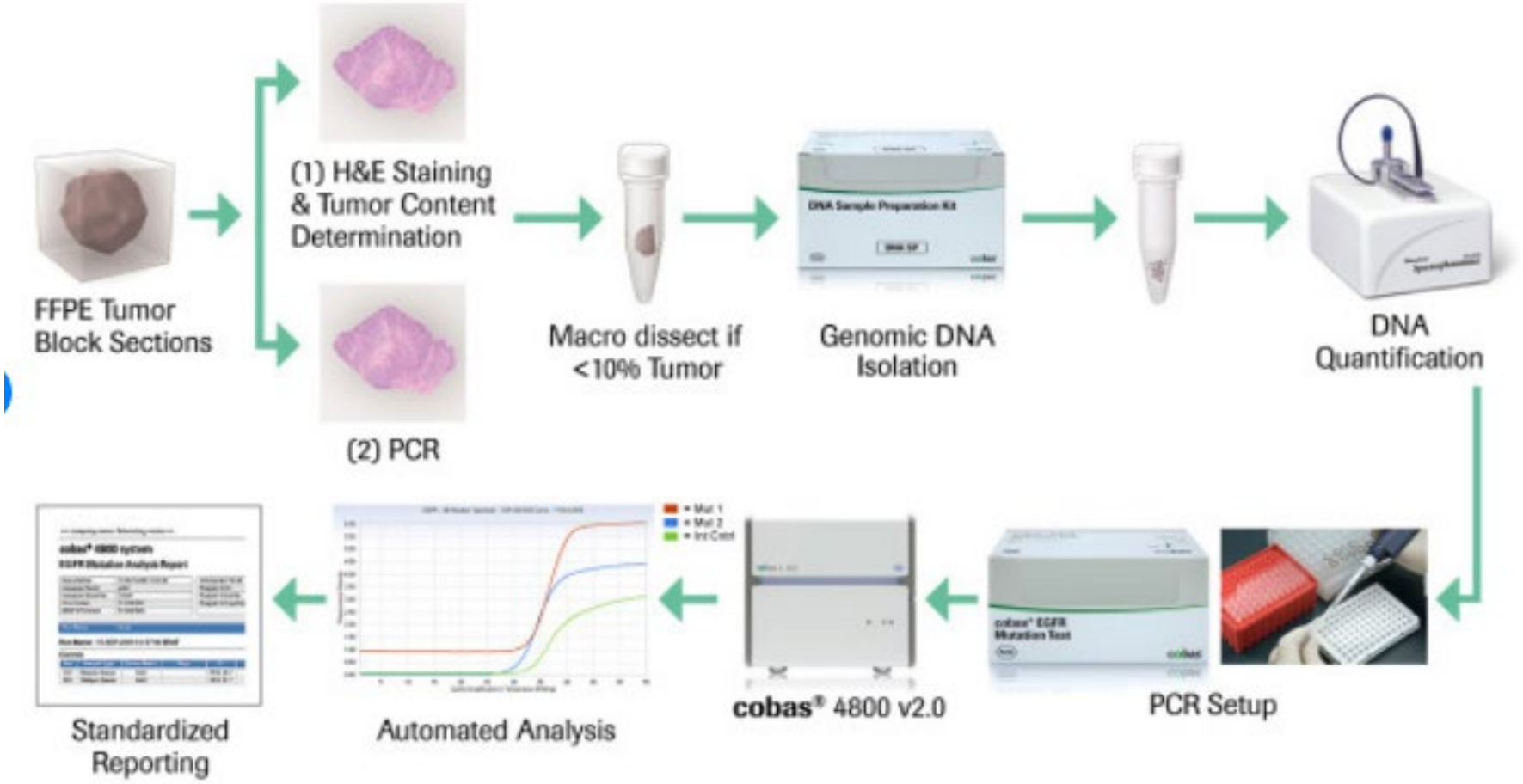
- Cheaper method, shorter turnaround time
- The target fusions must be known **(cannot detect new fusion partners)**

NGS *(next-generation sequencing)*

- High specificity and sensitivity, PREFERRED METHOD!
- The most reliable diagnostic test for gene fusions; the biggest advantage for the detection and identification of all known and potentially new ROS1, RET, NTRK rearrangements
- However, more expensive and often impractical in a local laboratory

1. Hsiao, et al. J Mol Diagn 2019; Marchio, et al. Ann Oncol 2019; 2. Naidoo and Drilon. Am J Hematol Oncol 2014; 3. Penault-Llorca, et al. J Clin Pathol 2019;
4. Solomon, et al. Ann Oncol 2019; 5. Wong, et al. Pathol and Oncol Res 2019 6. Kashima J et al. *Cancers (Basel)* 2019;11:599; 7. Lindeman NI et al. *Arch Pathol Lab Med* 2018;142:321–346; 7. Beadling C et al. *J Mol Diagn* 2016;18:165–175; 8. Ferrara R et al. *J Thorac Oncol* 2018;13:27–45; 9. FDA. Available at: https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools (accessed January 2021); 10. Mosele F et al. *Ann Oncol* 2020;31:1491–1505.

EGFR testing



EGFR testing

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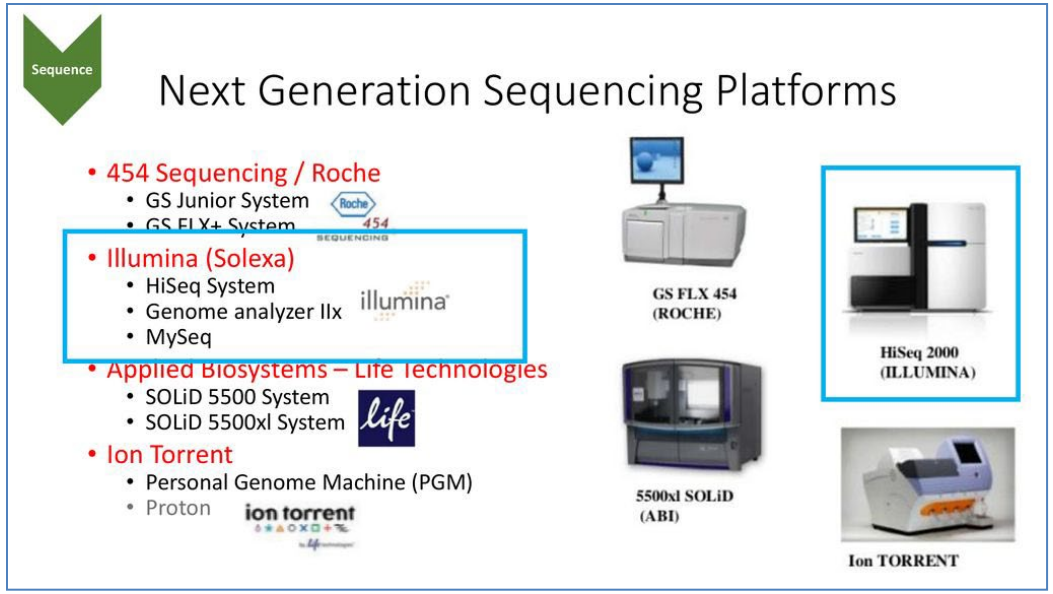

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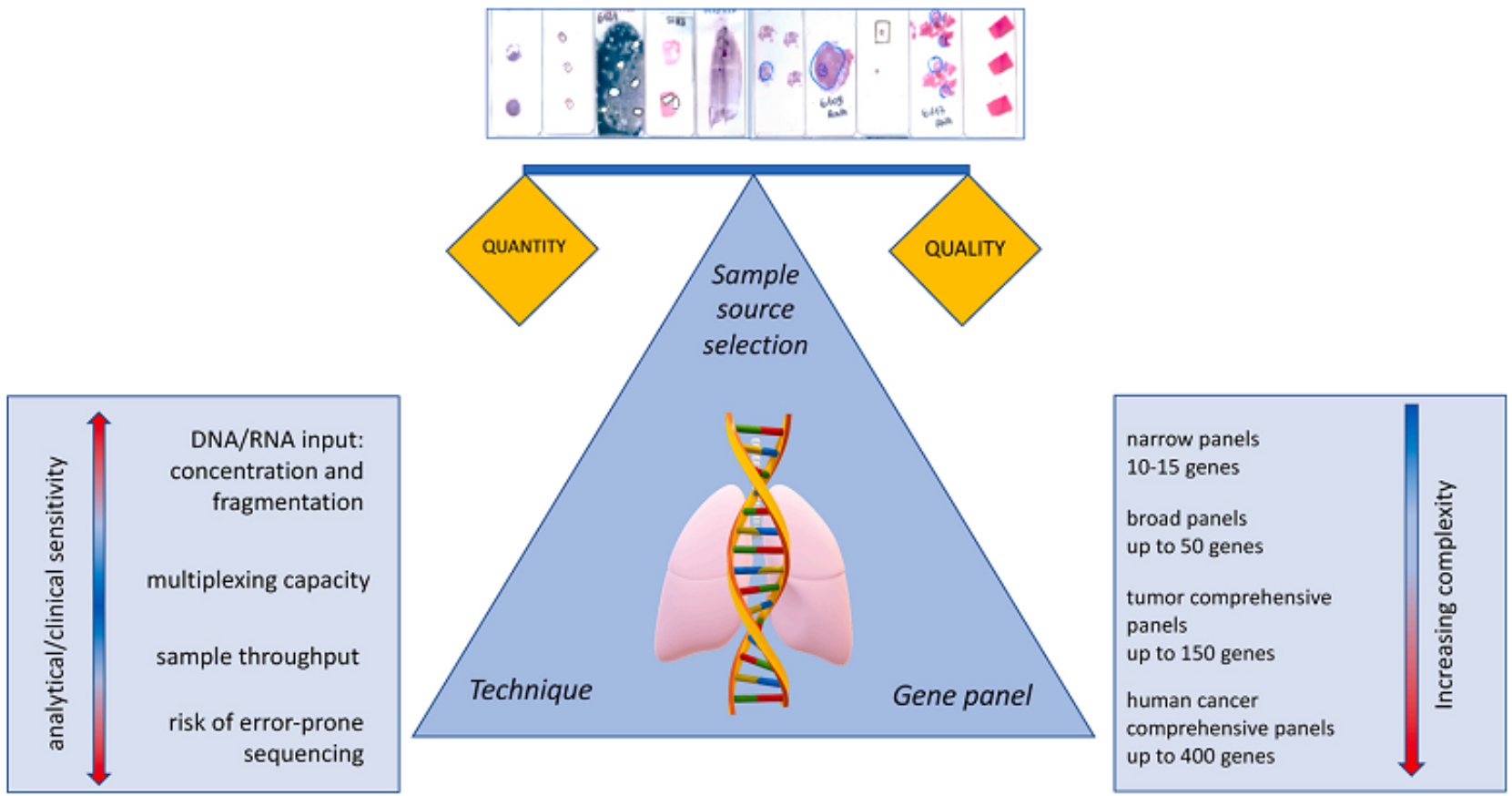
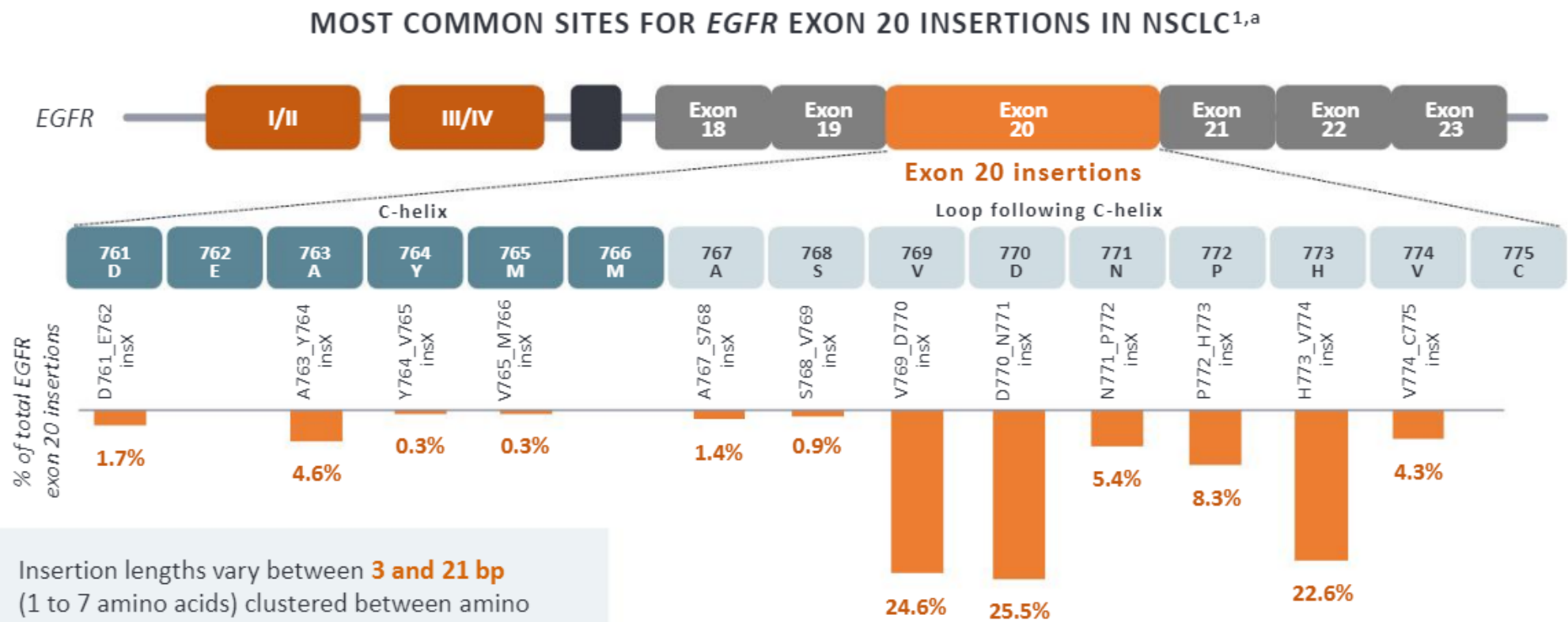
The storm of NGS in NSCLC diagnostic-therapeutic pathway: How to sun the real clinical practice

Giovanna De Maglio [a], Giulia Pasello [b,i,*], Mariella Dono [c], Michelangelo Fiorentino [d], Alessandro Follador [e], Marianna Sciortino [f], Umberto Malapelle [g,1], Marcello Tiseo [h,j,1]



EGFR Exon 20 Insertions Are a Heterogeneous Family of In-Frame Insertion and Duplication Mutations



Insertion lengths vary between **3 and 21 bp** (1 to 7 amino acids) clustered between amino acid positions 762 and 774 of the EGFR protein[1]

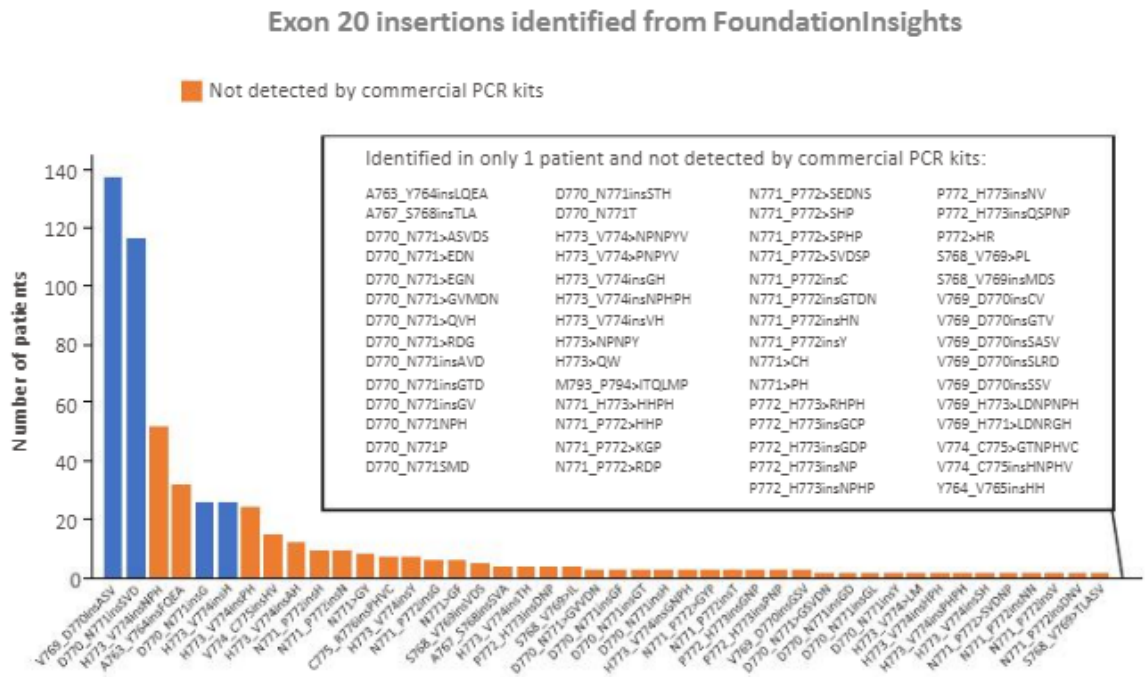
▪ Mutation frequency distribution was calculated using COSMIC v86 (http://cancer.sanger.ac.uk) after filtering for NSCLC adenocarcinomas harboring exon 20 insertions (N=349).[1]
bp, base pair; COSMIC, Catalogue of Somatic Mutations in Cancer.

1. Vyse S, Huang P. *Signal Transduct Target Ther*. 2019;4:5.

The GENIE and Foundation Insights databases showed that NGS can identify ≈50% of *EGFR* exon 20 insertions not detected by PCR test kits

	GENIE[a]	Foundation Insights[b]
Unique exon 20 insertion variants, n	40	102
Number of most common variants[c] that would have been detected by PCR, n/N (%)	4/9 (44)	4/17 (24)
Detection of patients with exon 20 insertions, n		
By PCR	89	305
By NGS	175	627
Cases missed by PCR that would have been identified by NGS, %	**49.1**	**51.4**

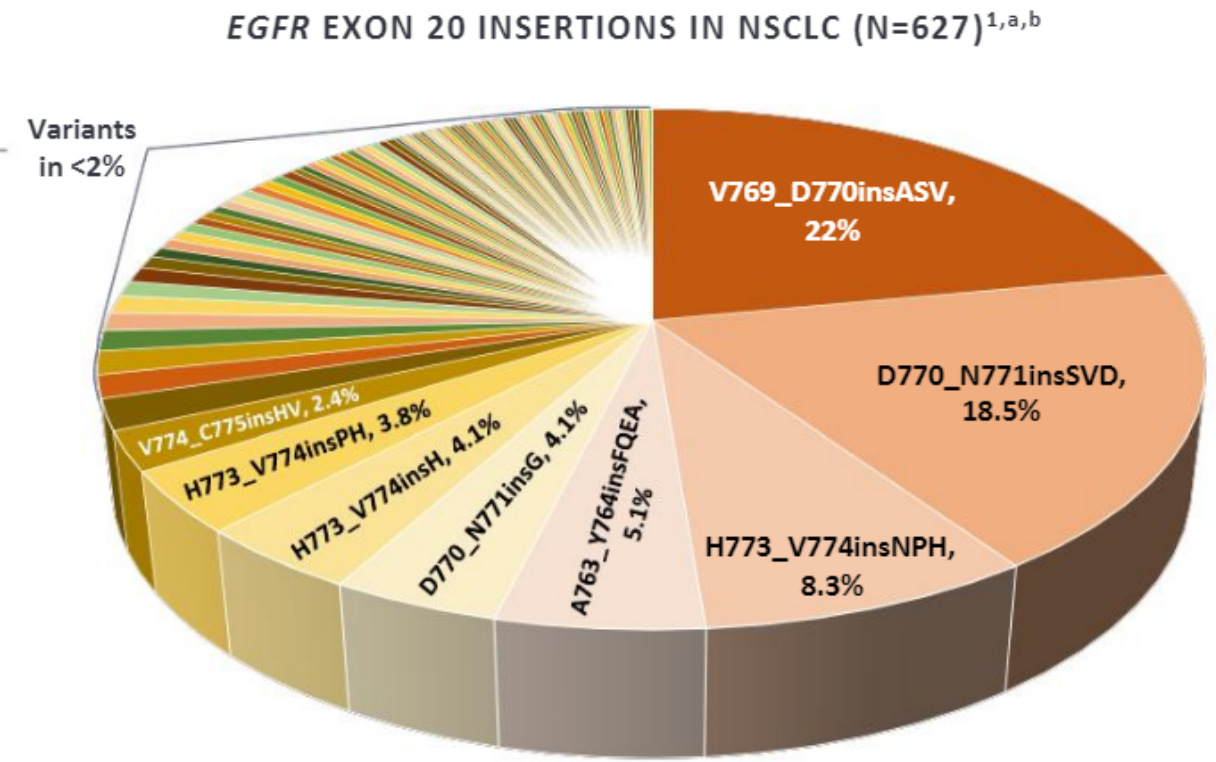
A variety of unique exon 20 insertion variants (40–102) were identified by NGS



Note: Total number of patients with NSCLC: N=68,879 (GENIE database: n=12,497; FoundationInsights database: n=56,382).
[a]GENIE is a real-world registry of cancer genomics data from leading cancer centers; NGS data were extracted from 13 participating US institutions. [b]FoundationInsights is a database from the FoundationCore knowledgebase of patient genomic profiles spanning >150 cancer types; [c]Present in ≥5% of patients.
EGFR: epidermal growth factor receptor; GENIE: Genomics Evidence Neoplasia Information Exchange; NGS: next-generation sequencing; PCR: polymerase chain reaction.
Baumi JM, et al. WCLC 2020. Abstract 3399.

Emerging Data Identified as Many as 102 *EGFR* Exon 20 Insertion Variants[1]

1. H773_V774insAH
2. N771_P772insH
3. N771_P772insN
4. N771>GY
5. C775_R776insPHVC
6. H773_V774insY
7. N771_P772insG
8. N771>GF
9. S768_V769insVDS
10. A767_S768insSVA
11. H773_V774insTH
12. P772_H773insDNP
13. S768_V769>IL
14. D770_N771>GVVDN
15. D770_N771insGF
16. D770_N771insGT
17. D770_N771insH
18. H773_V774insGNPH
19. N771_P772>GYP
20. N771_P772insT
21. P772_H773insGNP
22. P772_H773insPNP
23. V769_D770insGSV
24. D770_N771>GSVDN
25. D770_N771insGD
26. D770_N771insGL
27. D770_N771insY
28. H773_V774>LM
29. H773_V774insHPH
30. H773_V774insPHPH
31. H773_V774insSH
32. N771_P772>SVDNP
33. N771_P772insNN
34. N771_P772insV
35. N771_P772insVDN
36. S768_V769>TLASV
37. A763_Y764insLQEA
38. A767_S761insTLA
39. D770_N771>ASVDS
40. D770_N771>EDN
41. D770_N771>EGN
42. D770_N771>GVMDN
43. D770_N771>QVH
44. D770_N771>RDG
45. D770_N771insAVD
46. D770_N771insGTD
47. D770_N771insGV
48. D770_N771insNPH
49. D770_N771insP
50. D770_N771insSMD
51. D770_N771insSTH
52. D770_N771insT
53. H773_V774>NPNPYV
54. H773_V774>PNPYV
55. H773_V774insGH
56. H773_V774insNPHPH
57. H773_V774insVH
58. H773>NPNPY
59. H773>QW
60. M793_P794>ITQLMP
61. N771_H773>HHPH
62. N771_H772>HHP
63. N771_H772>KGP
64. N771_H772>RDP
65. N771_P772>SEDNS
66. N771_P772>SHP
67. N771_P772>SPHP
68. N771_P772>SVDSP
69. N771_P772insC
70. N771_P772insGTDN
71. N771_P772insHN
72. N771_P772insY
73. N771>CH
74. N771>PH
75. P772_H773>RHPH
76. P772_H773insGCP
77. P772_H773insGDP
78. P772_H773insNP
79. P772_H773insNPHP
80. P772_H773insNV
81. P772_H773insQSPNP
82. P772>HR
83. S768_V769>PL
84. S768_V769insMDS
85. V769_D770insCV
86. V769_D770insGTV
87. V769_D770insSASV
88. V769_D770insSLRD
89. V769_D770insSSV
90. V769_H773>LDNPNPH
91. V769_N771>LDNRGH
92. V774_C775>GTNPHVC
93. V774_C775insHNPHV
94. Y764_V765insHH

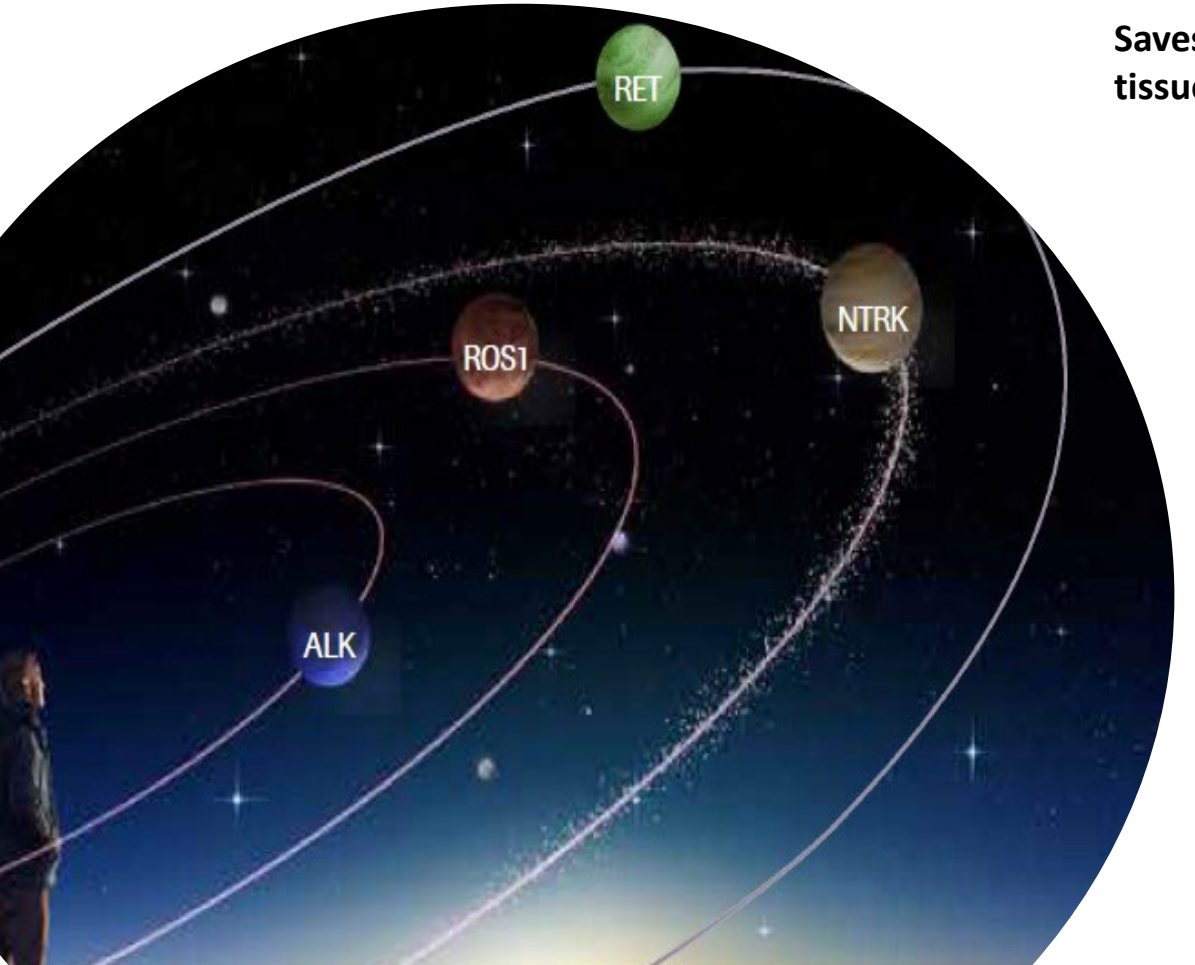


Published data has reported 64 unique *EGFR* exon 20 insertion variants[2,c], but emerging data from FoundationInsights identified as many as 102 variants[1,a]

•FoundationInsights is a database of patient genomic profiles. Of 56,382 NSCLC genomic profiles, 36,465 had lung adenocarcinoma, 8259 had *EGFR* mutations, and 627 had *EGFR* exon 20 insertion mutations;
[b] Variant prevalence are approximations; [c] Comprehensive genomic profiling performed on 14,483 NSCLC cases in the course of clinical care identified 2251 cases with *EGFR* mutations; 263 of these cases were *EGFR* exon 20 insertion mutations.

1. Bauml JM, et al. WCLC 2020 [abstract 3399]; 2. Riess JW, et al. *J Thorac Oncol*. 2018;13:1560-1568.

Comprehensive Genomic Profiling “CGP” (NGS-based)



Saves tissue

No miss mutations

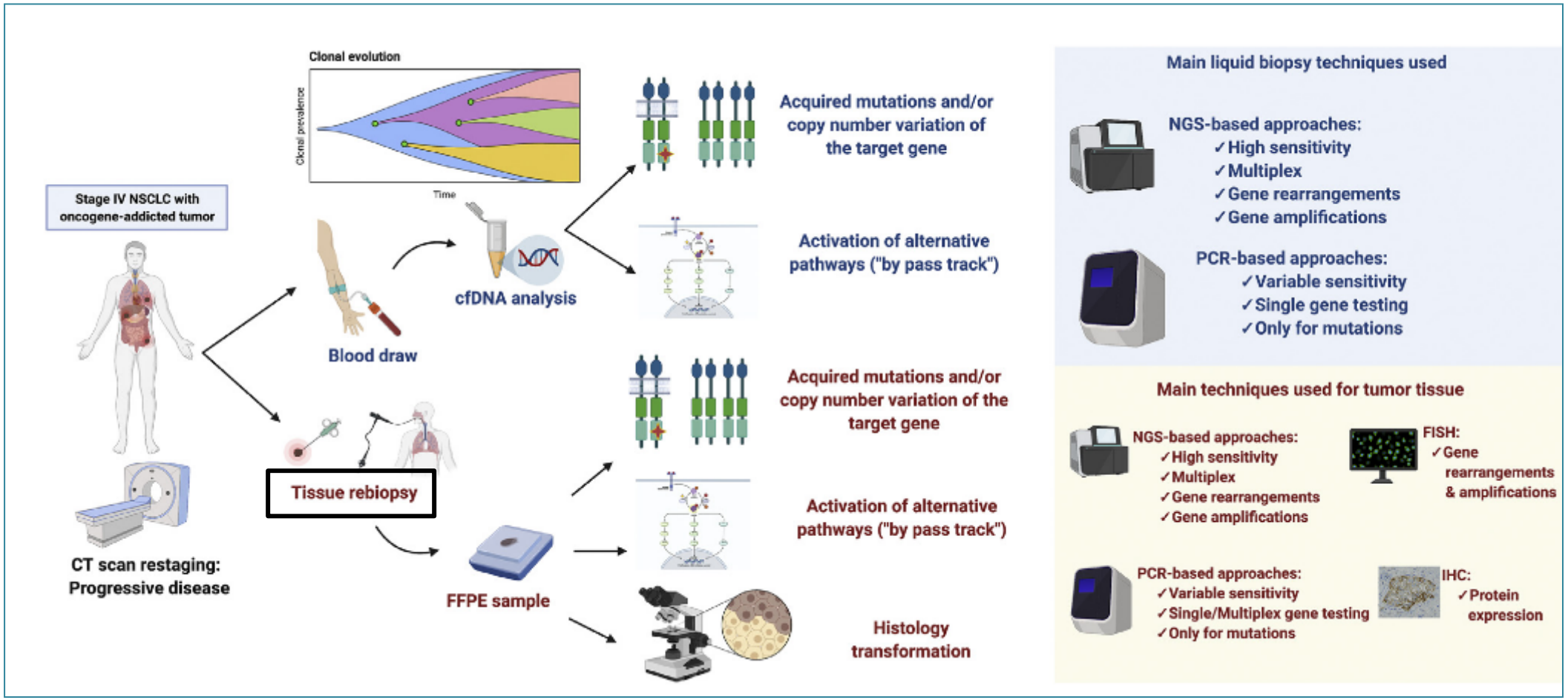
Saves time

Treatment decision

Timing of EGFR testing

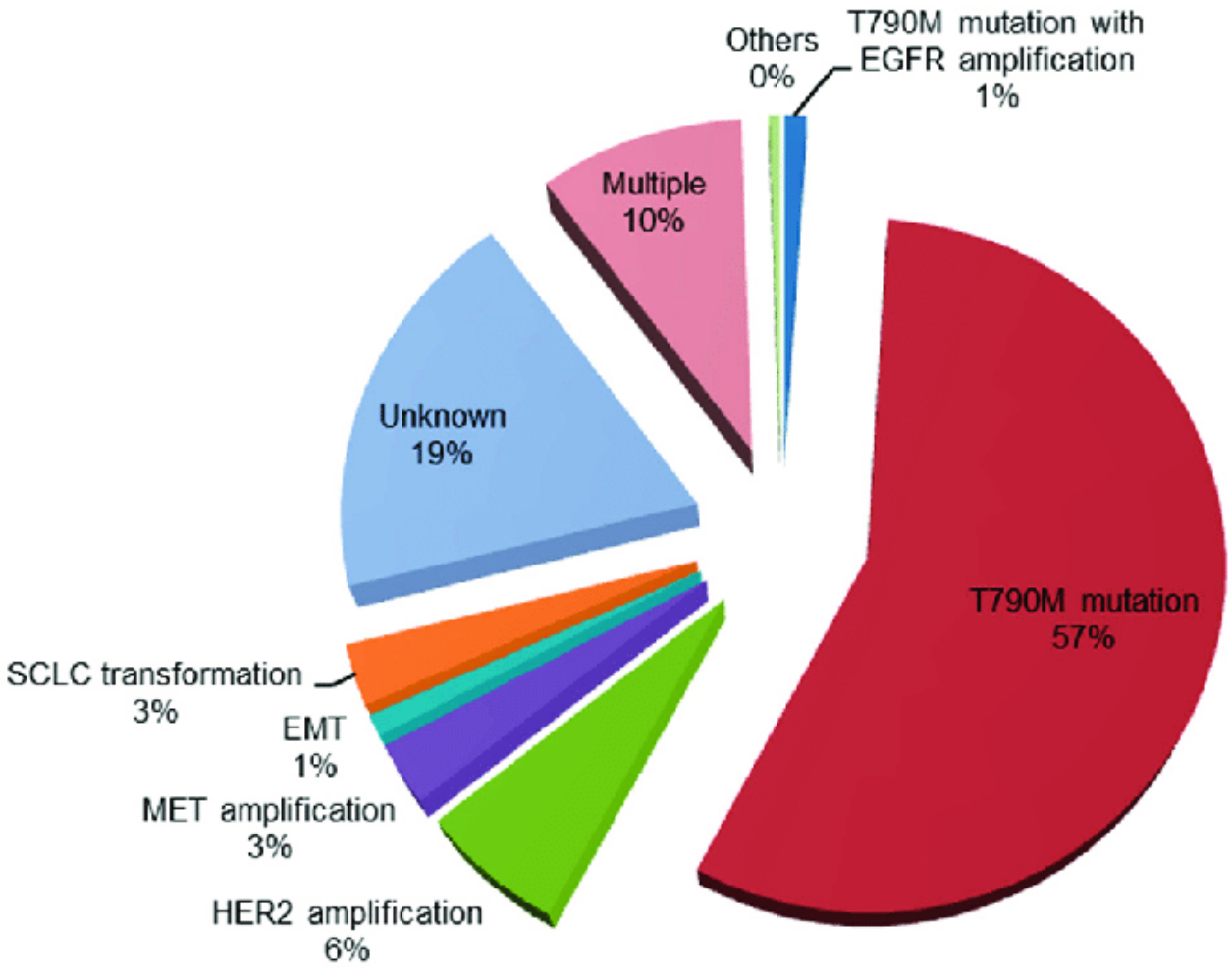
- ✓ ***Primary diagnosis***
- ✓ ***Dynamic monitoring-*** EGFR driver and resistance mutation status during treatment
- ✓ ***Disease progression***

Tissue versus Liquid Biopsy



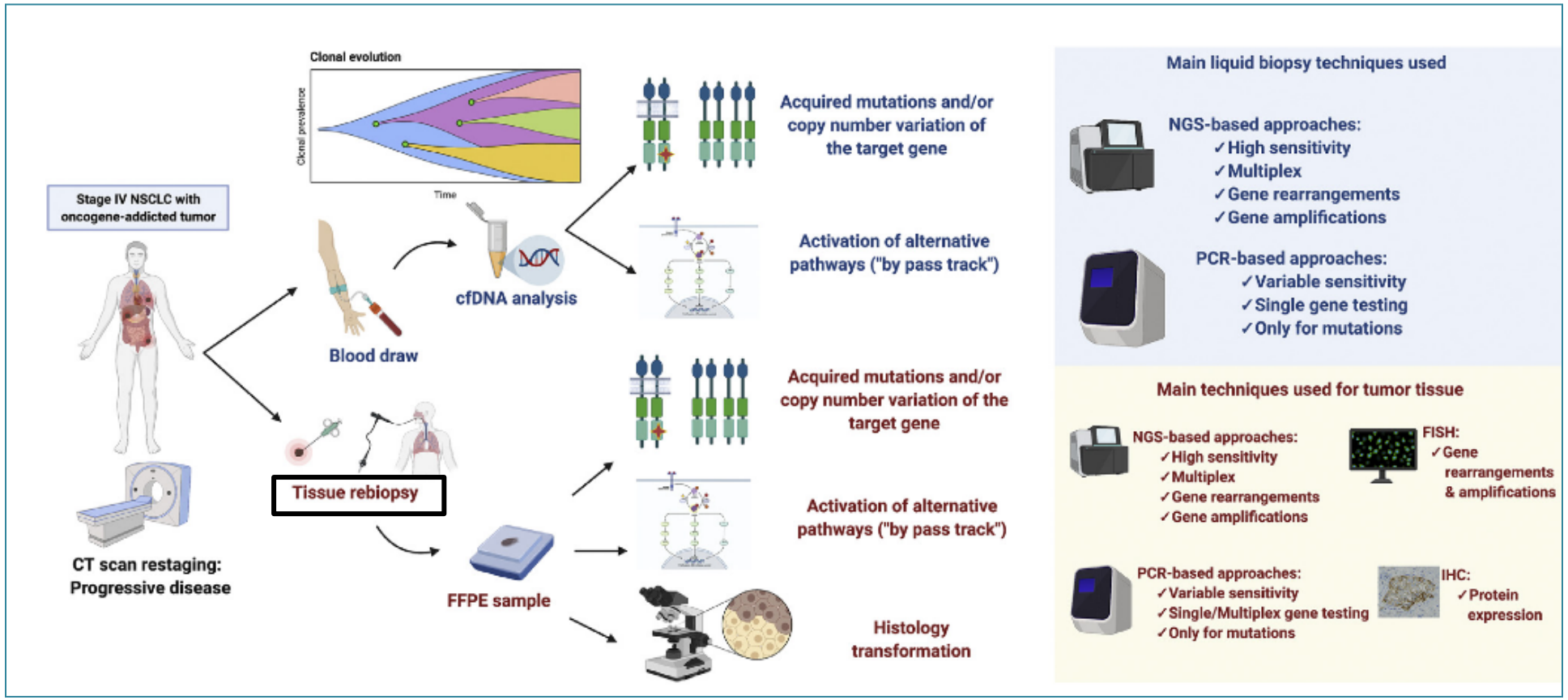
Rolfo C.et al.Liquid Biopsy for Advanced NSCLC: A Consensus Statement From the International Association for the Study of Lung Cancer. Journal of Thoracic Oncology Vol. 16 No. 10, October 2021, Pages 1647-1662

Mechanisms of acquired resistance to first-generation tyrosine kinase inhibitors (gefitinib and erlotinib)



- Westover D. et al.Mechanisms of acquired resistance to first-generation tyrosine kinase inhibitors (gefitinib and erlotinib). Annals of Oncology.Volume 29, Supplement 1, January 2018, Pages i10-i19

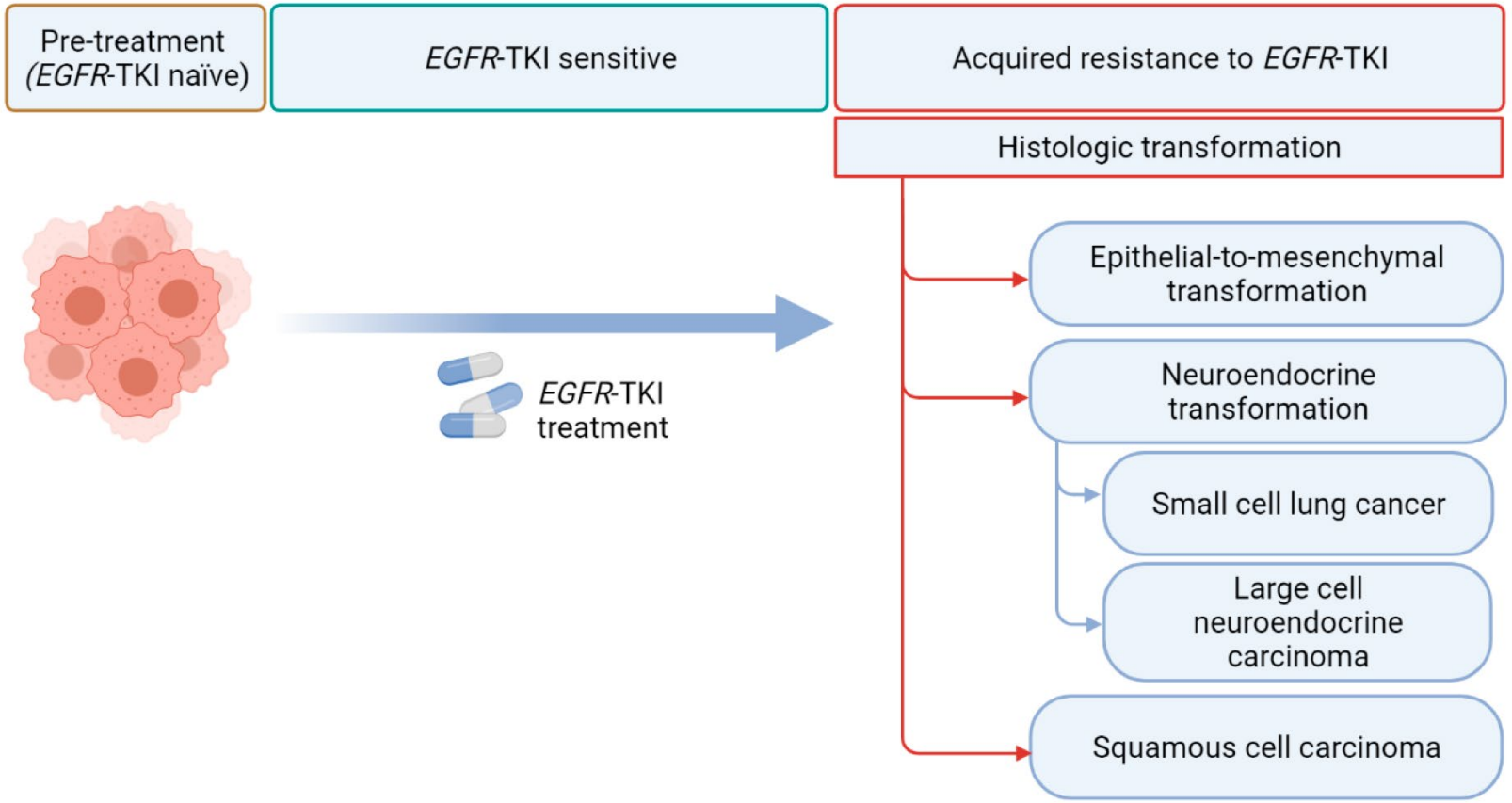
Tissue versus Liquid Biopsy



65.7% sensitivity and 99.8% specifity

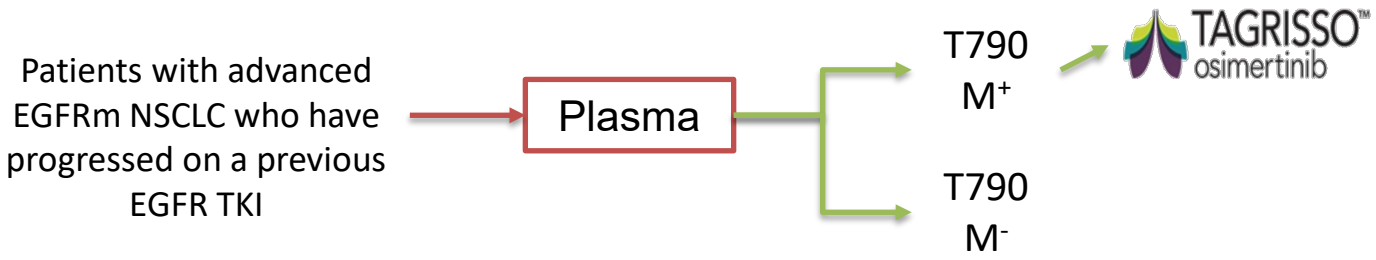
- Rolfo C.et al.Liquid Biopsy for Advanced NSCLC: A Consensus Statement From the International Association for the Study of Lung Cancer. Journal of Thoracic Oncology Vol. 16 No. 10, October 2021, Pages 1647-1662

Histologic Transformation in EGFR-Mutant Lung Adenocarcinomas



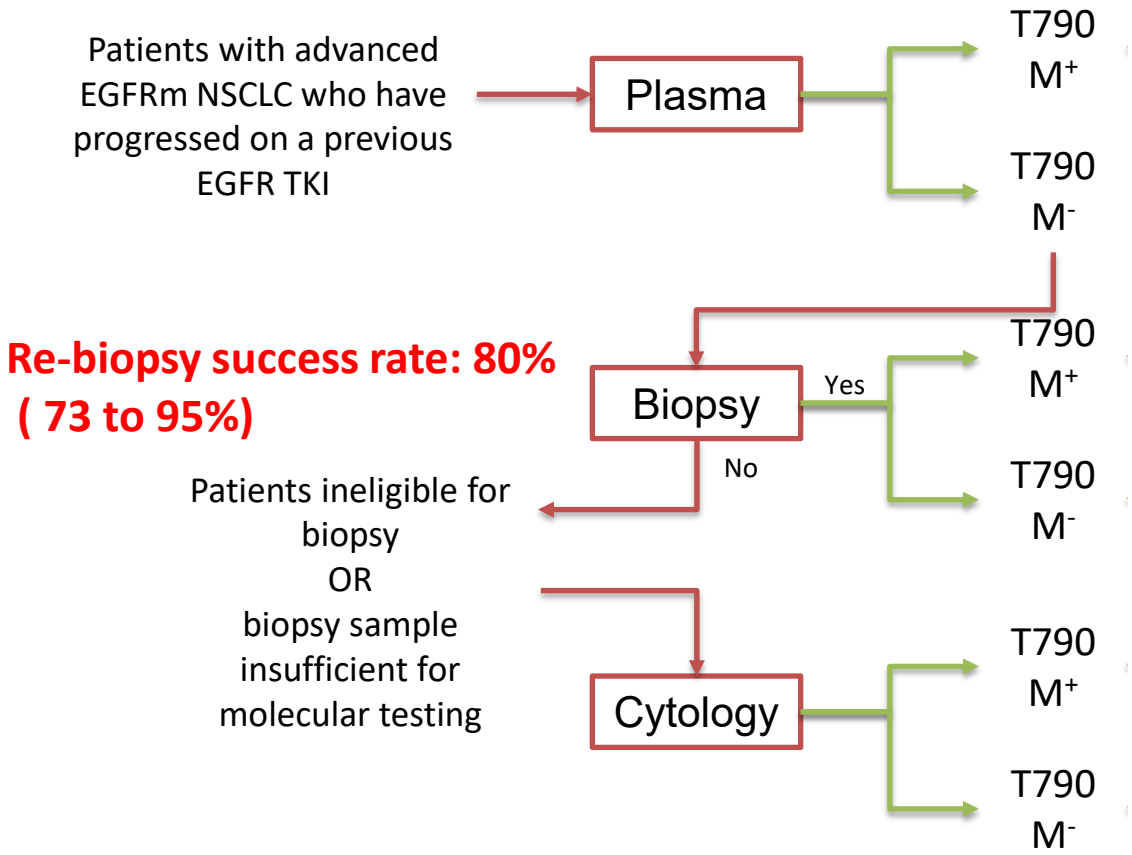
- Ranjan P. et al. Histologic Transformation in EGFR-Mutant Lung Adenocarcinomas: Mechanisms and Therapeutic Implications. Cancers 2021, 13, 4641

ALGORITHM FOR EGFR T790M MUTATION TESTING



- 1. Tagrisso Summary of product characteristics 2016

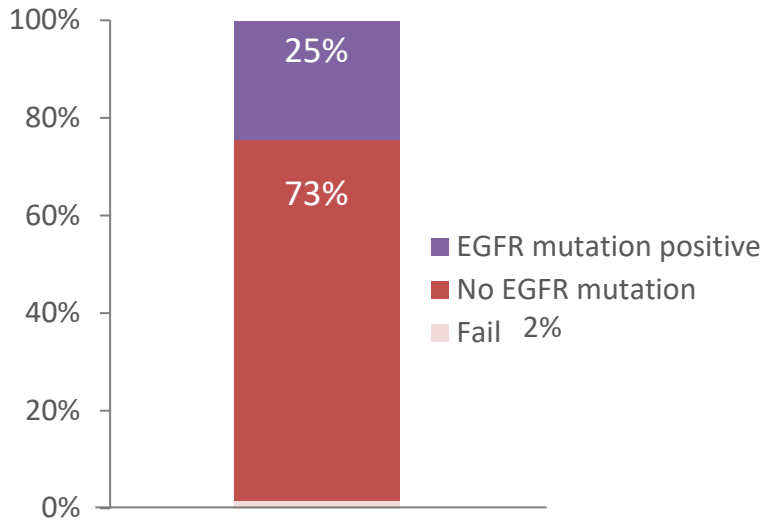
ALGORITHM FOR EGFR T790M MUTATION TESTING



1. Tagrisso Summary of product characteristics 2016

Different cytology samples can be successfully used for mutation testing

EGFRm test results



Of 128 cytological samples, 98% generated an EGFR test result, with an EGFRm frequency of 25%[1]

The EGFRm frequency was comparable to that detected in tissue biopsy specimens

EGFR T790M mutation test results

Of 26 cytological samples taken from patients resistant to EGFR TKIs (20 pleural effusion, 6 sputum), 42.3% harboured a EGFR T790M mutation[2]

A mutation frequency comparable to other studies[3]

- 1. Rekhtman N et al. J Thorac Oncol 2011;6:451–8 2. Lin Li et al. Chinese Journal of Cancer Research, 2015; 27 (3): 294-300 3. Lindeman NI, et al. Journal of Thoracic Oncology. 2013 Jul 31;8(7):823-59

EGFR testing

Table 2 FDA-approved companion diagnostic tests for NSCLC therapies

FDA-approved device	Manufacturer	Platform	Specimen	Therapy	Approximate turnaround time
therascreen EGFR RGQ PCR kit (47)	Qiagen	PCR	FFPE tumor tissue	Afatinib, gefitinib	1 to 7 days
FoundationOne CDx™ (48)	Foundation Medicine	NGS	FFPE tumor tissue	Afatinib, osimertinib, erlotinib, gefitinib, alectinib, crizotinib, ceritinib, dabrafenib plus trametinib	10 to 14 days
cobas *EGFR* Mutation Test v2 (49)	Roche	PCR	Plasma (K_2EDTA) or FFPE tumor tissue	Erlotinib, osimertinib	1 to 7 days
PD-L1 IHC 22C3 pharmDx (50)	Agilent Technologies	IHC	FFPE tumor tissue	Pembrolizumab	1 to 7 days
VENTANA *ALK* (D5F3) CDx Assay (51)	Roche/VENTANA Medical Systems	IHC	FFPE tumor tissue	Alectinib, crizotinib, ceritinib	1 to 3 days
Vysis *ALK* Break Apart FISH Probe Kit (52)	Abbott	FISH	FFPE tumor tissue	Alectinib, crizotinib, ceritinib	1 to 7 days
Oncomine™ Dx Target Test (53)	Thermo Fisher Scientific	NGS	FFPE tumor tissue	Crizotinib, dabrafenib plus trametinib, gefitinib	5 to 14 day

Reporting of EGFR mutation status using PCR platform

PRIMENJENE METODE:

Izolovanje DNK	QIAamp(R) DNA FFPE tissue kit
Genotipizacija	Easy(R)EGFR Diatech Pharmacogenetics (dokazivanje somatskih mutacija u humanom genomu primenom metode Real Time PCR na uređaju ABI 7500 - Applied biosystems)

OGRANIČENJA ISPITIVANJA

Analitička senzitivnost	Detekcija i identifikacija mutacija prisutnih u 1% ispitivanih ćelija (zavisno od mutacije)
Klinička specifičnost	Primenjenom metodom identifikuju se sve klinički/terapijski relevantne mutacije u *EGFR* genu.

ISPITIVANE MUTACIJE

Gen / egzon	Varijacije u proteinu (genu)
***EGFR* / egzon 18**	**Mix1***. .c.2155G>A p.(Gly719Ser); c.2155G>T p.(Gly719Cys); c.2156G>C p.(Gly719Ala).
***EGFR* / egzon 20**	**Mix2**. c.2369C>T p.(Thr790Met). **Mix3**.c.2303G>T p.(Ser768Ile). **Mix4***. c.2307_2308insGCCAGCGTG p.(Val769_Asp770insAlaSerVal); c.2310_2311insGGT p.(Asp770_Asn771insGly); c.2319_2320insCAC p.(His773_Val774insHis).
***EGFR* / egzon 21**	**Mix5**. c.2573T>G p.(Leu858Arg). **Mix6**. c.2582T>A p.(Leu861Gln).
***EGFR* / egzon 19**	**Mix7***: c.2235_2249del15 p.(Glu746_Ala750del); c.2236_2250del15 p.(Glu746_Ala750del); c.2240_2257del18 p.(Leu747_Pro753delinsSer); c.2239_2248delinsC p.(Leu747_Pro753delinsSer); c.2237_2255delinsT p.(Glu746_Ser752delinsVal); c.2240_2254del15 p.(Leu747_Thr751del); c.2239_2256del18 p.(Leu747_Ser752del); c.2237_2251 del15 p.(Glu746_Thr751delinsAla); c.2239_2253del15 p.(Leu747_Thr751del); c.2239_2251delinsC p.(Leu747_Thr751delinsPro); c.2239_2247del9 p.(Leu747_Glu749del); c.2235_2246del12 p.(Glu746_Glu749del); c.2239_2258delinsCA p.(Leu747_Pro753delinsGln); c.2240_2251del p.(Leu747_Thr751delinsSer); c.2237_2254del18 p.(Glu746_Ser752delinsAla); c.2238_2248delinsGC p.(Leu747_Ala750delinsPro); c.2238_2255del18 p.(Glu746_Ser752delinsAsp); c.2235_2252delinsAAT p.(Glu746_Thr751delinsIle); c.2238_2252>GCA p.(Leu747_Thr751delinsGln); c.2236_2253del18 p.(Glu746_Thr751del)

* ne mogu se međusobno razlikovati

REZULTATI PCR TESTIRANJA

U uzorku tkiva izdvojenog makrodisekcijom iz dostavljenog parafinskog kalupa **nisu detektovane mutacije *EGFR* gena**.

Rezultati ovog testa moraju biti interpretirani u kliničkom kontekstu zajedno sa drugim relevantnim podacima i ne mogu se samostalno koristiti za postavljanje dijagnoze maligniteta.

kraj izveštaja

Reporting Biomarker Findings

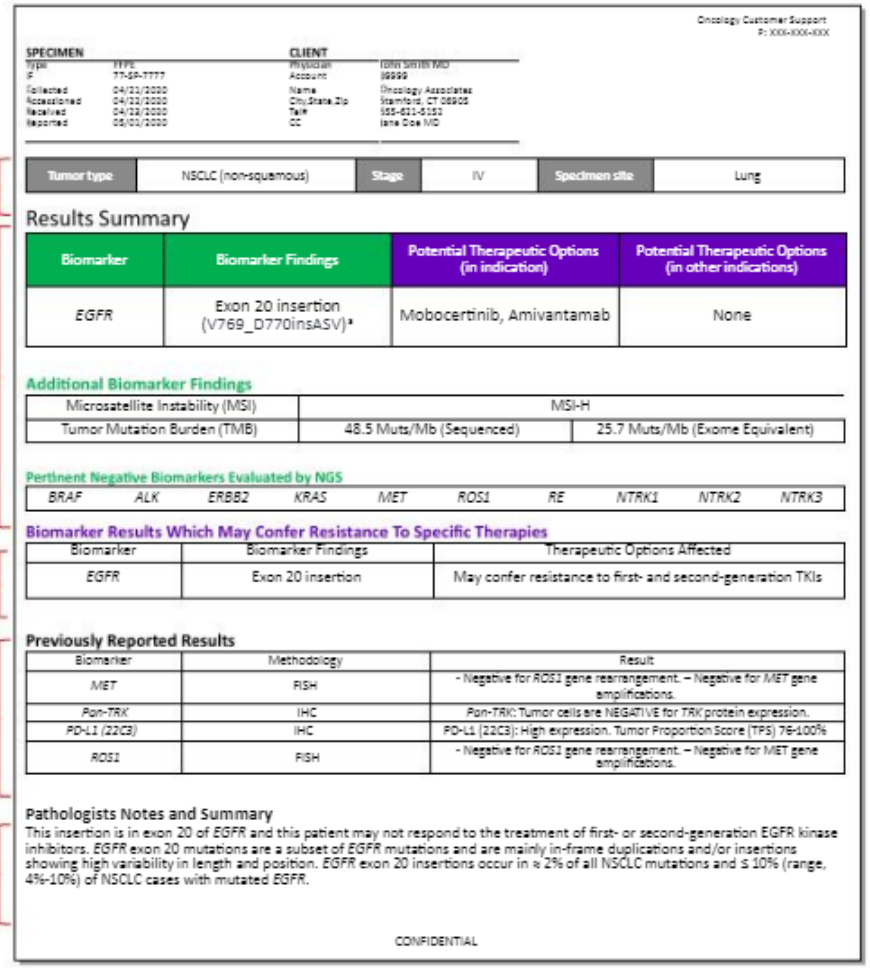
Clinically critical information (eg, tumor type, stage, specimen sites) at the beginning of the report is presented in a prominent manner

Actionable biomarkers (with the gene, alteration, and specific sequence of the variant) are reported, including reporting of negative biomarkers

Summary of biomarkers with potential resistance to therapies[b]

Summary of case-specific guideline-driven complementary testing results with details on methodology

Integrated Molecular Pathologist summary of the clinically relevant findings, including resistance to first- and second-generation TKIs[b]



Oncology Customer Support
P: XXX-XXX-XXX

SPECIMEN

Type	FFPE
#	77-SP-7777
Collected	04/21/2020
Accessioned	04/22/2020
Received	04/28/2020
Reported	05/01/2020

CLIENT

Physician	John Smith MD
Account	99999
Name	Oncology Associates
City,State,Zip	Stamford, CT 06905
Tel#	555-611-5151
cc	Jane Doe MD

Tumor type	NSCLC (non-squamous)	Stage	IV	Specimen site	Lung

Results Summary

Biomarker	Biomarker Findings	Potential Therapeutic Options (in indication)	Potential Therapeutic Options (in other indications)
EGFR	Exon 20 insertion (V769_D770insASV)[a]	Mobocertinib, Amivantamab	None

Additional Biomarker Findings

Microsatellite Instability (MSI)	MSI-H	
Tumor Mutation Burden (TMB)	48.5 Muts/Mb (Sequenced)	25.7 Muts/Mb (Exome Equivalent)

Pertinent Negative Biomarkers Evaluated by NGS

BRAF	*ALK*	*ERBB2*	*KRAS*	*MET*	*ROS1*	*RE*	*NTRK1*	*NTRK2*	*NTRK3*

Biomarker Results Which May Confer Resistance To Specific Therapies

Biomarker	Biomarker Findings	Therapeutic Options Affected
EGFR	Exon 20 insertion	May confer resistance to first- and second-generation TKIs

Previously Reported Results

Biomarker	Methodology	Result
MET	FISH	- Negative for *ROS1* gene rearrangement. – Negative for *MET* gene amplifications.
Pan-TRK	IHC	*Pan-TRK*: Tumor cells are NEGATIVE for *TRK* protein expression.
PD-L1 (22C3)	IHC	PD-L1 (22C3): High expression. Tumor Proportion Score (TPS) 76-100%
ROS1	FISH	- Negative for *ROS1* gene rearrangement. – Negative for MET gene amplifications.

Pathologists Notes and Summary

This insertion is in exon 20 of *EGFR* and this patient may not respond to the treatment of first- or second-generation EGFR kinase inhibitors. *EGFR* exon 20 mutations are a subset of *EGFR* mutations and are mainly in-frame duplications and/or insertions showing high variability in length and position. *EGFR* exon 20 insertions occur in ≈ 2% of all NSCLC mutations and ≤ 10% (range, 4%-10%) of NSCLC cases with mutated *EGFR*.

CONFIDENTIAL

Reporting guidelines recommend the use of colloquial nomenclature (eg, "exon 20 insertion") in addition to standard nomenclature for biomarkers to deliver a clear message to the physician reading the report

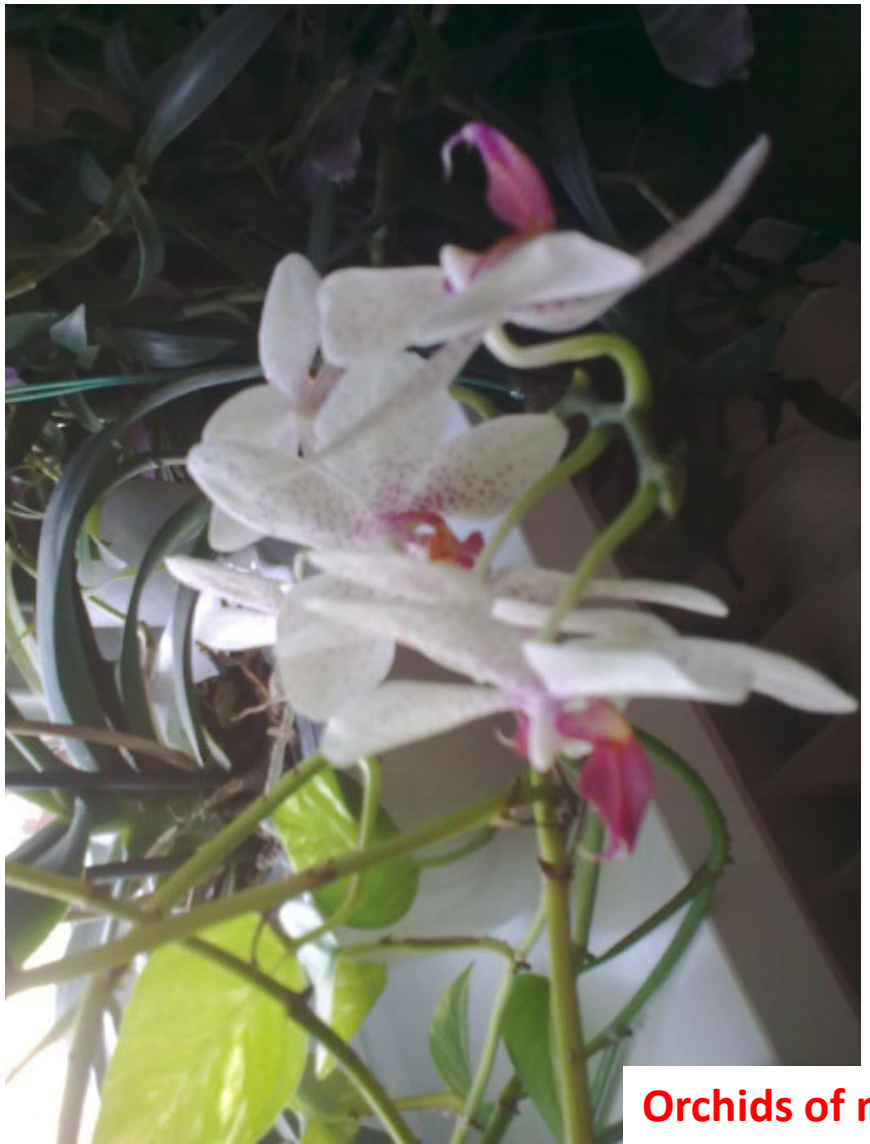
This is a sample report that incorporates templates from multiple laboratories.

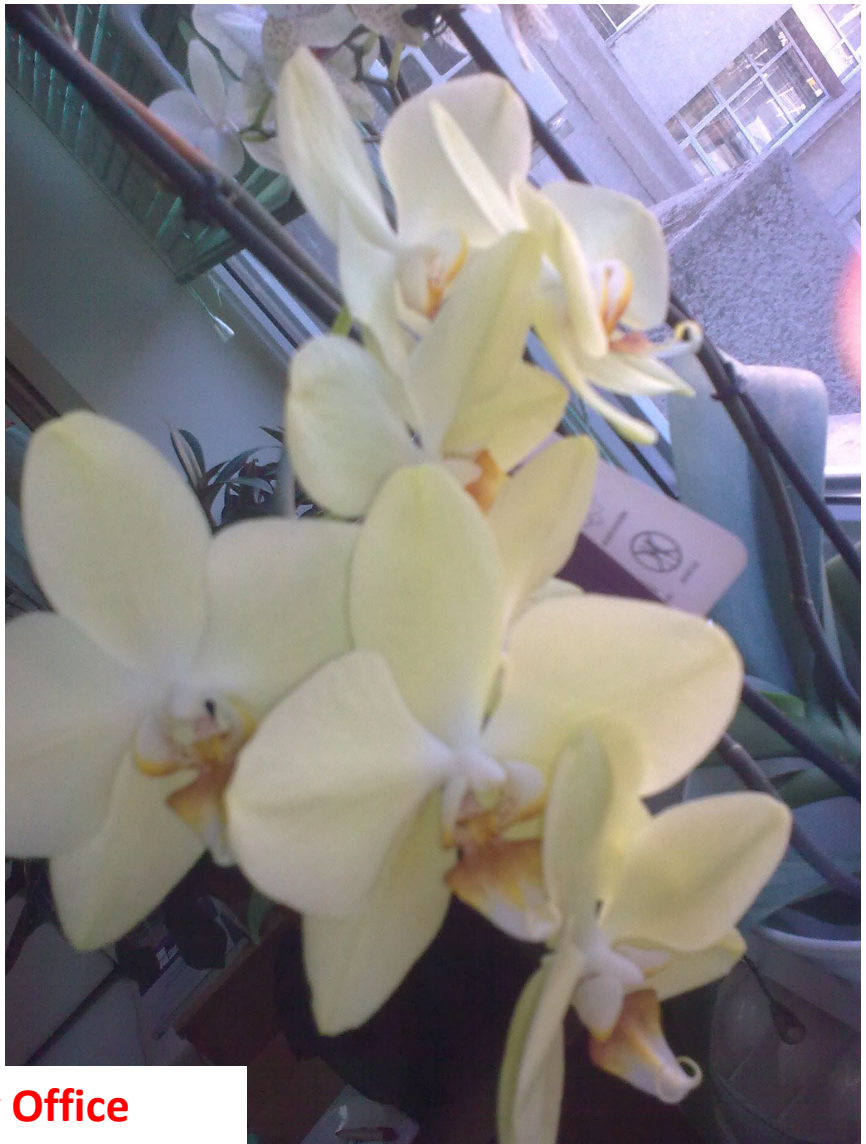
[a] This is one example of an exon 20 insertion mutation; [b] It is important to recognize that suitability for a treatment is based on many factors other than the diagnosis as written on a test requisition and the genotype discovered through testing.

TKI, tyrosine kinase inhibitor.

Li MM et al. *J Mol Diagn*. 2017;19:4-23.

Thank you for your attention





Orchids of my Office