GOOD SCIENCE
BETTER MEDICINE
BEST PRACTICE

ESMO PRECEPTORSHIP ON LUNG CANCER AND OTHER THORACIC MALIGNANCIES

Sequence for biomarker testing in advanced NSCLC (IHC, PCR, NGS and more)

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15-16 November 2024, Paris

DECLARATION OF INTERESTS

Prof Keith M Kerr

Consultancy

- AbbVie, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, Debiopharm, Diaceutics, Eli Lilly, Merck Serono, Merck Sharp & Dohme, Novartis, Pfizer, Regeneron, Roche, Roche Diagnostics/Ventana, Sanofi

Honoraria (speaker)

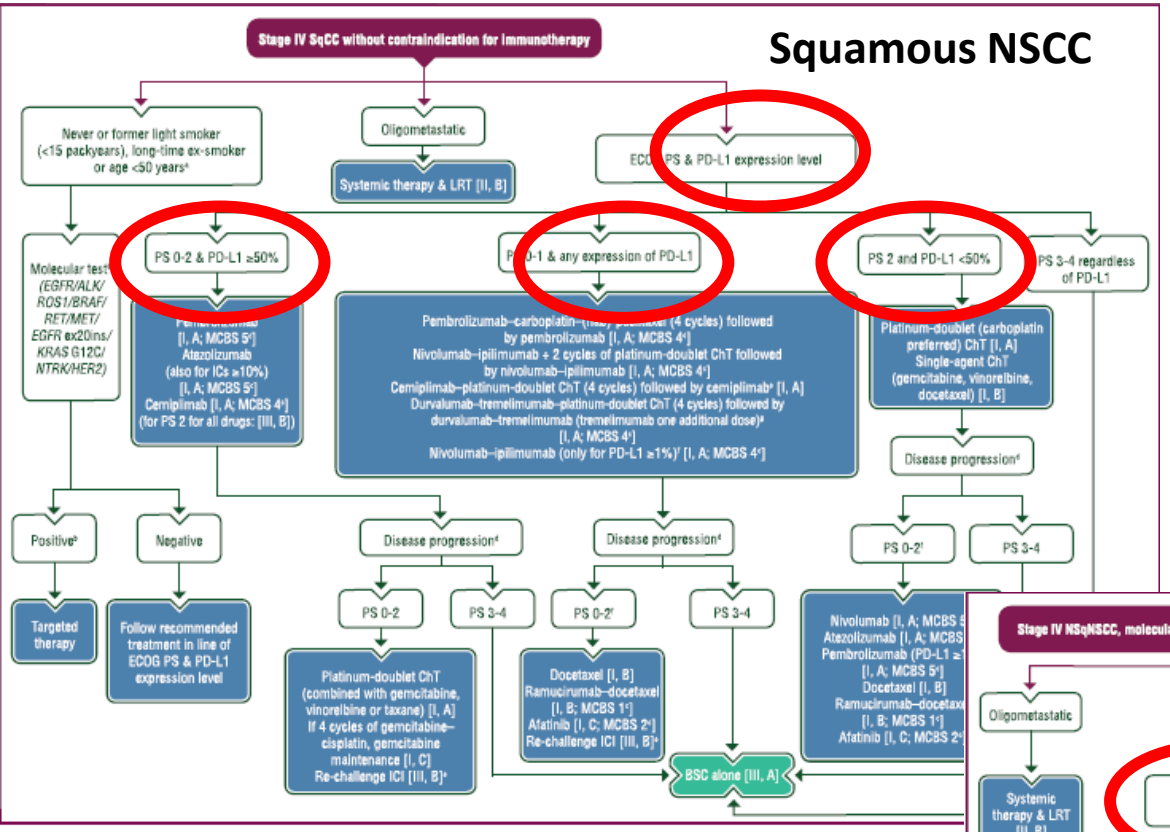
- AstraZeneca, Amgen, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Merck Serono, Merck Sharp & Dohme, Novartis, Pfizer, Roche, Roche Diagnostics/Ventana, Medscape, Prime Oncology

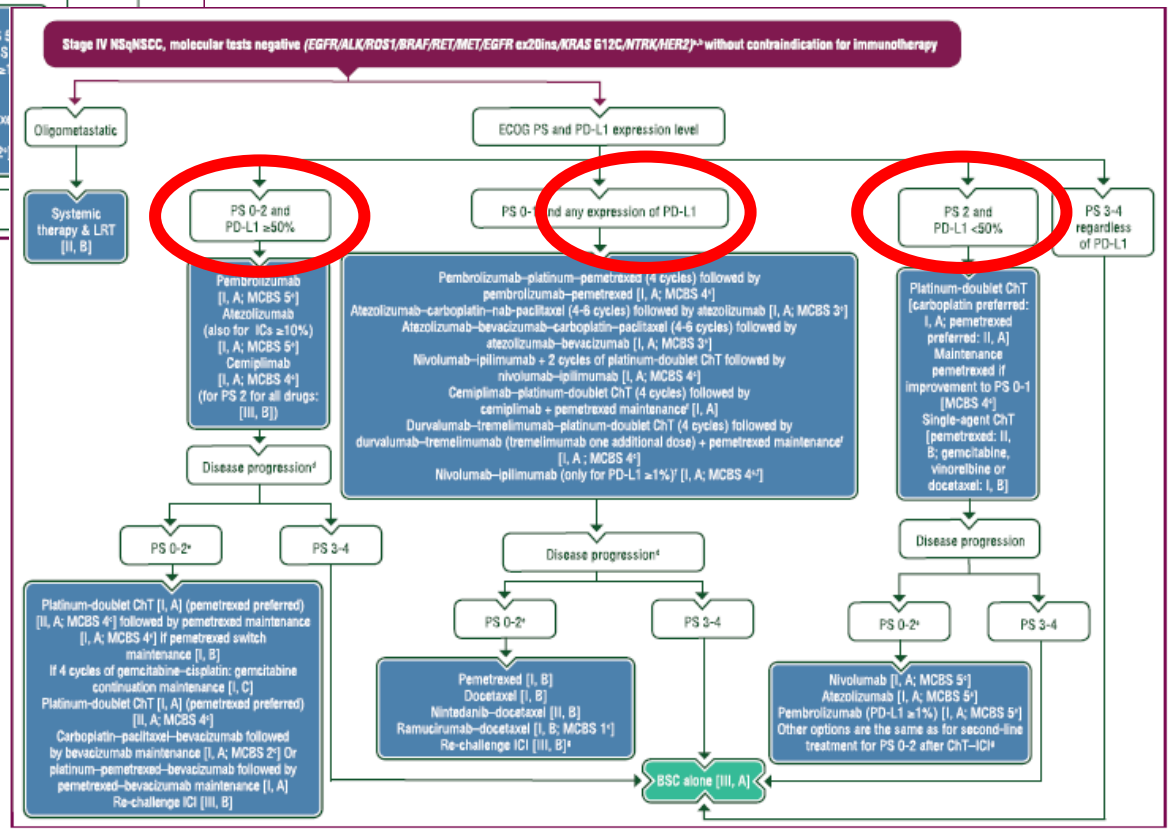
SEQUENCE FOR BIOMARKER TESTING IN ADVANCED NSCLC (IHC, PCR, NGS AND MORE)

Prof Keith M Kerr

ESMO Guidelines

Non-Oncogene-Addicted Stage 4 NSCLC

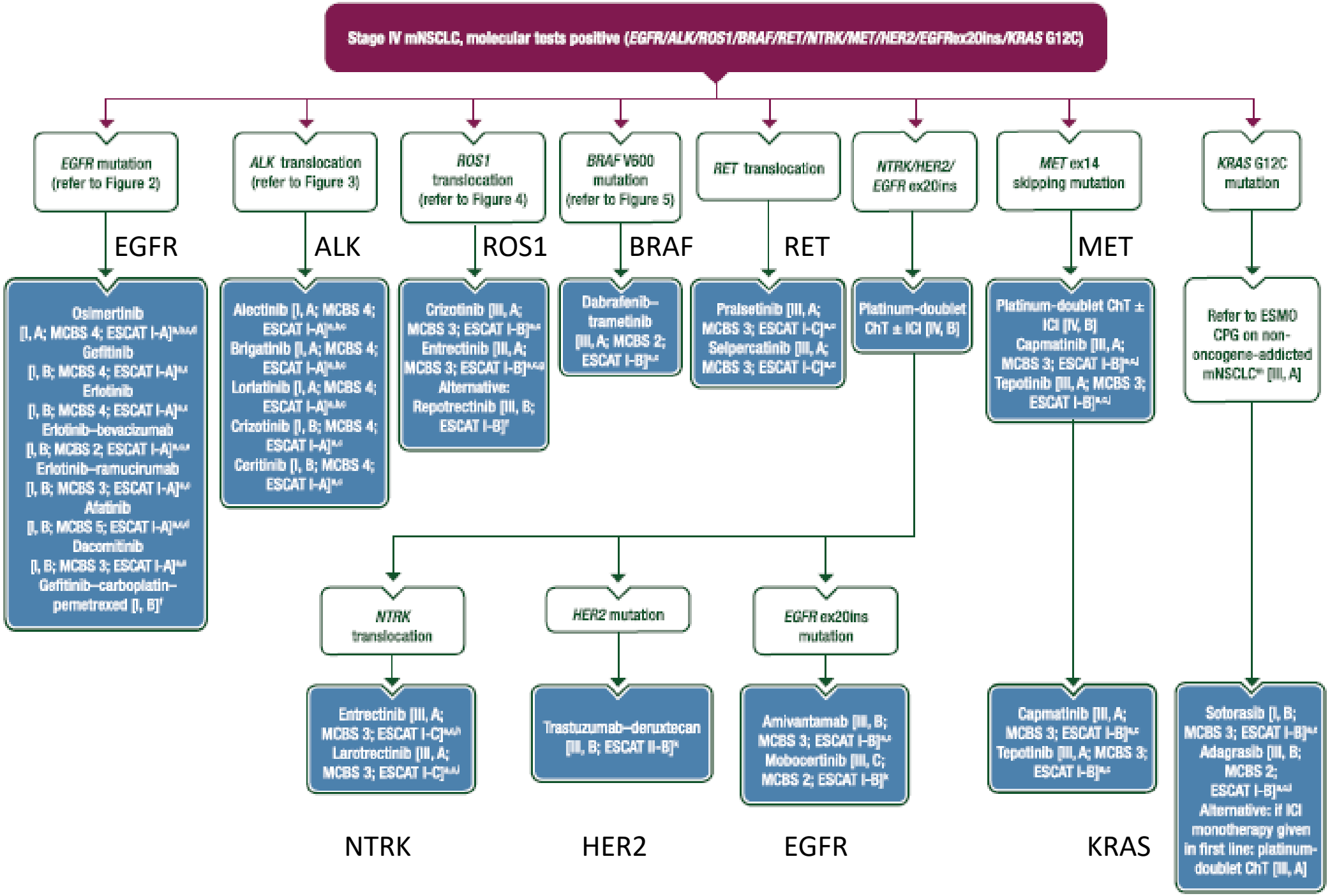




Squamous versus Non-squamous NSCC

PD-L1 IHC testing

Hendricks LE et al
Ann Oncol 2023



ESMO Guidelines

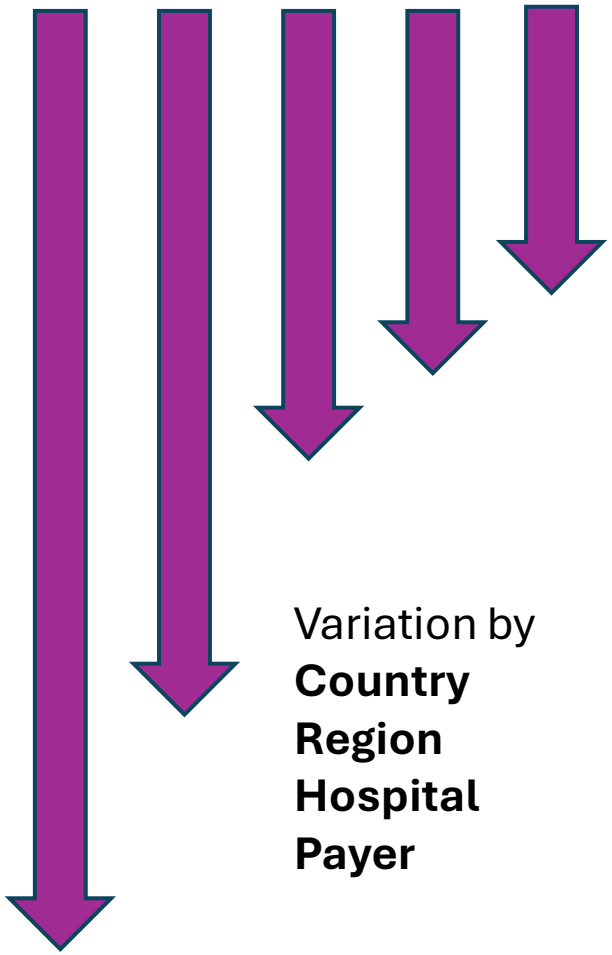
Oncogene-Addicted Stage 4 NSCLC

(largely a non-squamous population)

Hendricks LE et al
Ann Oncol 2023

Lung Cancer Testing Practice Is Variable in Different Health Systems

‘Routine’ Testing Package



Variation by
Country
Region
Hospital
Payer

- **PD-L1**
- **EGFR mutations**
- **ALK rearrangement**
- **ROS1 rearrangement**
- **BRAF mutations**
- **NTRK rearrangement**
- **RET rearrangement**
- **MET mutations**
- **HER2 mutation**
- **MET high amplification**
- **KRAS G12C mutation**
- **NRG1 rearrangement**
- **Other IO biomarkers (inflammation, TMB)**
- **Targets for Antibody-Drug Conjugates (ADCs)**

Clinical need

Patient wishes

ACCESS to:
Approved drugs
Clinical trials
Testing (NGS)

Knowledge and awareness

Guidelines →

Reimbursement

Use of NGS platforms

CAP/IASLC/AMP
ESMO
NCCN
ASCO
Pan-Asia
National or Regional

TESTING ISSUES TO CONSIDER

Making the initial diagnosis

Reflex versus Bespoke testing

Multiplex Parallel versus Sequential testing

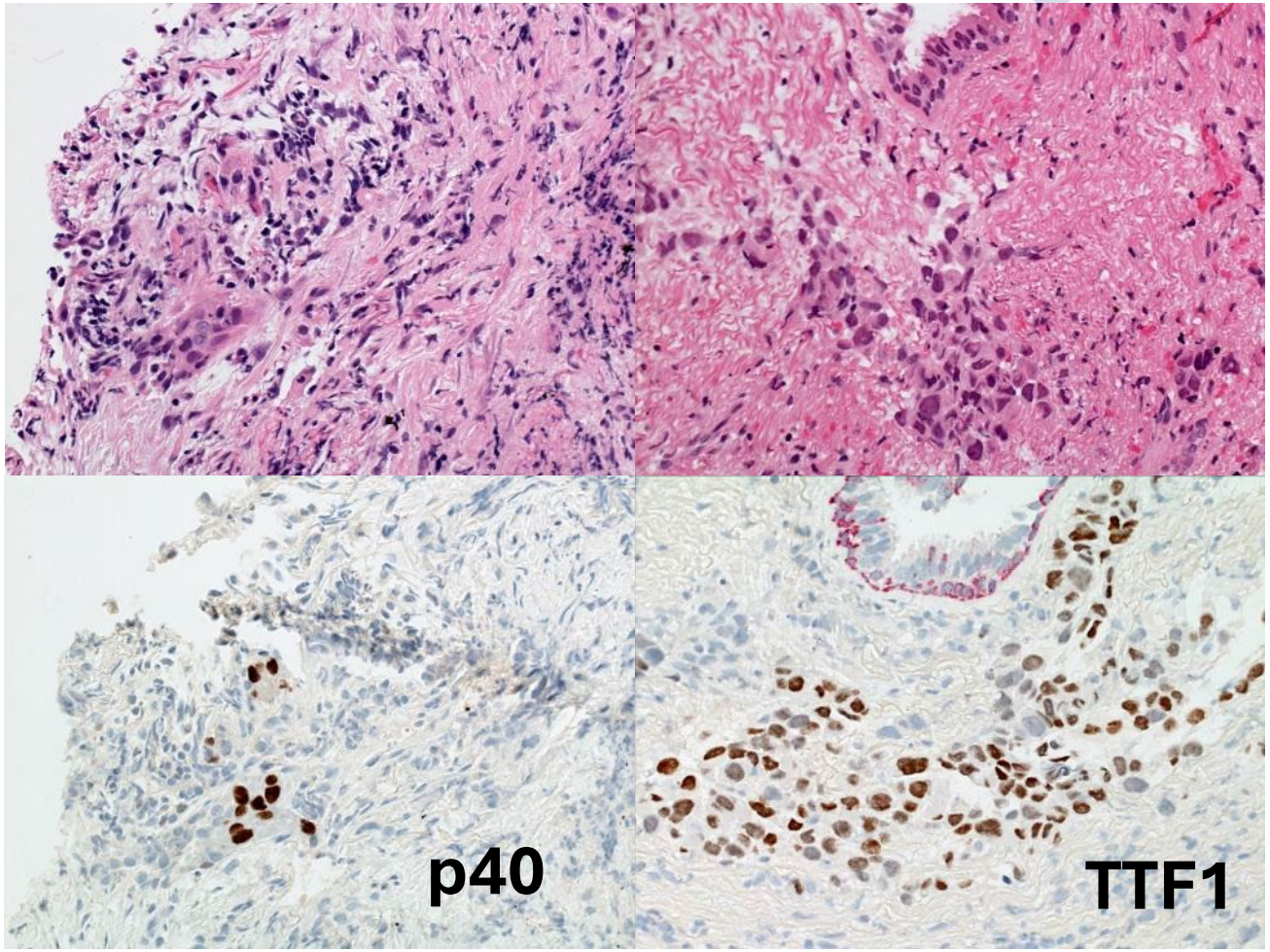
1L versus 2L+ therapy indications

Testing at disease relapse

MAKING THE INITIAL DIAGNOSIS

- Biopsy & Cytology
  - Most instances

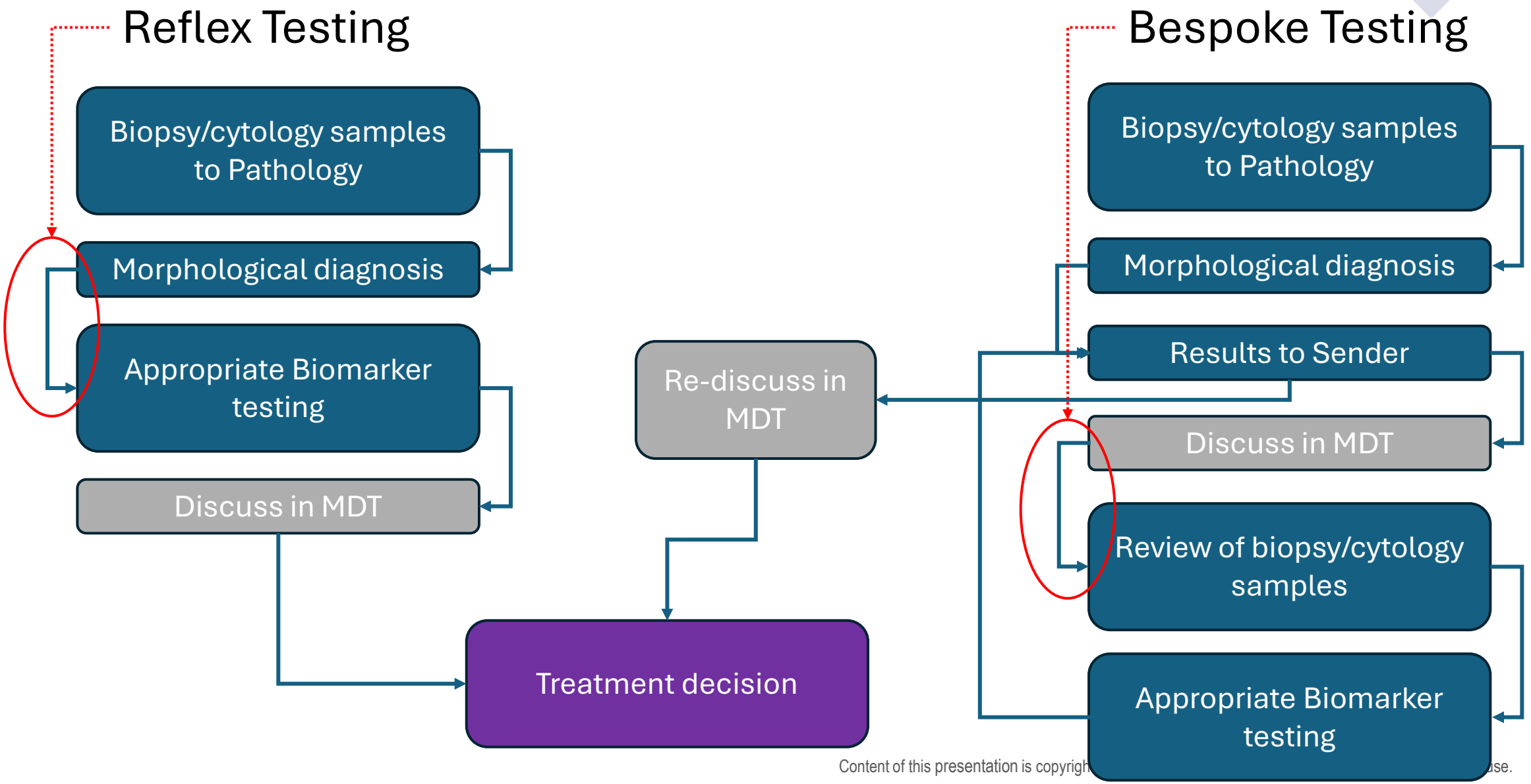
    DO NOT require immunohistochemistry
- Handle samples with care and with biomarkers in mind
- Don't waste tissue
  - Tissue handling protocols
- Don't overuse IHC
  - 25-40% of cases only
  - Only TWO markers needed



NSCC- probably squamous cell

NSCC – probably adenocarcinoma

REFLEX VERSUS BESPOKE (ON DEMAND) TESTING



Approaches to Biomarker testing matter

Reflex testing

- **Faster results**
- Patients less likely to be missed
- Best use of tissue

- Un-necessary testing
- Wasted lab time
- 2L biomarkers more difficult?

Bespoke / On-demand testing

- All tests are needed
- Less wasteful of lab resources

- **Slower results**
- Patients more likely to be missed?
- Tissue waste – higher test fails

Advanced disease
and
Early Stage

Gosney JR et al. ESMO Open 2023; Kerr KM et al. Histopathology 2024

HOW DO WE PURSUE MOLECULAR TESTING IN ADVANCED STAGE NSCLC?

Are ALL NSCLC tested for ALL biomarkers, or Selection based upon Histology?

- **PD-L1 testing** for **ALL** NSCLC

- Guidelines generally recommend **molecular** testing for all Non-squamous NSCLC
  - Caveat regarding never/long time ex-smokers, young patients, ethnic groups
- Testing strategy predicated on probability of finding something
  - Adenocarcinomas but…..
  - Clinical associations but……..

MULTIPLEX PARALLEL VERSUS SEQUENTIAL TESTING

Doing **all** the required tests at the same time is best:

IHC and nucleotide sequencing

Multiplex Parallel Sequencing

Multiplex Sequencing methodology

vs

Stand-alone Allele specific PCR

- Sometimes preferred for cost reasons
- How many targets are needed?
- Rapid testing (relatively speaking)
- Sensitivity & Specificity
- Rarer mutations in a gene may be missed or not covered

TISSUE STEWARDSHIP

Is the diagnostic sample good enough for ALL biomarker testing?

- Maximizing the yields from any diagnostic intervention whilst preserving patient safety
- Fixation and Processing in Pathology must be permissive of ALL the diagnostic techniques that MIGHT be needed
  - Only ONE CHANCE to do this properly
- Pathologists MUST preserve material for ALL anticipated stages of diagnosis

SPECIMEN ADEQUACY FOR BIOMARKER TESTING IS QUITE DIFFICULT TO PREDICT

Some things to consider

- Actual number of tumour cells in the sample – HOW MANY CELLS DO WE HAVE?
- The PROPORTION of the nucleated cells in the sample that are tumour

- DNA quality in the sample is VERY hard to predict
- So too with RNA except that it is likely to be worse!

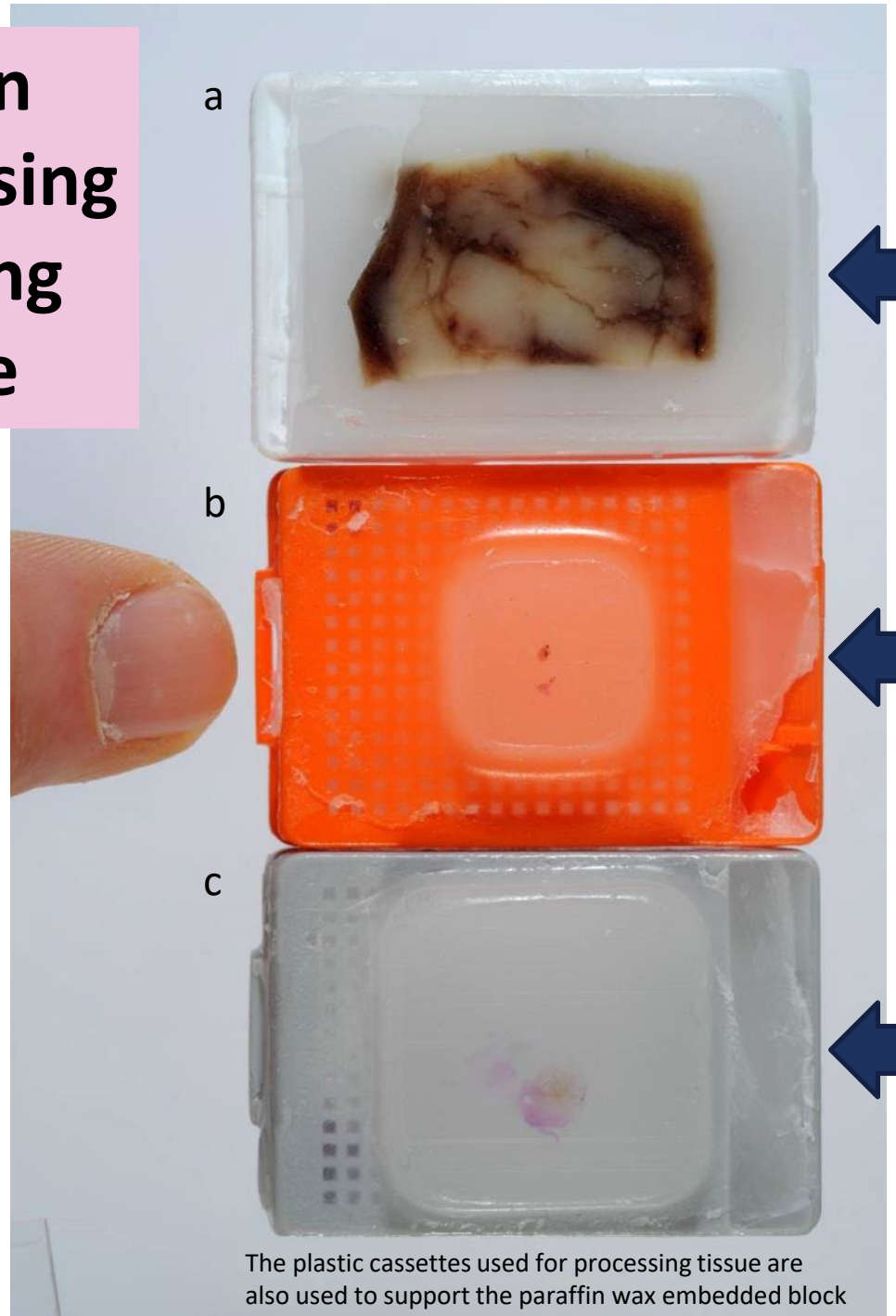
- Value of on-site assessment ROSE
- Warnings conveyed at the MDT
- **Give the patient the benefit of the doubt?**

100 cells for PD-L1 testing

Content limit (% TC) for sequencing

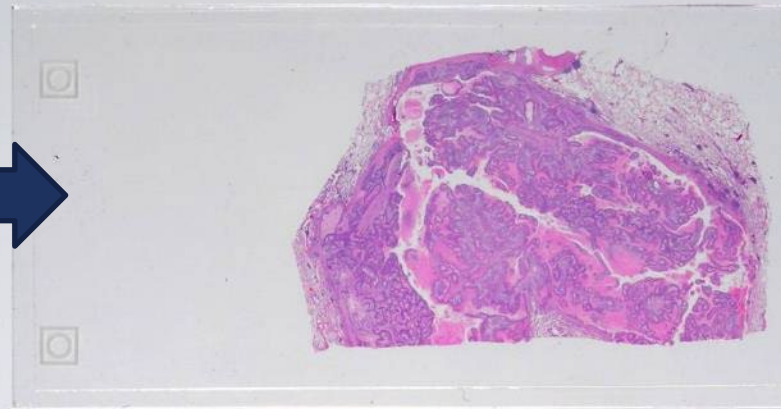
- **Lab (technology) dependant**
- **Some labs set 'high' limits**

Fixation
Processing
Handling
Storage

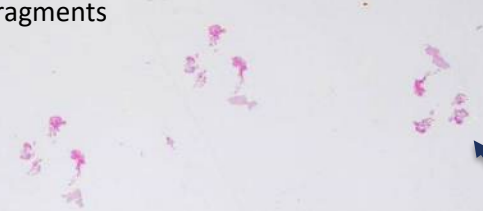


The plastic cassettes used for processing tissue are also used to support the paraffin wax embedded block

Abundant tumour tissue in a block taken from a resected tumour



Lung biopsy fragments
1mm or less



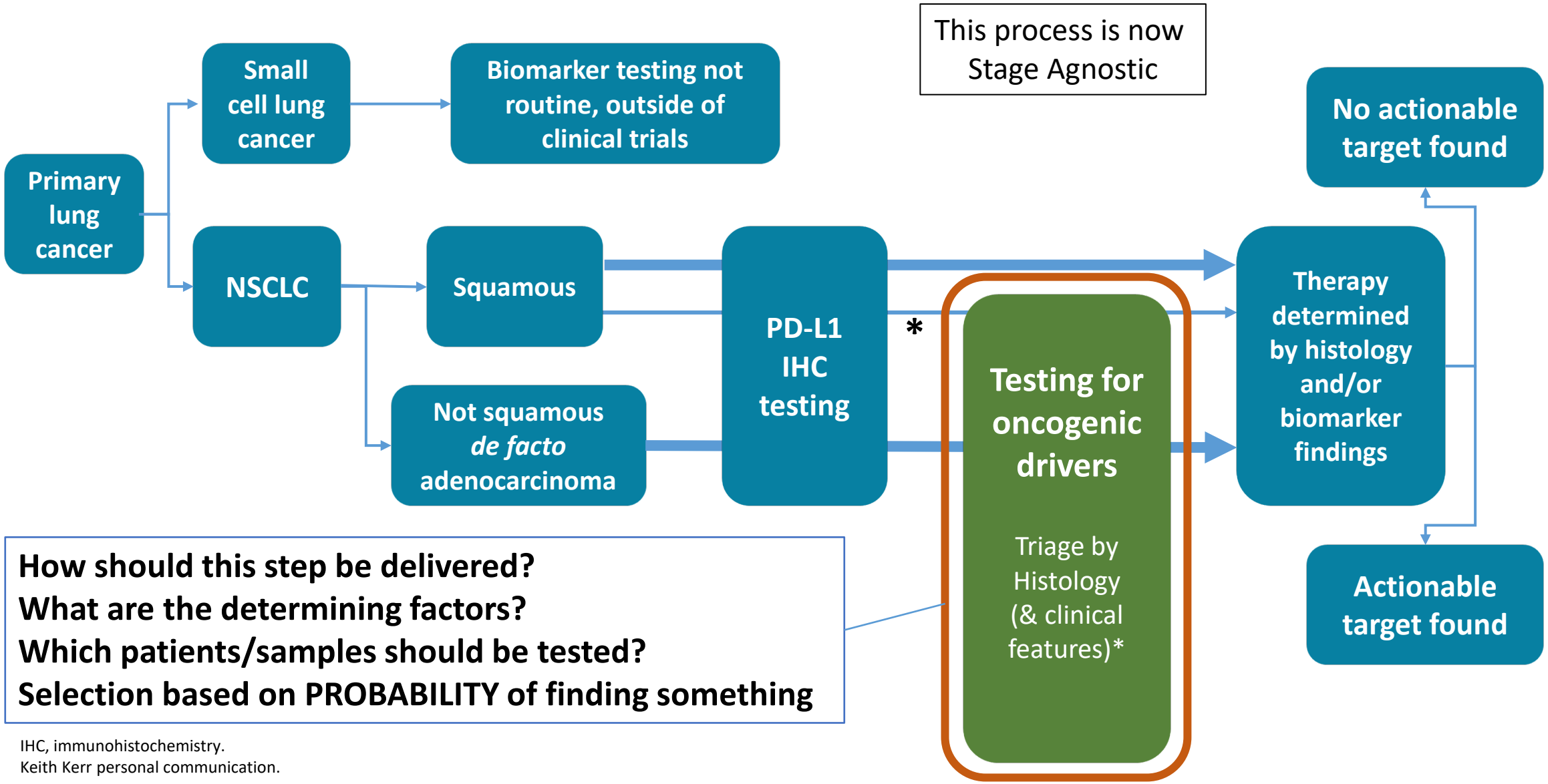
Although each section shows 5 fragments, only two remain in the block (left), after sections are cut for IHC and molecular testing

Cell pellet formed
from EBUS procedure

Sections cut from the block, mounted on glass slide and stained with Haematoxylin and eosin (H&E)

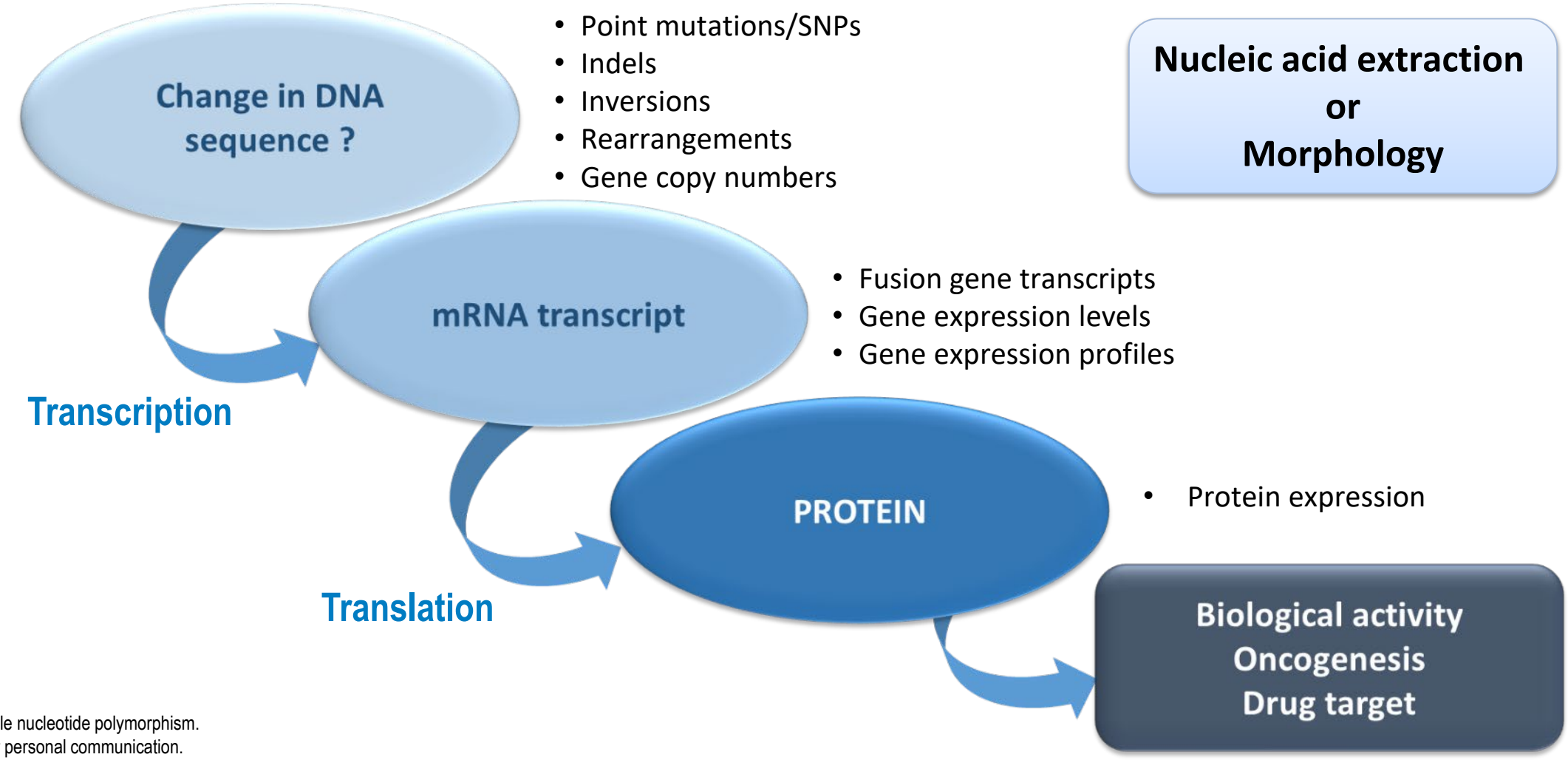
On average only 10-25% of this tissue is tumour!

NSCLC Diagnosis: Tumour Subtype and Biomarker Profiling



IHC, immunohistochemistry.
Keith Kerr personal communication.

Which Level to Test? How Will You Do It?



SNP, single nucleotide polymorphism.
Keith Kerr personal communication.

DIFFERENT BIOMARKERS: DIFFERENT TECHNOLOGY

Mutations[1,2]	Fusion genes[2]	Gene copy number[1,2,4–6]	Protein expression[2,7,8]
EGFR (many)	*ALK*	*MET*	PD-L1
KRAS G12C	*ROS1*		CD3, CD8
BRAF V600E	*NTRK1/2/3*		CD68
*MET*ex14 skipping	*RET*		ALK, ROS1, NTRK
HER2	*NRG1*		HER2, TROP2, MET?
	Technology		
DNA sequencing	RNA sequencing* FISH IHC screening	FISH CISH CGH NGS	IHC
DNA NGS ☺ ☺[3]	RNA NGS ☺[3]	DNA NGS ☹[3]	Nope![3]

- We need more than (just) NGS[3]
- But we do need NGS, for comprehensive coverage[3]
- IHC still has place[3]
- So does ISH[3]

ALK, anaplastic lymphoma receptor tyrosine kinase; *BRAF*, B-Raf proto-oncogene, serine/threonine kinase; CGH, comparative genomic hybridisation; CISH, chromogenic *in situ* hybridisation; DNA, deoxyribonucleic acid; *EGFR*, epidermal growth factor receptor; FISH, fluorescence *in situ* hybridisation; *HER2*, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridisation; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; *MET*ex14, mesenchymal epithelial transition factor exon 14; NGS, next generation sequencing; *NRG1,* neuregulin 1; *NTRK*, neurotrophic tyrosine kinase receptor; PD-L1, programmed death-ligand 1; *RET*, ret proto-oncogene; RNA, ribonucleic acid; *ROS1*, ROS proto-oncogene 1, receptor tyrosine kinase.

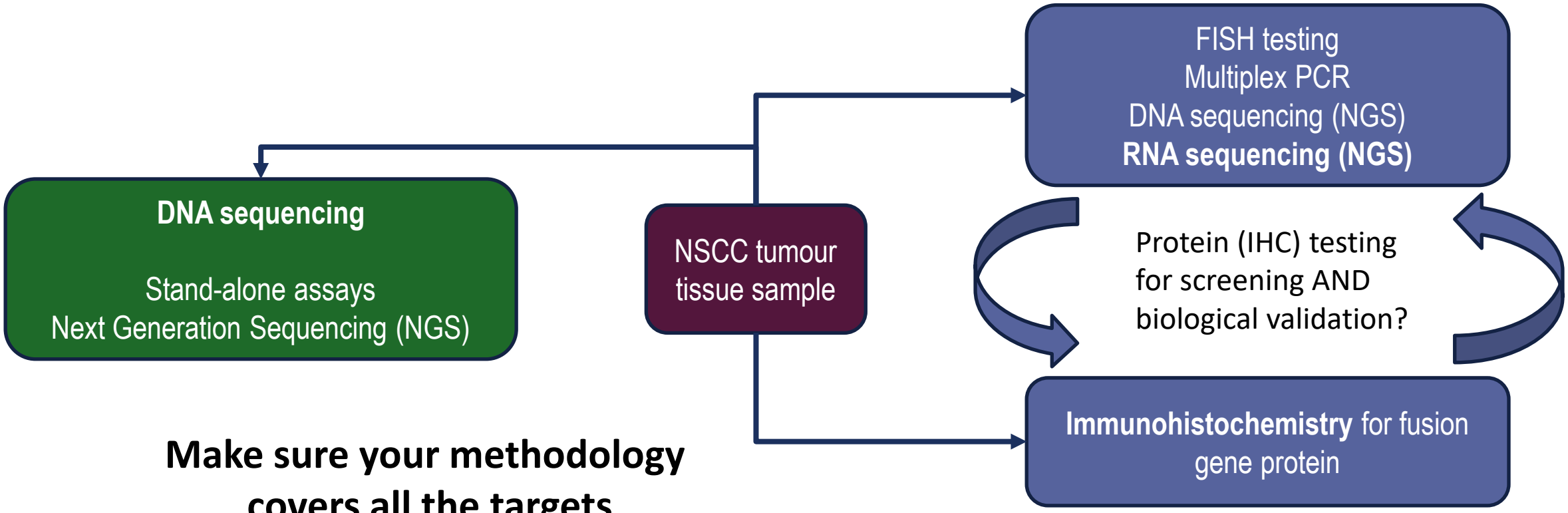
1. Kerr KM *et al. Lung Cancer* 2021; 154:161–175; 2. Penault-Llorca F et al. Virchows Arch 2022; 1–16; 3. Speaker's personal communications; 4. Dimou A *et al. PLoS One* 2014; 9:e107677; 5. Tchinda J, Lee C. *BioTechniques* 2006; 41:385–392; 6. Yoo SB *et al. Lung Cancer* 2010; 67:301–305; 7. Wang J *et al. J Exp Clin Cancer Res* 2014; 33:109; 8. Yaegashi LB *et al. Front Immunol* 2021; 12:714230

Multiplex Parallel (Simultaneous) testing of all required Biomarkers

Mutations
EGFR, KRAS, BRAF, HER2

MET exon14 mutations
MET amplification

Fusion genes
ALK, ROS1, NTRK, RET



Make sure your methodology covers all the targets you need to find!

MULTIPLEX SEQUENCING METHODOLOGY

Whole Genome Sequencing

Whole Exome Sequencing

Large panel targeted NGS

Smaller panel targeted NGS

Multiple gene ‘Black box’ rapid solutions (Biocartis *Idylla* rtPCR etc)

Next-generation sequencing (NGS)

Multiplex testing is certainly the way to go

Multiple mutations *and* fusion genes *and* gene copy number?

- **Good solution for the 'genomic biomarker shopping list'**
- **Relatively costly (depends where you practice)**
- **Requires more tissue?... and more time?**
- **Not equally good for all alterations (Mutations > Fusions >> Gene copy number)**
- **Generates lots of (sometimes incomprehensible) data**

How should we organize (prioritize?) testing?

- DNA sequencing: Targeted Hybrid-capture or Amplicon-based
  - Hybrid capture better for Fusion genes and METex14 mutations **if only DNA is used**
  - Amplicon-based methods often **require less DNA input**
- RNA sequencing (RNA NGS) identifies cases missed by Hybrid-capture sequencing but…**mRNA is a labile test moiety**

- "DNA plus RNA" or "DNA then RNA"?
  - Mutually exclusive oncogenic drivers
  - Cost saving, Delays fusion gene identification, Core target or Academic/Trials
  - Rapid PCR testing vs NGS?

- Future role for AI to predict mutation/fusion on H&E stained tissue sections?

IHC, immunohistochemistry; NGS, next-generation sequencing.

Benayed R, et al. Clin Cancer Res. 2019;25:4712-22.
Guo R, et al. Clin Cancer Res. 2021;27:799-806.
Socinski MA, et al. JCO Precis Oncol. 2021;5:PO.20.00516.
Subramanian J, Tawfik O. Expert Rev Anticancer Ther. 2021;21:877-86.

Single Gene/Sequential vs Parallel testing

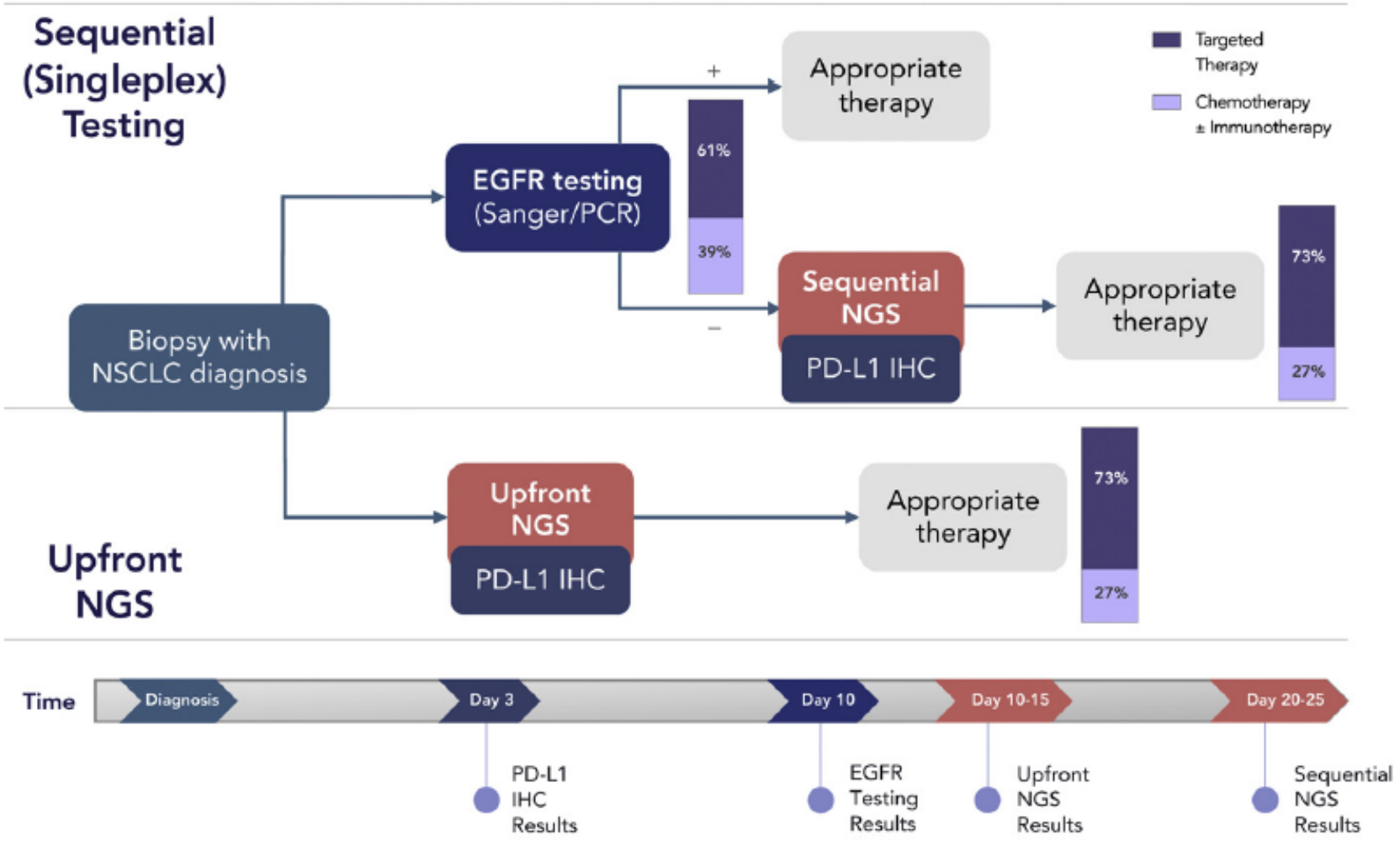


Fig. 4. Comparison of sequential testing versus upfront NGS.

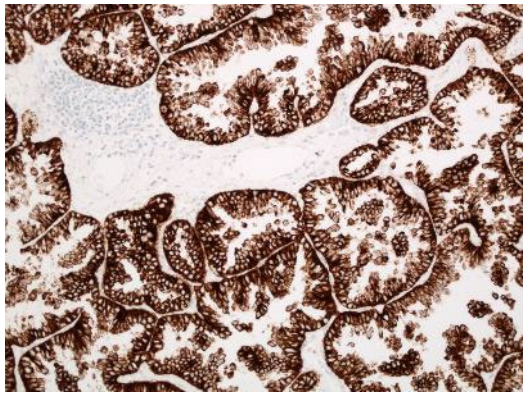
Strategy tailored to specific circumstances

High EGFR mutation prevalence

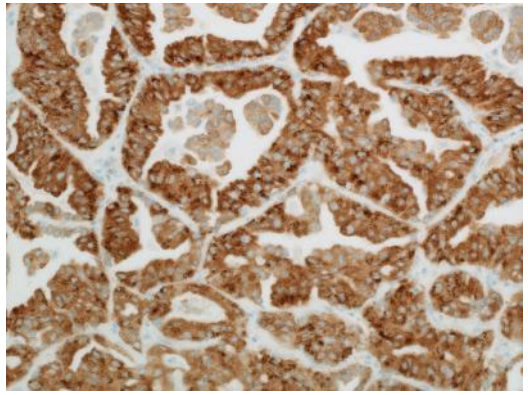
Access
Reimbursment
Clinical urgency

Tan AC et al Lung Cancer 2020

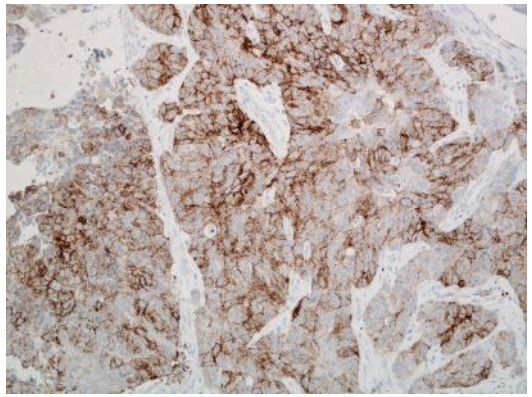
IHC for fusion gene products in NSCLC



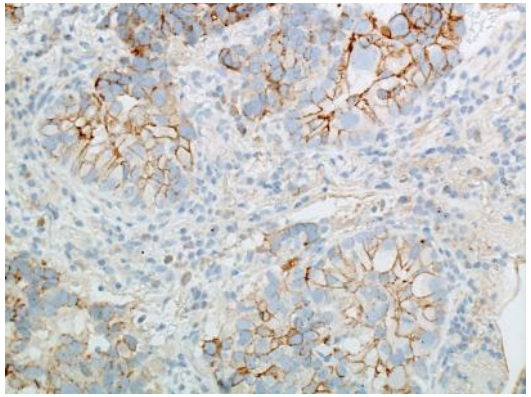
ALK: D5F3 assay



ROS1: SP384 assay



panNTRK: EPR17341 LDT



MET: SP44 assay

- Screening tool to select cases for molecular confirmation
- Therapy-determining companion diagnostic test (ALK D5F3 assay)
- Validation tool to confirm molecular test results: translation has occurred
- Rapid turn-around: early warning to the molecular laboratory

ALK, anaplastic lymphoma kinase; ROS1, c-ros oncogene 1; NTRK, neurotrophic-tropomyosin receptor kinase; LDT, laboratory developed tests
Keith Kerr personal communication
Images provided by author for educational purposes

1L VERSUS 2L+ THERAPY INDICATIONS

Upfront testing for ‘later’ use

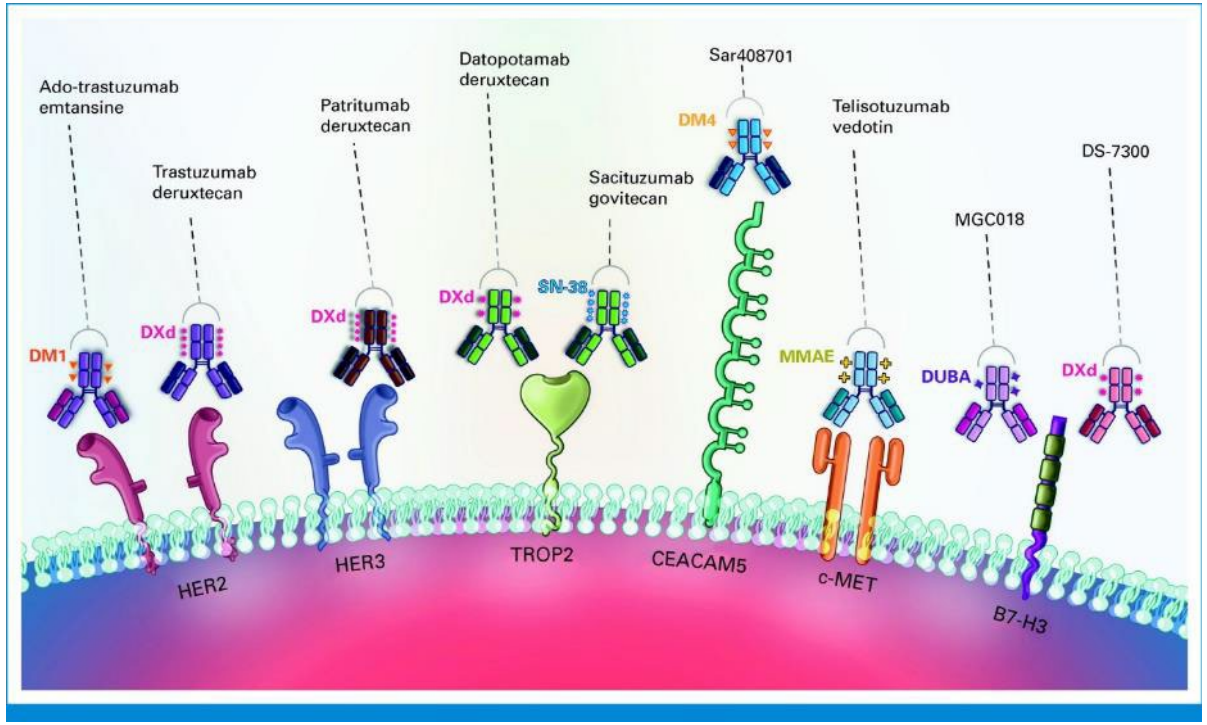
- Acceptable for driver mutations and fusion genes (*HER2, NTRK, EGFR*$_{exon20}$*, KRAS*$_{G12C}$)
- Less certain when protein biomarkers are considered (PD-L1 and ADC targets)
- Or if/when co-mutational status becomes an issue
- Trial design probably ‘blurs’ the picture

Testing at Relapse?

- Will new Pathologic information change what you do next?
- Old sample-pre 1L therapy (available, lost, exhausted, relevant?)
- Issues with rebiopsy
- Use of blood as a biomarker source

ADCs: Implications for diagnostics

Target	Drug	Payload
HER2	Trastuzumab-DM1	DM1
	Trastuzumab-DXd	Deruxtecan
HER3	Patritumab-DXd	Deruxtecan
TROP2	Datopotamab-DXd	Deruxtecan
	Sacituzumab govitecan	SN-38
CEACAM5	Tusamitamab ravtansine	DM4
c-MET	Telisotuzumab vedotin	MMAE
B7-H3	I-DXd (DS-7300a)	Deruxtecan
	MGC018	DUBA
CD56	Lorvotuzumab mertamsine	DM1
AXL	Enapotamab vedotin	MMAE
	Mecbotamab vedotin	MMAE
PK7	Cofetuzumab pelidotin	Auristatin-0101
PVRL4	Enfortumab vedotin	MMAE
TF	Tisotumab-vedotin	MMAE
EGFR	MRG003	MMAE
ROR2	Ozuriftamab vedotin	MMAE
NaPi2b	Upifitamab rilsodotin	AF-HPA
	Lifastuzumab vedotin	MMAE



Positivity Locality Quantity

The Pathologists' Conundrum

David L. Rimm, MD, PhD; Sanja Dacic, MD, PhD; Stuart J. Schnitt, MD

ADC, antibody-drug conjugate; AF-HPA, auristatin F- hydroxypropylamide; DUBA, deubiquitinating enzyme A; DXd, deruxtecan; I-DXd, ifinatamab deruxtecan; MMAE, monomethyl auristatin E.
Passaro A et al. J Clin Oncol. 2023;41(21):3747-3761.

Rimm DL, et al. *Arch Pathol Lab Med*. 2023;147(1):17-18.

TESTING AT DISEASE RELAPSE: RESISTANCE MECHANISMS FOR KINASE INHIBITORS

Acquired Resistance

- **On Target Alterations**
  - T790M, C797S, ALK mutations
- **Off Target Bypass Mechanisms**
  - Any alternative signaling which might maintain tumour growth
  - HER3 overexpression, MET amplification
- **Phenotypic Alteration from Adenocarcinoma**
  - Small cell carcinoma
  - Squamous cell carcinoma
  - Pleomorphic (sarcomatoid) carcinoma

MET amplification (or increased copy number)

Definitions – true amplification vs polysomy
Best identified by FISH or other ISH technique
Challenges with NGS approach
Even more challenges using blood

Primary 'Resistance'

- Co-mutational Status may confer resistance (TP53, KEAP1, STK11, SMARCA4…….)

DOES THE TEST REPRESENT THE PATIENT'S DISEASE BURDEN? DO THE REPORTED BIOMARKER FINDINGS MAKE SENSE?

- **Test accuracy and consistency**
  - Standardisation
  - Laboratory Accreditation
  - External Quality Assurance
- **The challenge of heterogeneity and sampling error**
  - Lack of test sensitivity?
  - Real biology
  - Trial samples
- **How are molecular findings reported to the treating Physician?**
  - Comprehensible reports
  - Enough detail for action
  - Avoid confusion
  - Drip feed test outcomes?
- **Role of the molecular MDT**
- **Role of the 'liquid biopsy'**

- MDT, multidisciplinary team.
- Speaker's personal communications.

ESMO

OPTIMAL PLATFORM AND SEQUENCE FOR BIOMARKER TESTING IN ADVANCED NSCLC: IHC, PCR, NGS AND MORE

- Making the primary diagnosis while Preserving material for biomarker testing
- Broad range of Biomarkers required for treatment decisions
- Broad range of Techniques required for delivery
- Tissue and Molecular Pathology should be integrated
- Reflex testing recommended
- Multiplex parallel testing (NGS) recommended
- IHC testing will increase
- Testing at relapse

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GOOD SCIENCE
BETTER MEDICINE
BEST PRACTICE

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