# The Multidimensional Nature of Cancer Classification: Consequences for Molecular Pathology

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World Health Organization

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# Declaration of Interests

- I am a pathologist, based at the International Agency for Research on Cancer, part of the World Health Organization

- All opinions expressed are personal, and not those of any of the organisations above.

CAROLI LINNÆI

EQUITIS DE STELLA POLARI,

ARCHIATRI REGII, MED. & BOTAN. PROFESS. UPSAL.;
ACAD. UPSAL. HOLMENS. PETROPOL. BEROL. IMPER.
LOND. MONSPEL. TOLOS. FLORENT. SOC.

# SYSTEMA NATURÆ

PER

*REGNA TRIA NATURÆ,*

SECUNDUM

CLASSES, ORDINES,
GENERA, SPECIES,

CUM

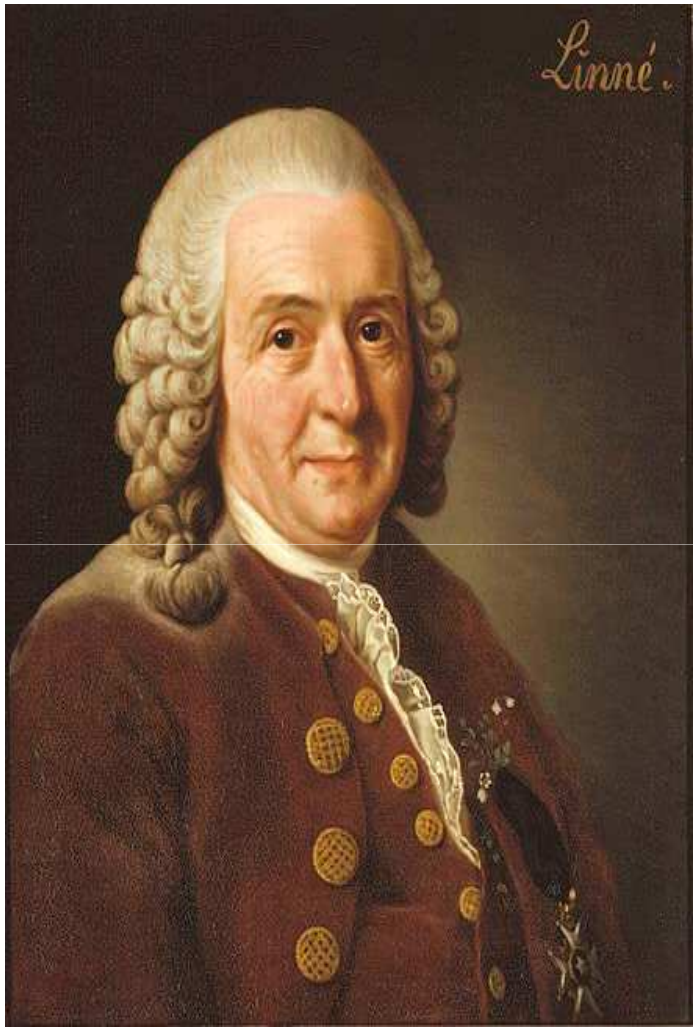
*CHARACTERIBUS, DIFFERENTIIS.*
*SYNONYMIS, LOCIS.*

TOMUS I.

EDITIO DECIMA, REFORMATA.

*Cum Privilegio S:æ R:æ M:tis Sveciæ.*

*HOLMIÆ,*
IMPENSIS DIRECT. LAURENTII SALVII,
1758.




Carl Linnaeus (1707 – 1778)

- *Taxonomy* is the science of defining and naming groups of biological organisms on the *basis of shared characteristics*

- *Cancer classification* is based on shared characteristics of cancers – currently mainly histology and genetics.

*WHA10.18 The Tenth World Health Assembly resolved, '…to continue work on formulating international definitions of nomenclature and statistical classification…' (May 1957)*

# WHO Blue Books

Scientific evidence

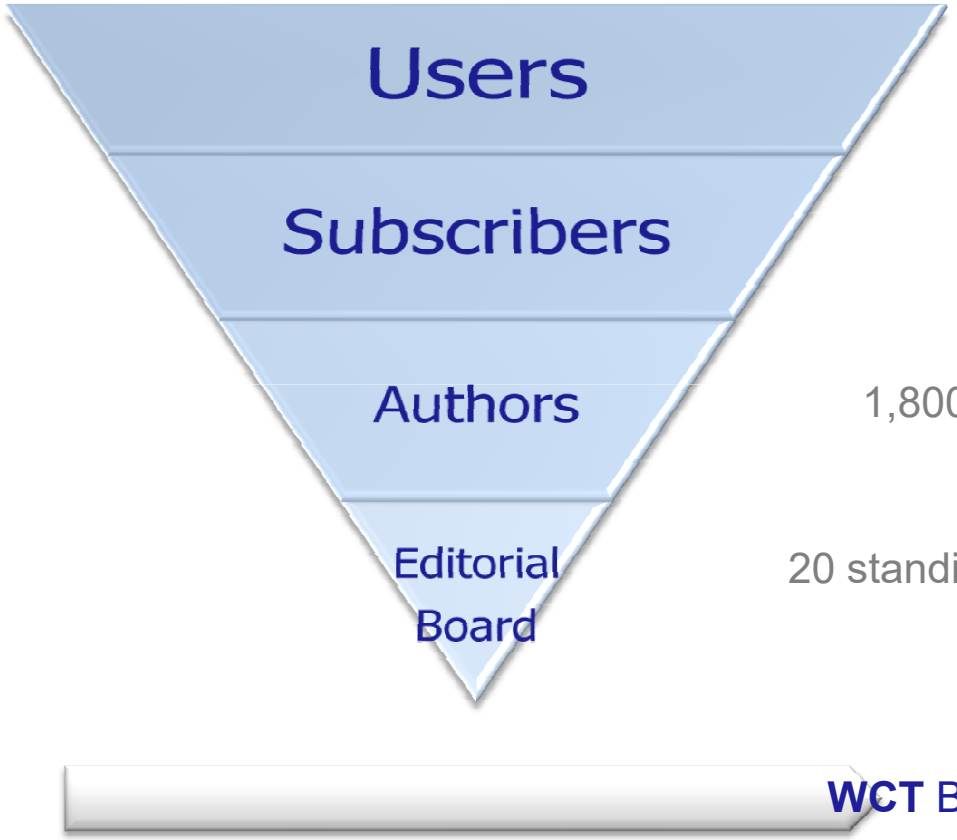
Illustrative cases


Diagnostic criteria

International Agency for Research on Cancer

World Health Organization

# WHO Blue Books
# Faculty

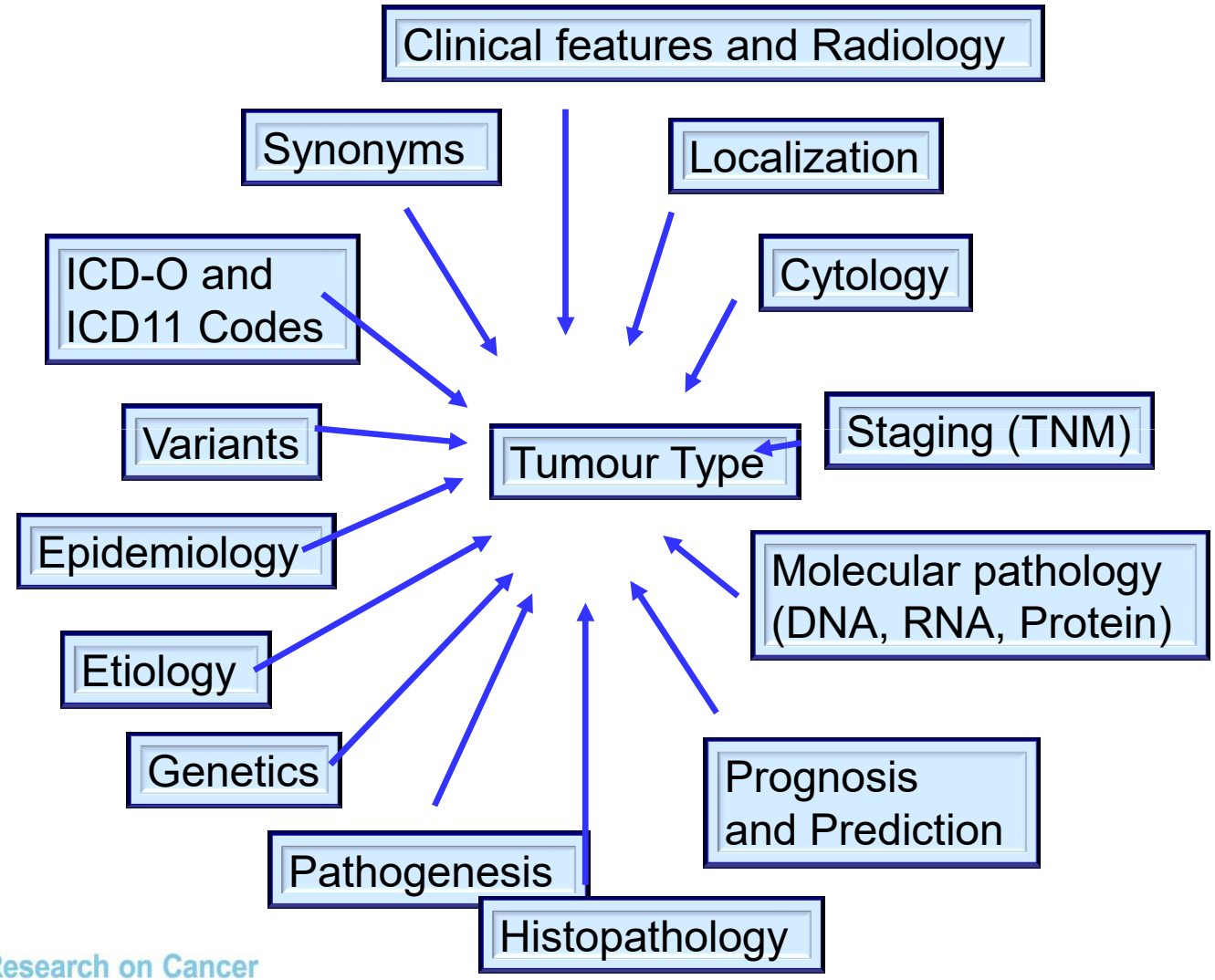


# *Classification terms*

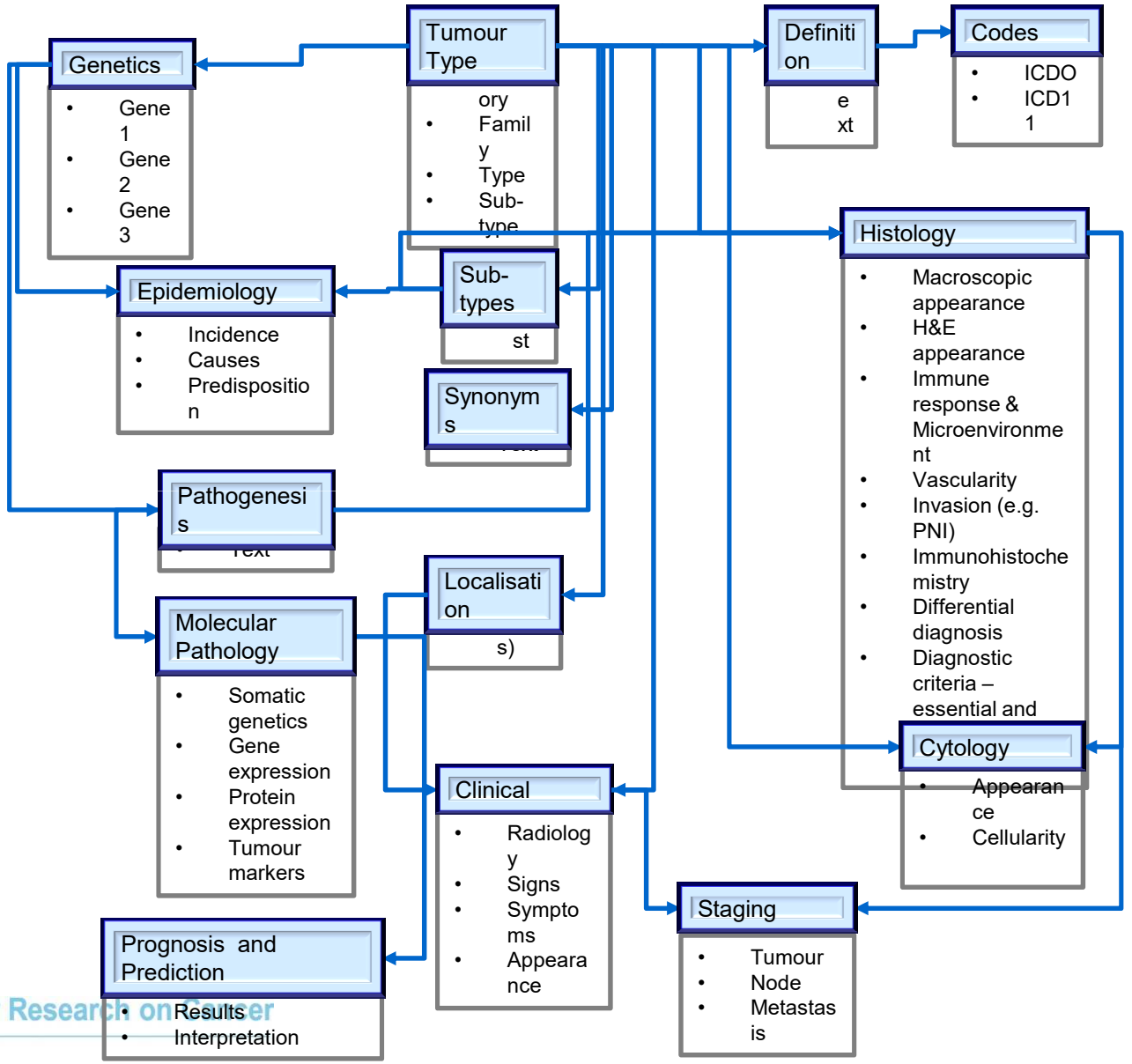
- *Site*, e.g. Stomach
- *Category*, e.g. Epithelial neoplasms
- *Family* (Class), e.g. Adenomas and other premalignant neoplastic lesions
- *Type*, e.g. Adenoma
- *Variant* (Sub-Type), e.g. Pyloric-gland type

Stage and Grade are dealt with separately….

# *The multi-dimensional nature of cancer classification*



# *The multi-dimensional nature of cancer classification*



# *WHO BB Layout (5th Series) DRAFT*

- Definition
- ICD-O and ICD11 Codes
- Related Terminology (Synonyms)
- Subtypes
- Localization
- Clinical features and Radiology
- Epidemiology
- Etiology
  - Causes
  - Predisposing factors (Genetics)
- Pathogenesis
- Macroscopic appearance
- Histopathology
  - H&E appearance
  - Immune response & Microenvironment
  - Vascularity
  - Invasion (e.g. PNI)
  - Immunohistochemistry
  - Differential diagnosis
- Cytology
- Molecular pathology
  - Somatic genetics
  - Gene expression
  - Protein expression
  - Tumour markers
- Diagnostic criteria – essential and desirable
- Staging (UICC TNM)
- Prognosis and Prediction
  - Prognostic factors
  - Predictive biomarkers
- Links to other resources
  - ICCR reporting guidance
  - TNM (UICC)

# *The 5th Series WHO Classification of Tumours*

- Digestive System Tumours
- Breast Tumours
- Soft Tissue and Bone Tumours
- Female Genital Tumours
- Thoracic Tumours
- Urinary and Male Genital Tumours
- Central Nervous System Tumours
- Head and Neck Tumours
- Endocrine Tumours
- Haematolymphoid Tumours
- Skin and Adnexa Tumours
- Eye and Orbit Tumours
- Paediatric Tumours
- Neuroendocrine Tumours
- Hereditary Tumours

http://whobluebooks.iarc.fr

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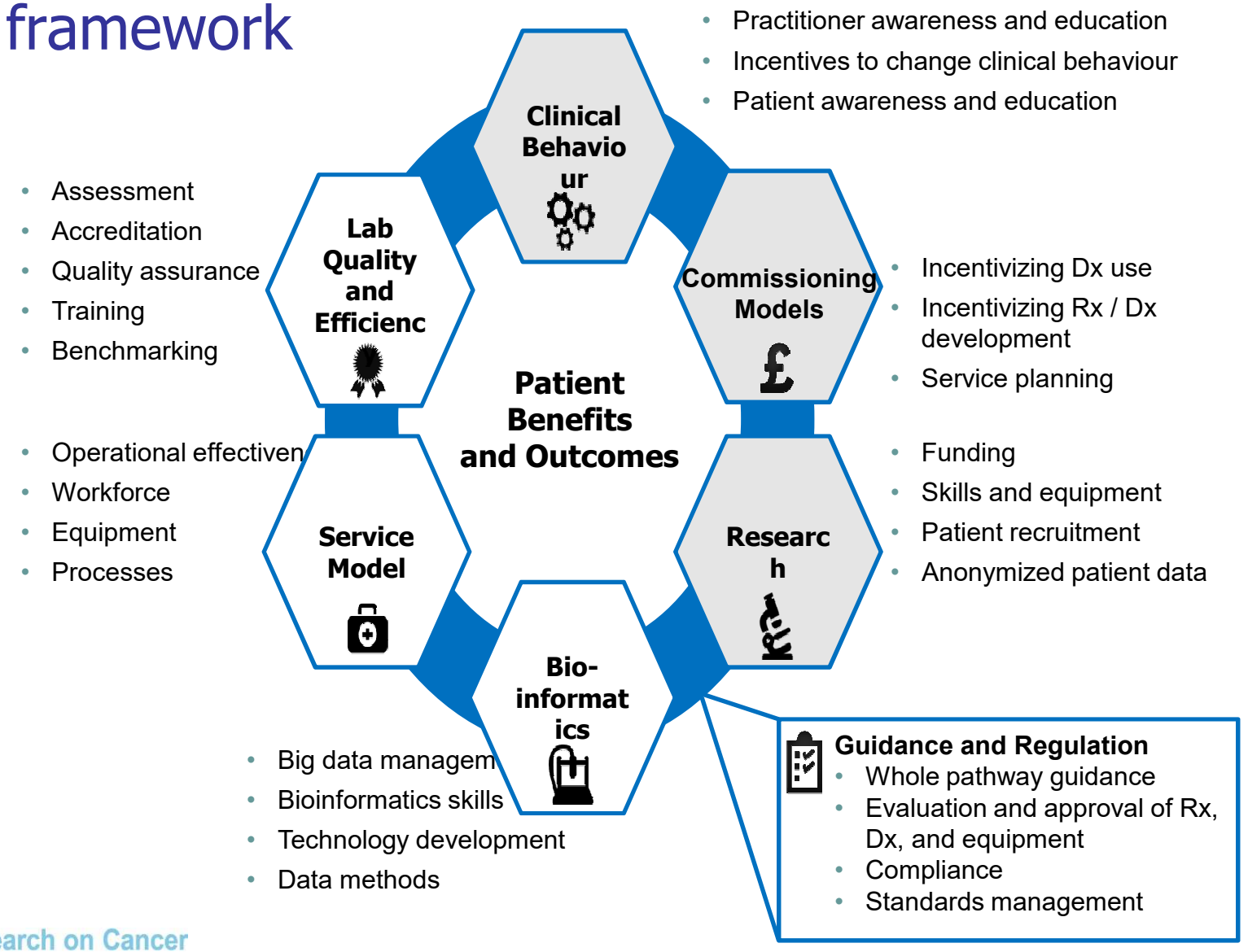
# *Pathology is changing…*

- Cancer classification has previously been based on consensus histopathological opinion with limited molecular input.
- Pathology is undergoing a rapid transformation due to the introduction of new technologies to practice.
- The understanding of cancer at a molecular level is now at a point where it needs to be integrated into its diagnosis.
- Digital pathology and image analysis are now also producing new insights, and providing quantitative justification of many existing diagnostic criteria, while challenging others.
- There is an urgent need to integrate these facets of diagnosis into cancer classification internationally.

# Stratified medicine ecosystem framework

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World Health Organization

JCP Online First, published on July 10, 2014 as 10.1136/jclinpath-2014-202404

Best practice

OPEN ACCESS

# Guidance for laboratories performing molecular pathology for cancer patients

Ian A Cree,[1,2] Zandra Deans,[3] Marjolijn J L Ligtenberg,[4] Nicola Normanno,[5] Anders Edsjö,[6] Etienne Rouleau,[7] Francesc Solé,[8] Erik Thunnissen,[9] Wim Timens,[10] Ed Schuuring,[10] Elisabeth Dequeker,[11] Samuel Murray,[12] Manfred Dietel,[13] Patricia Groenen,[4] J Han Van Krieken,[4] for the European Society of Pathology Task Force on Quality Assurance in Molecular Pathology and the Royal College of Pathologists

## *Key guidance for molecular pathology*

- Covers every stage of the process
- Produced by EQA providers
- Endorsed by the RCPath and ESP

## *Key guidance for clinical use of NGS in cancer*

- Covers every stage of the process
- Produced by EQA providers (IQN Path)
- Endorsed by the RCPath and ESP

Virchows Arch
DOI 10.1007/s00428-016-2025-7

REVIEW AND PERSPECTIVES

# Integration of next-generation sequencing in clinical diagnostic molecular pathology laboratories for analysis of solid tumours; an expert opinion on behalf of IQN Path ASBL

Zandra C Deans[1] · Jose Luis Costa[2] · Ian Cree[3] · Els Dequeker[4] · Anders Edsjö[5] · Shirley Henderson[6] · Michael Hummel[7] · Marjolijn JL Ligtenberg[8] · Marco Loddo[9] · Jose Carlos Machado[2] · Antonio Marchetti[10] · Katherine Marquis[9] · Joanne Mason[6] · Nicola Normanno[11] · Etienne Rouleau[12] · Ed Schuuring[13] · Keeda-Marie Snelson[9] · Erik Thunnissen[14] · Bastiaan Tops[8] · Gareth Williams[9] · Han van Krieken[8] · Jacqueline A Hall[15,16] · On behalf of IQN Path ASBL

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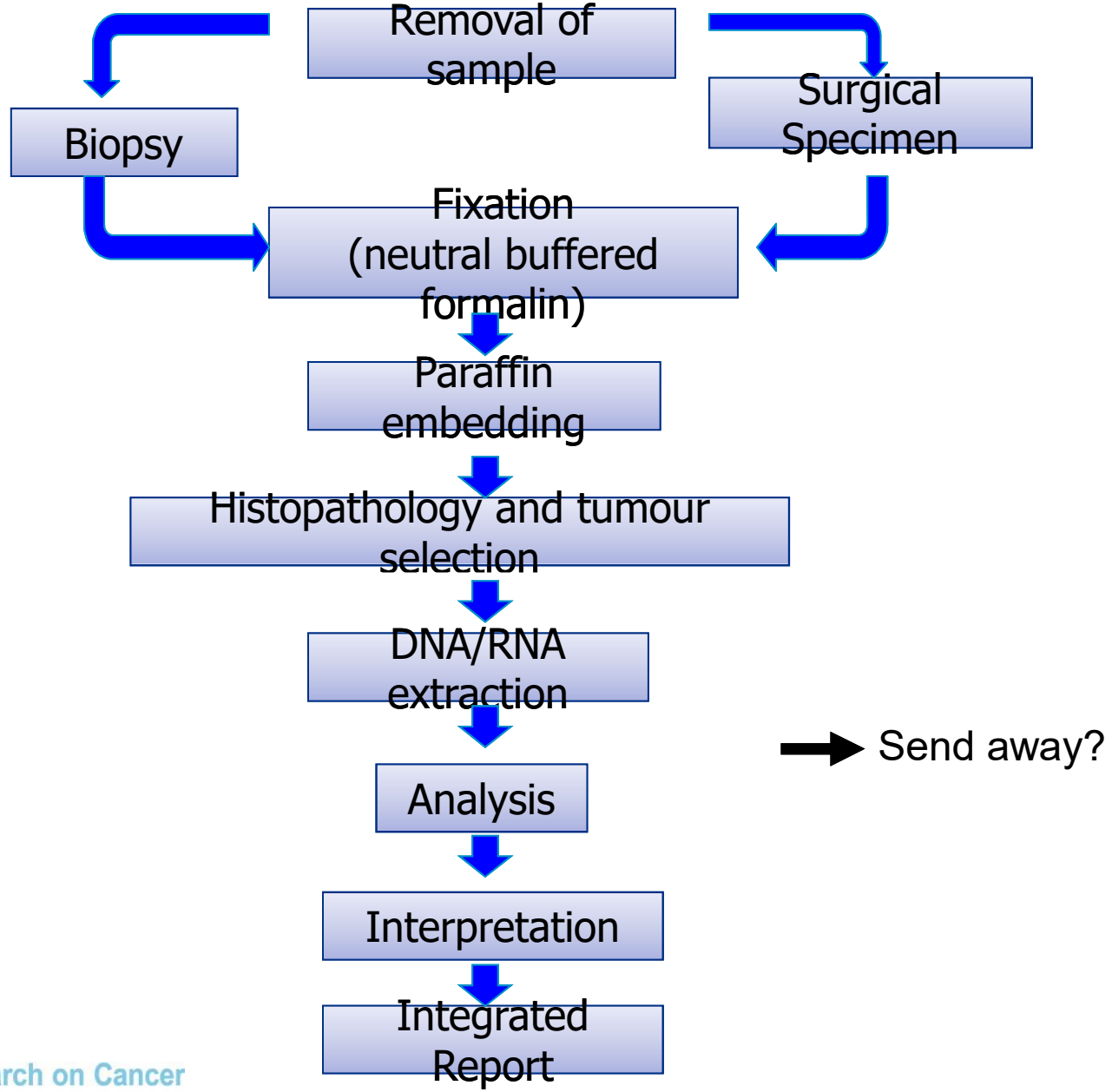

Removal of sample
Biopsy
Surgical Specimen
Fixation (neutral buffered formalin)
Paraffin embedding
Histopathology and tumour selection
DNA/RNA extraction
Analysis
Interpretation
Integrated Report
Send away?

# *The problem….*

- Tissue Safe

## *Sample receipt and handling*

- Dedicated SOPs required
- Defined processing – changes can affect DNA and RNA recovery
- The use of fixed tissue that has been previously frozen is not advised, and decalcification is likely to reduce DNA and particularly RNA recovery
- Close collaboration between clinical teams and histopathology essential
- Contamination during cutting – issues exist: mitigate
- Microdissection or punches, not laser capture routinely
- Estimate percentage neoplastic cells present

## *DNA and RNA extraction*

- Methods described – no preference for manual or automated systems
- Storage of DNA and RNA should be controlled carefully. Accession logs or bar-coded vials can be used to prevent sample mis-identification.
- Temperature logs should be maintained - good practice:
  - Store extracted DNA and RNA samples, clearly labelled, at -20 C or -80 C respectively.
  - Store PCR products in a separate freezer at -20 C or -80 C
  - Store sequencing libraries at -20 C or -80 C

***Choice of analytical method***

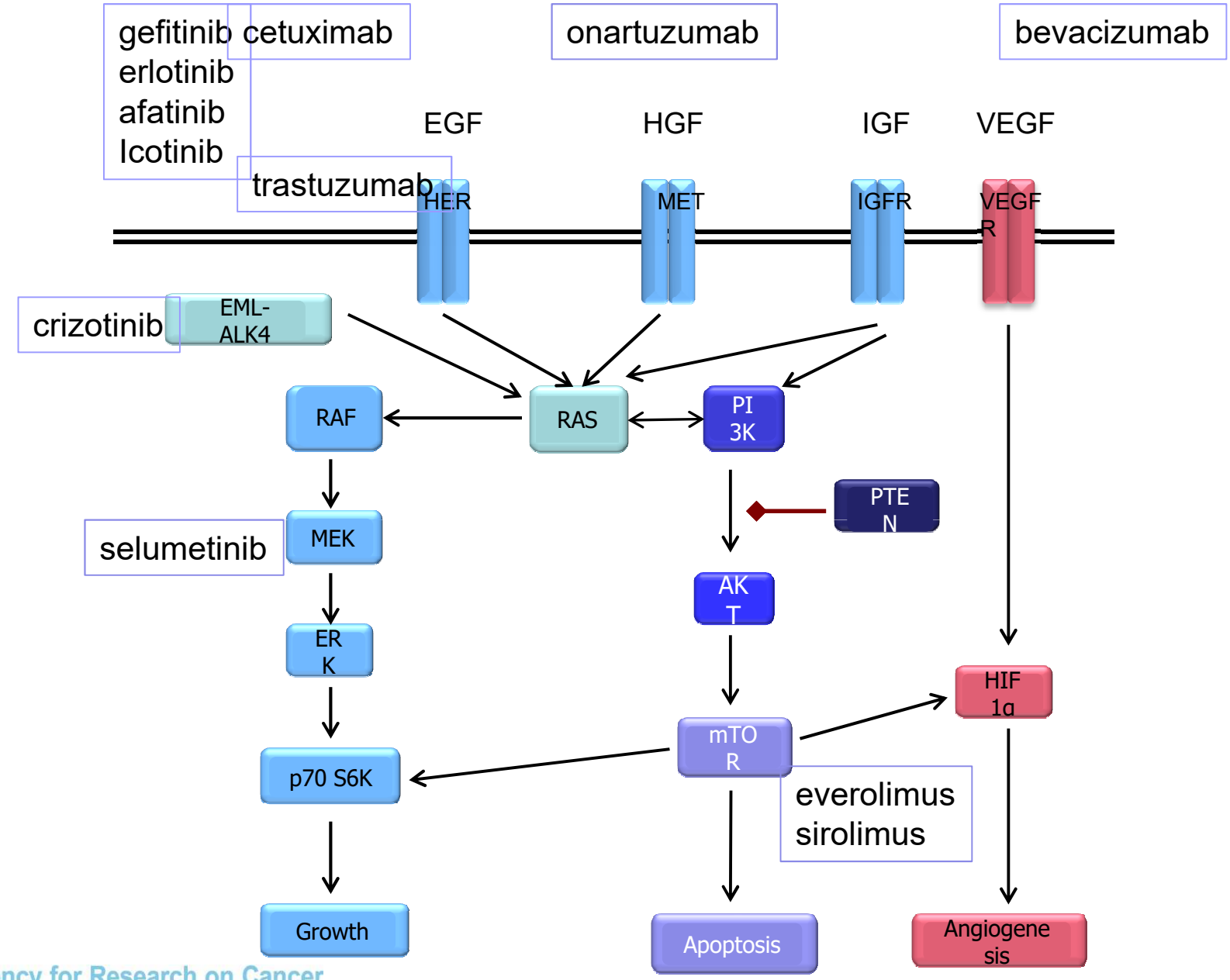
- Testing requirements are defined by clinical need
- Panel testing is now recommended for some cancers
- Servicing and training costs a consideration

***Performance of molecular tests***

- Monitor the performance against confidence intervals
- Use externally sourced controls where possible for in house tests
- Turnaround time v. timeliness
- Continuity planning

# *Mutation testing by Polymerase Chain Reaction (PCR)*

- Defined mutations in hotspots
- High sensitivity – use <150 bp primers for FFPE
- Not comprehensive
- Simple set up and performan
- Rapid – results in hours, not
- Inexpensive
- Can be automated and multi


gefitinib
erlotinib
afatinib
Icotinib
cetuximab
trastuzumab
onartuzumab
bevacizumab
EGF
HGF
IGF
VEGF
HER
MET
IGFR
VEGF R
crizotinib
EML-ALK4
RAF
RAS
PI 3K
PTE N
selumetinib
MEK
AK T
ER K
HIF 1α
mTO R
p70 S6K
everolimus
sirolimus
Growth
Apoptosis
Angiogene sis

Pathogenesis 3 (2016) 1–8

Contents lists available at ScienceDirect

Pathogenesis

journal homepage: http://www.pathogenesisjournal.com/

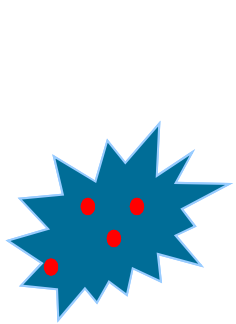
Original Article

# Development and validation of a TaqMan Array for cancer mutation analysis

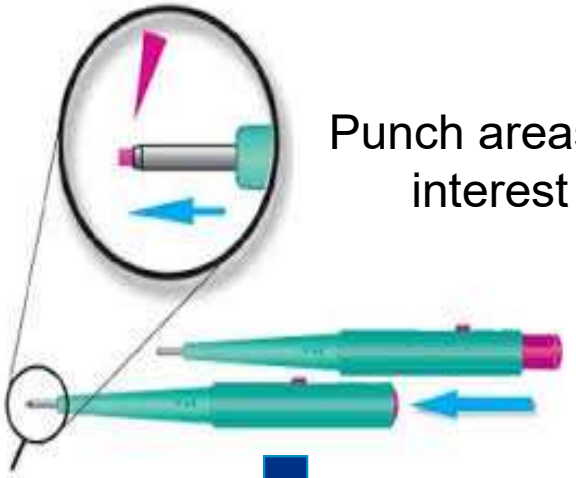
Hugh Kikuchi [a,b], Anne Reiman [a,b], Jenifer Nyoni [a], Katherine Lloyd [c], Richard Savage [d], Tina Wotherspoon [a], Lisa Berry [a], David Snead [a,b], Ian A. Cree [a,e,f,*]

[a] Department of Pathology – Coventry and Warwickshire Pathology Services (CWPS), University Hospitals Coventry and Warwickshire, Coventry CV2 2DX, UK
[b] Warwick Medical School, University of Warwick, University Hospitals Coventry and Warwickshire, Coventry CV2 2DX, UK
[c] MOAC DTC, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK
[d] Systems Biology Centre, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK
[e] Institute of Ophthalmology, University College London, Bath Street, London EC1V 9EL, UK
[f] Centre for Technology Enabled Health Research (CTEHR), Faculty of Health & Life Sciences, Coventry University, Coventry CV1 5FB, UK

# *Taqman array PCR panel testing: Ras – EGFR – BRAF (REB) arrav*



Mark tumour



Punch areas of interest

Extract DNA

Taqman Array

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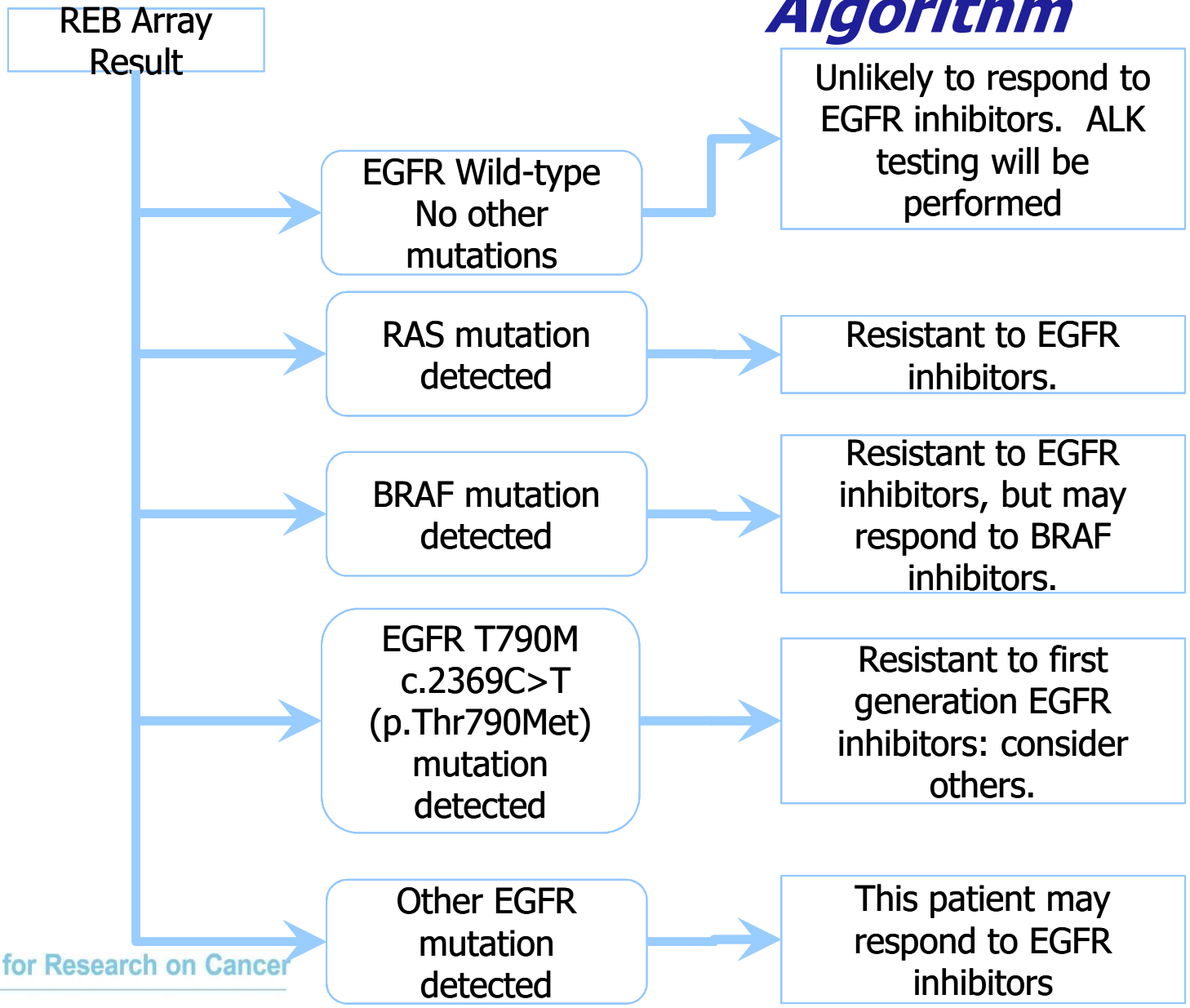
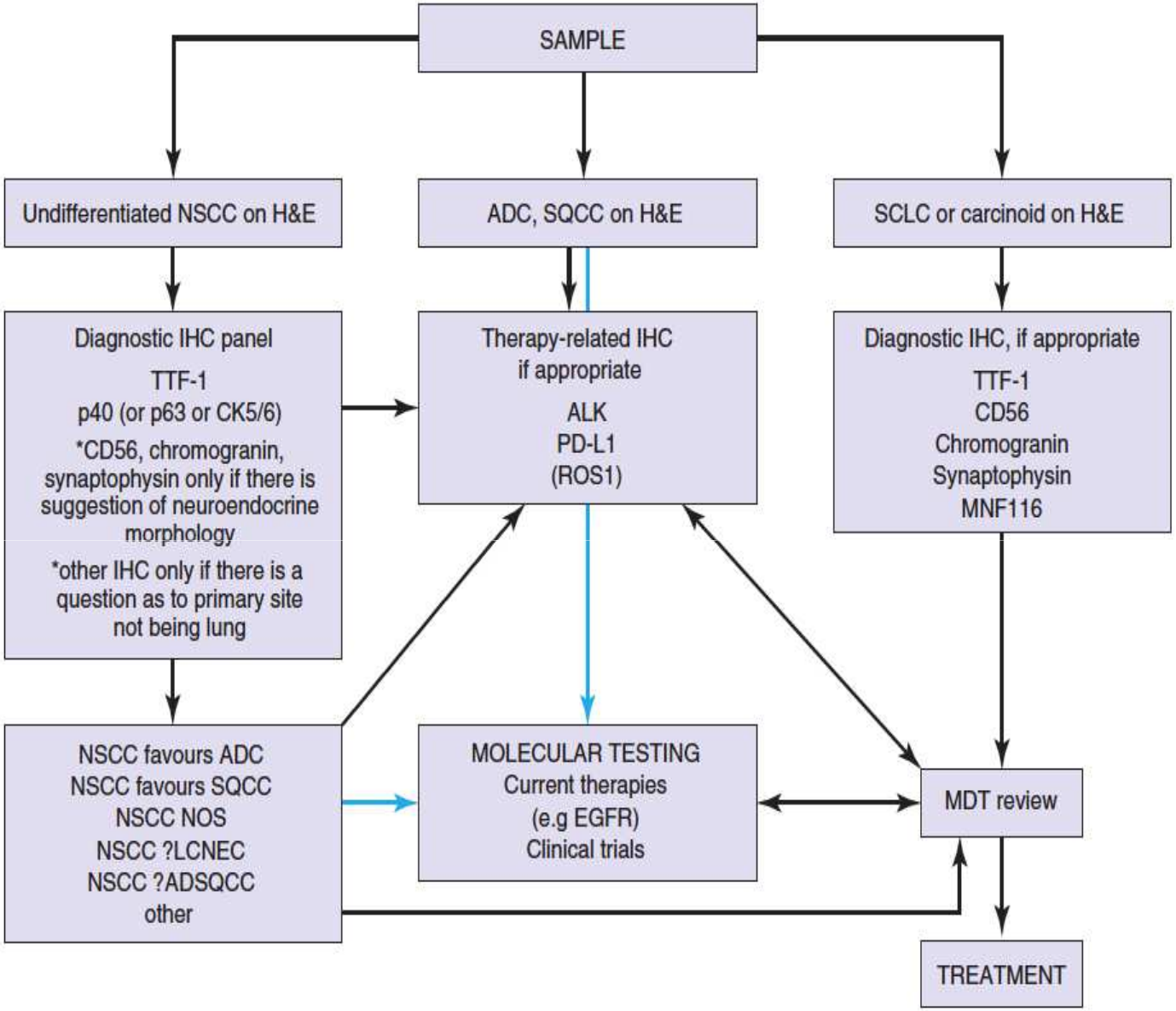
World Health Organization

Kikuchi H, et al. Development and validation of a TaqMan Array for cancer mutation analysis. Pathogenesis. 2016 (3): 1–8.

# NSLSC Algorithm



# NSCLC algorithm






Cree IA, et al. PD-L1 testing for lung cancer in the UK: recognizing the challenges for implementation. Histopathology. 2016; 69(2): 177-86

# IonTorrent next-generation

# Ion Chef™ System
## *Workflow Automation for Sequencing*

- Simple to use
  - Automated template prep AND chip loading – *library in, loaded chips out*
  - Simple reagent and consumables loading– *minutes of hands-on time*
  - Minimizes potential sources of user error and sequencing variability
- High throughput
  - Processes 2 chips and multiple barcoded samples within hours
- Flexible
  - Supports all Ion™ systems, chips, read lengths, and

# Melanoma panel

| Gene | Reference | Codons included | Number of Mutations |
|---|---|---|---|
| BRAF | NM_004333.4 | 466, 469, 583, 584, 586, 592, 594, 595, 597, 600, 601, 605, 614, 618 | 29 |
| GNAQ | NM_002072.3 | 209, 359 | 6 |
| GNA11 | NM_002067.2 | 183, 209, 223 | 3 |
| NRAS | NM_002524.4 | 12, 13, 18, 50, 59, 61, 68 | 29 |
| c-KIT | NM_000222.2 | 553, 557, 559, 560, 566, 569, 576, 642, 655, 816, 820, 822, 823, 829, 853 | 18 |
| KRAS | NM_033360 | 12, 61 | 5 |
| MEK1/MAP2K1 | NM_002755.2 | 111, 124, 203, 264 | 5 |

Reiman A, Kikuchi H, Scocchia D, Smith P, Tsang YW, Snead D, Cree IA. Validation of an NGS mutation detection panel for melanoma. BMC Cancer. 2017 Feb 22;17(1):150.

# *Accreditation and Quality assurance – Internal and External*

- All laboratories providing molecular pathology services should have laboratory **accreditation** according to ISO 15189 or equivalent
- All laboratories performing molecular tests for cancer patients should be part of an external quality assessment (**EQA**) scheme
- Internal quality assessment (IQA) - the use of **control** materials within each run is recommended
- **Monitor** to assure end to end performance
- Implement changes when **errors** occur and pre-empt by looking at others' mistakes!

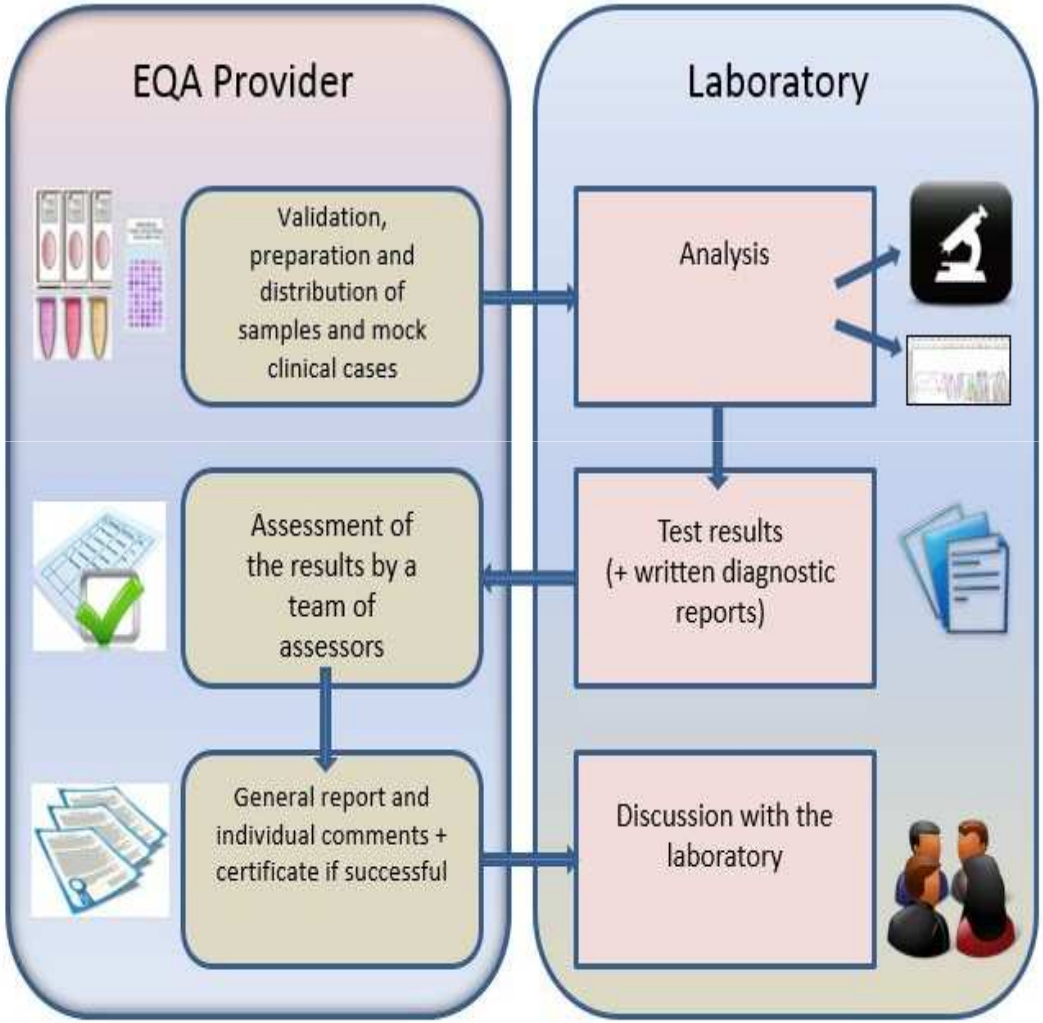
# How does EQA help with testing quality?

IQNPath
International Quality Network for Pathology

**External Quality Assessment**

(WHO definition)

A system for objectively checking the laboratory's performance using an external agency or facility




Picture by Veronique Tack, ESP EQA

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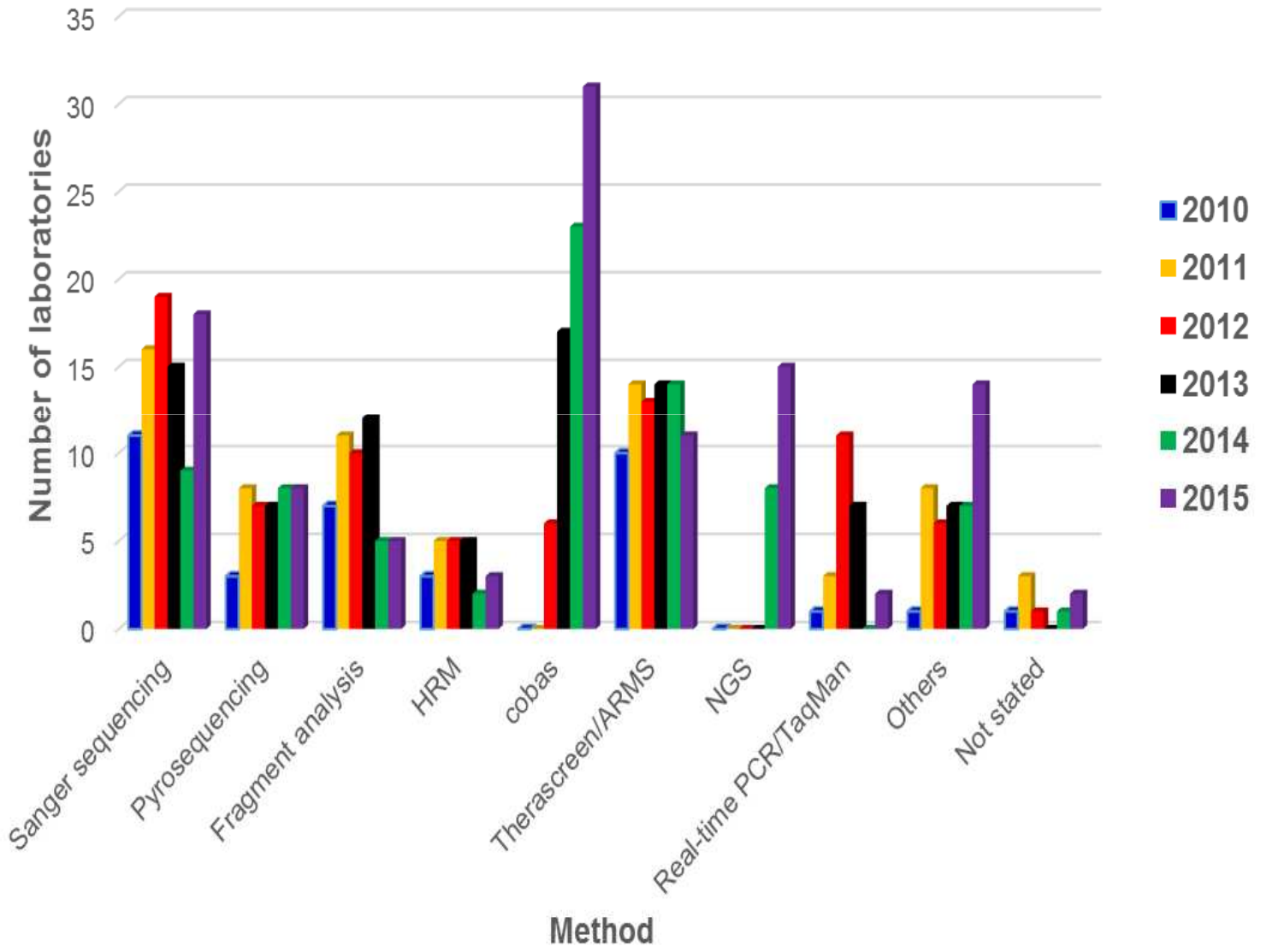
# Molecular analysis of lung cancer EQA

UK NEQAS
Molecular Genetics

***EGFR* testing methodologies**

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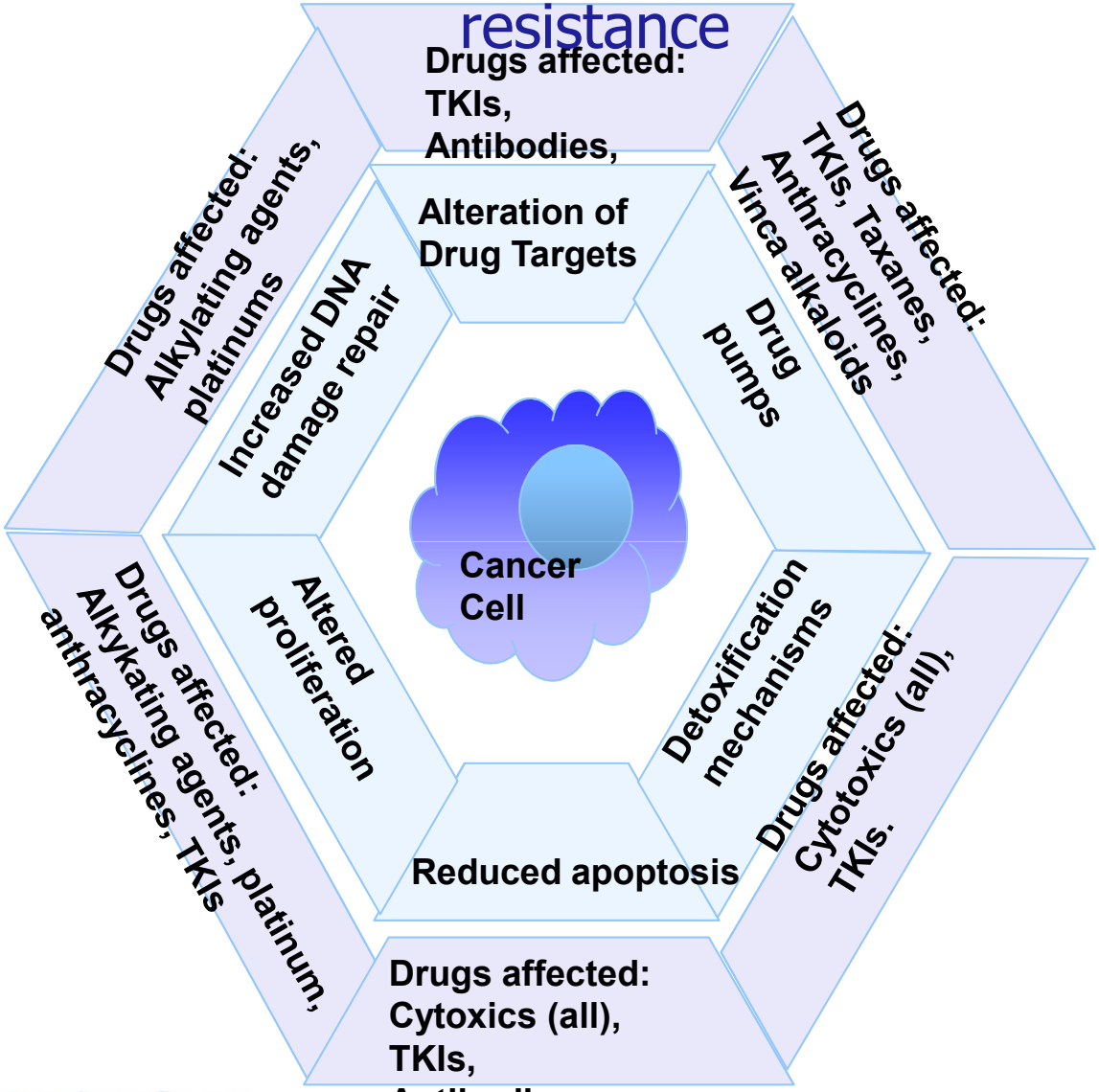
# Molecular chess: Hallmarks of anti-cancer drug resistance

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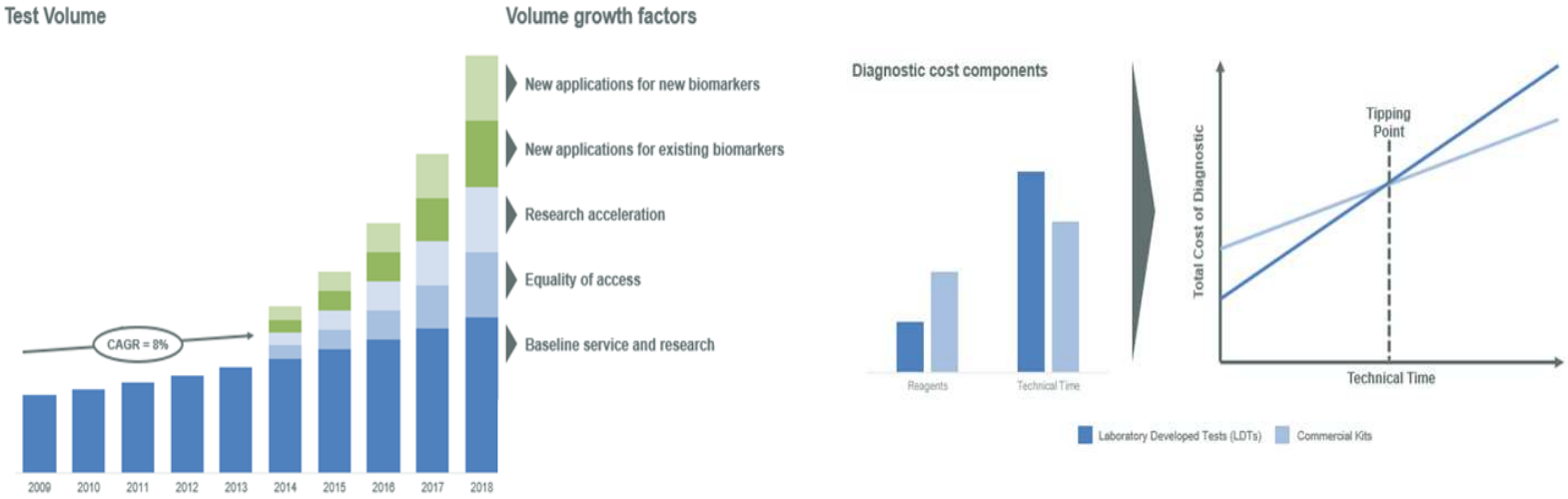
World Health Organization

Cree IA, Charlton P. Molecular chess? Hallmarks of anti-cancer drug resistance. BMC Cancer. 2017; 17: 10

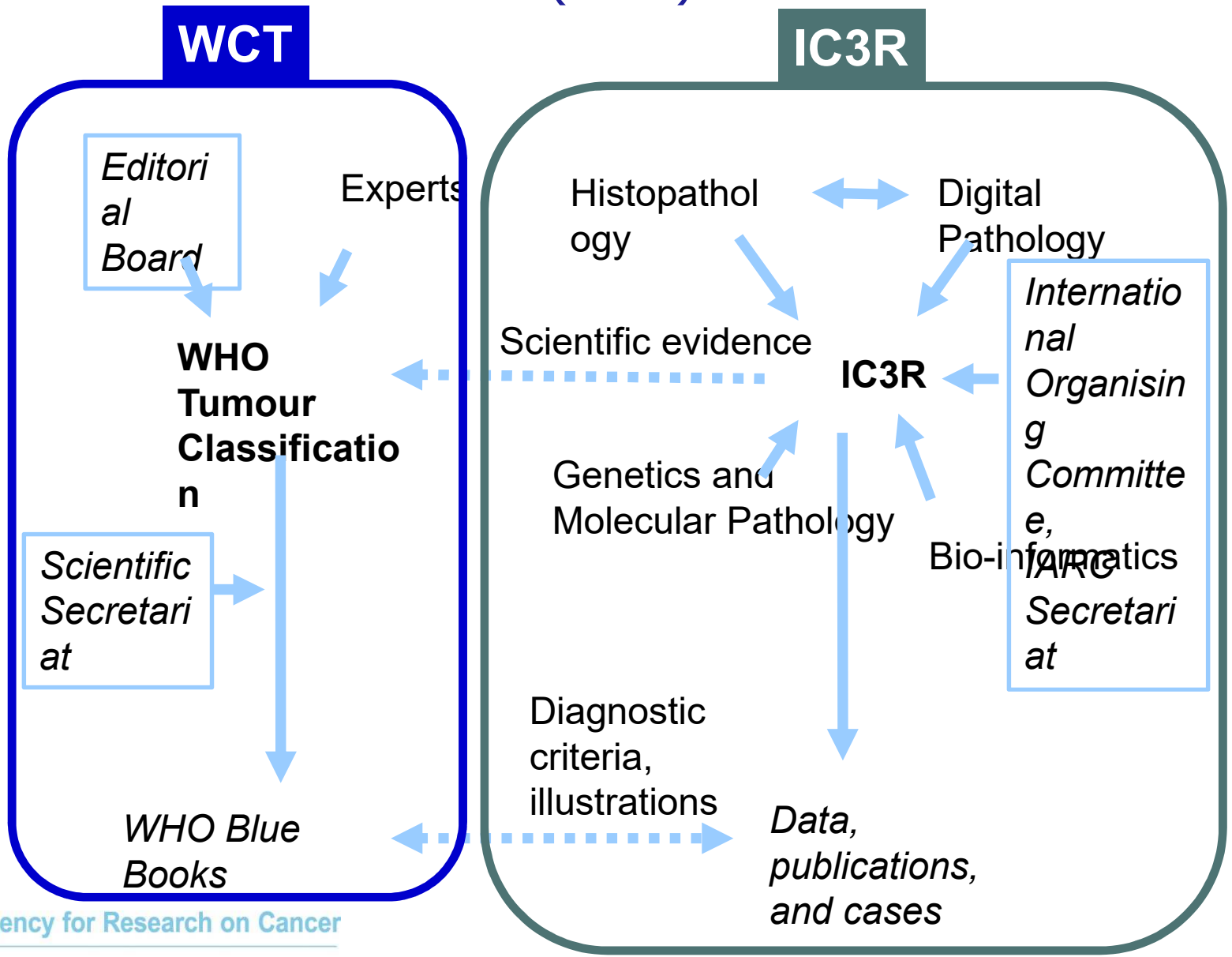
# *CMD ImPACT business planning tool*

The tool is a simple Microsoft Excel programme that allows the costs and income to be modelled against a wide range of funding or demand scenarios, from current requirements to expansion of existing molecular diagnostic services.

***www.ropathi.org/cmd-impact***



# Collaboration for Cancer Classification and Research (IC3R)

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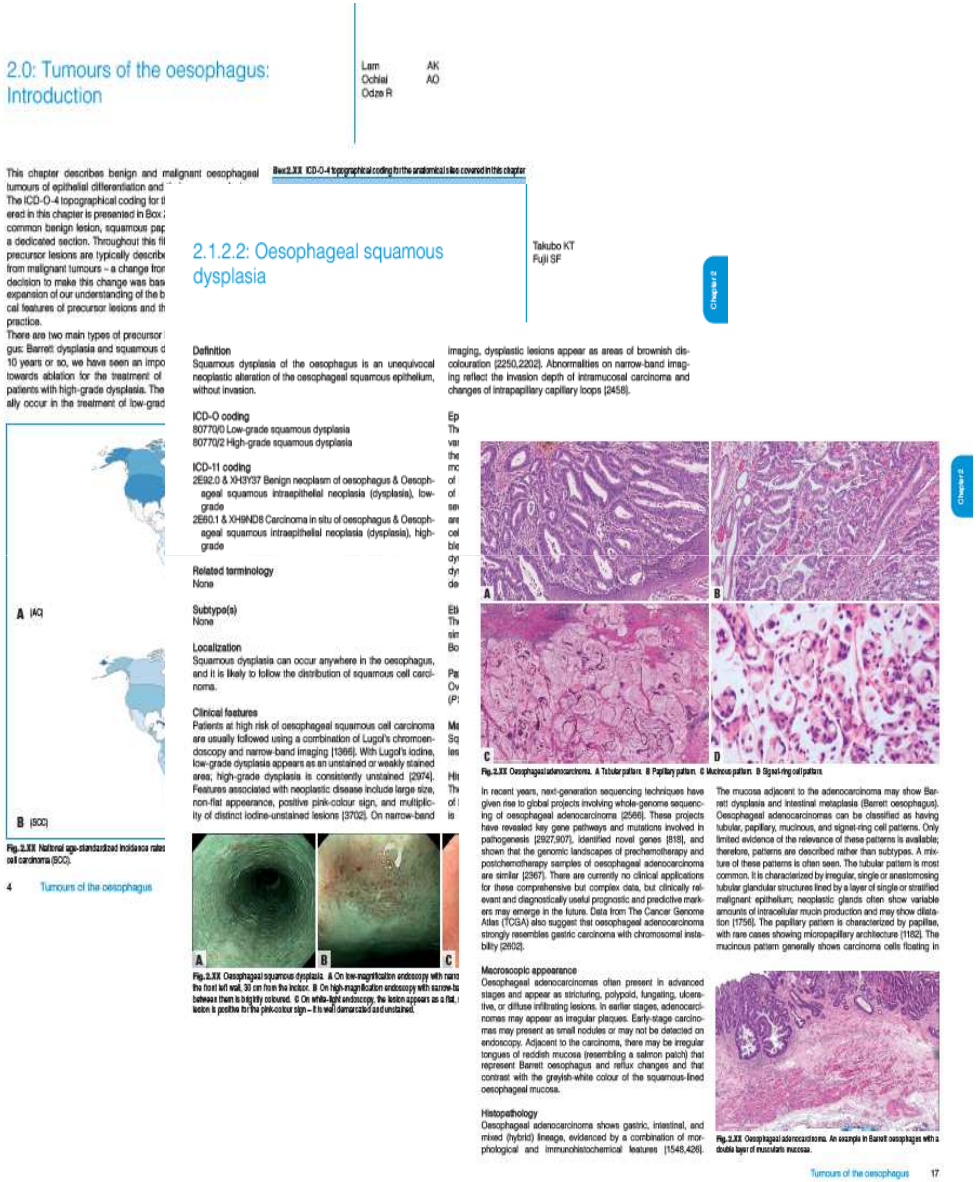
# Proofs



2.0: Tumours of the oesophagus: Introduction

Lam AK
Ochiai AO
Odze R

This chapter describes benign and malignant oesophageal tumours of epithelial differentiation and ... The ICD-O-4 topographical coding for t... ered in this chapter is presented in Box ... common benign lesion, squamous pap... a dedicated section. Throughout this fi... precursor lesions are typically describ... from malignant tumours – a change fro... decision to make this change was bas... expansion of our understanding of the b... cal features of precursor lesions and th... practice.
There are two main types of precursor ... gus: Barrett dysplasia and squamous d... 10 years or so, we have seen an impo... towards ablation for the treatment of ... patients with high-grade dysplasia. The... ally occur in the treatment of low-grad...

Box 2.XX ICD-O-4 topographical coding for the anatomical sites covered in this chapter

2.1.2.2: Oesophageal squamous dysplasia

Takubo KT
Fujii SF

Chapter 2

**Definition**
Squamous dysplasia of the oesophagus is an unequivocal neoplastic alteration of the oesophageal squamous epithelium, without invasion.

**ICD-O coding**
80770/0 Low-grade squamous dysplasia
80770/2 High-grade squamous dysplasia

**ICD-11 coding**
2E92.0 & XH3Y37 Benign neoplasm of oesophagus & Oesophageal squamous intraepithelial neoplasia (dysplasia), low-grade
2E60.1 & XH9ND8 Carcinoma in situ of oesophagus & Oesophageal squamous intraepithelial neoplasia (dysplasia), high-grade

**Related terminology**
None

**Subtype(s)**
None

**Localization**
Squamous dysplasia can occur anywhere in the oesophagus, and it is likely to follow the distribution of squamous cell carcinoma.

**Clinical features**
Patients at high risk of oesophageal squamous cell carcinoma are usually followed using a combination of Lugol's chromoendoscopy and narrow-band imaging [1366]. With Lugol's iodine, low-grade dysplasia appears as an unstained or weakly stained area; high-grade dysplasia is consistently unstained [2974]. Features associated with neoplastic disease include large size, non-flat appearance, positive pink-colour sign, and multiplicity of distinct iodine-unstained lesions [3702]. On narrow-band imaging, dysplastic lesions appear as areas of brownish discolouration [2250,2202]. Abnormalities on narrow-band imaging reflect the invasion depth of intramucosal carcinoma and changes of intrapapillary capillary loops [2458].

A (AC)
B (SCC)

Fig. 2.XX National age-standardized incidence rates ... cell carcinoma (SCC).

4 Tumours of the oesophagus

Fig. 2.XX Oesophageal squamous dysplasia. A On low-magnification endoscopy with narro... the front left wall, 30 cm from the incisor. B On high-magnification endoscopy with narrow-ba... between them is brightly coloured. C On white-light endoscopy, the lesion appears as a flat, ... lesion is positive for the pink-colour sign – it is well demarcated and unstained.

Fig. 2.XX Oesophageal adenocarcinoma. A Tubular pattern. B Papillary pattern. C Mucinous pattern. D Signet-ring cell pattern.

In recent years, next-generation sequencing techniques have given rise to global projects involving whole-genome sequencing of oesophageal adenocarcinoma [2566]. These projects have revealed key gene pathways and mutations involved in pathogenesis [2827,907], identified novel genes [818], and shown that the genomic landscapes of prechemotherapy and postchemotherapy samples of oesophageal adenocarcinoma are similar [2367]. There are currently no clinical applications for these comprehensive but complex data, but clinically relevant and diagnostically useful prognostic and predictive markers may emerge in the future. Data from The Cancer Genome Atlas (TCGA) also suggest that oesophageal adenocarcinoma strongly resembles gastric carcinoma with chromosomal instability [2802].

**Macroscopic appearance**
Oesophageal adenocarcinomas often present in advanced stages and appear as stricturing, polypoid, fungating, ulcerative, or diffuse infiltrating lesions. In earlier stages, adenocarcinomas may appear as irregular plaques. Early-stage carcinomas may present as small nodules or may not be detected on endoscopy. Adjacent to the carcinoma, there may be irregular tongues of reddish mucosa (resembling a salmon patch) that represent Barrett oesophagus and reflux changes and that contrast with the greyish-white colour of the squamous-lined oesophageal mucosa.

**Histopathology**
Oesophageal adenocarcinoma shows gastric, intestinal, and mixed (hybrid) lineage, evidenced by a combination of morphological and immunohistochemical features [1548,426].

The mucosa adjacent to the adenocarcinoma may show Barrett dysplasia and intestinal metaplasia (Barrett oesophagus). Oesophageal adenocarcinomas can be classified as having tubular, papillary, mucinous, and signet-ring cell patterns. Only limited evidence of the relevance of these patterns is available; therefore, patterns are described rather than subtypes. A mixture of these patterns is often seen. The tubular pattern is most common. It is characterized by irregular, single or anastomosing tubular glandular structures lined by a layer of single or stratified malignant epithelium; neoplastic glands often show variable amounts of intracellular mucin production and may show dilatation [1756]. The papillary pattern is characterized by papillae, with rare cases showing micropapillary architecture [1182]. The mucinous pattern generally shows carcinoma cells floating in

Fig. 2.XX Oesophageal adenocarcinoma. An example in Barrett oesophagus with a double layer of muscularis mucosae.

Tumours of the oesophagus 17

# *Conclusions*

- Change is inevitable, but translation of research findings, and then implementation are difficult.
- In pathology, molecular and digital methods are entering practice.
- This has implications for the way departments are organised, patient pathways, and cost effectiveness.
- Quality control is essential, with clear standard operating procedures.

- The potential gains for patients are considerable!

*Thank you!*