Hospital Convention 2016

Recent Advancement in
Anatomical Pathology

Molecular Pathology Tests for Targeted Cancer Therapy

Wong Wing Sze,
Anatomical Pathology, Queen Mary Hospital

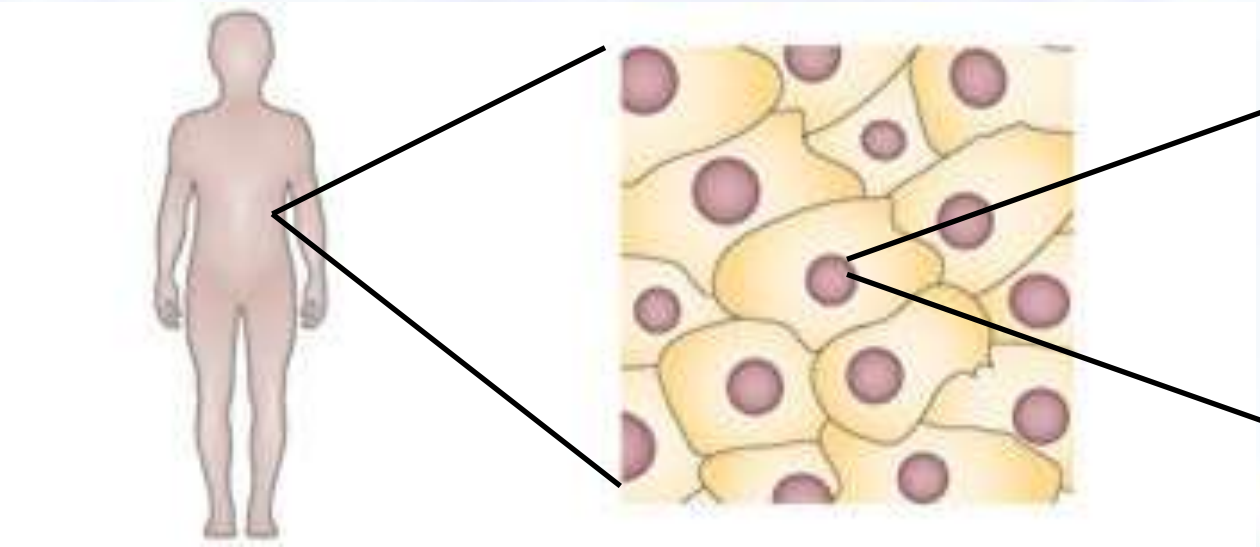
3rd May, 2016

Overview

1980s
Anatomical classification

1990s- 2000s
Histological classification

2010s
Molecular classification



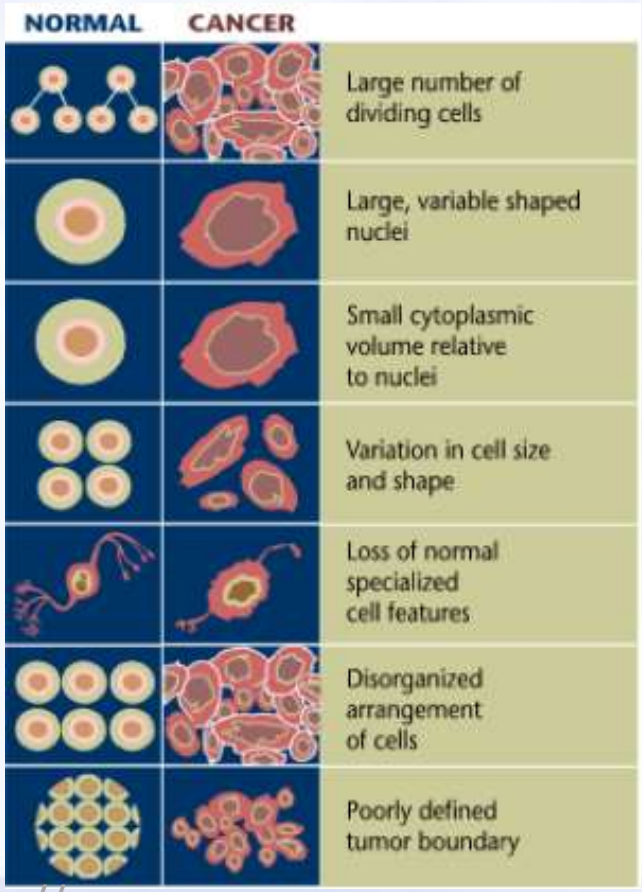
Old model
Origin of tumour dictates therapy

New model
Molecular signature dictates therapy

http://www.nature.com/nrclinonc/journal/v9/n11/full/nrclinonc.2012.160.html

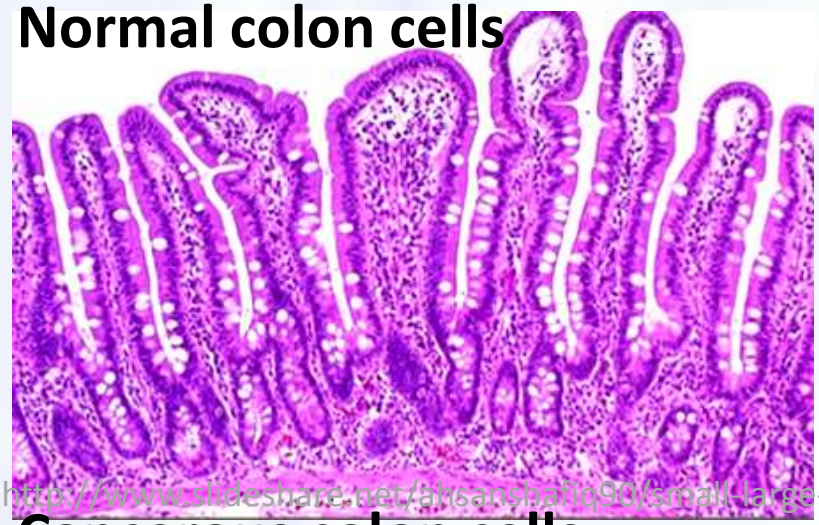
Histological classification

- haematoxylin : nucleus
- Eosin : cytoplasm



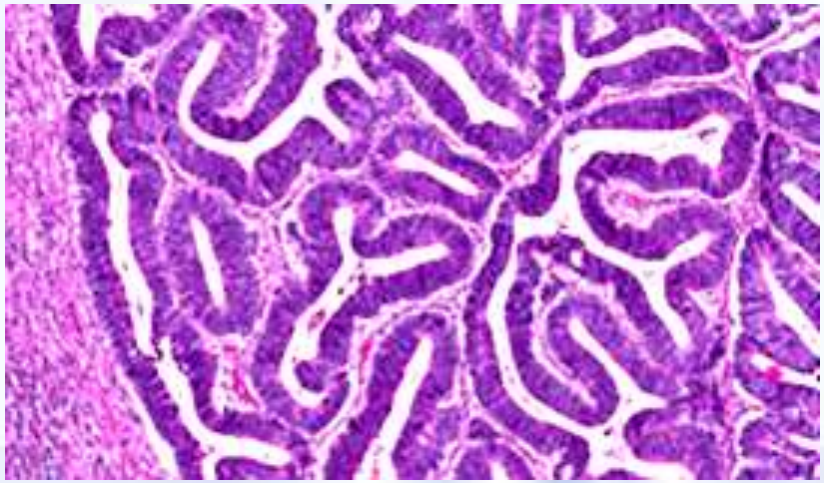
http://lungcancer.about.com/od/Biology-of-Cancer/a/Cancer-Cells-Normal-Cells.htm

Normal colon cells



http://www.slideshare.net/ahsanshafiq90/small-large-gut

Cancerous colon cells

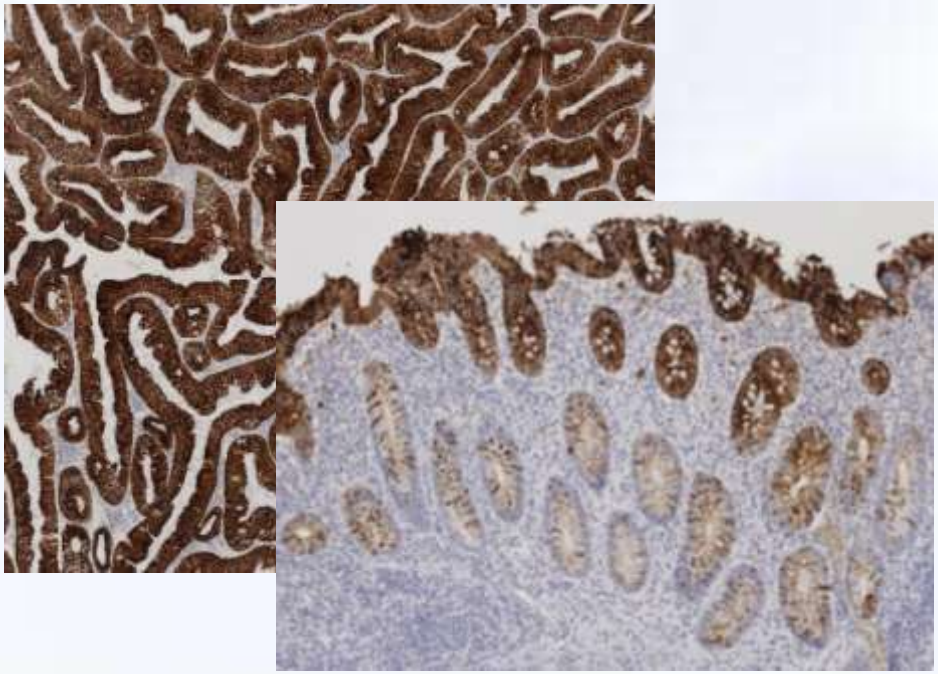


http://atlasgeneticsoncology.org/Tumors/colonID5006.html

Histological classification

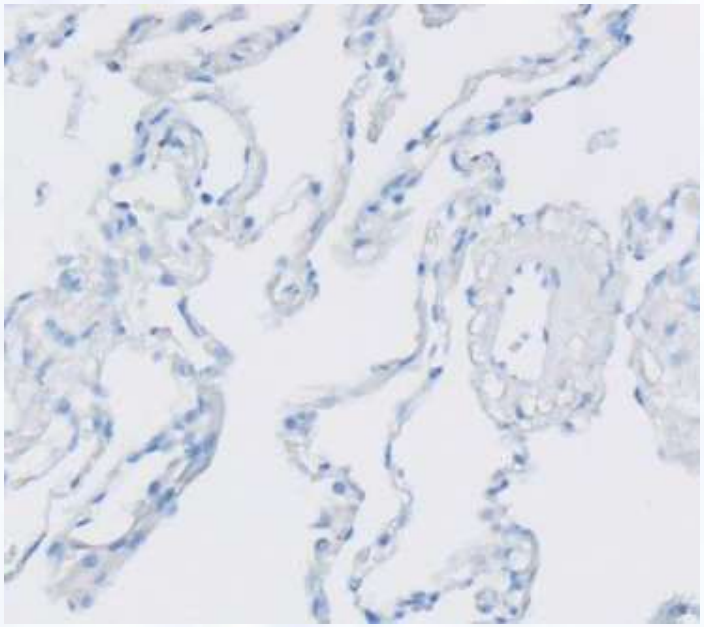
- Immunohistochemisty: specific tissue detection by chemical reaction with specific antibodies
- E.g CK20 for colon

Colon, CK20 positive



http://virtualslides.leica-microsystems.com/dih/webViewer.php?snapshotId=13468515525466

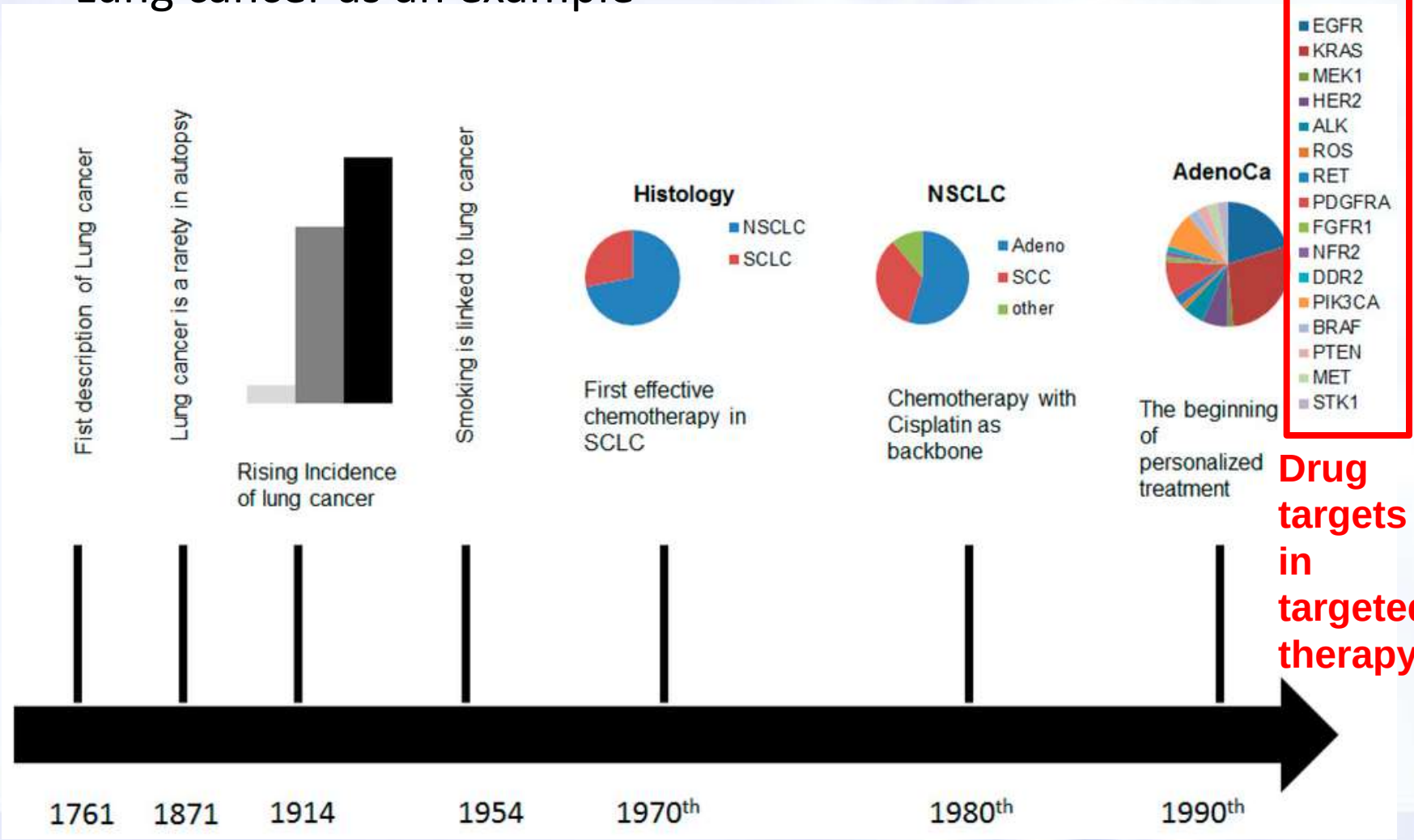
Lung, CK20 negative



http://www.newcomersupply.com/product/uro-2-cocktail-multi-tissue-control-slides

Development of Molecular Pathology

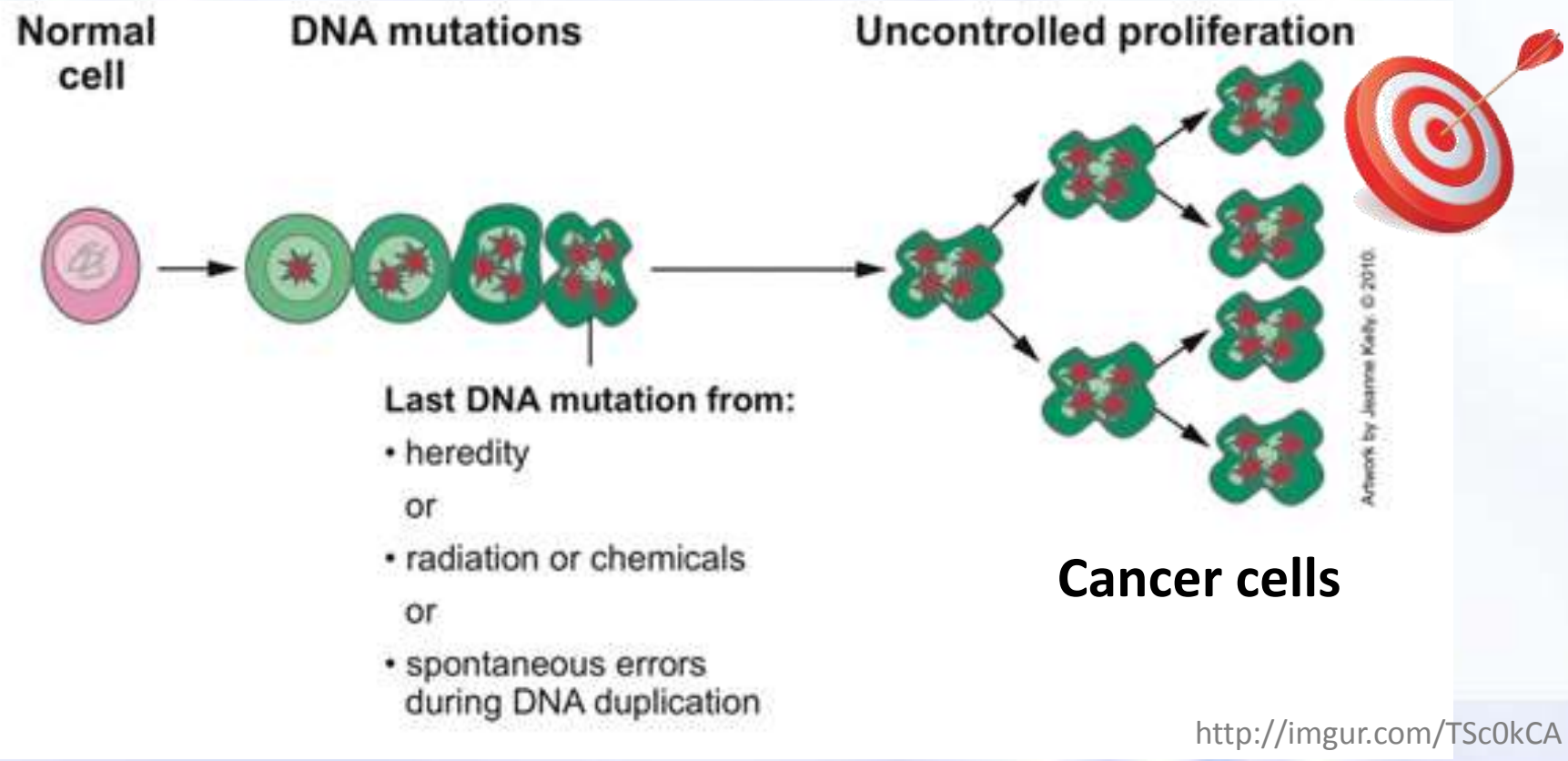
- Lung cancer as an example



Plone T, 2016

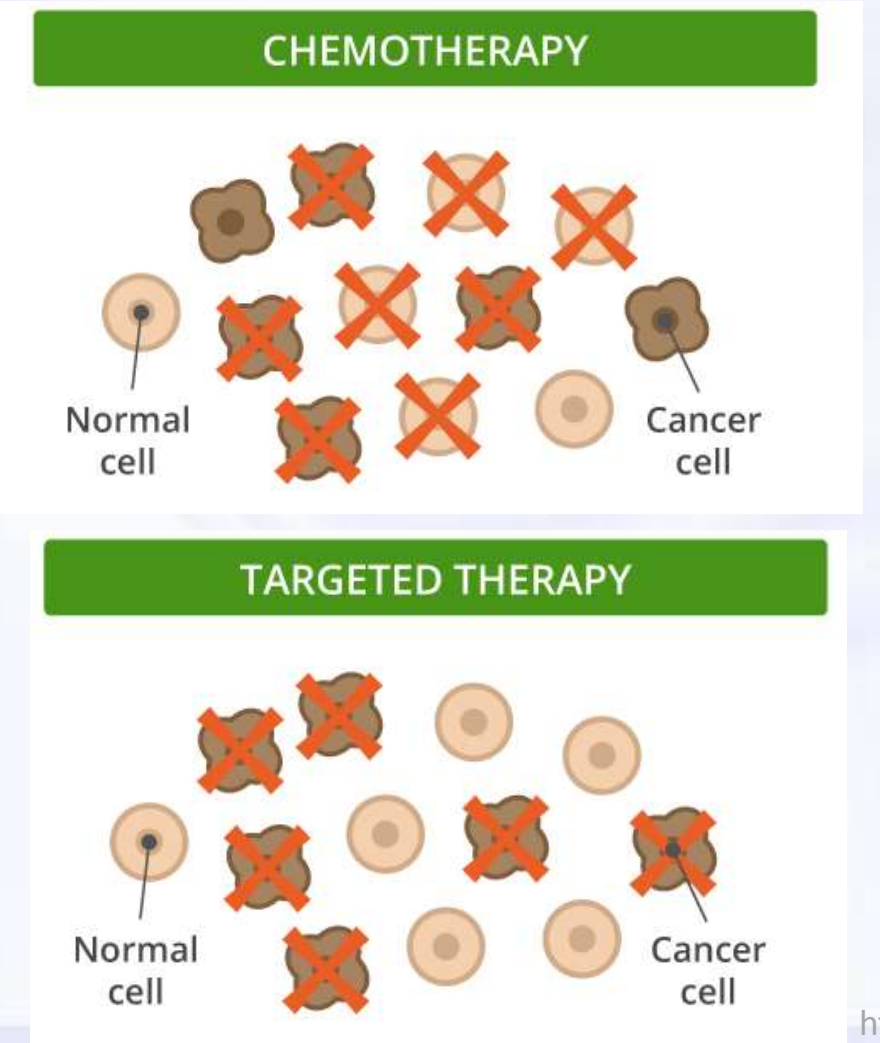
DNA mutations in cancer cells

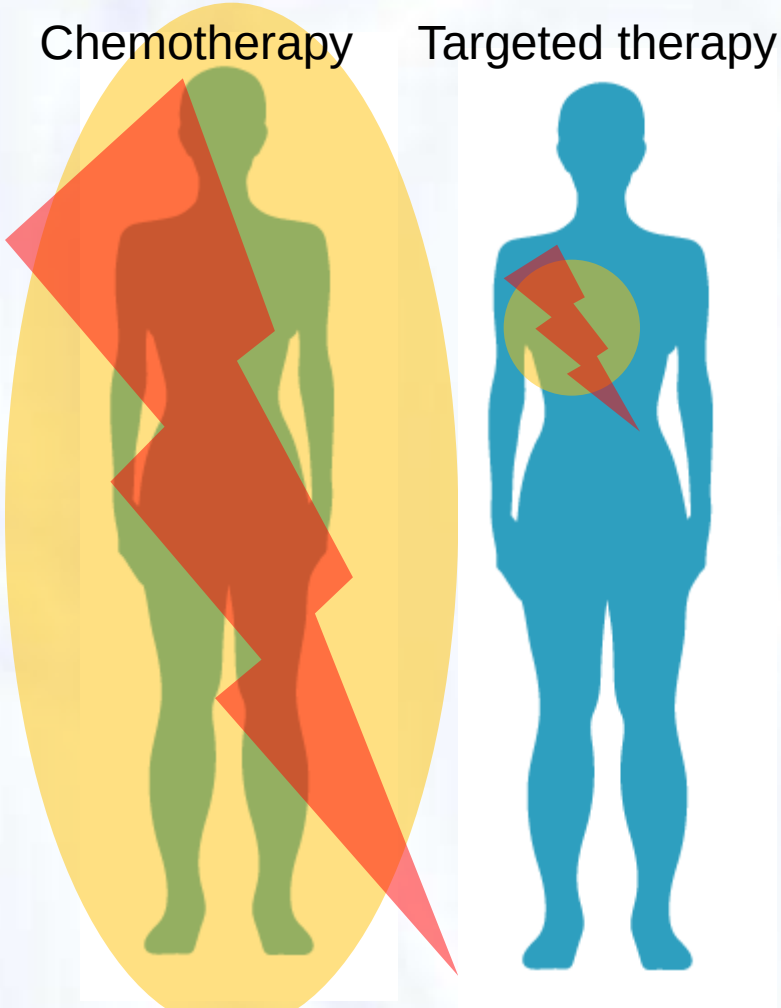
- DNA mutations as cancer markers
- Some of these mutations are the targets explored in targeted therapy development



http://imgur.com/TSc0kCA

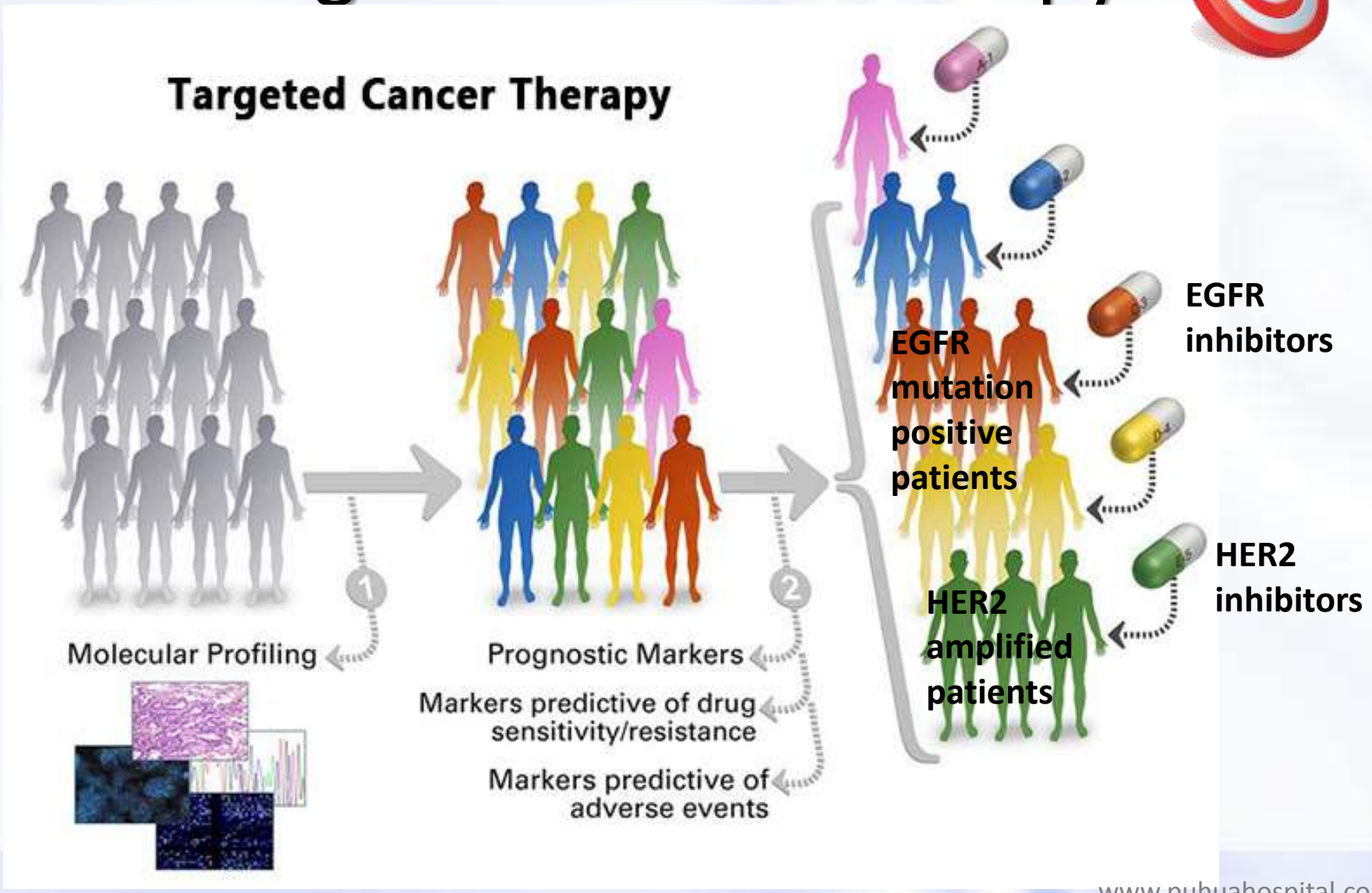
Traditional vs Targeted cancer therapy



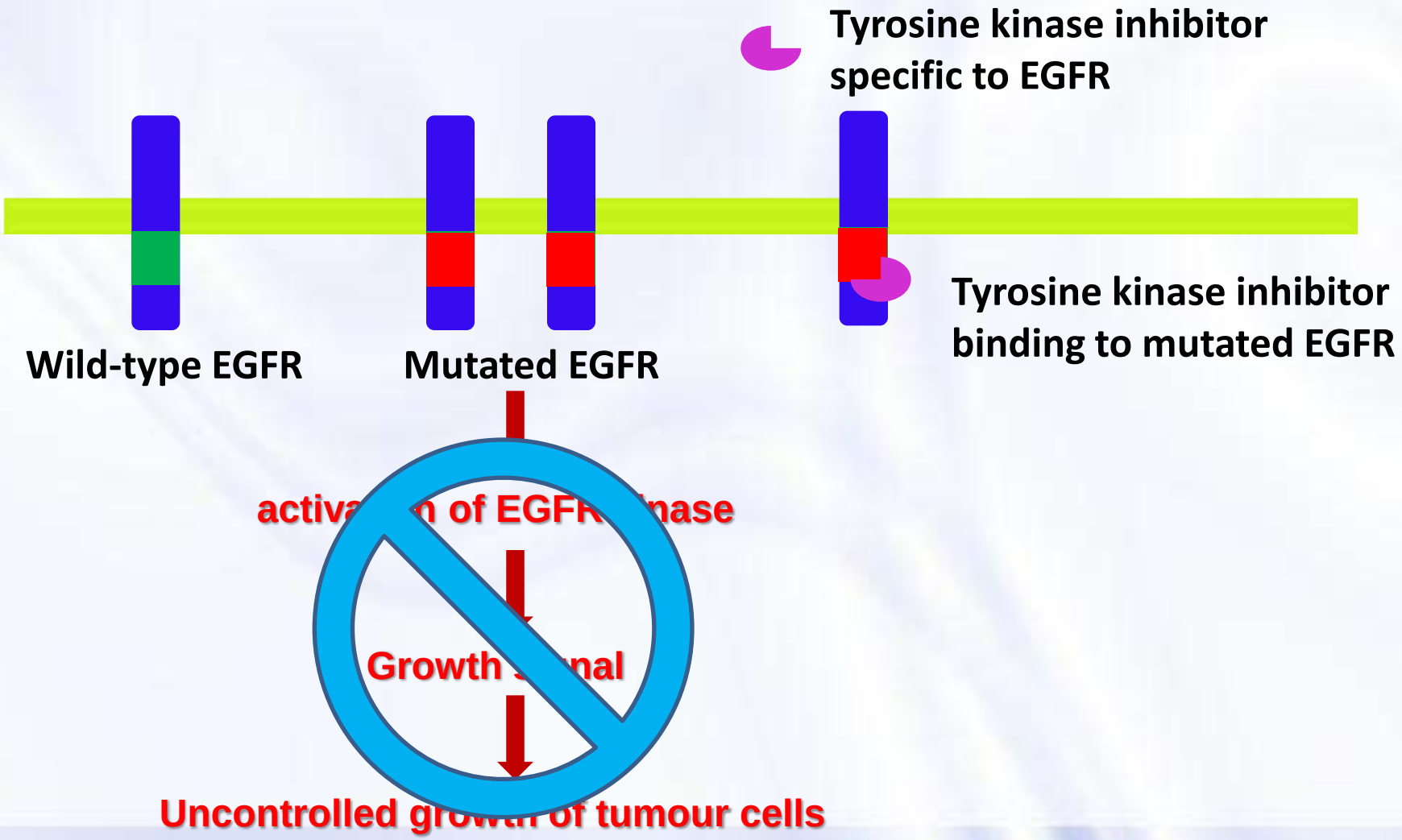


http://www2.lifewithlungcancer.info/experts/Diagnosing_and_Treating_Lung_Cancer/What_is_a_lung_biopsy_and_what_procedures_might_I_have_to_undergo/98/index.html

Targeted Cancer Therapy



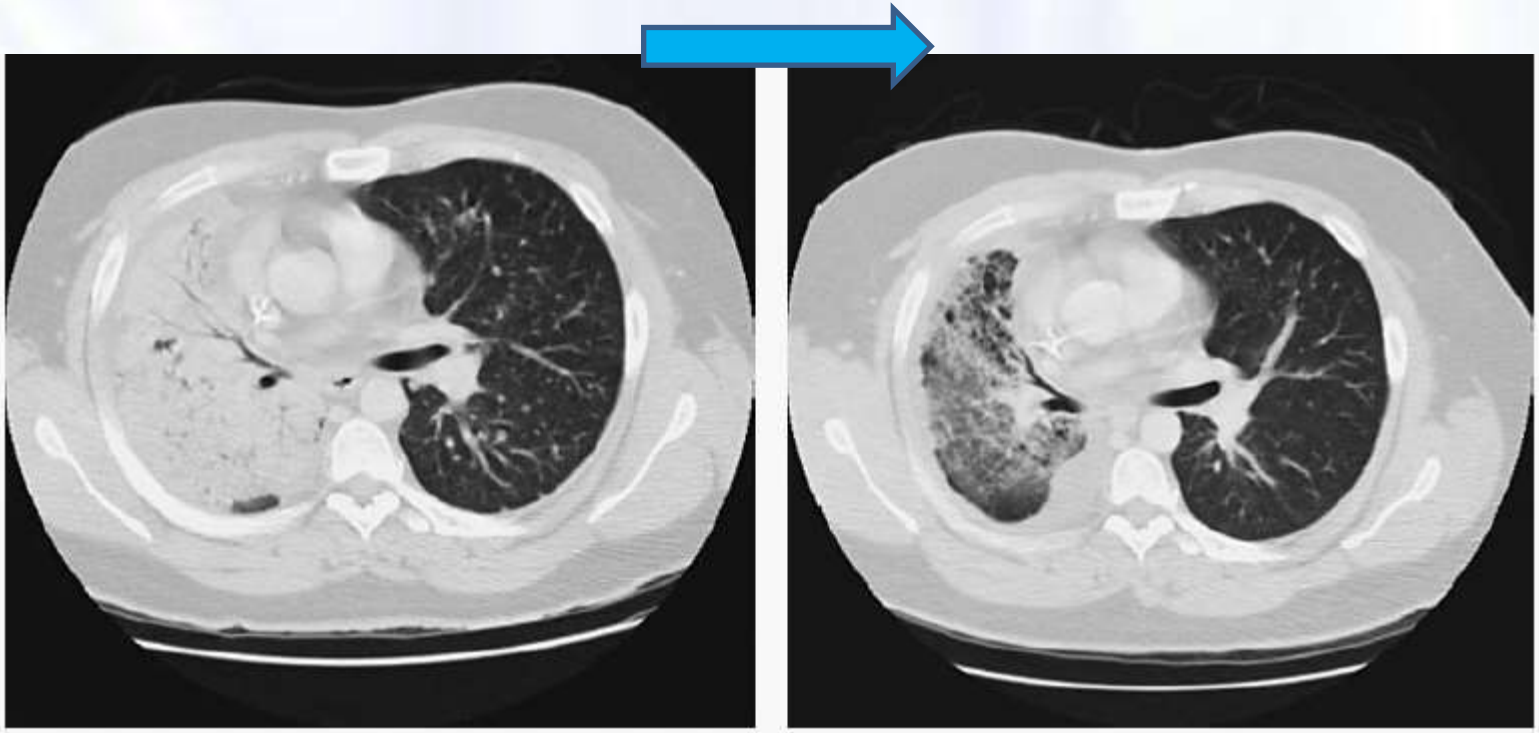
Targeted therapy for EGFR mutation



Targeted Cancer Therapy

- Effective treatment response with targeted therapy

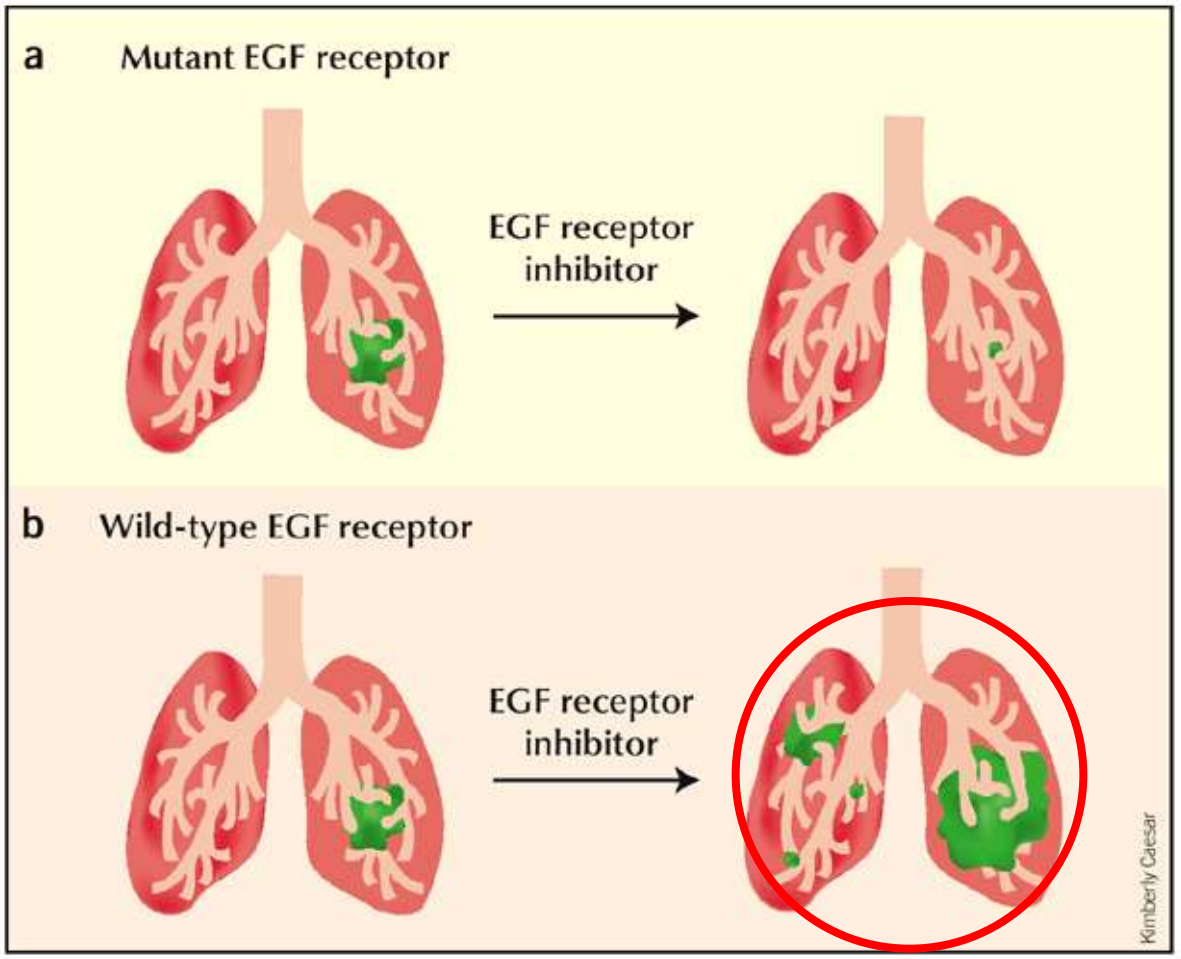
Six weeks after treatment with getifinib



Lynch TJ, 2004

Selection is required for targeted therapy

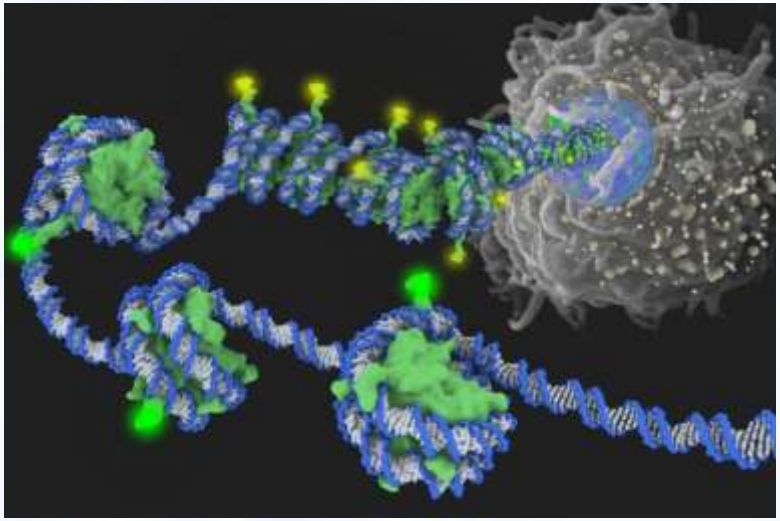
- Targeted drugs do not benefit patients without mutations of the targeted genes
- Selection is required for targeted therapy



http://www.nature.com/nm/journal/v10/n6/fig_tab/nm0604-577_F1.html

Importance of Molecular Pathology Tests

- Histological classification cannot be applied for selecting patient for targeted therapy
- The need of molecular testing to select patients for targeted therapy



From microscopic examination to genomic examination of tumour cells

http://www.cancer.gov/research/areas/diagnosis

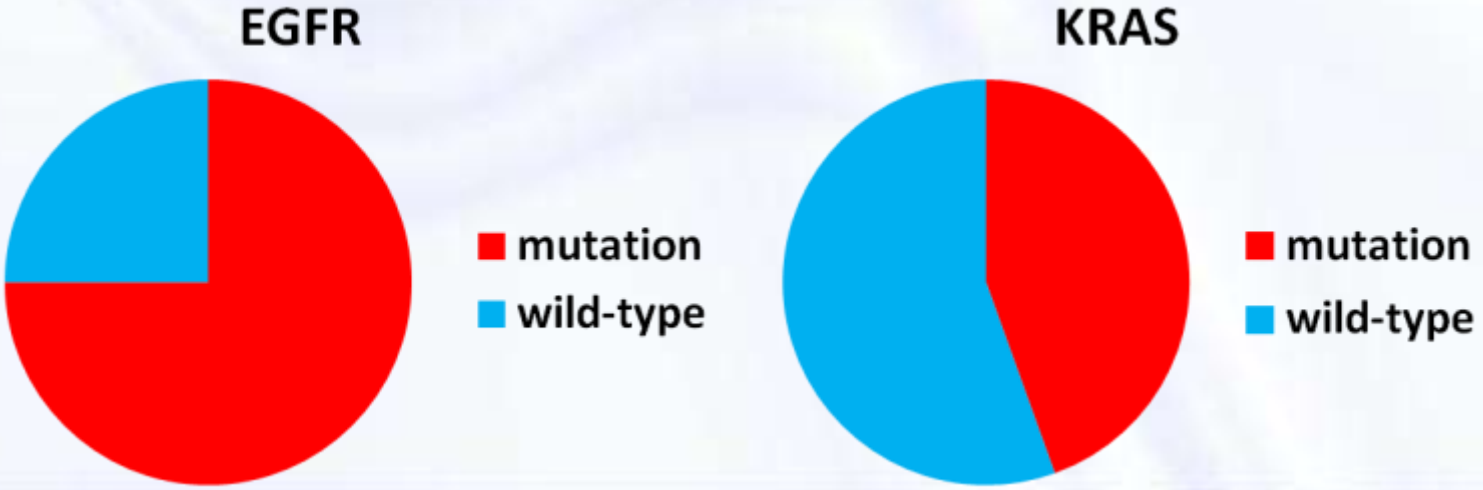
https://www.mskcc.org/blog/setting-cells-right-path-new-leukemia-drug-shows-growing-promise

Common Molecular Pathology tests for targeted cancer therapy in the Hospital Authority

- *EGFR* mutation test for lung cancer
- *KRAS* and *NRAS* mutation test for colon cancer
- *HER2* gene amplification test for breast and gastric cancers
- *ALK* translocation test for lung cancer

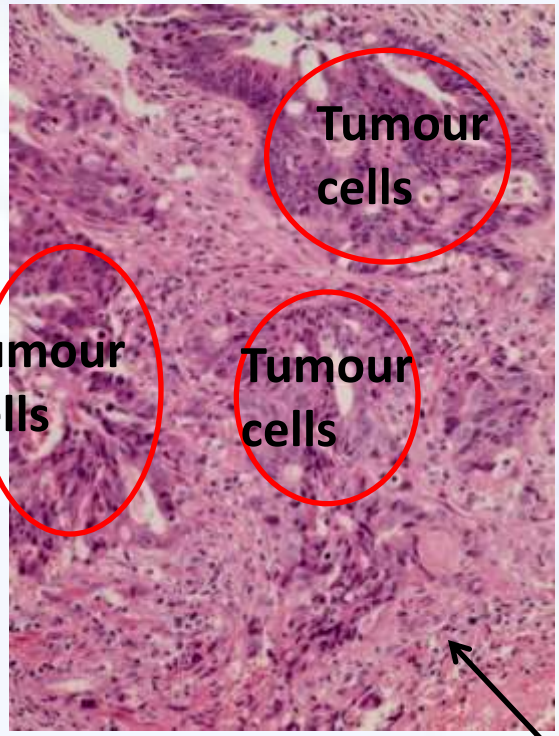
Mutation Test

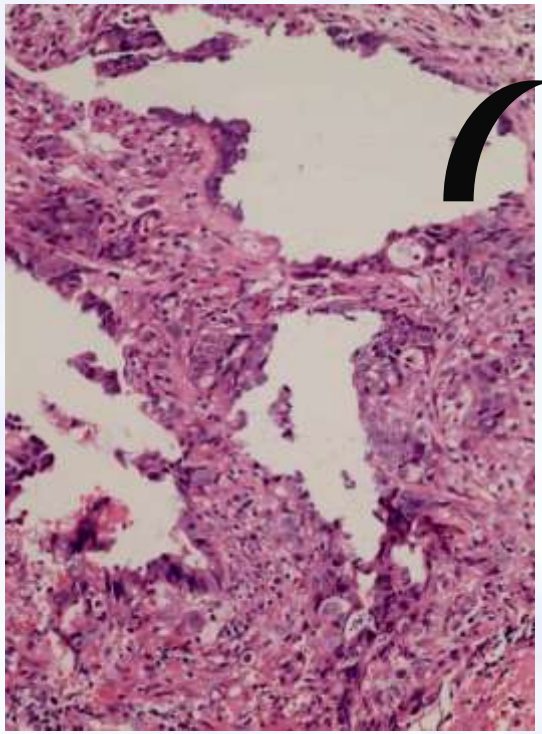
- Mutation of EGFR in 75% of Hong Kong non-smokers with lung adenocarcinomas (Tam IY, 2006)
- Mutation of KRAS in 44.5% of patients with colorectal cancer in Hong Kong (Tong JH, 2014)
- Patients suitable for targeted therapy can be selected after mutation testing

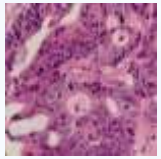


Mutation Test

- Tumour cells are collected by micro-dissection under the microscope



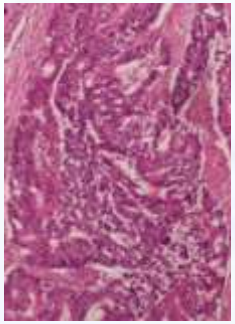


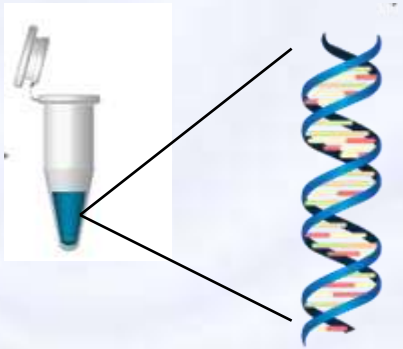


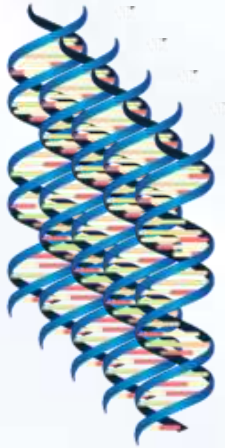
Tumour cells collected

Mutation Test

- DNA are extracted from the tumour cells
- polymerase chain reaction (PCR) for DNA amplification







Tumour cells collected

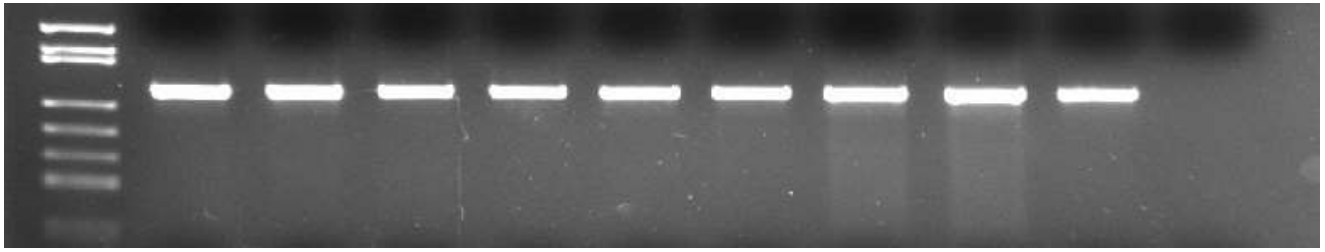
DNA extraction

DNA amplification by PCR

Millions of DNA copies

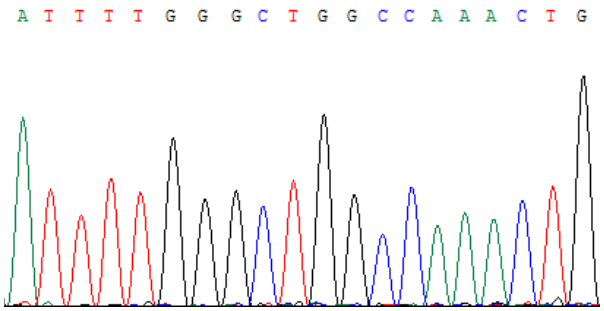
Mutation Test

- PCR products examined by gel electrophoresis



- DNA are sequenced for mutation detection

DNA sequencer

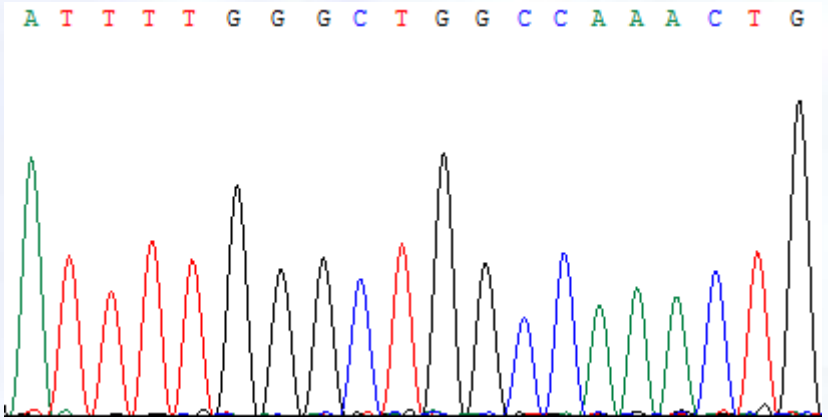


DNA sequences

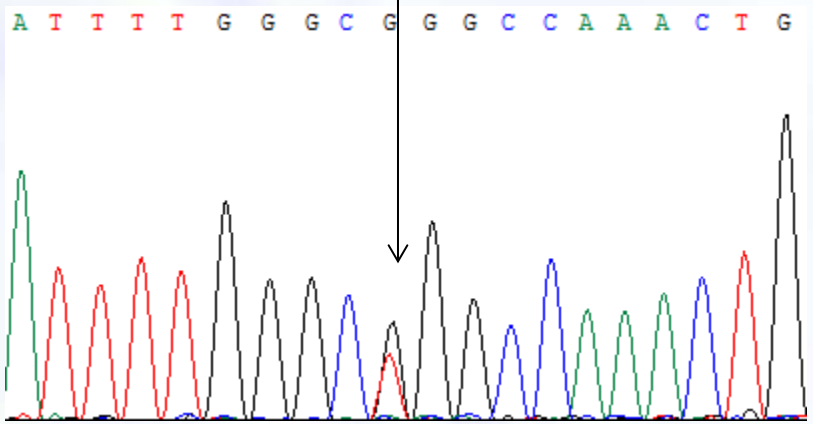
Mutation test

- CTG → CGG
- L858R mutation in EGFR

Normal cells DNA



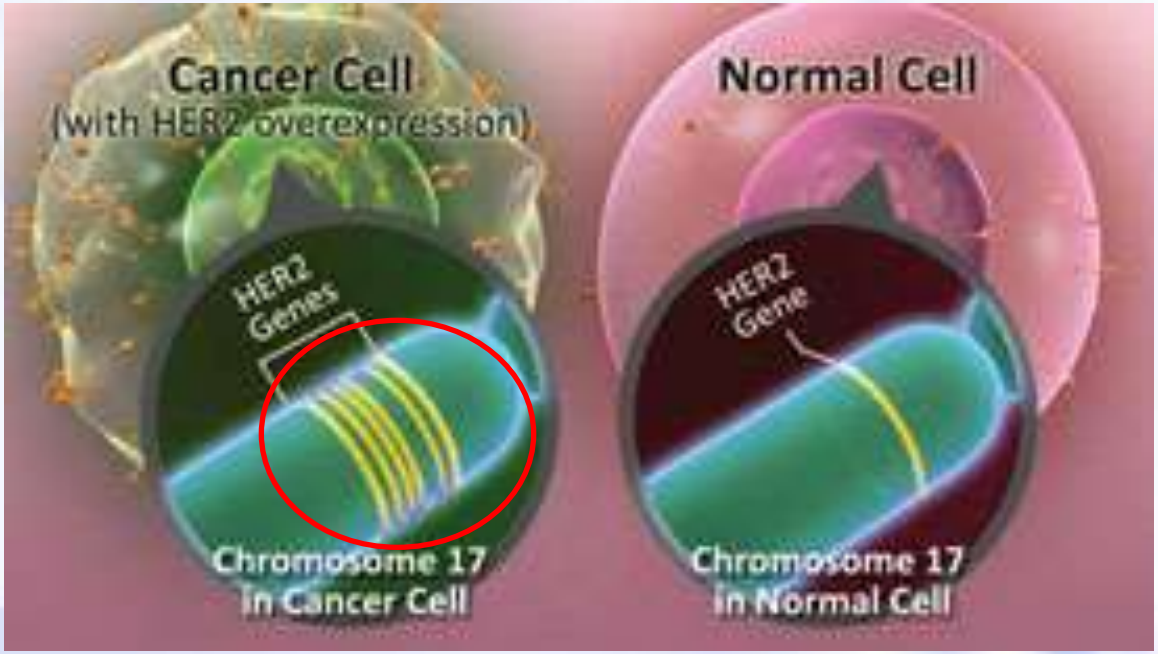
Tunour cells DNA



L858R

HER2 Amplification

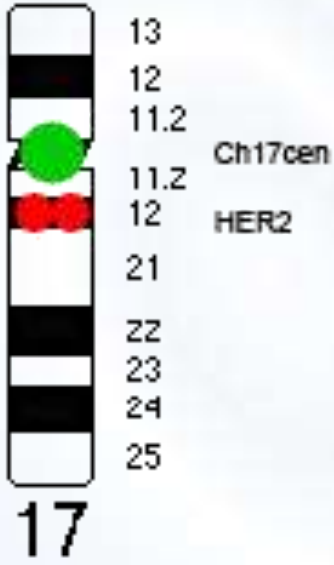
- more copies of the *HER2* gene on chromosome 17 in cancer cells compared to normal cells.
- 21% HER2 overexpression (by immunohistochemistry) in Hong Kong breast cancer patents (Yau T, 2008)
- *HER2* Fluorescence *in situ* hybridization (FISH) test is developed to identify patients with *HER2* amplification



http://global.onclive.com/publications/Oncology-live/2011/november-2011/Emerging-Biomarker-Science-Presents-Practical-Questions

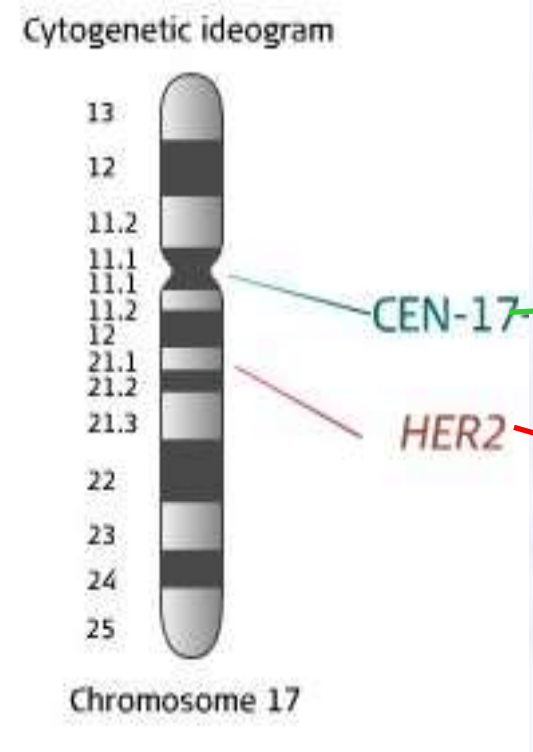
HER2 Amplification Test

- Chromosome 17 centromere is labeled with green fluorescent probe
- *HER2* gene is labeled with red fluorescent probe
- Fluorescent probes hybridized to the tumour cells on the slides (Fluorescence *in situ* hybridization, FISH)
- Tumour cells examined under fluorescence microscope

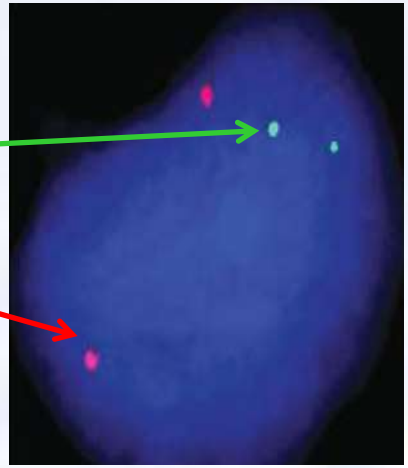


http://www.timesofisrael.com/fish-key-to-israeli-firms-lung-cancer-detection-tech/

HER2 Amplification Test

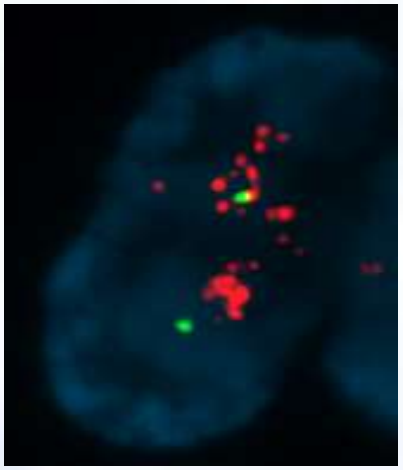


Normal cell



2 red, 2 green

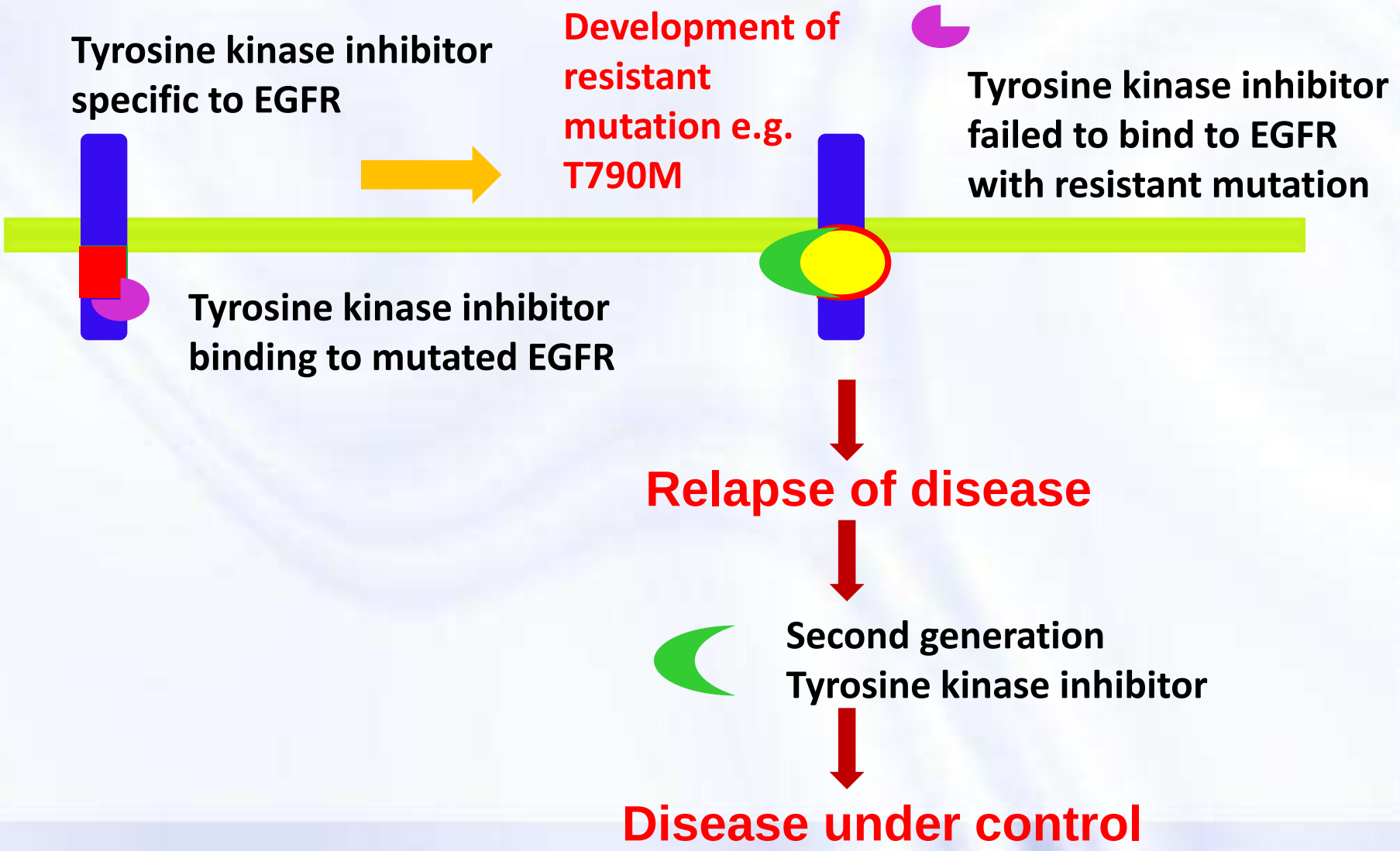
Cancer cell



More copies of HER2 gene (red probes) over chromosome 17 centromere (green probes)

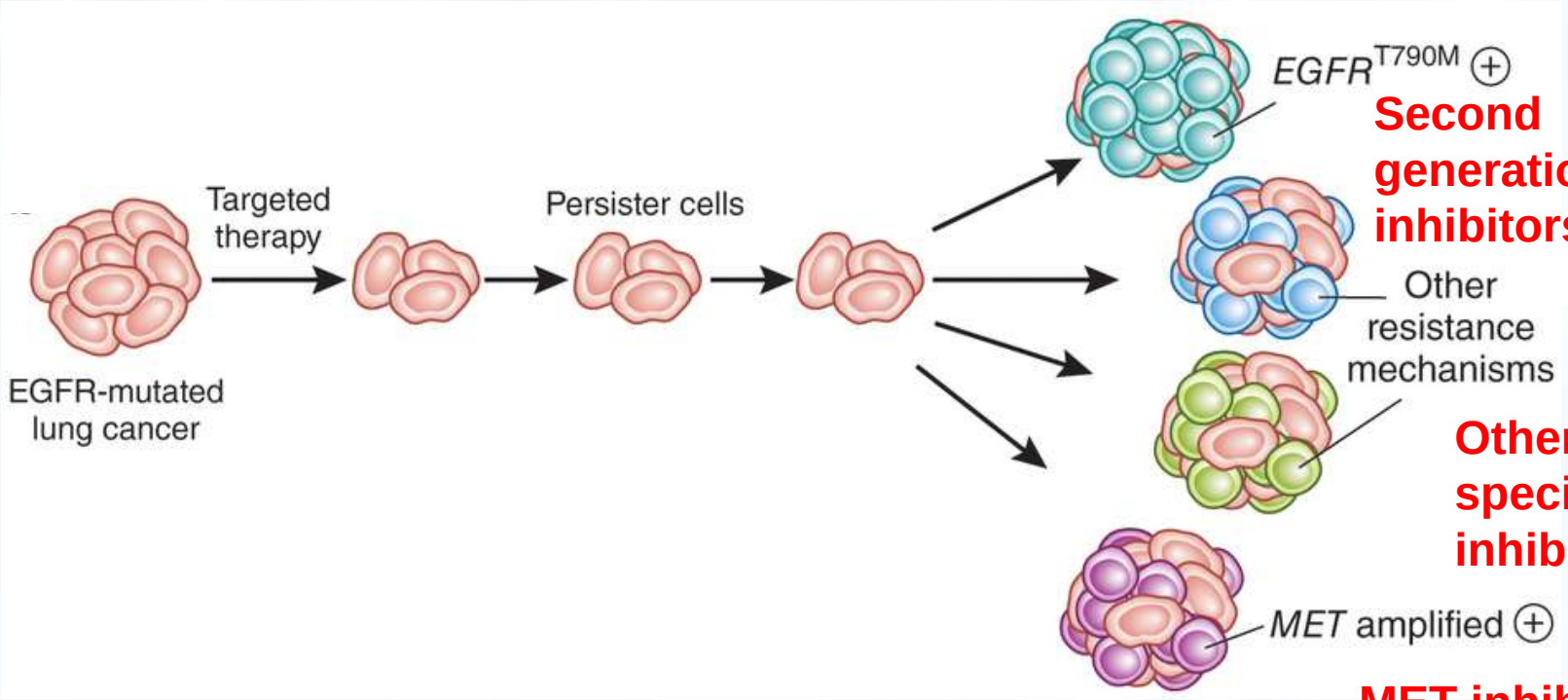
http://dako.marketport.dk/userfiles/image/TEJ/Cytogenetic%20ideogram.jpg

Targeted therapy: a continued battle



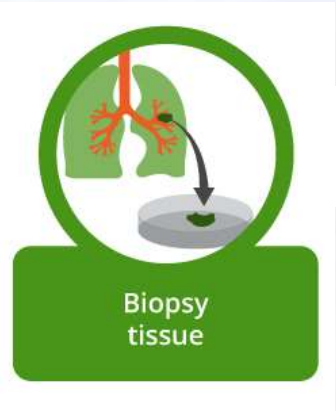
Targeted therapy: a continued battle

- Mechanisms of acquired resistance to first generation EGFR inhibitors for patients with *EGFR* mutations



Oxnard GR, 2016

Targeted therapy: a continued battle



If it is mutation **positive** → targeted cancer therapy

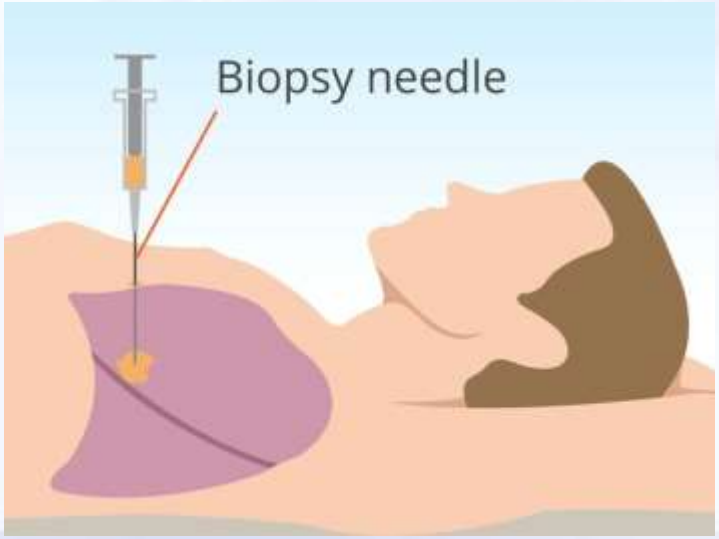
Identification of resistant mechanism → another targeted inhitbitors

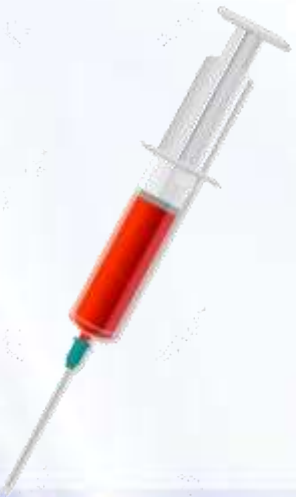
If there is disease relapse, re-biopsy is needed

http://www2.lifewithlungcancer.info/experts/Diagnosing_and_Treating_Lung_Cancer/What_is_a_lung_biopsy_and_what_procedures_might_I_have_to_undergo/98/index.html

More advanced molecular technologies under development

- Repeated biopsy taking, an invasive procedure to patients
- Circulating tumour DNA in blood
- Next Generation Sequencing





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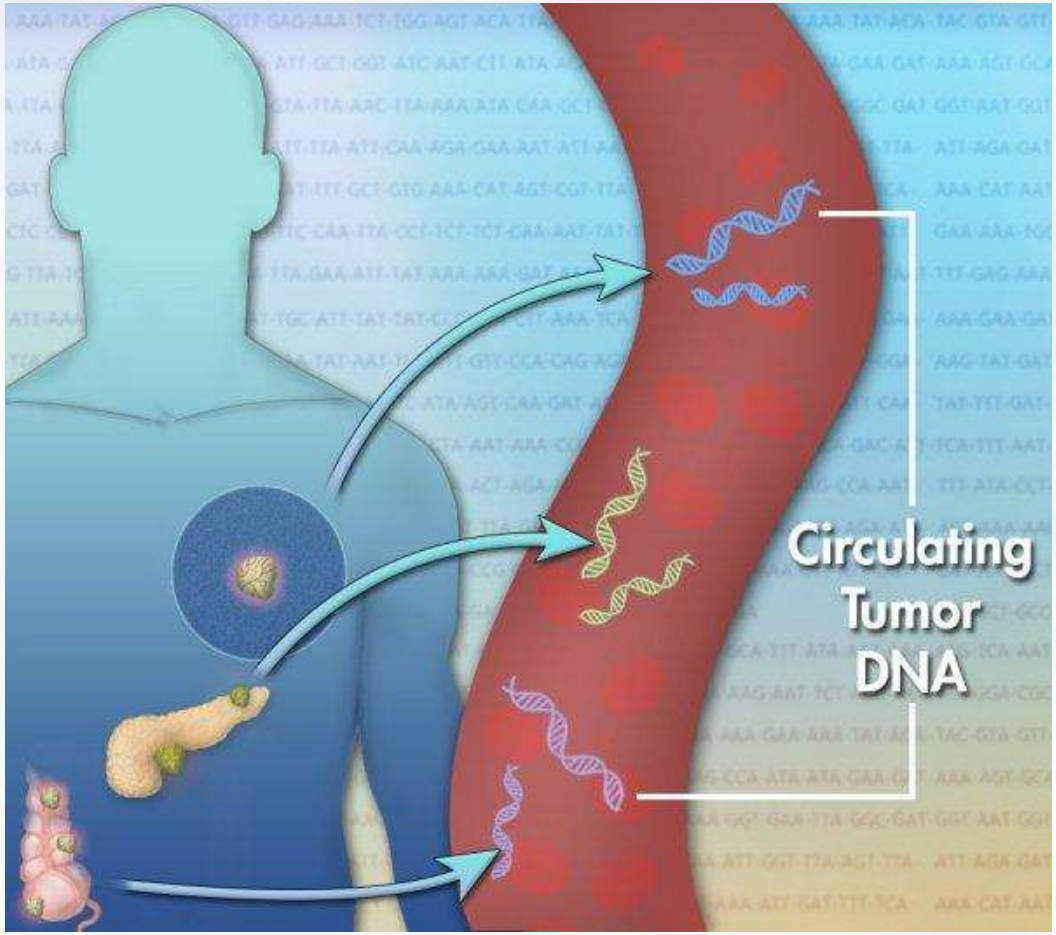
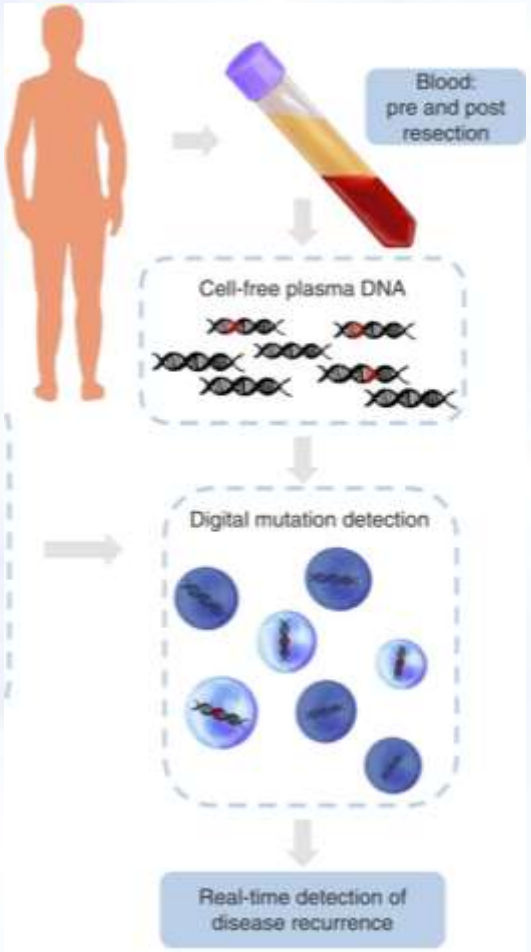
Circulating tumour DNA in blood



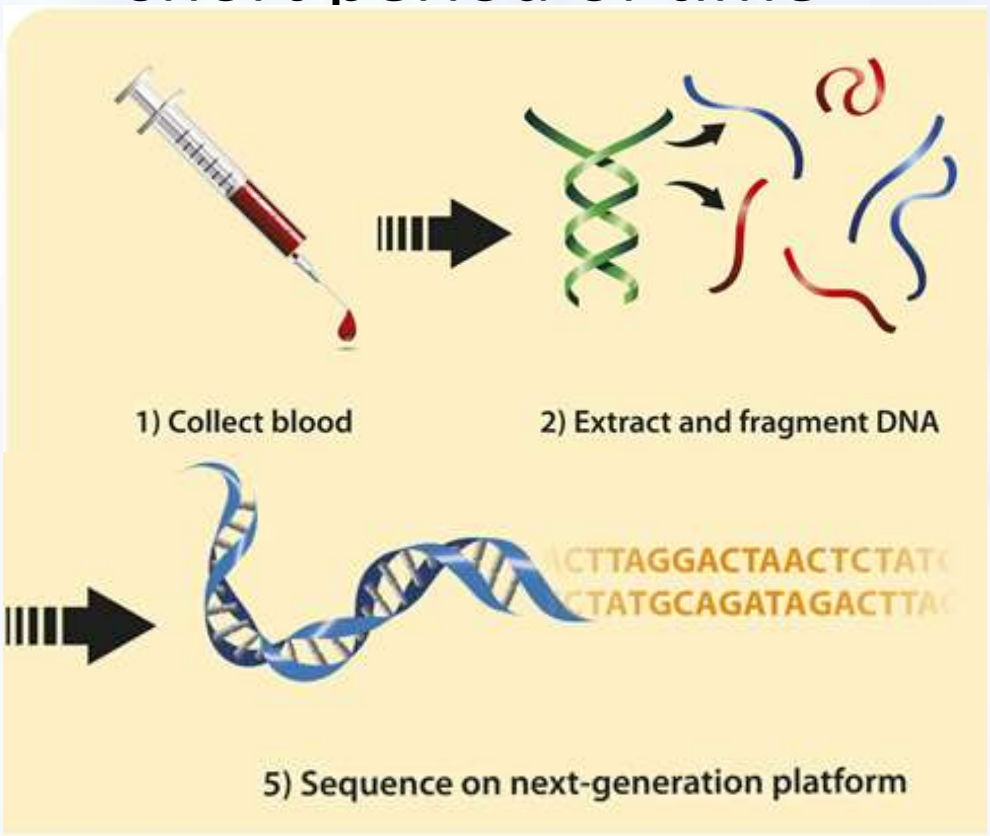


https://www.genome.gov/dmd/img.cfm?node=Photos/Graphics&id=92951

http://www.nature.com/ncomms/2015/150707/ncomms8686/fig_tab/ncomms8686_F1.html

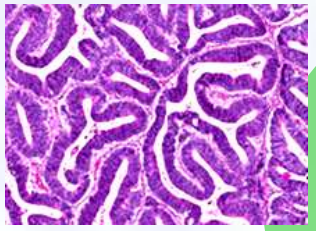
Next generation sequencing

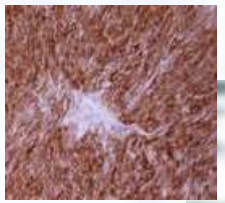
- Massively sequence the genome in a relatively short period of time



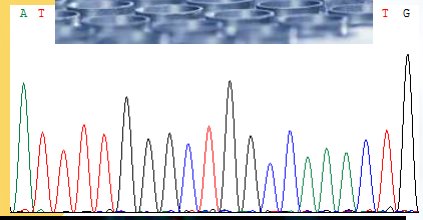
http://epilepsyu.com/blog/quest-diagnostics-to-provide-whole-exome-sequencing-service-to-diagnose-neurological-disorders/

Summary

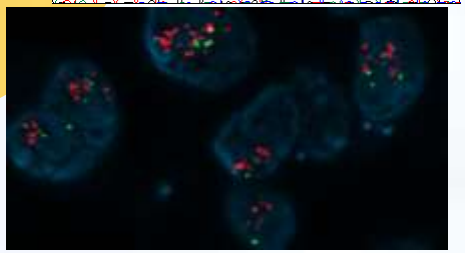




Histological aspect
H&E staining
Special stain
Immunohistochemistry



Molecular aspect
Mutation test
Gene amplification / translocation test



Disease diagnosis

Tumour histology?
Tumour type?
Grading?
Staging?

mutation positive tumour ?
Tumour with gene amplification?
Translocated tumour?

Pathologists

Scientists

Personalized Targeted Cancer Therapy

Oncologists

Acknowledgements

- Diagnostic Molecular Pathology Laboratory , Division of Anatomical Pathology, Department of Pathology, Queen Mary Hospital

Dr LP Chung

Mr Eddie Lo

Ms Kathy Cheung

Ms Anka Lin

Mr Kwan Pak Shing

Ms Priscilla Lo

Ms Allison Chow

Ms Yeung Lu

Mr Jason Cheung


Thank You
Queen Mary Hospital
瑪麗醫院