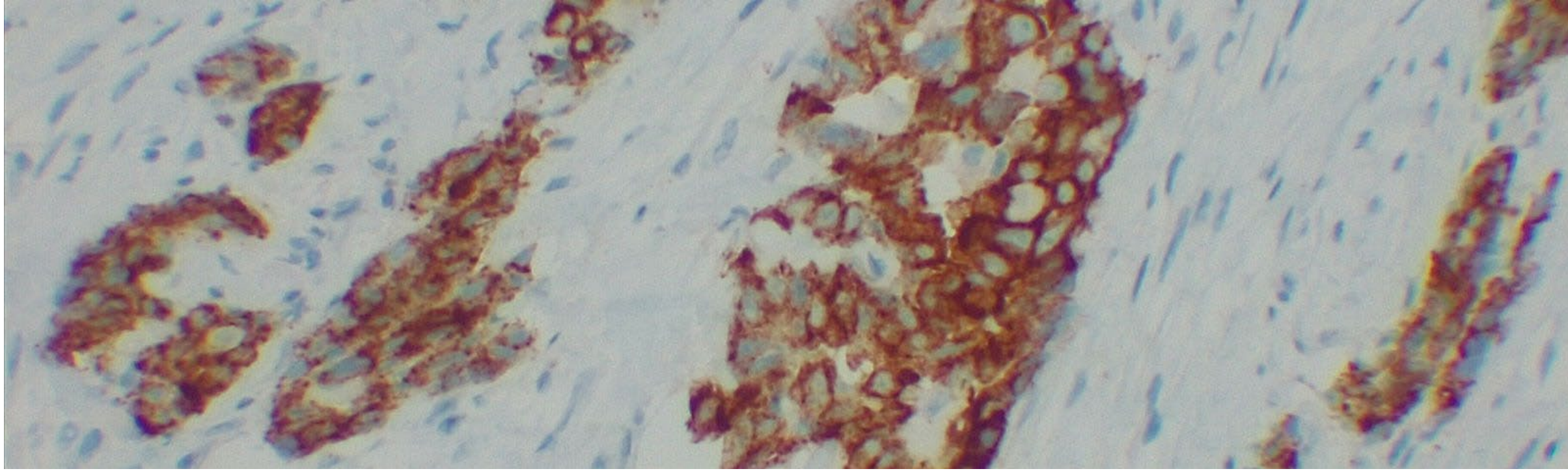


Značaj testiranja i uloga patologa u dijagnozi ALK+ NSCLC

Prof.dr Sofija Glumac, patolog
Institut za patologiju, Medicinski fakultet u Beogradu

Only for Healthcare Professionals

The meeting is initiated, organized and funded by Takeda

nSCLC; Non-small cell lung cancer

ONCOLOGY

The management of patients with cancer is becoming ever more dependant on a knowledge of the pathology of each patient’s disease-

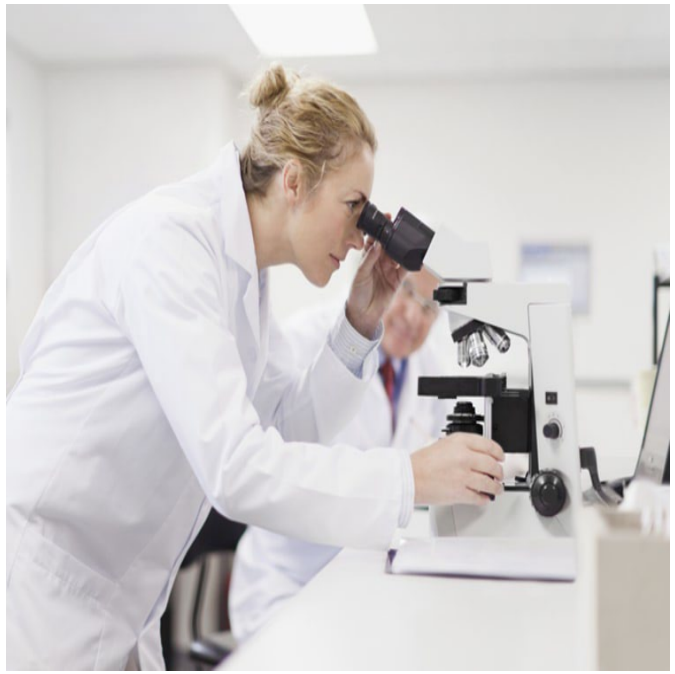
"Know your enemy"

- The role of pathologists in routine diagnosis of lung cancer has significantly changed, during the past decade.

- All essential components of both pathologic workflow and diagnosis are:
  - optimal processing of biopsy tissues,
  - stratification of the cut sections, and
  - prioritization of biomarker analysis.

Diagnostic pathology
A critical role in cancer diagnosis

The pathologist of today…

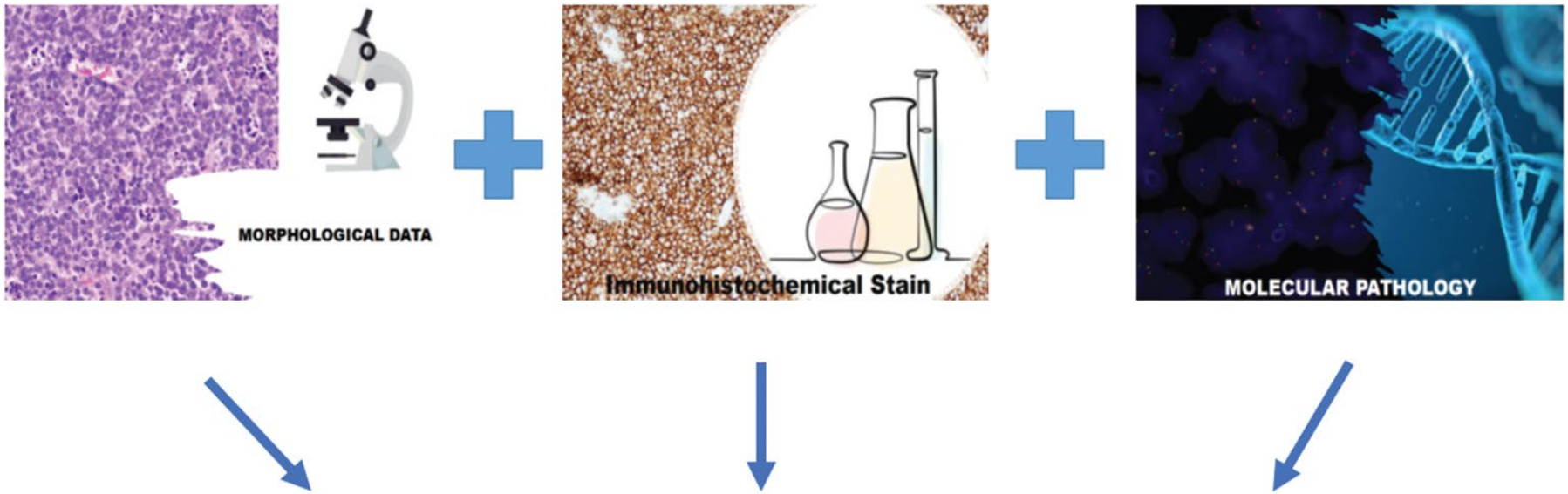


…uses histological and cytological methods to diagnose tumours and evaluate their stage and grade[1–3]

…interprets a range of laboratory tests[2]

…works with multi-disciplinary teams, including molecular tumour boards, to help guide treatment decisions for each patient[2–4]

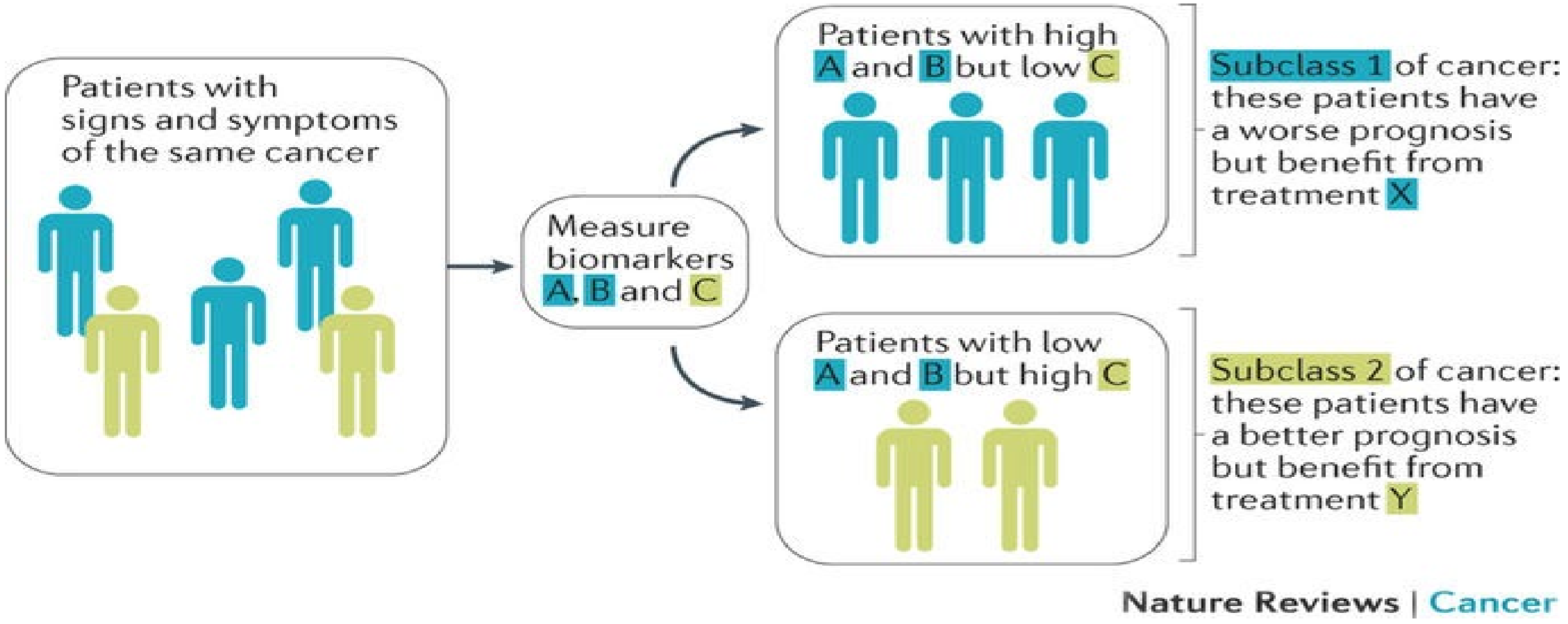
1. Royal College of Pathologists. Histopathology fact sheet (Accessed 11 August 2021); 2. D'Abbronzo G, Franco R. *Exp Rev Precis Med Drug Dev* 2021 (3. Angerilli V *et al. Diagnostics* 2021;11 ; 4. Luchini C *et al. Trends Cancer* 2020;6:738–44

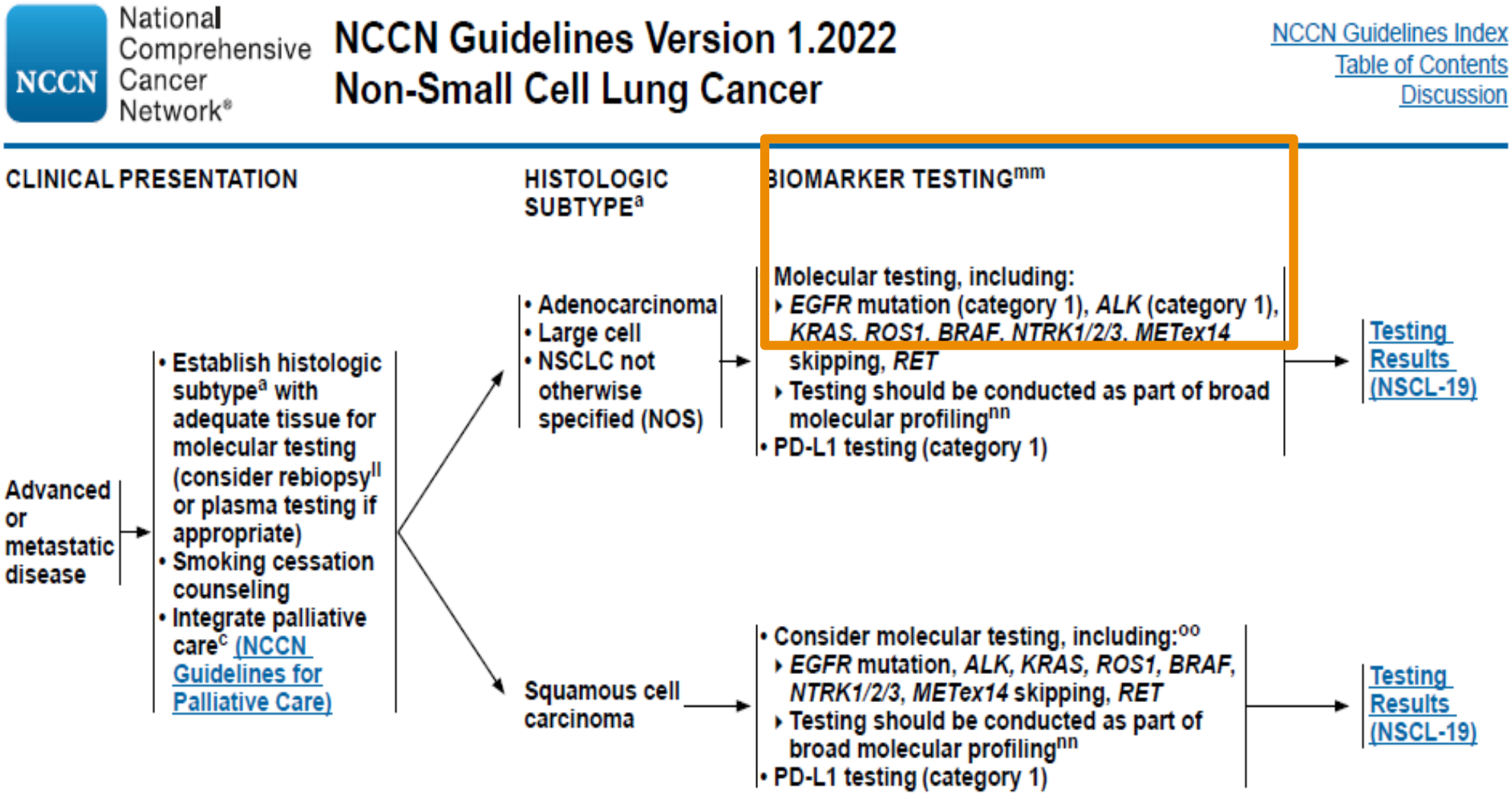


Integrated report in modern pathology

Modern pathologist provides reports, integrating morphological, immunophenotypically, and molecular data in a complete diagnosis useful for the most correct therapeutic choice.

Precision medicine and personalized therapy



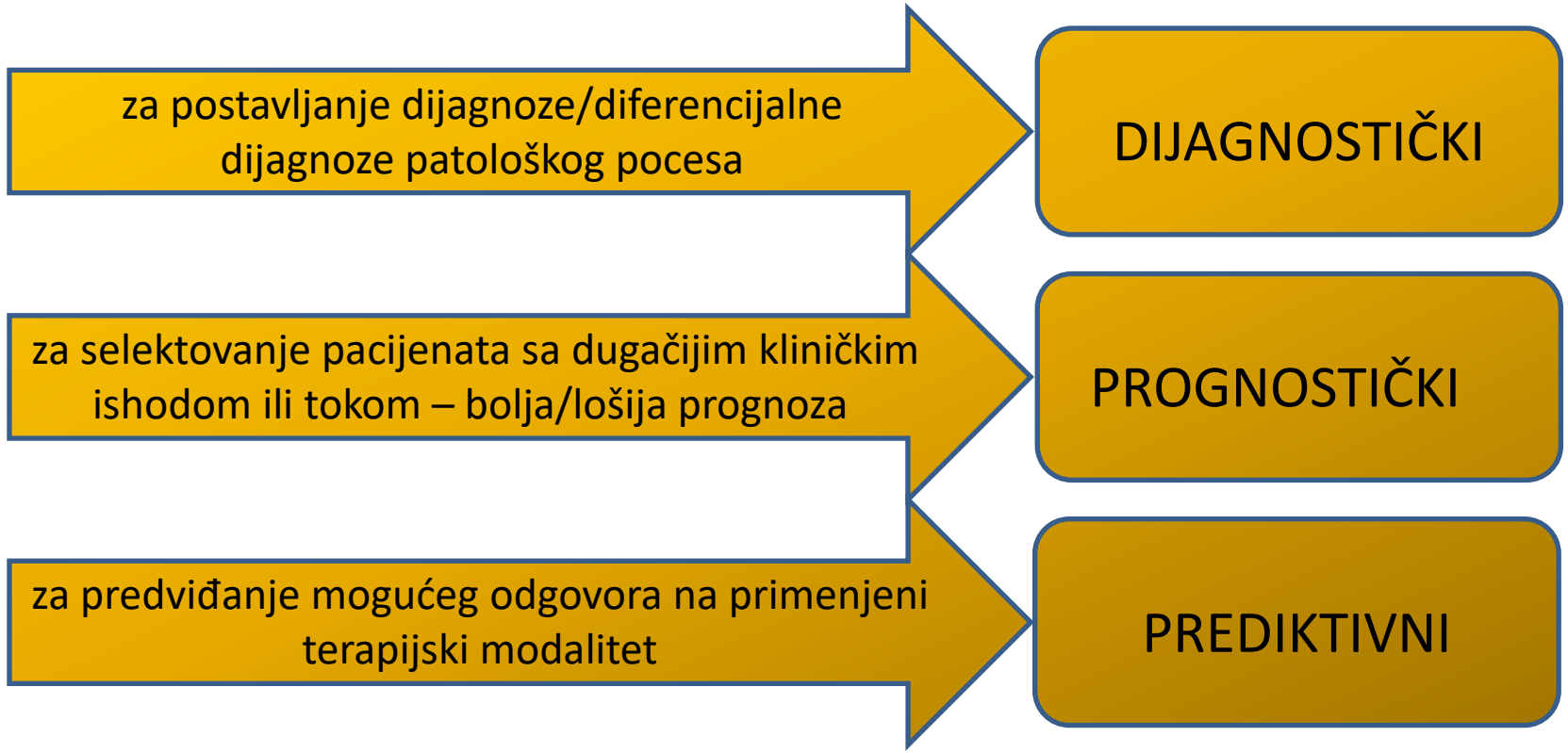


NCCN, National Comprehensive Cancer Network; NGS, sekvenciranje nove generacije; NSCLC, nesitnoćelijski karcinom pluća; EGFR, receptor epidermalnog faktora rasta; KRAS, KRAS proto onkogen; ALK, kinaza anaplastičnog limfoma; BRAF, B-raf proto-onkogen;; NTRK, neurotrofna receptor tirozin kinaza; METex14, mezenhimalno-epitelialni tranzitni faktor exona 14; RET, rearranged during transfection; ROS1, c-ros onkogen 1 kinaza; PD-L1, ligand 1 programirane ćelijske smrti

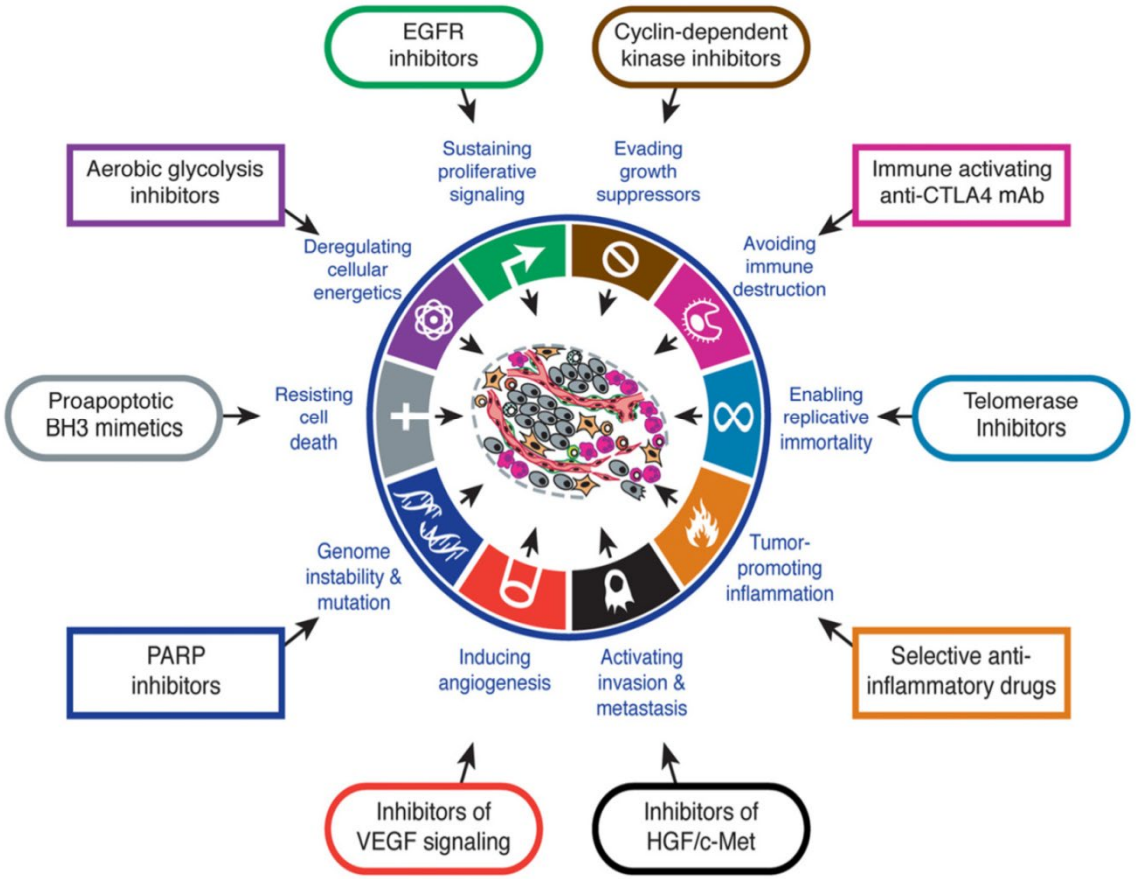
NCCN guidelines. Non-small cell lung carcinoma. Version 1. 2022. Available at: https://www.nccn.org/professionals/physician_gls/PDF/nscl.pdf (accessed March 2022).

Biomarker

Biomolekul koji se objektivno može kvantifikovati.
Prisutan je u telesnim tečnostima i tkivima (u/na ćeliji).
Pokazatelj je fiziološkog ili patološkog procesa.



The hallmarks of cancer



Virtually every new therapy for NSCLC in last 10 years is biomarker-dependant

Hanahan D, Weinberg RA. Cell 144; 2011

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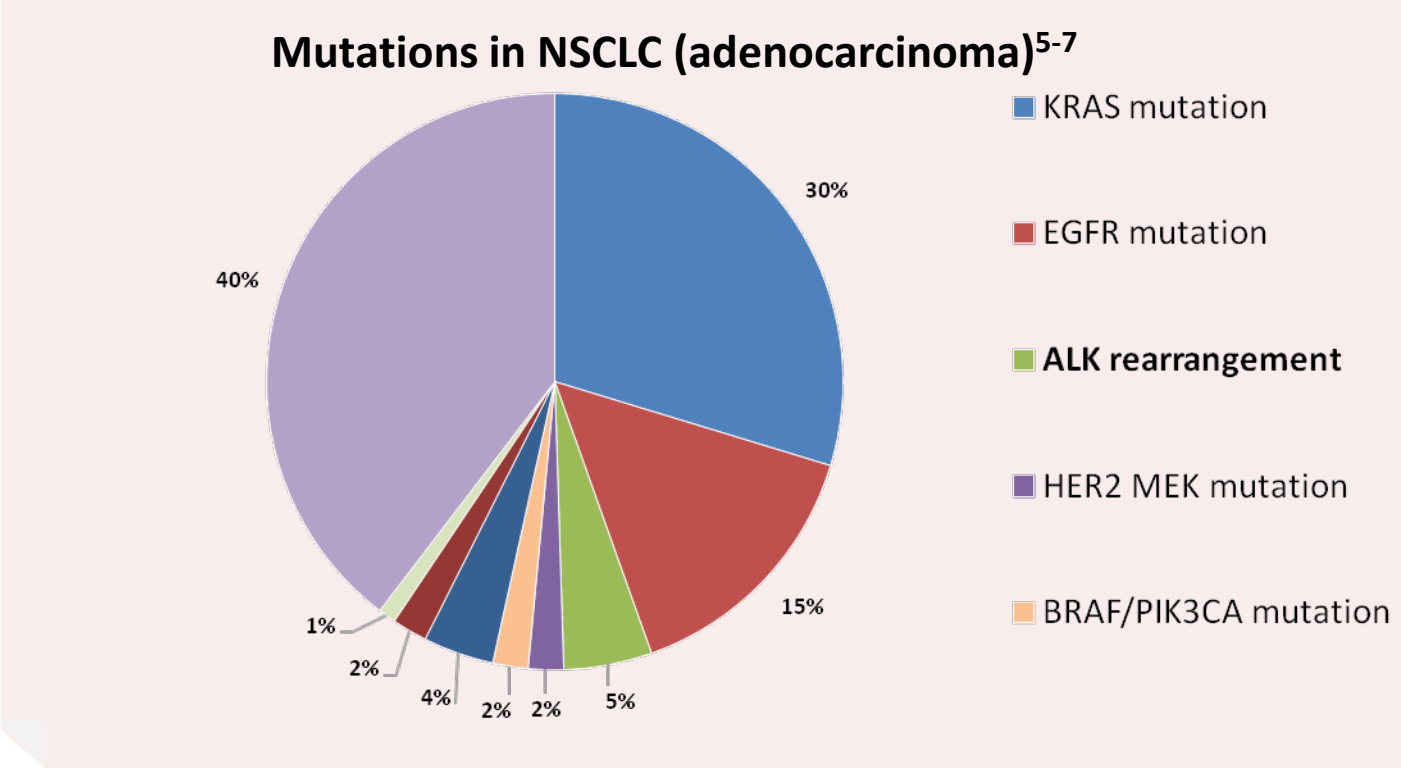
ALK Rearrangements in NSCLC

***ALK* rearrangements occur in 3% to 7% of NSCLC adenocarcinomas, with ≈ 40,000 cases worldwide each year[1-4]**



1. Gainor JF, et al. *Clin Cancer Res*. 2013;19:4273-4281; 2. Wong DW, et al. *Cancer*. 2009;115:1723-1733; 3. Koivunen JP, et al. *Clin Cancer Res*. 2008;14:4275-4283; 4. Chia PL, et al. *Clin Epidemiol*. 2014;6:423-432; 5. Chan BA, Hughes BGM. *Transl Lung Cancer Res*. 2015;4:36-54; 6. Liang H, et al. *Onco Targets Ther.* 2020;13:2491-2510; 7. Reungwetwattana T, et al. *Lung Cancer.* 2017;103:27-37.

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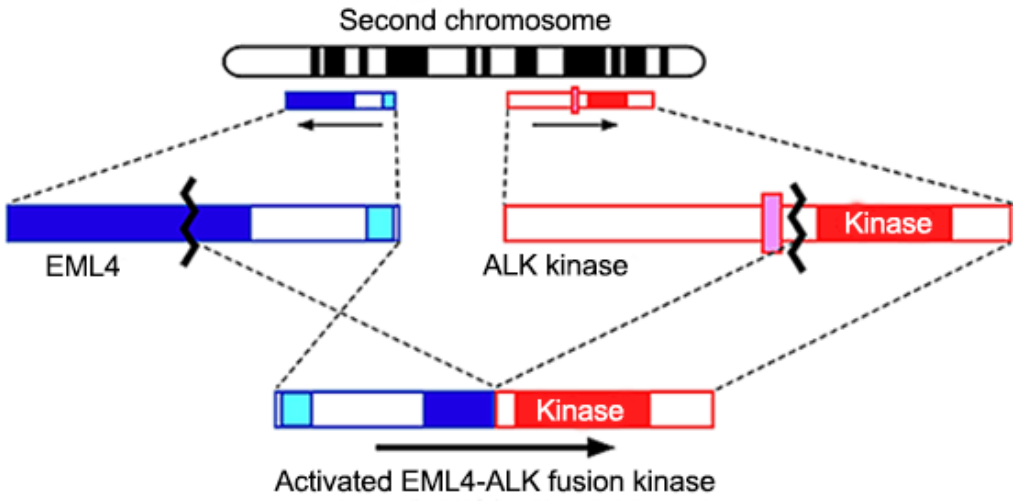
ALK u NSCLC

- ***A****naplastic* ***L****ymphoma* ***K****inase* gen na kratkom kraku hromozoma 2 koji pripada familiji insulinskih transmembranskih tirozin - kinaznih receptora.

- Prevalencija driver/onkogene aberacije ALK gena u NSCLC-u iznosi **3-7%**, prvenstveno u **adenokarcinomima**.

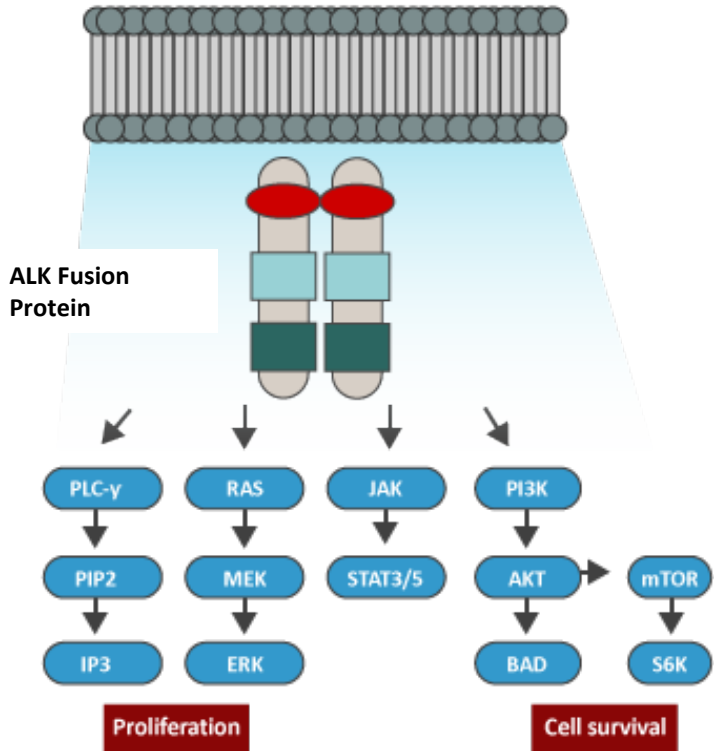


Soda M et Al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007 Aug 2;448(7153):561-6. doi: 10.1038/nature05945. Epub 2007 Jul 11. PMID: 17625570.

ALK Fusion Proteins and Their Pathological Role in NSCLC

- In NSCLC, ALK receptor tyrosine kinase has been found fused to > 20 to 90 different partners[1,2]
- The most common ALK fusion partner in NSCLC is EML4 accounts for ≈ 30% of all ALK fusion proteins[2]
- The ALK rearrangements activate multiple oncogenic signaling pathway RAS, PLC, JAK, and PI3K pathways which play pivotal roles in cell cycle progression, survival, and proliferation[3,4]
- The EML4-ALK fusions were mutually exclusive with mutations in the EGFR and KRAS genes[5]

***ALK* Fusion Protein–Mediated Signaling Pathway[5]**



1. Hsiao SJ, Ou SHI. *J Thorac Oncol.* 2020;15:1103-1105; 2. Chia PL, et al. *Clin Epidemiol.* 2014;6:423-432;3. Grande E, et al. *Mol Cancer Ther.* 2011;10:569-579; 4. Roskoski R, et al. *Pharmacol Res.* 2013;68:68-94; 5. OncLive. http://www.onclive.com/publications/Oncology-live/2012/august-2012/ALK-Inhibitors-Moving-Rapidly-From-Discovery-to-Clinical-Approval-and-Beyond. Accessed April 7, 2021; 6. Takahashi T, et al. *Ann Surg Oncol.* 2010;17(3):889-897.

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Clinical Features of ALK+ NSCLC

Patient characteristics largely associated with *ALK* rearrangements include the following:

Age

- More common in younger patients—median age of 52 years

Histology

- More common in patients with adenocarcinomas
- However, patients with mixed squamous cell histology may harbor ALK rearrangements

Smoking History

- More common in never or light smokers

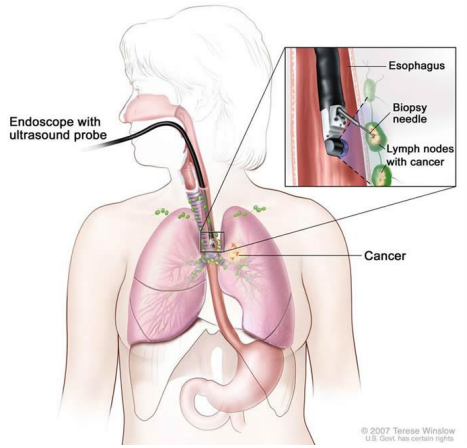
Chia PL, et al. Clin Epidemiol. 2014. Soda M, et al. Clin Cancer Res. 2012. NCCN Clinical Practice Guidelines. Non-Small Cell Lung Cancer. V7.2017

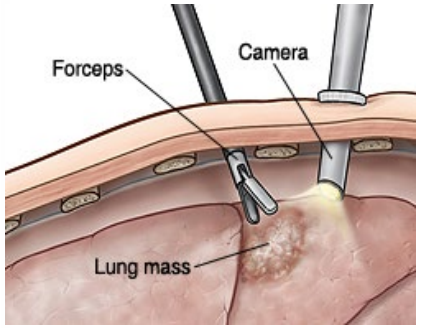
Sample for ALK test[1-6]

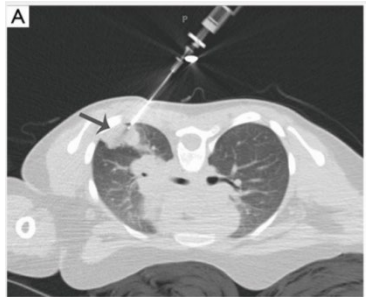
- Both cytological samples and tissue biopsy may be tested for Alk rearragements

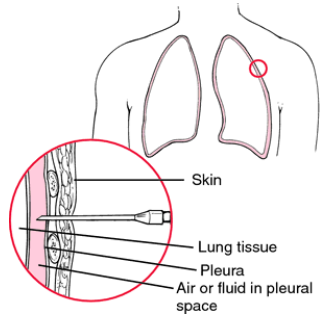
- Lung cell/ cytological samples can be collected using:
  - Pleural Fluid
  - Bronchoscopy (Broncoalveolar lavage (brush/wash))

- Lung tissue can be collected using biopsy procedures:
  - Fine needle aspirations
  - Bronchoscopy (transbronchial / endobronchial)
  - Core needle biopsy
  - Video assisted thoracoscopic surgery
  - Thoracotomy









1.Shukla S, et al. 2015. J Cytol;32(3):159-164; 2.https://www.cancer.org/treatment/understanding-your-diagnosis/tests/testing-biopsy-and-cytology-specimens-for-cancer/cytology-types.html/accessed August 22, 2021; 3. https://www.cancer.org/cancer/lung-cancer/detection-diagnosis-staging/how-diagnosed.html/ accessed August 22, 2021; 4.https://www.lhsc.on.ca/thoracic-surgery/endobronchial-ultrasound-ebus/accessed August 22, 2021; 5.https://jtd.amegroups.com/article/view/6158/html/accessed August 22, 2021; 6.https://medical-dictionary.thefreedictionary.com/thoracocentesis/accessed August 22, 2021

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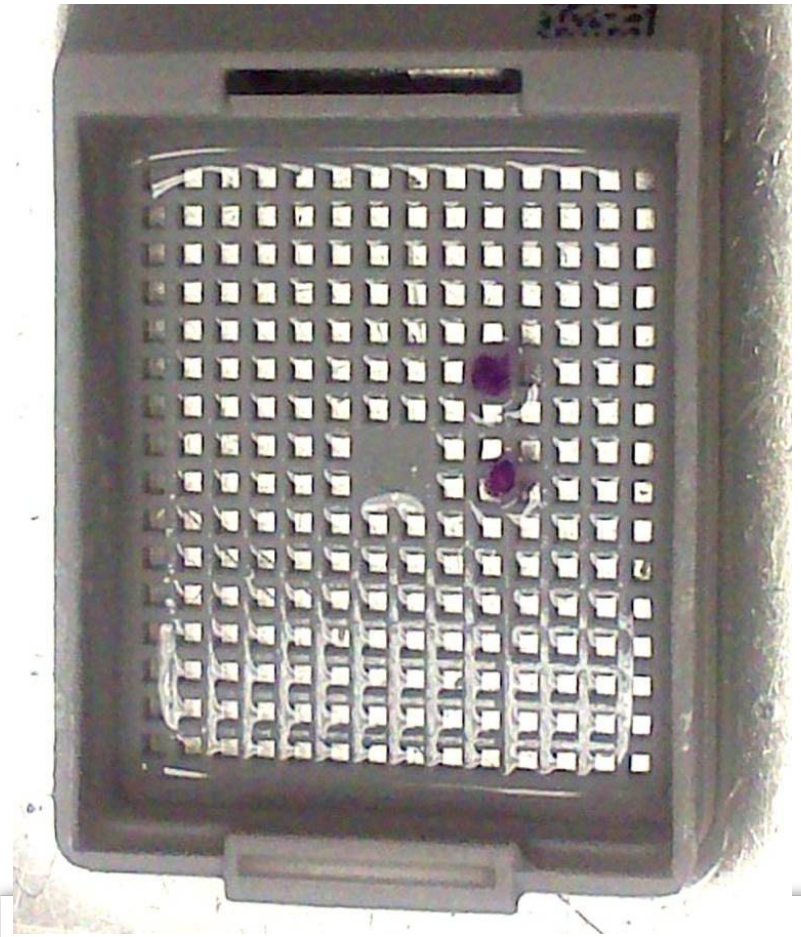
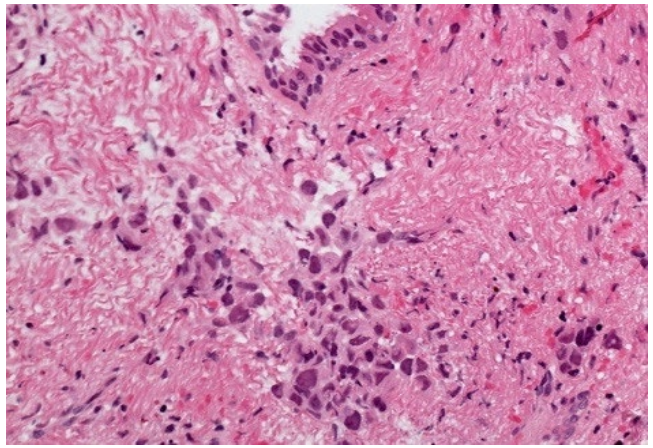
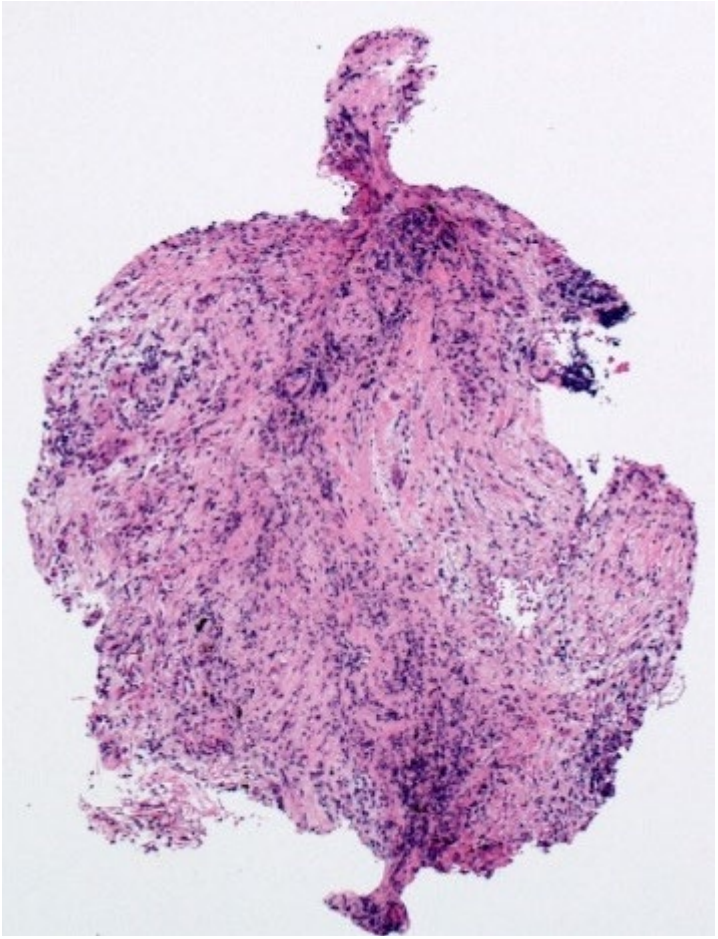
Is there enough material for all diagnosis?





On average only 10-25% of this tissue is tumour!

- ✓Morphologic diagnosis
- ✓Immunohistochemistry
- ✓ Molecular testing
- ✓ Conserve tissue
- ✓ Don’t waste



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Recommended Testing Metods for ALK+ NSCLC

Guidelines	Description
NCCN[1,a]	***ALK* testing should be conducted as part of broad molecular profiling, before selection of first-line therapy for metastatic NSCLC, if clinically feasible**
ESMO[2]	**Routine testing for *ALK* rearrangements is standard of care and should be:** ▪ **Conducted in patients with advanced, non squamous NSCLC** ▪ **Assessed in parallel with *EGFR* mutation analysis** ▪ **Performed before first-line treatment** ▪ Patients who are never/former light smokers with a confirmed diagnosis of squamous cell carcinoma may be tested for *ALK* rearrangements
IASLC[3]	***EGFR*, *ALK*, and *ROS1* testing should be conducted in all patients with lung cancer**
PNPK Kanker Paru[4]	**EGFR, KRAS, and EML4-ALK testing should be conducted before selection of targeted therapy for adenocarcinoma if facility and testing materials meet the requirement**

- NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) recommend that repeat **panel testing or selected biomarker testing be considered at progression on first-line therapy** if a complete assessment for all actionable biomarkers was not done before starting therapy in eligible patients with metastatic NSCLC[1]

AMP, Association for Molecular Pathology; CAP, College of American Pathologists; ESMO, European Society for Medical Oncology; IASLC, International Association for the Study of Lung Cancer; NCCN®, National Comprehensive Cancer Network®.

[a] The NCCN Guidelines for NSCLC provide recommendations for certain individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories. NCCN makes no warranties of any kind whatsoever regarding their content, use, or application and disclaims any responsibility for their application or use in any way.

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.5.2021. © National Comprehensive Cancer Network, Inc. 2021. All rights reserved. Accessed June 22, 2021. To view the most recent and complete version of the guideline, go online to NCCN.org; 2. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2018;29(suppl 4):iv192-iv237. (Updated September 15, 2020 by ESMO Guidelines Committee); 3. Lindeman NI, et al. Arch Pathol Lab Med. 2013;137:828-860; 4. Lindeman NI, et al. J Thoracic Oncol. 2018;20:129-159; 4. http://kanker.kemkes.go.id/guidelines/PNPKParu.pdf/accessed August 22, 2021.

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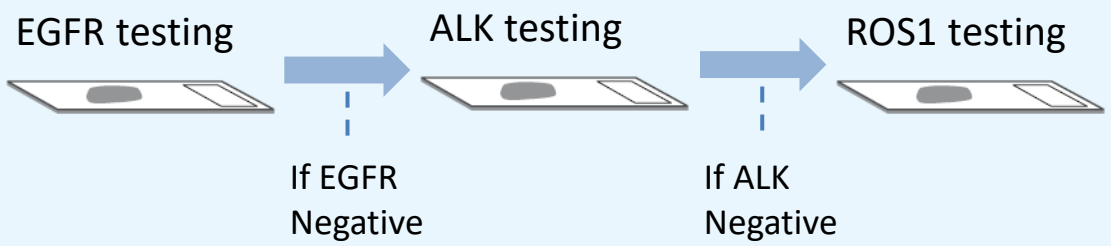
Sequential Vs Parallel Testing

Sequential Testing



- Cost saving and to some extent could spare some tumor tissue from unnesscecary test.
- Time for sequential test may be too long for a sick patient.
- Sequential testing strategy is associated with a risk of using up all available tissue.

Parallel Testing

- Parallel testing is recommended.
- This approach is usually more efficient, especially for a less frequent oncogenic driver such us ALK rearrangement.
- Physician could treat patient quickly.
- Could identify those extremely rare patients who have more than one molecular alteration in their tumor.

1. Tsao MS, et al, eds. *IASLC Atlas of ALK and ROS1 Testing in Lung Cancer*. Aurora, CO: IASLC Press; 2016.

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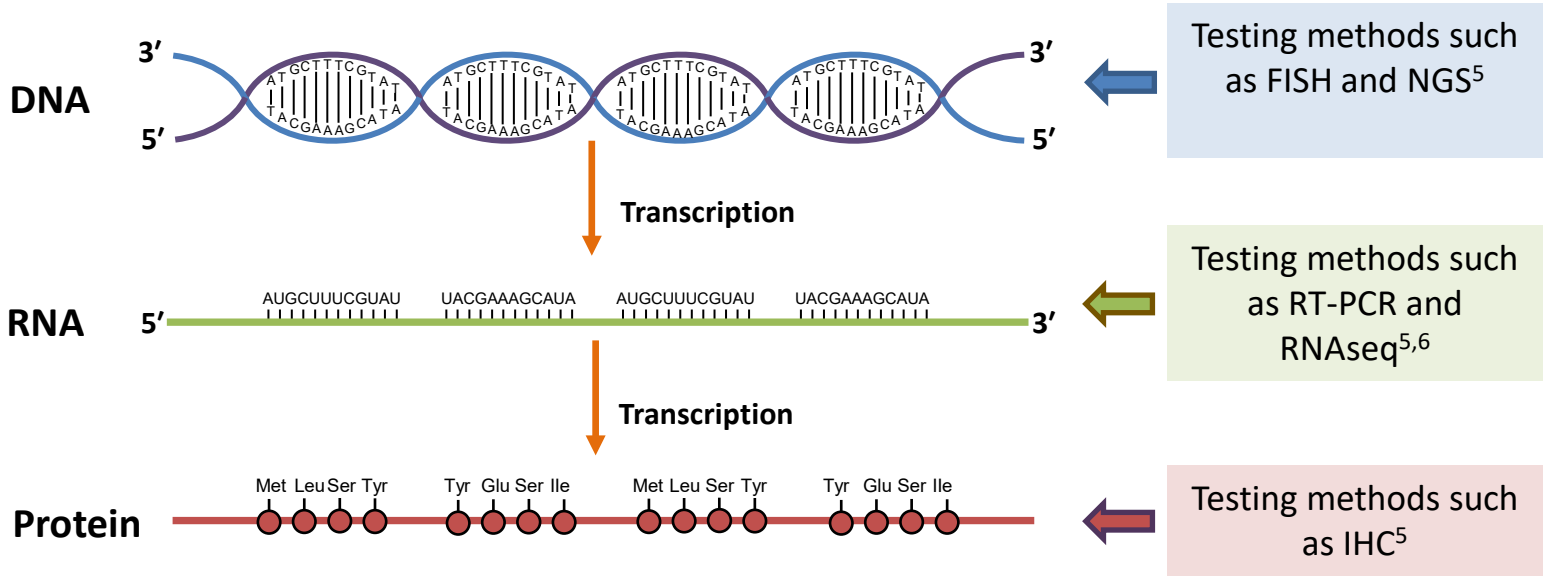
Recommended Testing Metods for ALK+ NSCLC

ALK rearrangements can be tested for using a variety of methods.
Testing methods acknowledged in current guidelines are:

Guidelines	Testing Methods
NCCN[1,a]	• FISH • IHC • NGS • PCR (unlikely to detect fusions with novel partners)
ESMO[2]	Any appropriate, validated method • FISH • IHC • NGS (emerging)
IASLC/CAP /AMP[3,4]	• FISH • IHC

***ALK* testing can occur at the DNA, RNA, and protein levels by various methods**



Testing methods such as FISH and NGS[5]

Testing methods such as RT-PCR and RNAseq[5,6]

Testing methods such as IHC[5]

FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; PCR, polymerase chain reaction; RNAseq, RNA sequencing; RT-PCR, reverse transcriptase PCR.

[a] NCCN makes no warranties of any kind whatsoever regarding their content, use, or application and disclaims any responsibility for their application or use in any way.

1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.5.2021. © National Comprehensive Cancer Network, Inc. 2021. All rights reserved. Accessed June 22, 2021. To view the most recent and complete version of the guideline, go online to NCCN.org; 2 Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2018;29(suppl 4):iv192-iv237. (Updated September 15, 2020 by ESMO Guidelines Committee); 3. Lindeman NI, et al. *J Thorac Oncol.* 2018;142:321-346; 4. Lindeman NI, et al. *J Thorac Oncol.* 2013;8:823-859; 5. Tsao MS, et al, eds. *IASLC Atlas of ALK and ROS1 Testing in Lung Cancer*. Aurora, CO: IASLC Press; 2017; 6. Benayed R, et al. *Clin Cancer Res.* 2019;25:4712-4722.

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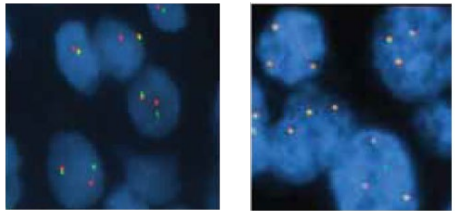
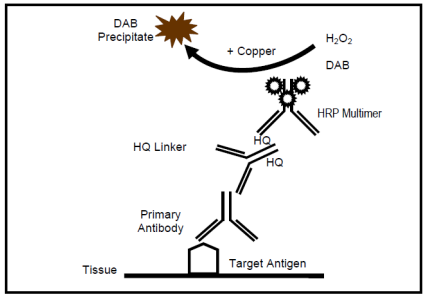
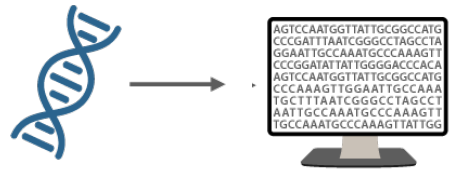
Comparison between FDA Approved Companion Diagnostic Test for ALK Testing[1-3]

	FISH (Vysis ALK Breakapart)	IHC (Ventana ALK (D5F3))	NGS (Foundation One)
Sample	FFPE	FFPE	FFPE, Peripheral whole blood
Principle	 **ALK positive** **ALK negative** Image source: *IASLC Atlas of ALK Testing in Lung Cancer*. Tsao MS, et al, eds. Aurora, CO: IASLC Press; 2013.		
Turn around time	18 – 20 hours	4.5 hours	days - weeks
FDA approved companion diagnostic test	Crizotinib, Brigatinib	Crizotinib, Ceritinib Alectinib, Lorlatinib	Crizotinib, Ceritinib Alectinib

FFPE: Formalin Fixed Parafilm Embedded

1. Tsao MS, et al, eds. IASLC Atlas of ALK and ROS1 Testing in Lung Cancer. Aurora, CO: IASLC Press; 2016.; 2. Abbott. Vysis ALK break apart FISH kit. https://www.molecular.abbott/sal/en-us/staticAssets/ALK%20US%20CE%20Clinical%20PI_R3_mw001_3060.pdf. Accessed June 15, 2017.; 3.https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools;

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Diagnostic Vs Companion Diagnostic (CDx) Test[1]

Similarities between diagnostic and companion diagnostic tests

- Throughly validated
- Recognized by regulatory agencies
- Helps HCPs to determine whether a particular therapeutic product's benefits to patients

Diagnostic tests

- Not associated with a drug treatment

Companion Diagnostic tests

- use with a specific group or class of therapeutic products, the companion diagnostic's intended use should name the specific group or class of therapeutic products, rather than specific products
- Patient selection via the diagnostic test is a prerequisite for use of the drug
- Multiple companion diagnostic test may be approved for use with the same drug

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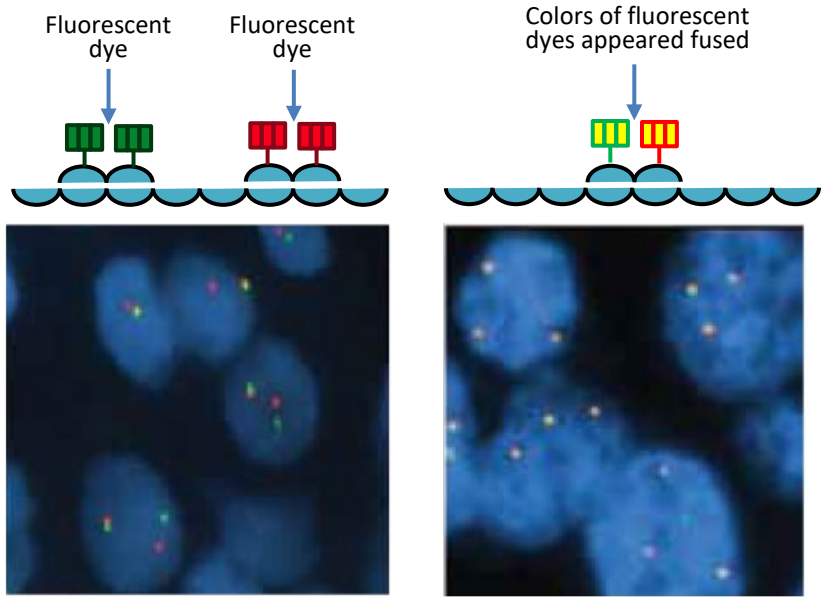
https://www.fda.gov/medical-devices/in-vitro-diagnostics/companion-diagnostics/accessed August 22, 2021.

Fluorescence in Situ Hybridization[1,2]

- The break-apart FISH probe is designed to **detect gene fusions** resulting from interchromosomal translocations
- The FISH probe labels different ends of the fusion breakpoint
- Using 2 fluorochromes (**1 green**, **1 orange**)
  - **ALK positive: colors are separated** if the gene is rearranged
  - **ALK Negative: colors appear fused** (single yellow) if the sample is negative for ALK rearrangements



ALK positive **ALK negative**

Image source: *IASLC Atlas of ALK Testing in Lung Cancer*. Tsao MS, et al, eds. Aurora, CO: IASLC Press; 2013.

1. Vysis ALK Break Apart FISH Probe Kit Package insert; 2. Tsao MS, et al, eds. IASLC Atlas of ALK and ROS1 Testing in Lung Cancer. Aurora, CO: IASLC Press; 2017

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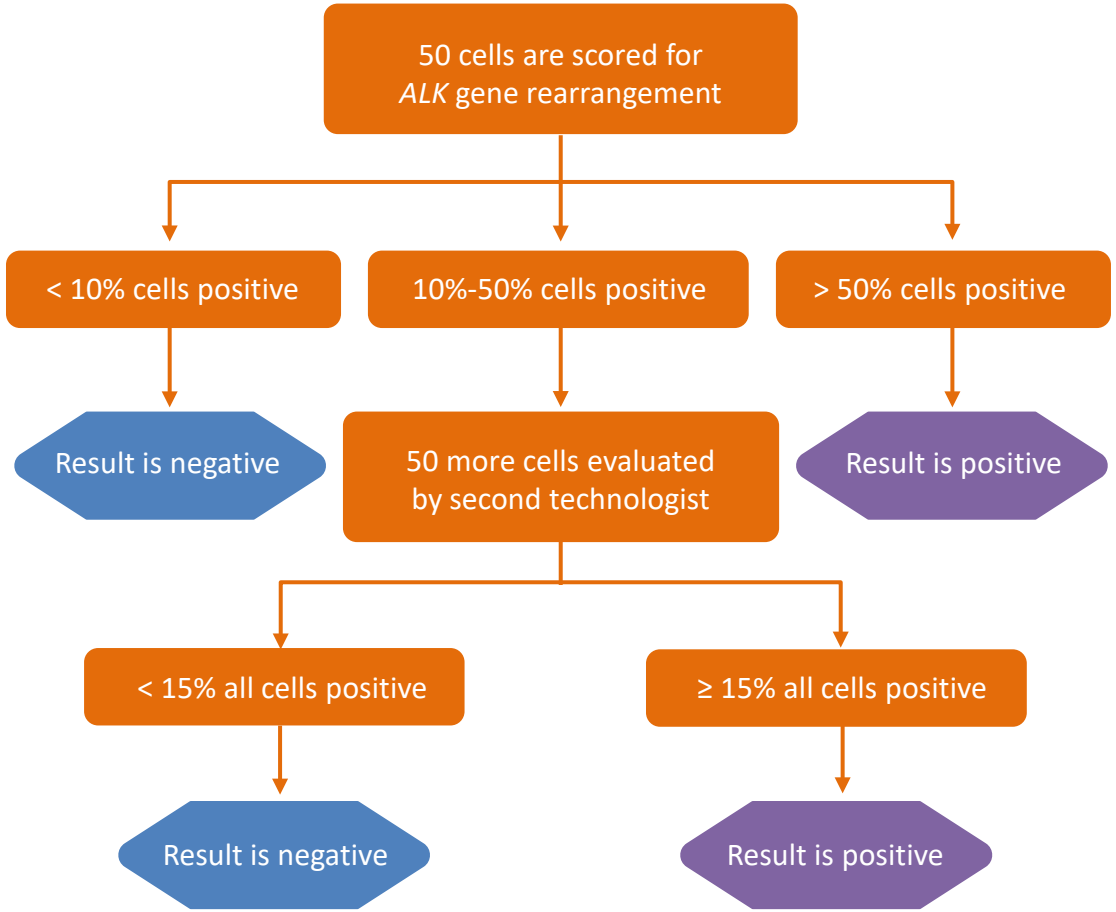
FISH Scoring Algorithm[1]



- The tumor is classified as ***ALK* positive** **if ≥ 50%** of cells show *ALK* rearrangements
- If the score is 10% to 50%, a second technician confirms positivity or negativity.
- After scoring 100 cells cut off for positivity is ≥15% positive cells

TKI, tyrosine kinase inhibitor.

1. Tsao MS, et al, eds. IASLC Atlas of ALK and ROS1 Testing in Lung Cancer. Aurora, CO: IASLC Press; 2017; 2. FDA. https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools. Accessed April 7, 2021.

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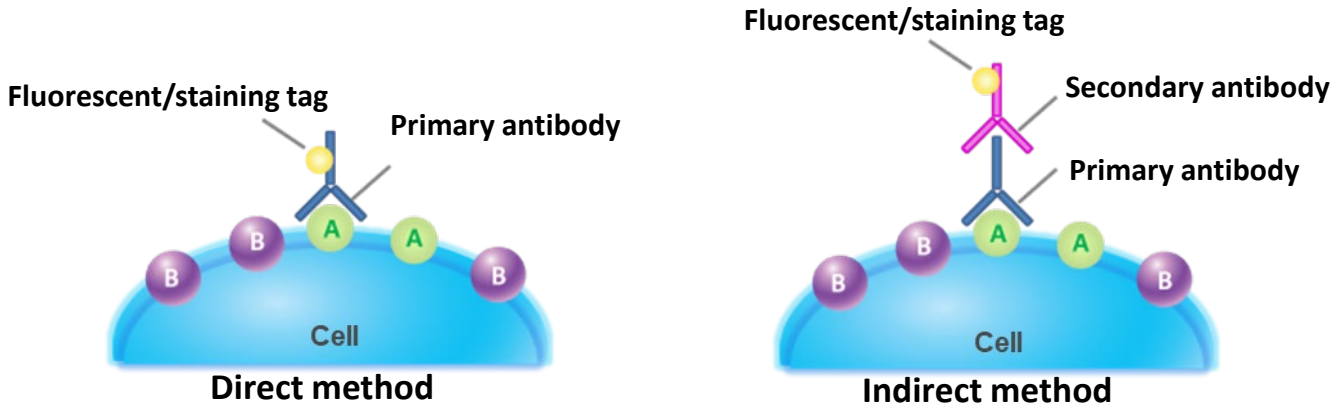
Immunohistochemistry[1]

- Immunohistochemistry (IHC) is a technique that uses **labeled antibodies to recognize specific protein** within a tissue sample
- IHC can be used to detect **NSCLC driver mutation, such as ALK**
- IHC assays **may use a direct or indirect method of detection**
  - The direct method **uses 1 antibody** with fluorescent tag to detect the protein of interest
  - The indirect method **uses a secondary antibody** resulting in an amplified signal



1. Tsao MS, et al, eds. IASLC Atlas of ALK and ROS1 Testing in Lung Cancer. Aurora, CO: IASLC Press; 2017.

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Antibody Sensitivity in IHC Assays

- The Sensitivity of the IHC assay depends on the antibodies used[1,2]

Clone	Clone Type	Isotype	Sensitivity	Specificity
D5F3- Ventana	Rabbit Monoclonal	IgG	100%	99%
5A4- Novocastra	Mouse Monoclonal	IgG1	100%	98%

- Clone: a specific cell line that was used to produce the antibody.[3]
- Clone type: refer to the species host where human antigen (for example: ALK) is introduced to develop the antibody (for example: ALK).[3]
- Isotype: will be used as negative control, which is an antibody that does not recognize the target.[4]

1. Tsao Ms, et al, eds. IASLC Atlast of ALK and ROS1 Testing in Lung Cancer. Aurora, CO: IASLCPress; 2017; 2. Wynes MW, Mw, et al. J. Thorac Oncol. 2014,; 9:631-638; 3 https://www.sigmaaldrich.com/ID/en/technical-documents/technical-article/protein-biology/elisa/generation-of-antibodies/accessed August 08, 2021; 4.Hewitt SM, et al. 2014; J Histochem Cytochem 62:693-697.

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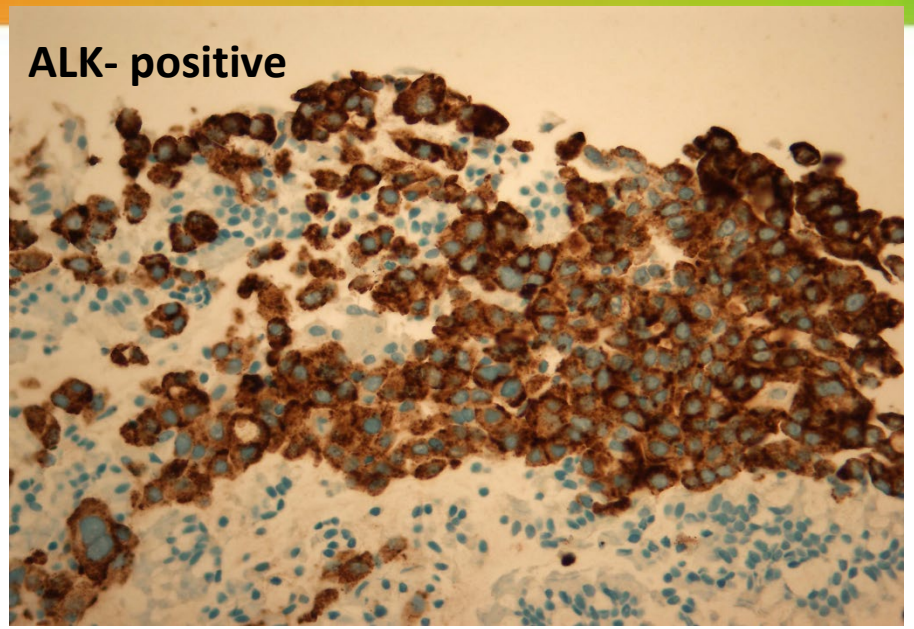
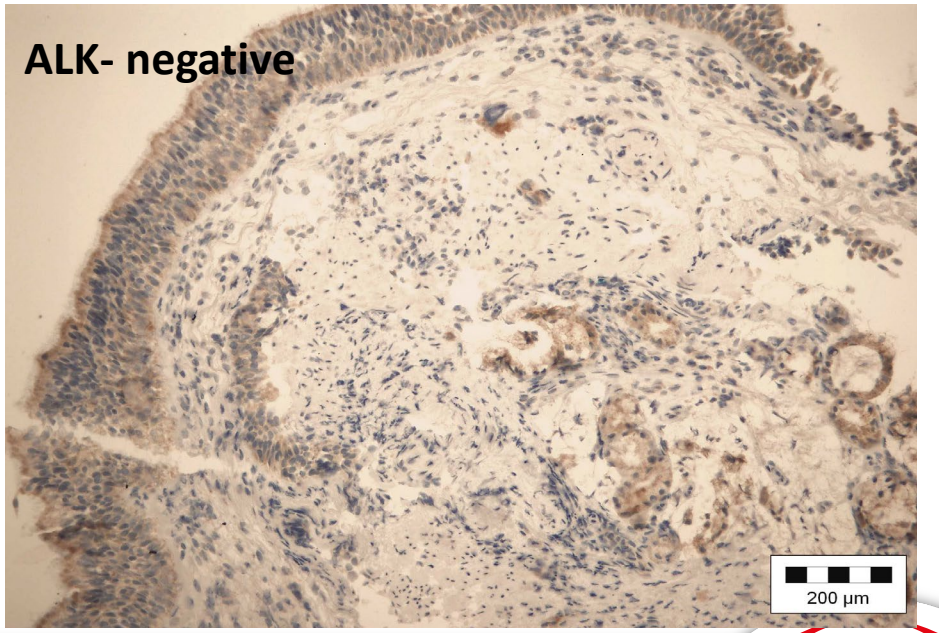
Ventana ALK (D5F3) IHC Assay

- The Ventana ALK (D5F3) IHC assay detects the expression of ALK
- Ventana used a range of human NSCLC tissue specimens in developing the ALK IHC assay, including:
  - Formalin-fixed Paraffin-embedded (FFPE) of primary and metastatic NSCLC tumors (resection, needle biopsies, bronchial biopsies)
  - Cell blocks (FNA)
- The Ventana ALK (D5F3) utilize D5F3 clone as the primary recombinant rabbit monoclonal antibody
- Result are qualitative either positive or negative

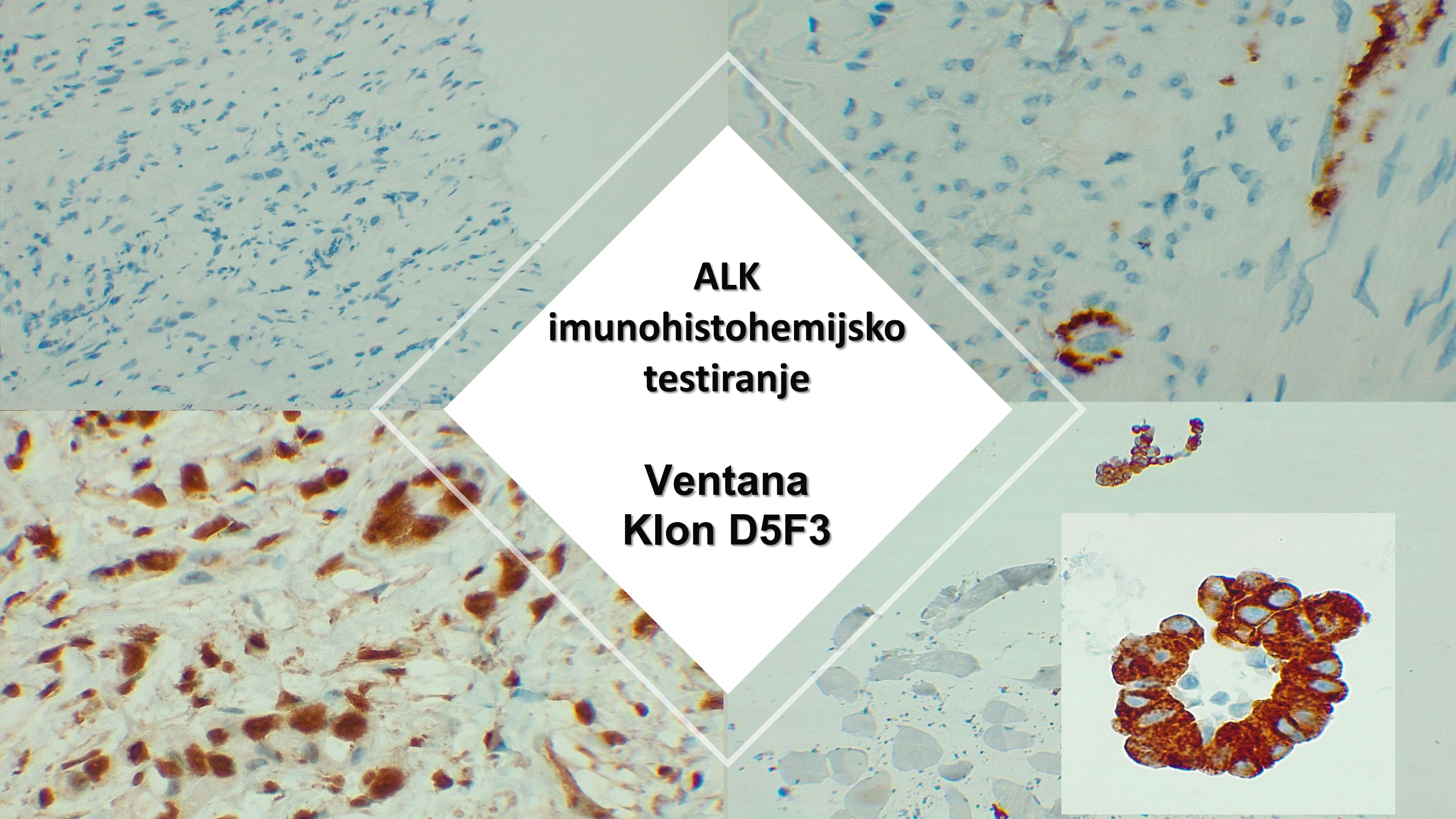




Ventana ALK (D5F3) CDx Assay package insert 1011645US Rev G

ALK imunohistohemijsko testiranje

Ventana Klon D5F3



Pogrešne interpretacije IHH analize.

Mucin produkujuće ćelije	Ukoliko je citoplazma maskirana intracelularnim mucinom postoji odsustvo ALK proteina, a negativno i marginalno membranozno bojenje predstavlja lažno negativni rezultat.
Membranozno bojenje	Nespecifično, apikalno, membranozno bojenje može da se vidi u normalnim pneumocitima (tip II), ali ne i u ćelijama tumora.
Neuroendokrine ćelije	Pojedini tumori (skvamozni karcinom, krupnoćelijski neuroendokrini karcinom) i netumorske ćelije (ganglijske ćelije) mogu da budi obojene.
Nespecifično bojenje mucina	Ekstracelularni mucin u pozadini, citoplazmi alveolarnih makrofaga i bronhijalnih ćelija, zavisno od pojačivača signala, može da bude obojen.

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High Concordance for IHC (D5F3) and FISH Assays

Reference	No. of specimens	IHC Sensitivity	IHC Specificity
Mino-Kenudson et al, 2010[1]	153	100%	99%
Martinez et al, 2013[2]	79	83%	100%
Minca et al, 2013[3]	231	94%	100%

1. Mino-Kenudson M, et al. Clin Cancer Res. 2010; 16:1561-1571; 2. Martinez P, et al. Plos One. 2013;8:e52261; 3. Minca EC, et al. J Mol Diagn. 2013; 15:341-346

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National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2022
Non-Small Cell Lung Cancer

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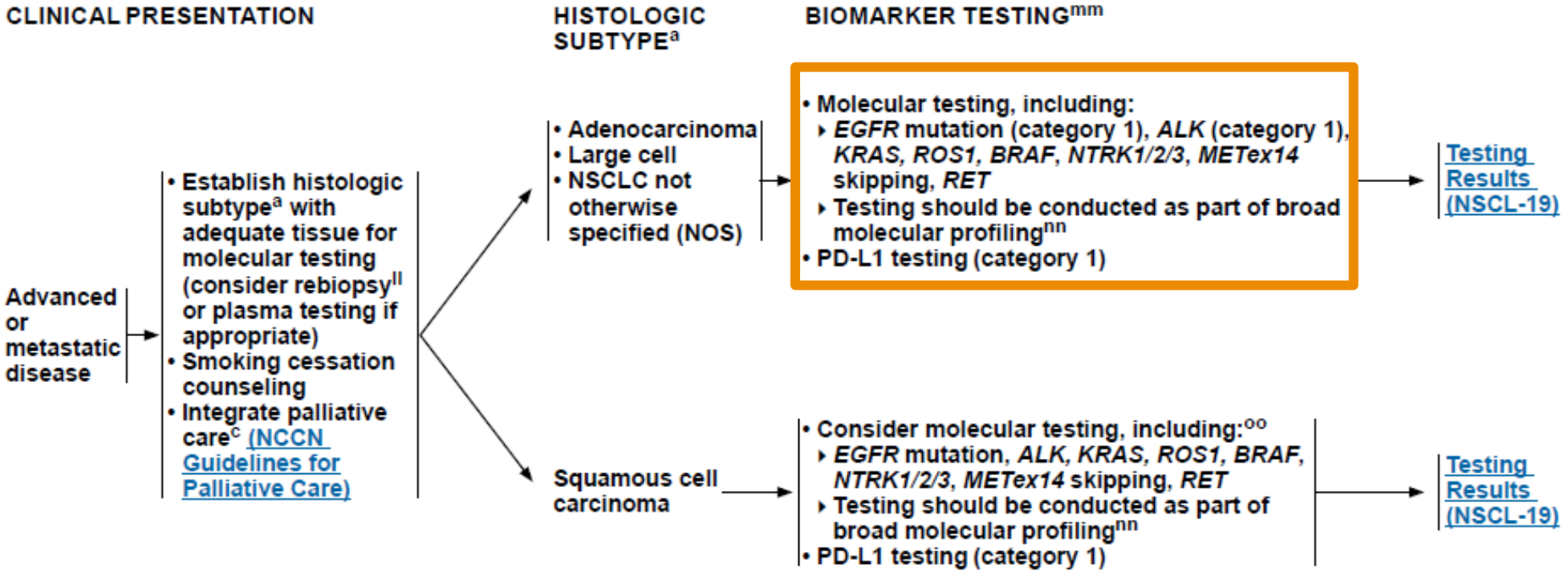


Preporučuje se široko molekularno profilisanje primenom NGS na prisustvo sledećih biomarkera:

***EGFR, ALK, KRAS, ROS1, BRAF, NTRK 1/2/3, MET*ex14 skiping i *RET* alteracije,**

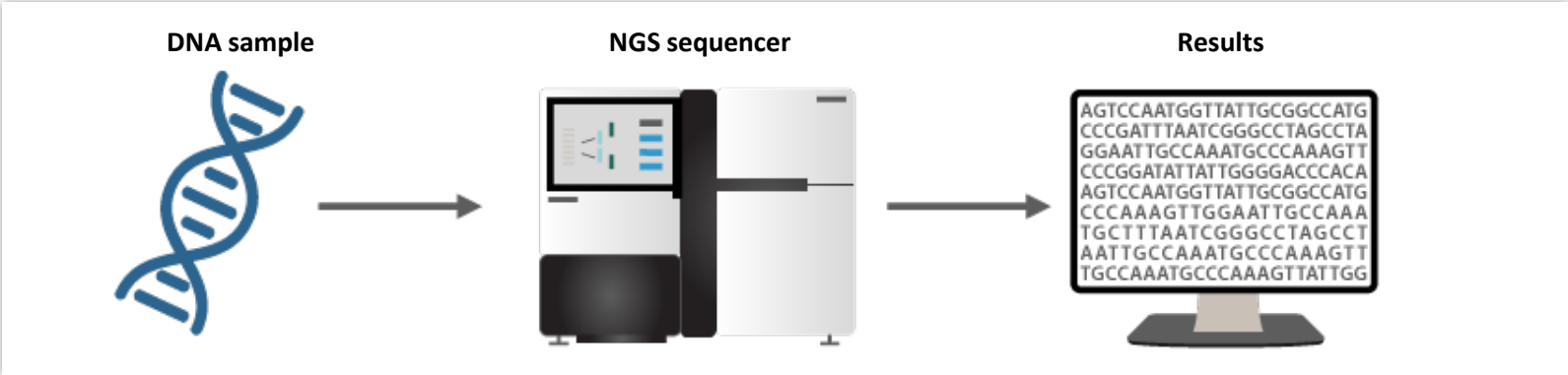
kao i PD-L1 testiranje imunohistohemijskom metodom

NCCN, National Comprehensive Cancer Network; NGS, sekvenciranje nove generacije; NSCLC, nesitnoćelijski karcinom pluća; EGFR, receptor epidermalnog faktora rasta; KRAS, KRAS proto onkogen; ALK, kinaza anaplastičnog limfoma; BRAF, B-raf proto-onkogen;; NTRK, neurotrofna receptor tirozin kinaza; METex14, mezenhimalno-epitelialni tranzitni faktor exona 14; RET, rearranged during transfection; ROS1, c-ros onkogen 1 kinaza; PD-L1, ligand 1 programirane ćelijske smrti

NCCN guidelines. Non-small cell lung carcinoma. Version 1. 2022. Available at: https://www.nccn.org/professionals/physician_gls/PDF/nscl.pdf (accessed March 2022).

Next-Generation Sequencing

- Next-generation sequencing (NGS) analyzes millions of DNA fragments simultaneously to provide information regarding an individual's genome[1]
- Different NGS platforms exist, reporting accuracies of ≤ 99.9%[2]
- NGS can detect **multiple biomarkers at once**, for example: ALK, EGFR, BRAF V600E, MET[3]
- NGS detected a complex *ALK* rearrangement that was not identified by FISH[4]



1. Behjati S, Tarpey PS. *Arch Dis Child Educ Pract Ed*. 2013;98:236-238.; 2. Liu L, et al. *J Biomed Biotechnol*. 2012;251364; 3. https://www.foundationmedicine.com/test/foundationone-cdx/accessed August 27, 2021, 4. Peled N, et al. *J Thorac Oncol*. 2012;7:e14-e16.

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Summary

- ALK rearrangements are driver mutations in NSCLC that activate downstream oncogenic signaling pathways.
- NCCN, ESMO, IASLC/CAP/AMP, and PNPK Kanker Paru guidelines for NSCLC recommend to test ALK rearrangements prior to targeted therapy selection.
- Currently Indonesia doesn’t associate certain targeted therapy such as anti-ALK with approved companion diagnostic tets.
- There are various ALK tests including FISH, IHC, RT-PCR and NGS.
- Ventana ALK (D5F3) IHC assay is one of ALK test that widely used.
- There is a high concordance between FISH and IHC test.
- ALK test can be performed as a sequential or as a pararell test with other driver mutations.

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