Interpreting NGS Reports: Clinical Applications and Personalized Medicine

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20241123

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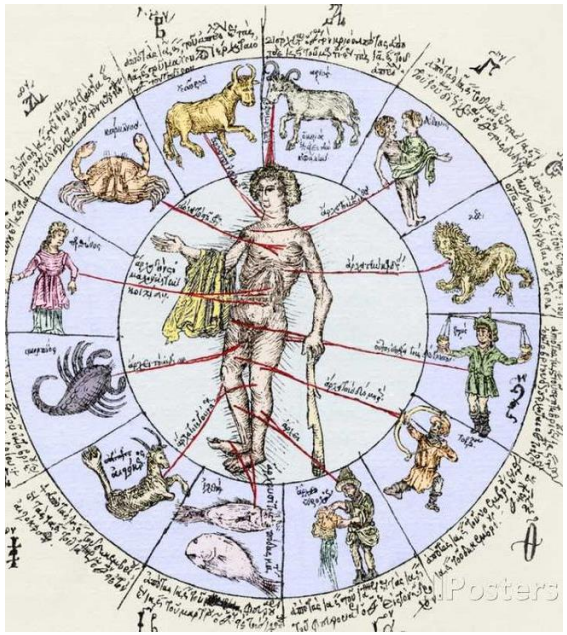
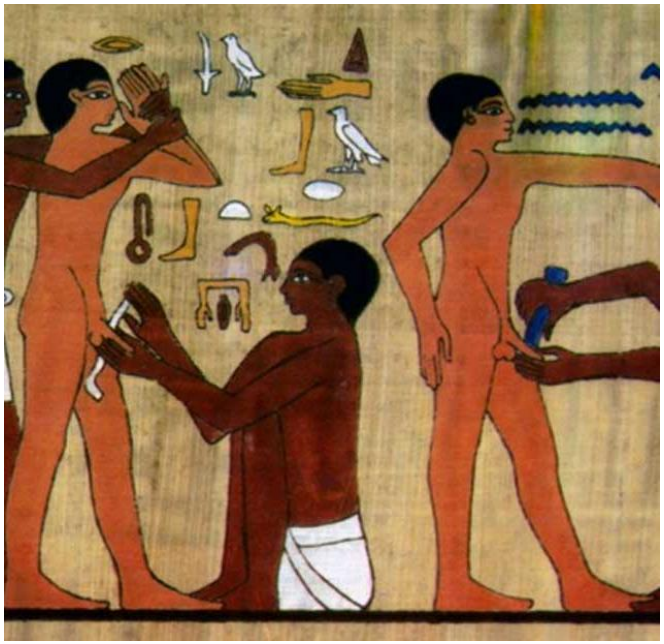


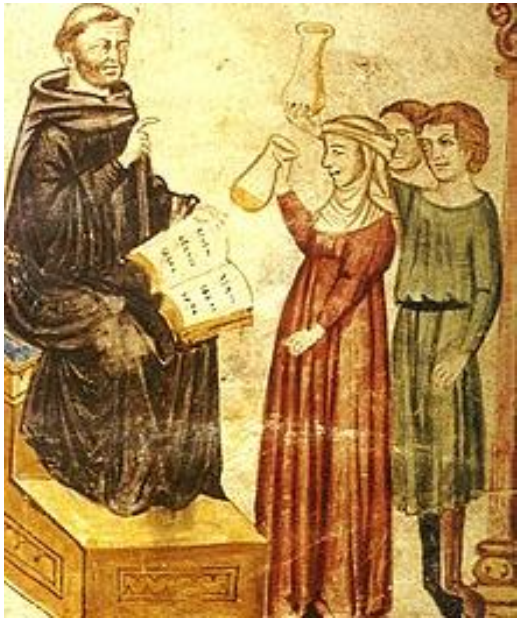


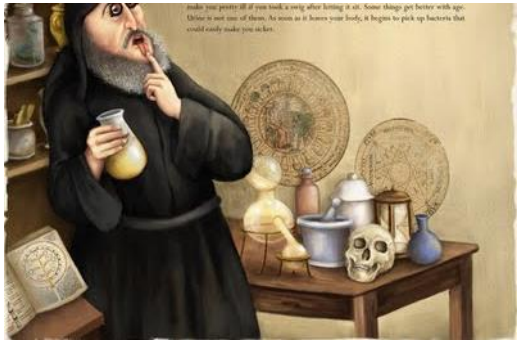
Pathology is the study of the **causes and effects** of disease or injury - Clinical Use

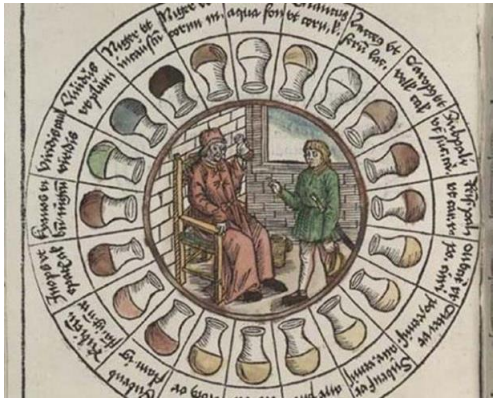
Diagnosis-Treatment-Prevention

Biomarkers

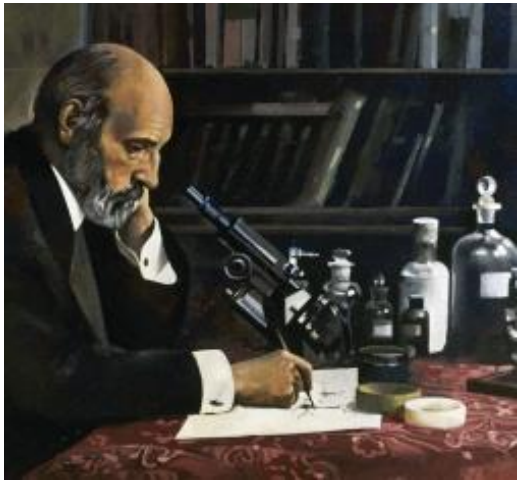
Anatomical Pathologist







Laboratory medicine
Clinical Pathologists



醫學實驗室 (Medical laboratory)

Clinical Laboratory: a laboratory where tests are carried out on clinical specimens to obtain information about the health of a patient to aid in diagnosis, treatment, and prevention of disease.

Types

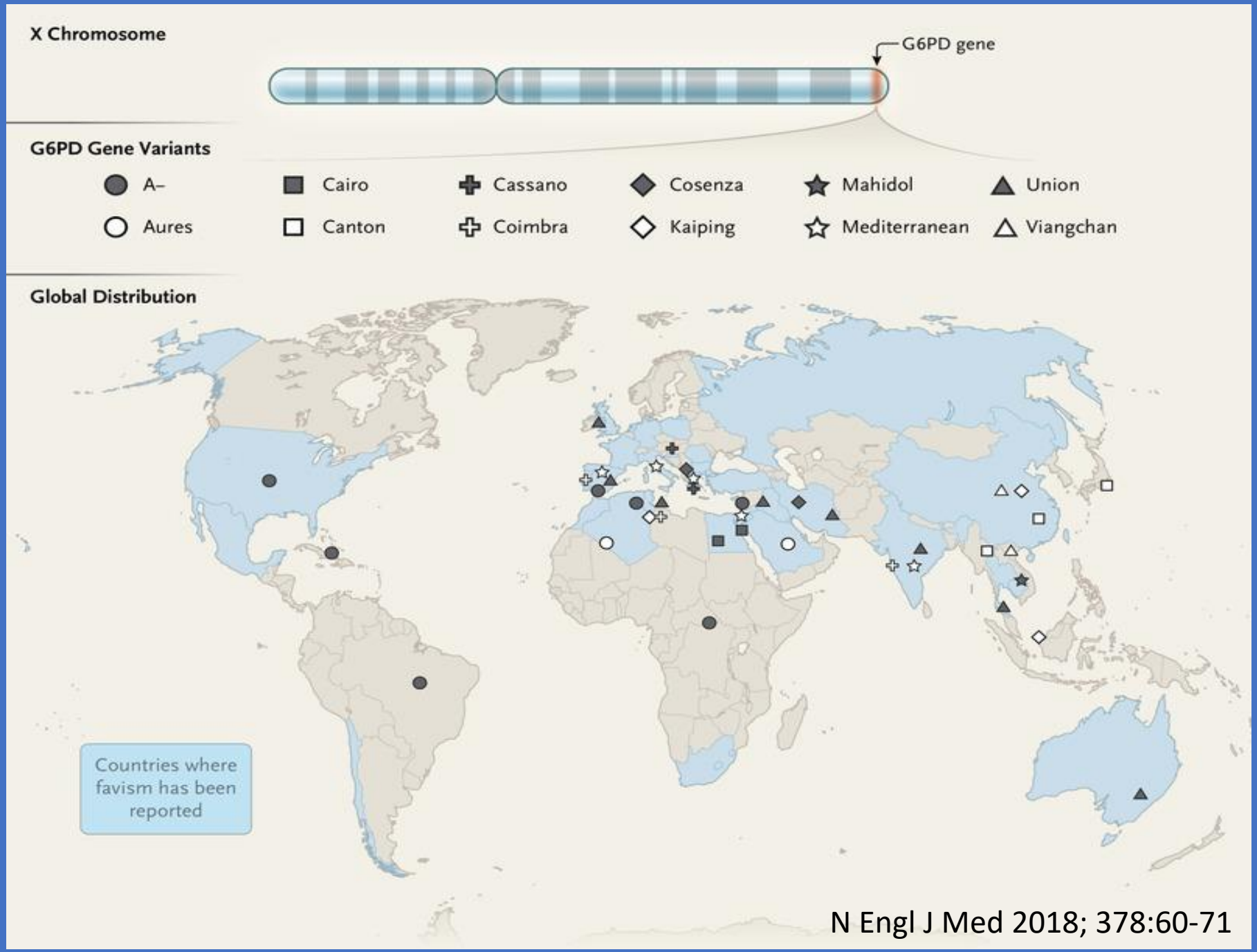
Hospital/Medical center: comprehensive tests

Clinics/Nursing home/Long-term care…: basic tests

Commercial Lab: **independent**; providing test that is otherwise not provided in other settings due to low test volume or complexity

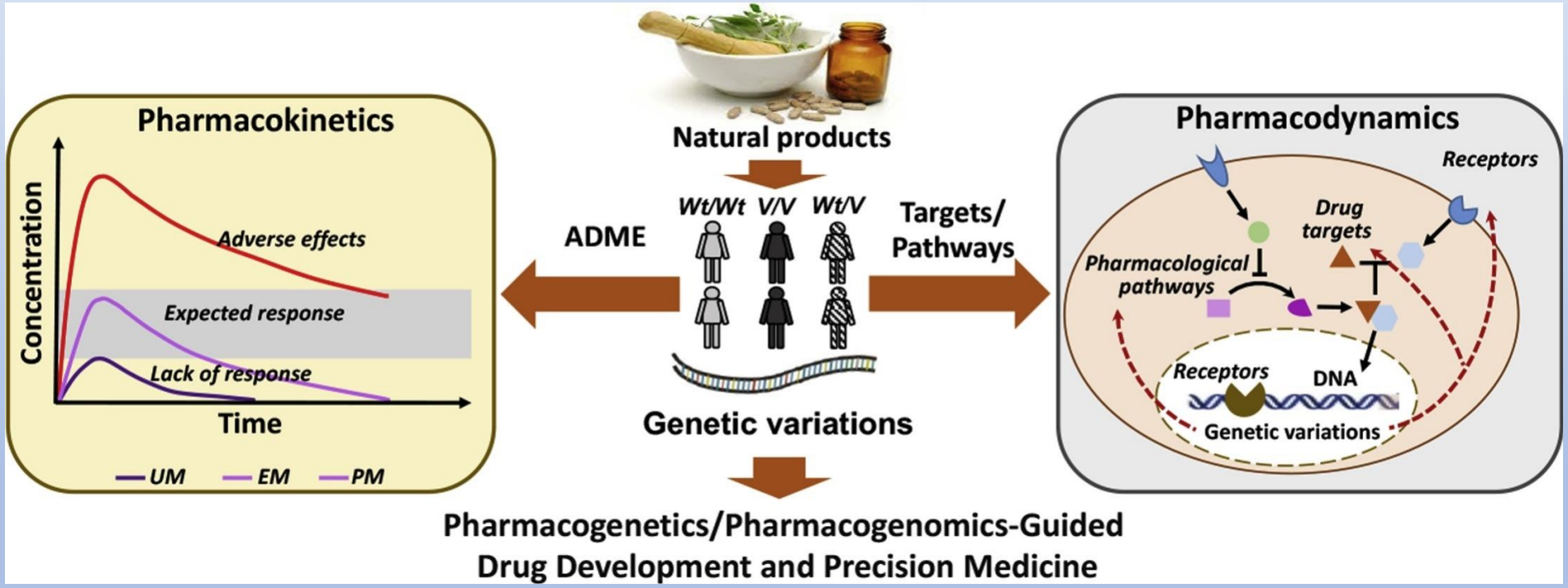
Pharmacogenetics History

- 510B.C
  - When Pythagoras noted that ingestion of fava beans resulted in a potentially fatal reaction in some, but not all, individuals
  - A connection between fava bean ingestion and hemolytic anemia: a blood disorder that causes weakness and fatigue.
  - This connection worried him so much that he forbade his followers from ever eating the Vicia faba. He declared the sacredness of the bean, admonishing those who dared to consider eating it.



N Engl J Med 2018; 378:60-71

- Drug metabolism (pharmacokinetics)
- Efficacy (pharmacodynamics)
- Toxicity



ADME: absorption, distribution, metabolism and excretion

Drug safety - Immune Related

Table 2 | Examples of immune-mediated adverse reactions associated with HLA alleles

Reactions	Drug	HLA class I	HLA class II
SCAR	Allopurinol	*HLA-B*58:01*[129]	NA
	Carbamazepine	*HLA-A*31:01*[47,130] *HLA-B*15:02*[131] *B*15:21*[132] *B*57:01*[133]	NA
	Dapsone	*HLA-B*13:01*[134,135]	NA
	Nevirapine	*HLA-C*04:01*[136,137]	NA
	Phenytoin	*HLA-B*15:02*[138]	NA
DRESS	Abacavir	*HLA-B*57:01*[139]	NA
	Vancomycin	*HLA-A*32:01*[140]	NA
DILI	Amoxicillin-Clavulanate	*HLA-A*02:01*[141]	*HLA-DRB1*15:01-DRB5*01:01-DQB1*06:02* haplotype[142,143]
	Flucloxacillin	*HLA-B*57:01*[46]	NA
	Ticlopidine	*HLA-A*33:03*[144,145]	NA
Agranulocytosis	Clozapine	*HLA-B*38* *HLA-B*39* *HLA-B*67*[146] *HLA-Cw7-B18* *HLA-Cw7-B39* haplotype[147]	*HLA-DRB5*02:01*[147]
Type I hypersensitivity reaction	β-Lactam antibiotics	NA	*HLA-DRB1*10:01*[102] *HLA-DRA* rs7192[148]

DILI, drug-induced liver injury; DRESS, drug reactions with eosinophilia and systemic symptoms; NA, not applicable; SCAR, serious cutaneous adverse reactions (includes Stevens–Johnson syndrome, toxic epidermal necrolysis and DRESS).

Toxicity
還有很多是來自
免疫系統的影響

Nature Reviews Genetics **volume 24**, pages350–362 (2023)

- Precision Medicine

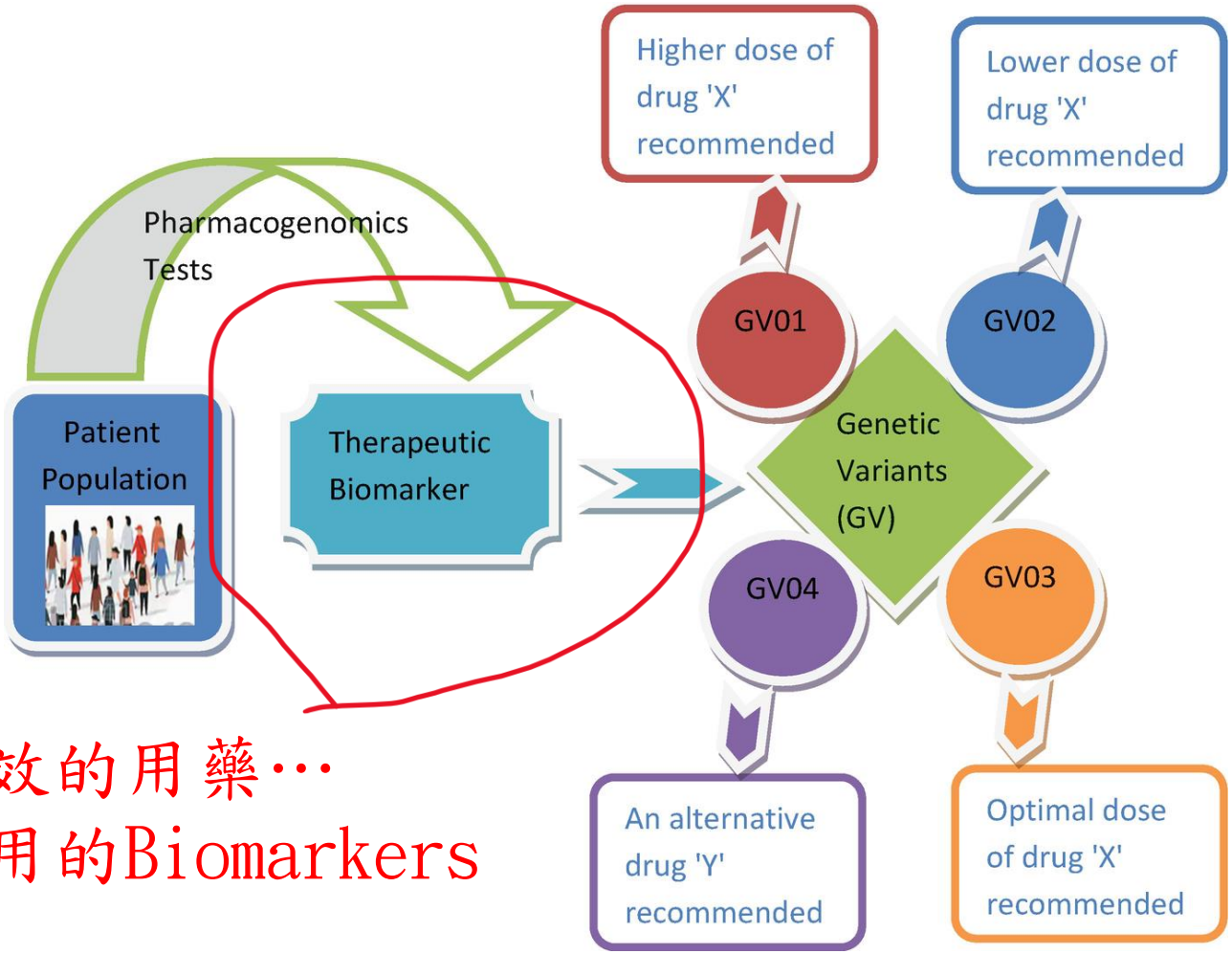
- Precision Medicine / Right Drug (test) for the Right Patient

正確有效的用藥(檢測)…

…以減少不必要的資源浪費

…以減少不必要的傷害/副作用

To deliver the Right drug to the Right person at the Right dose



正確有效的用藥…

需要好用的Biomarkers

U.S. FOOD & DRUG ADMINISTRATION

Companion Diagnostics

A companion diagnostic is a medical device, often an in vitro diagnostic (IVD), which provides information that is essential for the safe and effective use of a corresponding drug or biological product.

Companion diagnostics can:

- identify patients who are most likely to benefit from a particular therapeutic product,
- identify patients likely to be at increased risk for serious side effects as a result of treatment with a particular therapeutic product; or
- monitor response to treatment with a particular therapeutic product for the purpose of adjusting treatment to achieve improved safety or effectiveness.

If the diagnostic test is inaccurate, then the treatment decision based on that test may not be optimal.

COMPANION VS COMPLEMENTARY DIAGNOSTICS[1]

Companion Diagnostic

- Medical device, often an in vitro diagnostic, which:
  - Provides information that is ***essential*** for the safe and effective use of a corresponding drug or biological product
  - Can help identify patients in whom benefits from a particular therapeutic product outweighs any potential side effects or risks[2]

Complementary Diagnostic

- Type of in vitro diagnostic that can ***lend information*** about the risk/benefit of a drug
  - Biomarker is NOT a prerequisite for receiving the drug
  - May inform on enhanced benefit in a subpopulation of patients (even when therapeutic benefit has been shown in all populations)

1. Scheerens H. *Clin Transl Sci.* 2017; 10(2): 84–92. 2. US FDA. Companion Diagnostics. [website]

List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools)

Content current as of:
10/29/2024

Regulated Product(s)
Medical Devices

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A *companion diagnostic device* can be *in vitro* diagnostic (IVD) device or an imaging tool that provides information that is essential for the safe and effective use of a corresponding therapeutic product. Below is a table of cleared or approved companion diagnostic devices (in vitro and imaging).

The use of an IVD companion diagnostic device is stipulated in the instructions for use in the labeling of the diagnostic device. The IVD companion diagnostic devices included in the first table below are indicated for a specific therapeutic product(s). Some IVD companion diagnostic devices are indicated for a specific group of oncology therapeutic products (see FDA's guidance document, Developing and Labeling In vitro Companion Diagnostic Devices for a Specific Group of Oncology Therapeutic Products, for more information on group labeling). The second table below lists companion diagnostic devices with group labeling indications.

Please submit any questions to DICE@fda.hhs.gov.

List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools)

Search: BRAF

Export Excel

Show 10 entries

Diagnostic Name (Manufacturer)	Indication - Sample Type	Drug Trade Name (Generic) NDA / BLA	Biomarker(s)	Biomarker(s) (Details)	PMA / 510(k) / 513(f)(2) / HDE (Approval / Clearance / Grant Date)
cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc.)	Melanoma - Tissue	Zelboraf (vemurafenib) NDA 202429	*BRAF*	V600E	P110020 (08/17/2011)
cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc.)	Melanoma - Tissue	Cotellic (cobimetinib) NDA 206192 in combination with Zelboraf (vemurafenib) NDA 202429	*BRAF*	V600E or V600K	P110020/S016 (11/07/2016)
FoundationOne CDx (Foundation Medicine, Inc.)	Non-Small Cell Lung Cancer (NSCLC) - Tissue	Tafinlar (dabrafenib) NDA 202806 in combination with Mekinist (trametinib) NDA 204114	*BRAF*	V600E	P170019 (11/30/2017)
FoundationOne CDx (Foundation Medicine, Inc.)	Melanoma - Tissue	Mekinist (trametinib) NDA 204114	*BRAF*	V600E and V600K	P170019 (11/30/2017) Group

FoundationOne CDx (Foundation Medicine, Inc.)	Melanoma - Tissue	Tecentriq (atezolizumab) BLA 761034 in combination with Cotellic (cobimetinib) NDA 206192 and Zelboraf (vemurafenib) NDA 202429	*BRAF*	*BRAF* V600 mutations	P170019/S030 (01/19/2022)
FoundationOne CDx (Foundation Medicine, Inc.)	Non-Small Cell Lung Cancer (NSCLC) - Tissue	BRAFTOVI (encorafenib) NDA210496 in combination with MEKTOVI (binimetinib) NDA210498	*BRAF*	*V600E*	P170019/S039 (10/11/2023)
FoundationOne Liquid CDx (Foundation Medicine, Inc.)	Non-Small Cell Lung Cancer (NSCLC) - Plasma	BRAFTOVI (encorafenib) NDA210496 in combination with MEKTOVI (binimetinib) NDA210498	*BRAF*	V600E	P190032/S011 (10/11/2023)
FoundationOne Liquid CDx (Foundation Medicine, Inc.)	Metastatic Colorectal Cancer (mCRC) - Plasma	BRAFTOVI (encorafenib) NDA 210496 in combination with cetuximab BLA 125084	*BRAF*	*BRAF V600E* alteration	P190032/S010 (06/08/2023)
Oncomine Dx Target Test (Life Technologies Corporation)	Non-Small Cell Lung Cancer (NSCLC) - Tissue	Tafinlar (dabrafenib) NDA 202806 in combination with Mekinist (trametinib) NDA 204114	*BRAF*	V600E	P160045 (06/22/2017)
Oncomine Dx Target Test (Life Technologies Corporation)	Anaplastic Thyroid Cancer (ATC) - Tissue	Tafinlar (dabrafenib) NDA 202806 in combination with Mekinist (trametinib) NDA 204114	*BRAF*	*BRAF* V600E mutations	P160045/S025 (09/29/2023)

Diagnostic Name (Manufacturer)	Indication - Sample Type	Drug Trade Name (Generic) NDA / BLA	Biomarker(s)	Biomarker(s) (Details)	PMA / 510(k) / 513(f)(2) / HDE (Approval / Clearance / Grant Date)
therascreen BRAF V600E RGQ PCR Kit (QIAGEN GmbH)	Colorectal Cancer - Tissue	Braftovi (encorafenib) NDA 210496 in combination with Erbitux (cetuximab) BLA 125084	*BRAF*	V600E	P190026 (04/15/2020)
THXID BRAF Kit (bioMérieux Inc.)	Melanoma - Tissue	Mekinist (trametinib) NDA 204114	*BRAF*	V600E or V600K	P120014 (05/29/2013)
THXID BRAF Kit (bioMérieux Inc.)	Melanoma - Tissue	Tafinlar (dabrafenib) NDA 202806	*BRAF*	V600E	P120014 (05/29/2013)
THXID BRAF Kit (bioMérieux Inc.)	Melanoma - Tissue	Braftovi (encorafenib) NDA 210496 in combination with Mektovi (binimetinib) NDA	*BRAF*	V600E or V600K	P120014/S008 (06/27/2018)

Device Indication for a Specific Group of Oncology Therapeutic Products

Diagnostic Name (Manufacturer)	Indication(s) - Sample Type	PMA (Approval Date)	Device Indication for a Specific Group of Oncology Therapeutic Products and Trade Name (Generic) – NDA/BLA
cobas EGFR Mutation Test v2 (Roche Molecular Systems, Inc.)	Non-Small Cell Lung Cancer (NSCLC) - Tissue or Plasma	P120019/S031 (10/27/2020)	Non-small cell lung cancer (tissue): "Identifying patients with NSCLC whose tumors have *EGFR* exon 19 deletions or exon 21 (L858R) substitution mutations and are suitable for treatment with a tyrosine kinase inhibitor approved by FDA for that indication" List of tyrosine kinase inhibitors approved by FDA for this indication: • Tarceva (erlotinib) - NDA 021743 • Tagrisso (osimertinib) - NDA 208065 • Iressa (gefitinib) - NDA 206995 • Gilotrif (afatinib) - NDA 201292 • Vizimpro (dacomitinib) - NDA 211288 • Lazcluze (Lazertinib) - NDA 219008 as part of a combination therapy Non-small cell lung cancer (plasma): "Identifying patients with NSCLC whose tumors have *EGFR* exon 19 deletions or exon 21 (L858R) substitution mutations and are suitable for treatment with a tyrosine

FoundationOne CDx (Foundation Medicine, Inc.)	Non-Small Cell Lung Cancer (NSCLC) - Tissue	P170019/S033 (03/16/2022)	"Identifying patients with NSCLC whose tumors have *EGFR* exon 19 deletions or exon 21 (L858R) substitution mutations and are suitable for treatment with a tyrosine kinase inhibitor (TKI) approved by FDA for that indication" List of tyrosine kinase inhibitors approved by FDA for this indication: • Tarceva (erlotinib) - NDA 021743 • Tagrisso (osimertinib) - NDA 208065 • Iressa (gefitinib) - NDA 206995 • Gilotrif (afatinib) - NDA 201292 • Vizimpro (dacomitinib) - NDA 211288 • Lazcluze(Lazertinib) - NDA 219008 as part of a combination therapy

FoundationOne CDx (Foundation Medicine, Inc.)	Melanoma - Tissue	P170019/S025 (11/10/2021)	"Identifying patients with melanoma whose tumors have BRAF V600E and are suitable for treatment with BRAF Inhibitors approved by FDA for that indication" List of BRAF Inhibitors approved by FDA for this indication: • Tafinlar (dabrafenib) - NDA 202806 • Zelboraf (vemurafenib) - NDA 202429 "Identifying patients with melanoma whose tumors have *BRAF* V600E and V600K and are suitable for treatment with BRAF/MEK Inhibitor Combinations approved by FDA for that indication" List of BRAF/MEK Inhibitor Combinations approved by FDA for this indication: • Cotellic (cobimetinib) - NDA 206192 in combination with Zelboraf (vemurafenib) - NDA 202429 • Braftovi (encorafenib) - NDA 210496 in combination with Mektovi (Binimetinib) - NDA 210498 • Tafinlar (dabrafenib) - NDA 202806in combination with Mekinist (trametinib) - NDA 204114

醫療器材許可證查詢

查詢結果共4筆

NO	許可證/登錄字號	有效日期	中文品名	英文品名	醫療器材商	製造業者	限制項目
1	衛署醫器輸字第024721號	117-02-22	羅氏BRAF V600基因突變核酸檢驗試劑	Cobas 4800 BRAF V600 Mutation Test	台灣羅氏醫療診斷設備股份有限公司	ROCHE MOLECULAR SYSTEMS, INC.	R02 輸 入
2	衛署醫器輸壹字第012661號	117-01-31	〝亞培〞艾西斯BRAF探針(未滅菌)	〝Abbott〞Vysis BRAF FISH Probe Kit (Non-sterile)	美商亞培股份有限公司台灣分公司	ABBOTT MOLECULAR INC.	R02 輸 入
3	衛部醫器輸字第034679號	115-07-21	埃迪拉NRAS-BRAF基因突變檢測套組	Idylla NRAS-BRAF Mutation Test	芮弗士科技有限公司	BIOCARTIS NV	R02 輸 入 R06 QMS/QSD
4	衛部醫器輸壹字第013690號	117-12-18	“羅氏”抗BRAF V600E (VE1)鼠單株一級抗體 (未滅菌)	“Roche” Anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody (Non-sterile)	台灣羅氏醫療診斷設備股份有限公司	VENTANA MEDICAL SYSTEMS, INC.	R02 輸 入

每頁數量: 10 1 ~ 4 / 4

中文品名	羅氏BRAF V600基因突變核酸檢驗試劑
英文品名	Cobas 4800 BRAF V600 Mutation Test
效能	本產品的主要效能是偵測從福馬林固定石蠟包埋的人類黑色素瘤(melanoma)組織中萃取之DNA內BRAF V600的突變。對人類黑色素瘤而言，本試劑的使用目的是用來幫助篩選腫瘤帶有BRAF V600突變的病人接受Zelboraf(vemurafenib)治療。(以下空白)

中文品名	〝亞培〞艾西斯BRAF探針(未滅菌)
英文品名	〝Abbott〞Vysis BRAF FISH Probe Kit (Non-sterile)
效能	限醫療器材分類分級管理辦法「免疫病理組織化學試劑與套組(B.1860)」第一等級鑑別範圍。

中文品名	“羅氏”抗BRAF V600E (VE1)鼠單株一級抗體 (未滅菌)
英文品名	“Roche” Anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody (Non-sterile)
效能	限醫療器材分類分級管理辦法「免疫病理組織化學試劑與套組(B.1860)」第一等級鑑別範圍。

1 名稱

產品名稱: 埃迪拉NRAS-BRAF基因突變檢測套組
Idylla NRAS-BRAF Mutation Test (Cartridge and TTP (版次： NRAS-BRAF_IVD/1.0))

產品型號: A0030/6

2 效能 / 適應症

2.1 效能

僅供體外診斷使用。
限專業人員使用。

需搭配Biocartis Idylla System(衛部醫器輸字第033691號)使用。

本產品是一種體外診斷檢測試劑，用於對*NRAS*致癌基因的密碼子12、13、59、61、117、146及*BRAF*致癌基因的密碼子600中的突變進行定性檢測。本產品涵蓋從FFPE採樣到結果產出的整個流程，包括從福馬林固定、石蠟包埋(FFPE) 的人體大腸直腸癌的組織切片樣品中釋出DNA、Real-Time PCR擴增與檢測。

2.2 適應症

本產品適用於測定轉移性大腸直腸癌病患在診斷時的腫瘤NRAS突變狀態，以及輔助跨科別團隊的治療決策。腫瘤BRAF突變狀態評估係搭配腫瘤RAS突變狀態評估確定病患的預後。本產品不適用於診斷大腸直腸癌。醫師必須一併考量病患的突變狀態及其他疾病因素，才做成治療決定。

查詢結果共11筆

NO	許可證/登錄字號	有效日期	中文品名	英文品名	醫療器材商	製造業者	限制項目
1	衛署醫器輸字第020836號	119-03-02	羅氏抗表皮生長因子受體(5B7)兔子單株一級抗體	Roche CONFIRM anti-EGFR (5B7) Rabbit Monoclonal Primary Antibody	台灣羅氏醫療診斷設備股份有限公司	VENTANA MEDICAL SYSTEMS, INC	R02 輸 入
2	衛署醫器輸字第024527號	112-01-30	羅氏抗人類表皮生長因子受體E746-A750 del(SP111)-兔子單株一級抗體	Anti-EGFR E746-A750 del (SP111) Rabbit Monoclonal Primary Antibody	台灣羅氏醫療診斷設備股份有限公司	VENTANA MEDICAL SYSTEMS, INC.	R02 輸 入
3	衛署醫器輸字第024529號	112-01-31	羅氏抗人類表皮生長因子受體L858R(SP125)-兔子單株一級抗體	anti-EGFR L858R(SP125) Rabbit Monoclonal Primary Antibody	台灣羅氏醫療診斷設備股份有限公司	VENTANA MEDICAL SYSTEMS, INC.	R02 輸 入
4	衛署醫器輸壹字第008741號	119-04-19	亞培艾西斯EGFR/CEP 7探針（未滅菌）	Abbott Vysis EGFR/CEP 7 FISH Probe Kit（Non-Sterile）	美商亞培股份有限公司台灣分公司	ABBOTT MOLECULAR INC.	R02 輸 入 R06 QMS/QSD
5	衛部醫器製字第004963號	114-11-03	台塑生醫EGFR基因突變檢測套組	Formosa EGFR Mutation Detection Kit	台塑生醫科技股份有限公司	台塑生醫科技股份有限公司宜蘭廠	R01 國 產
6	衛部醫器輸字第028935號	115-10-27	羅氏EGFR基因突變檢驗套組第二代	cobas EGFR Mutation Test v2	台灣羅氏醫療診斷設備股份有限公司	Roche Molecular Systems, Inc.	R02 輸 入
7	衛部醫器輸字第030485號	117-01-19	“凱杰” 表皮生長因子接受器擴增反應突變檢驗試劑組	“QIAGEN” therascreen EGFR RGQ PCR kit	凱杰生物科技有限公司	QIAGEN GMBH	R02 輸 入
8	衛部醫器輸字第033692號	114-10-22	埃迪拉 EGFR 基因突變檢測套組	Idylla EGFR Mutation Test	芮弗士科技有限公司	BIOCARTIS NV	R02 輸 入 R06 QMS/QSD
9	衛部醫器輸字第036209號	117-08-08	"凱杰" 表皮生長因子接受器擴增反應突變檢驗試劑組加強型	"QIAGEN" therascreen EGFR plus RGQ PCR Kit	凱杰生物科技有限公司	QIAGEN GMBH	R02 輸 入 R06 QMS/QSD
10	衛部醫器陸輸字第000668號	115-08-03	“麗賽生醫”人類EGFR基因突變檢測試劑盒(螢光PCR法)	AmoyDx EGFR 29 Mutations Detection Kit	麗賽生醫股份有限公司	Amoy Diagnostics Co., LTD.	R02 輸 入 R03 中國貨品

衛生福利部食品藥物管理署
藥品仿單查詢平台

2 適應症

Zelboraf可用於治療BRAF V600突變陽性且無法以手術切除或轉移性的成人黑色素瘤。

5.1.2 BRAF野生型黑色素瘤的腫瘤促進

在體外試驗已顯示BRAF野生型細胞暴露於BRAF抑制劑時，反而會活化MAP激酶的訊息傳遞及增加細胞增生。在開始使用Zelboraf治療前，需確認腫瘤檢體有BRAF V600E突變的證據。*[見適應症(2)及用法及用量(3)]*。

FDA U.S. FOOD & DRUG ADMINISTRATION

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use ZELBORAF safely and effectively. See full prescribing information for ZELBORAF.

ZELBORAF® (vemurafenib) tablet for oral use

Initial U.S. Approval: 2011

---------- INDICATIONS AND USAGE----------

- ZELBORAF® is a kinase inhibitor indicated for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E mutation as detected by an FDA-approved test. (1.1, 2.1)
- ZELBORAF® is indicated for the treatment of patients with Erdheim-Chester Disease with BRAF V600 mutation. (1.2, 2.1)

Limitation of Use: ZELBORAF is not indicated for treatment of patients with wild-type BRAF melanoma (2.1, 5.2)

----------DOSAGE AND ADMINISTRATION----------

- Confirm the presence of BRAF V600E mutation in tumor specimens prior to initiation of treatment with ZELBORAF. (2.1)
- Recommended dose: 960 mg orally twice daily taken approximately 12 hours apart with or without a meal. (2.2)

---------- DOSAGE FORMS AND STRENGTHS----------

Tablet: 240 mg (3)

---------- CONTRAINDICATIONS ----------

None

Safe and Effective

US FDA, FFDCA…

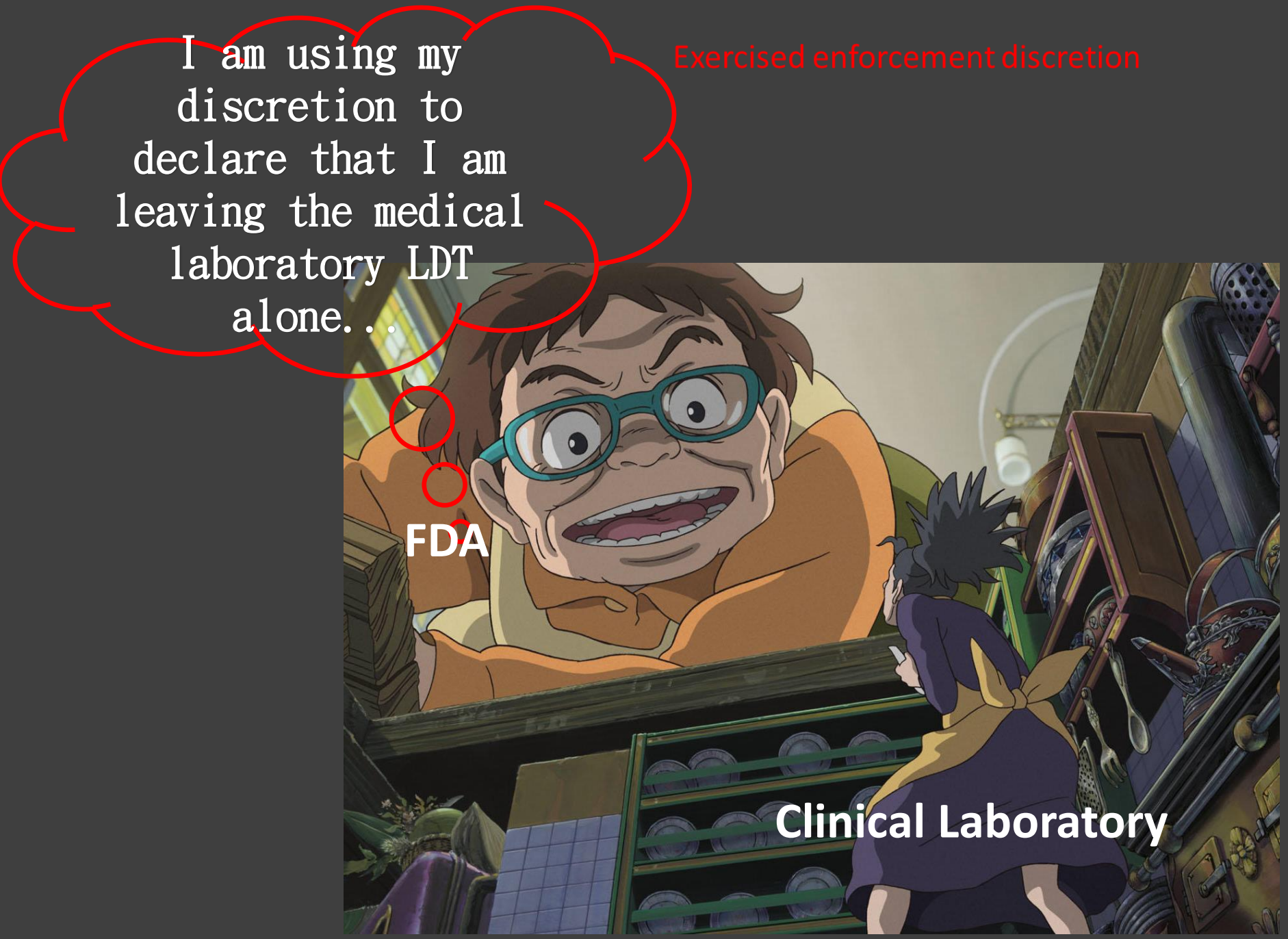
安全及有效

Accurate and Reliable

CMS, CLIA 88

正確及可靠

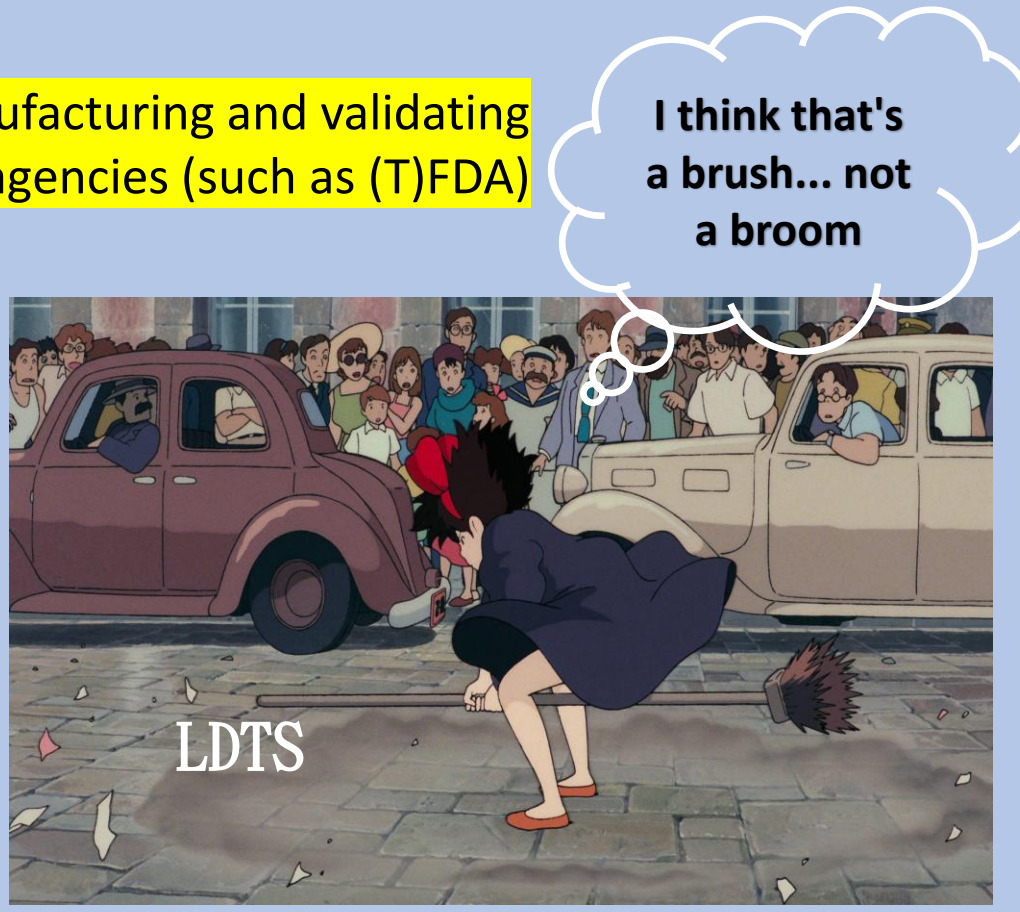
Exercised enforcement discretion



這個是法律，在美國的醫學實驗室
都需要 CLIA 認證

In Vitro Diagnostic Device

The manufacturer is responsible for manufacturing and validating
Approved for marketing by government agencies (such as (T)FDA)



Laboratory Developed Test
Laboratories are responsible for their own validation

IVD

CE IVD

d) Criteria that make a test an LDT

An LDT is not usually a self-developed product but rather a procedure that uses self-developed devices in the test sub-steps. However, it is not usually clear to the patients or the physicians treating them whether a test is carried out with an LDT or a CE-IVD device (see Figure 2).

以德國的醫學實驗室為例

用IVD，實驗室要負責查證 Verification

用LDT，實驗室要負責確證 Validation

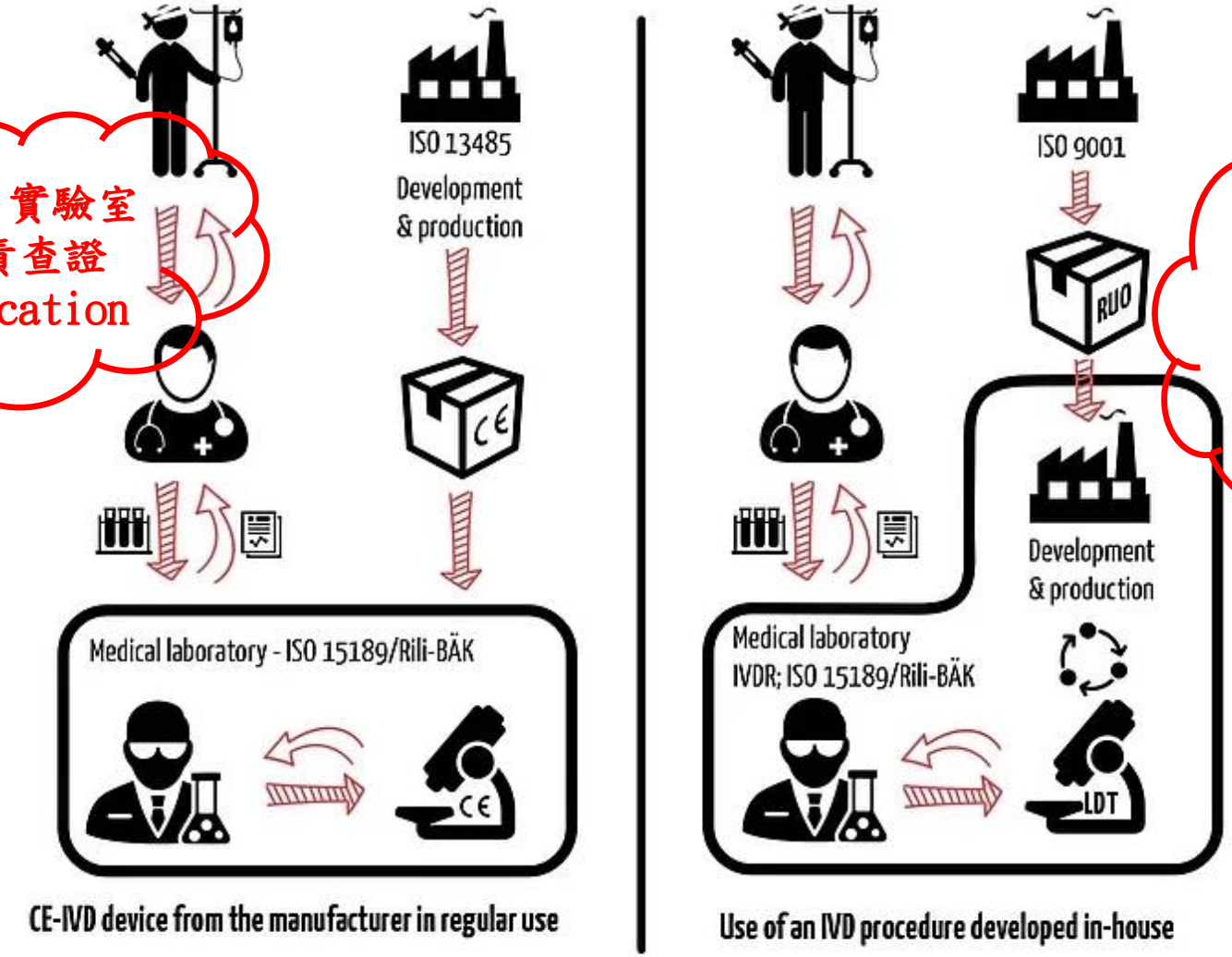
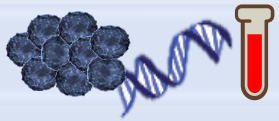


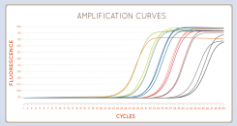
Fig. 3: Comparison of the use of CE-IVD devices and LDTs in medical laboratories

Different Methods of Assessing Pharmacogenetic Variants

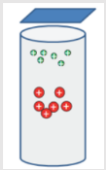


Extraction of DNA from patient sample

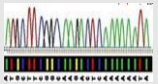
RT-PCR



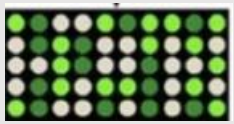
MALDI-TOF



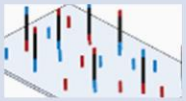
Sanger Sequencing PyroSequencing



Genotyping Array



NGS



+

- Cost-effective
- Short turnaround time
- Familiar lab work flow
- Distributable test kits/widely available
- Easy implementation for clinical diagnostics
- Lower sample input

−

- Can only detect a limited set of variants

+

- Analyzes multiple genes in a single assay
- Detects virtually all types of genomic DNA alterations, including single nucleotide variants, insertions and deletions, copy number changes, and chromosomal aberrations
- Enable identification of rare variants

−

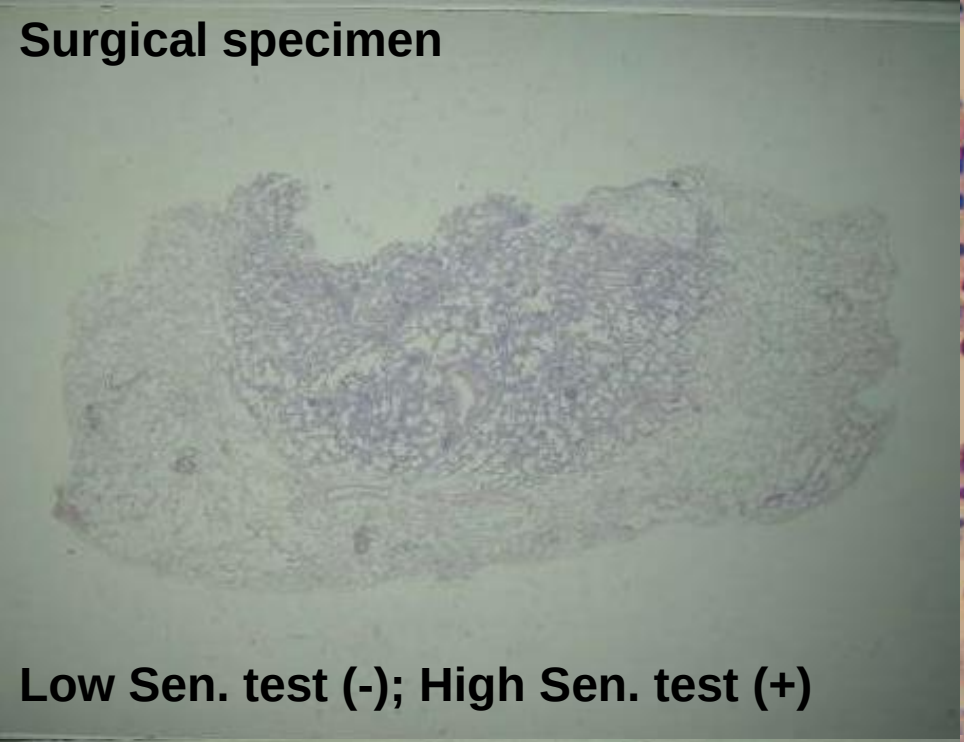
- Relatively expensive for sequencing low number of targets
- Relatively time-consuming

NGS, Next-Generation Sequencing; PCR, Polymerase chain reaction; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

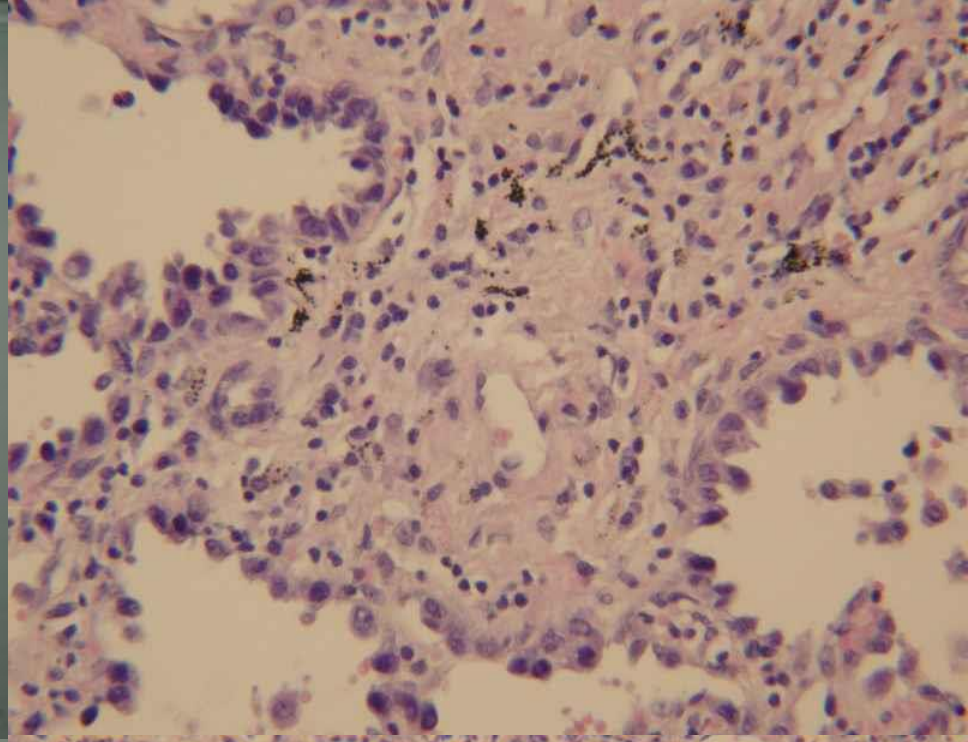
Problems from clinical specimen

Fresh/frozen specimen v.s. Formalin-fixed Paraffin-embedded (FFPE) tissue/cell block	• The fresh/frozen tissue is the best way to get DNA/RNA • However, clinically, it is not so realistic • FFPE: most of the clinical tests, real world
Cytology smear Blood: good DNA/RNA	• RNA from the mutated EGFR gene expression
Standard operation procedure (SOP)	• **over-fixation?** • The efficiency of PCR (cycle, DNA loading amount…)

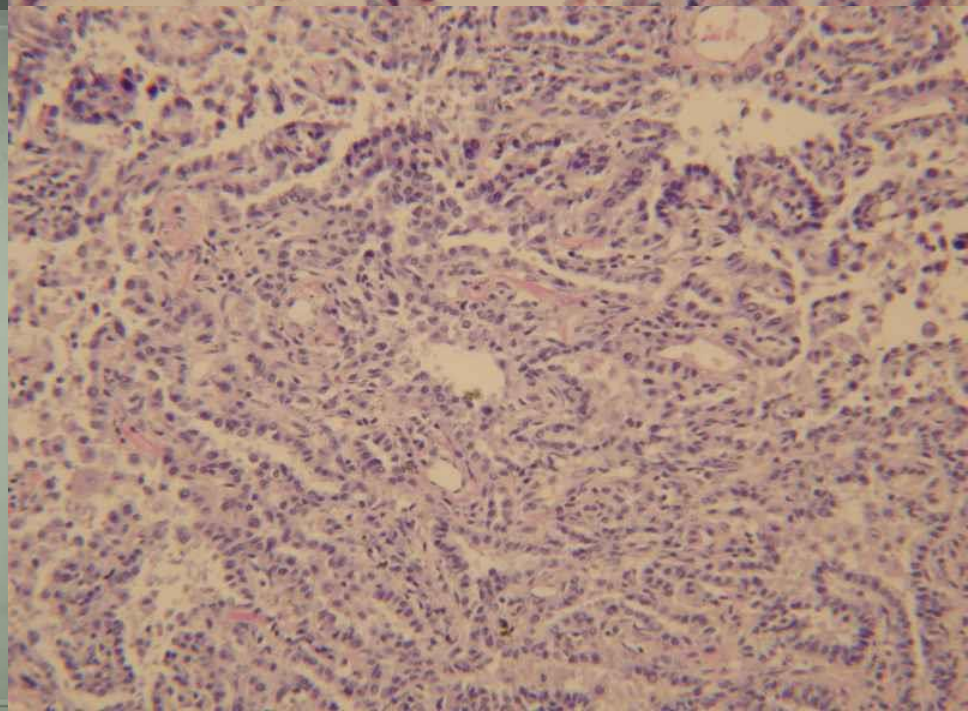
Surgical specimen



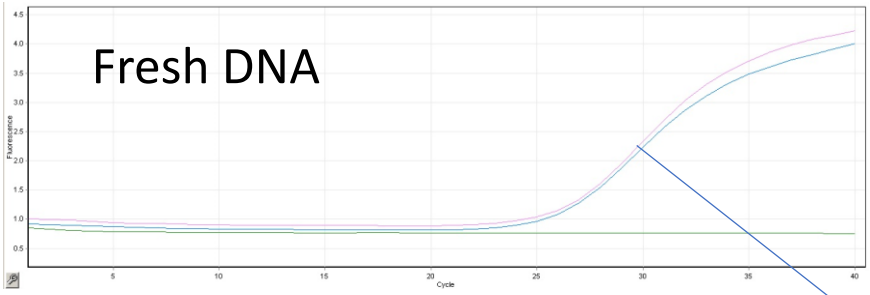
Low Sen. test (-); High Sen. test (+)

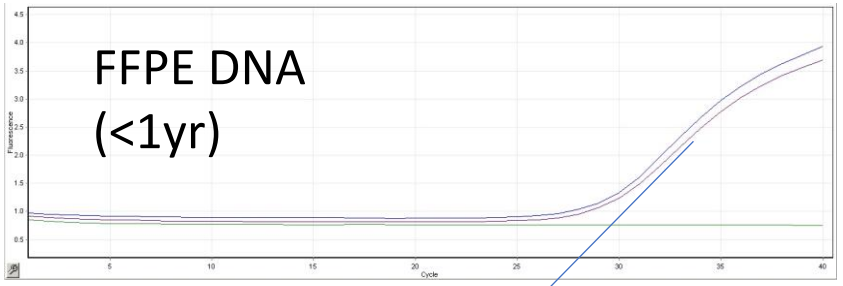


Low Sen. test (+); High Sen. test (+)

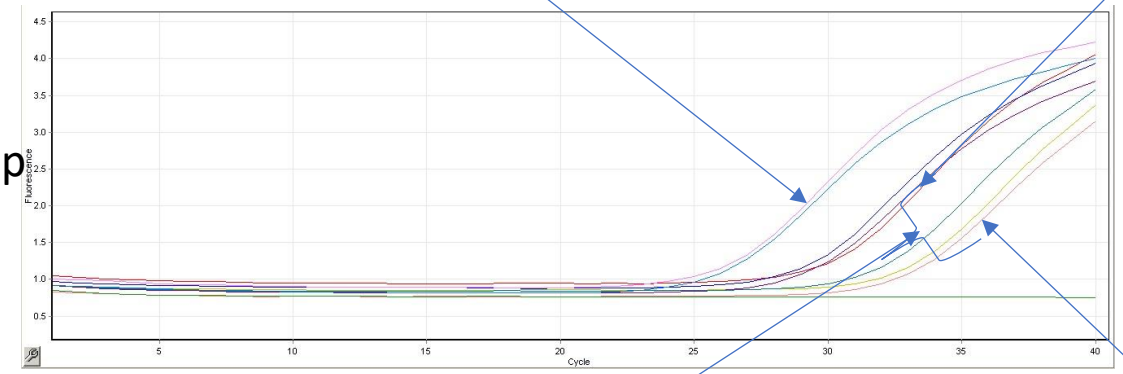


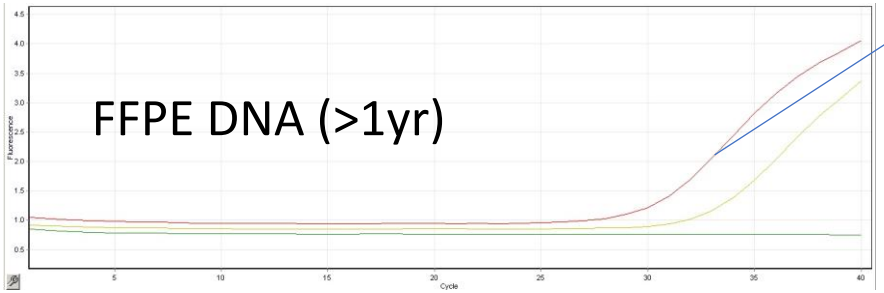
Amplifiability

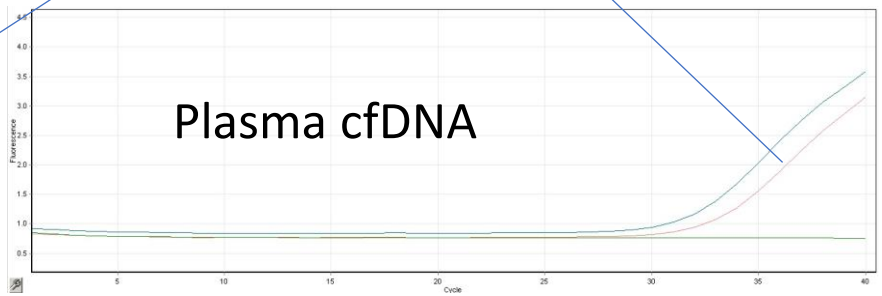




10ng each (by OD)
PCR product ~180bp







Data form Taipei Institute of Pathology

Summary of Methods

Table 1. Methods for detecting *EGFR* mutations in lung cancer specimens

Technique	Reference	Sensitivity (% mutant DNA)	Mutations identified	Comprehensive detection of deletions and insertions?
Direct sequencing	Multiple	25	Known and new	Yes
PCR-SSCP	(10)	10	Known and new	Yes
TaqMan PCR	(11, 12)	10	Known only	No
Loop-hybrid mobility shift assay	(13)	7.5	Known only	Yes
Cycleave PCR	(14)	5	Known only	Yes
PCR-RFLP and length analysis	(15)	5	Known only	Yes
MALDI-TOF MS–based genotyping	(16)	5	Known only	No
PNA-LNA PCR clamp	(17)	1	Known only	No
Scorpions ARMS	(18)	1	Known only	No
dHPLC	(19–21)	1	Known and new	Yes
Single-molecule sequencing	(22)	0.2	Known and new	Yes
Mutant-enriched PCR	(23)	0.2	Known only	No
SMAP	(8)	0.1	Known only	No

Abbreviations: SSCP, single-strand conformation polymorphism; PNA-LNA: peptide nucleic acid–locked nucleic acid; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; ARMS, amplified refractory mutation system; dHPLC, denaturing high performance liquid chromatography.

Genotyping Platform Comparison

	MassARRAY	qPCR	NGS (T-Seq)	microarray
Best SNP Throughput	10-500	1-10	1000-10000	1000-10000
Best Sample Throughput	8-768/run	1-384/run	8-96/run	24/96/run
TAT	24hr	4hr	48hr	144hr
Cost per Sample	Low	Low	High	High
Cost per SNP	Low	Mid	Mid	Low
SNP Assay	Flexible	Flexible	Flexible*	Fix
Assay Design	Fast and Low Cost	Fast but high cost	Slow and high cost	Slow and high cost

NTRK (<1%)

FDA approved
- Larotrectinib (VITRAKVI), Bayer
- Entrectinib (ROZLYTREK), Roche

- DS-6051b

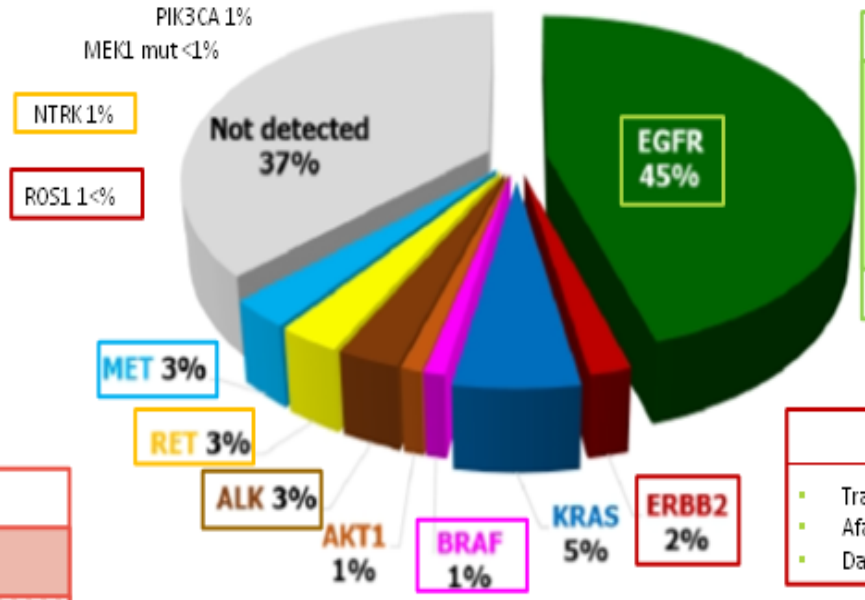
MET exon 14 skipping

Japan approved
- Tepotinib (Tepotinib), Merck
  2020/03/25

FDA approved
- Capmatinb (Temmetko), Novartis
  2020/05/06

RET fusion

FDA approved
- Selpercatinib (Retevmo), Phizer
  2020/05/08



EGFR mut

TFDA approved
- Gefitinib (Iressa), AZ
- Erlitinib (Tarceva), Roche
- Afatinib (Giotrif), BI
- Dacomitinib (Vizimpro) Pfizer
- Osimertinib (Tagrisso), AZ

- Rociletinib, Clovis

HER2
- Trastuzumab
- Afatinib
- Dacomitinib

ROS1 fusion

TFDA approved
- Crizotinib (XALKORI)

FDA approved
- Lorlatinib (LORVIQUA /LORBRENA), Pfizer
- Entrectinib (ROZLYTREK), Roche

- Ceritinib, Alectinib , Brigatinib, Cabozantinib, Repotrectinib, DS-6051b

ALK fusion

TFDA approved
- Crizotinib (XALKORI), Phizer
- Ceritinib (Zykadia), Nvartis
- Alectinib (ALECENSA), Chugai
- Brigatinib (Alunbrig), Takeda
- Lorlatinib (LORVIQUA /LORBRENA), Pfizer

BRAF	MEK

TFDA approved
- Dabrafenib (Tafinlar) + Trametinib (Mekinist), Novartis

- Vemurafenib (Zelboraf) + Cobimetinib (Cotellic), Roche
- Encorafenib (Braftovi) + Binimetinib (Mektovi), Pfizer

Figure adapted from 2020 Matsumoto S, et al.
ASCO#9605 LC-SCRUM-ASIA

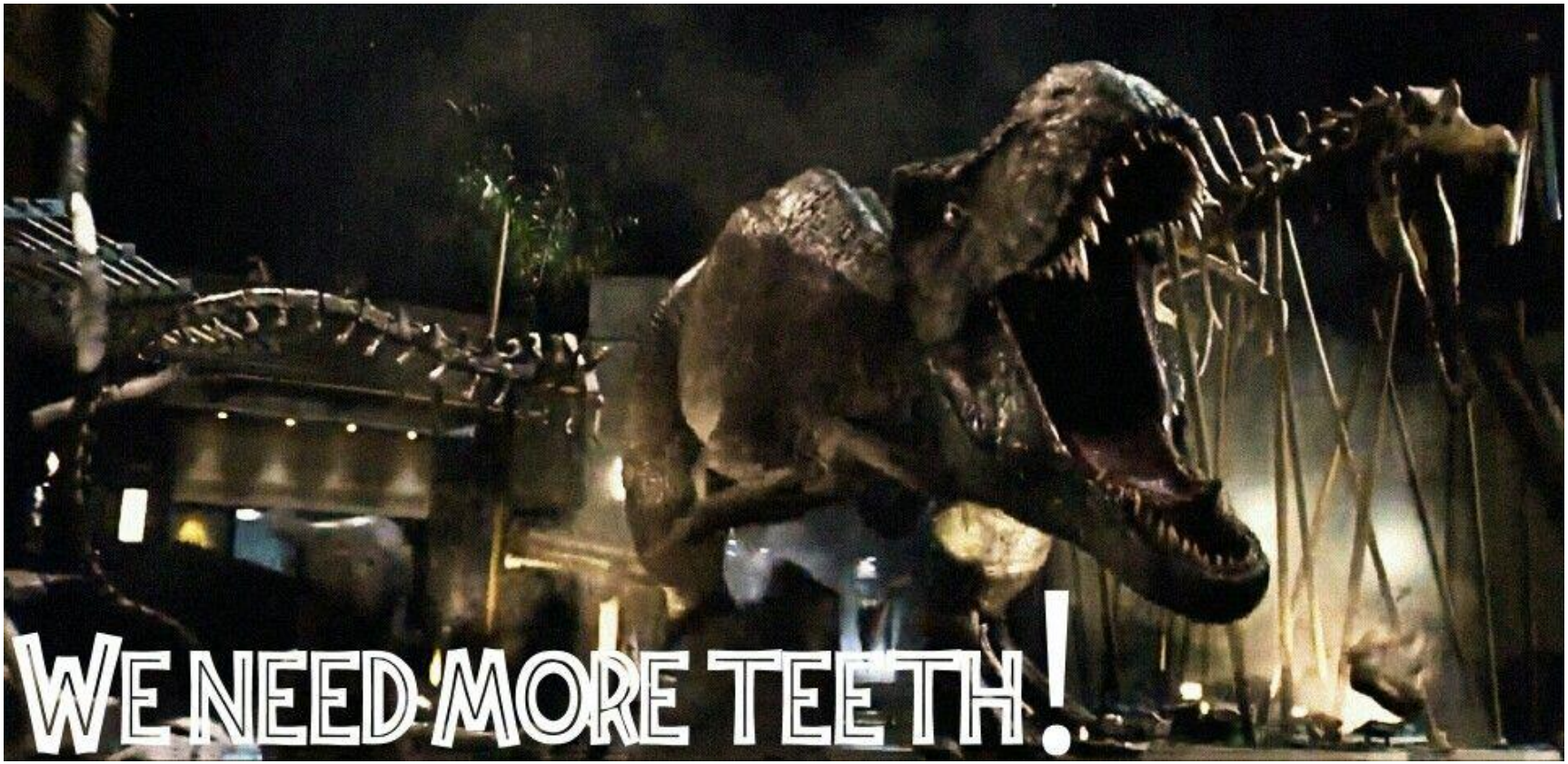
Table 2 Brigatinib activity against various *ALK* mutations

ALK mutation	Gainor et al, cancer discovery 2016[19]				Zhang et al, AACR 2015 abstract 781[26]		
	ALK phosphorylation mean IC_{50} (nmol/L)						
	Ceritinib	Alectinib	Brigatinib	Lorlatinib	Ceritinib	Alectinib	Brigatinib
EML4–ALK	5	11	11	2	37	25	14
C1156Y	5	12	5	5	195	67	45
I1171N	8	398	26	49	119	724	124
I1171S	4	177	18	30	ND	ND	ND
I1171T	4	34	6	12	ND	ND	ND
F1174C	38	27	18	8	109	31	58
F1174L	ND	ND	ND	ND	117	44	55
F1174V	ND	ND	ND	ND	121	46	64
V1180L	ND	ND	ND	ND	16	597	11
L1196M	9	118	27	34	67	133	41
L1198F	196	42	14	15	697	84	82
L1152R	ND	ND	ND	ND	437	62	11
L1152p	ND	ND	ND	ND	451	48	20
G1202R	124	707	130	50	354	690	184
G1202R del	50	59	96	5	ND	ND	ND
D1203N	35	28	35	11	159	42	79
E1210K	6	32	24	2	80	59	107
G1269A	0	25	ND	10	29	56	9
D1203N + F1174c	238	75	123	70	ND	ND	ND
D1203N + E1210K	98	83	136	27	ND	ND	ND
T1151Tins	ND	ND	ND	ND	283	201	114

ND = not done	IC_{50} <50	IC_{50} 50–200	IC_{50} >200

Notes: The in vitro activity of brigatinib is shown relative to the *ALK* inhibitors alectinib, ceritinib, and brigatinib. Results from two independent studies are summarized in this table.
Abbreviations: AACR, American Association of Cancer Research; ALK, anaplastic lymphoma kinase.

Next Generation Sequencing



Next Generation Sequencing

Current Generation Sequencing?

Lab ID: 1095

-Molecular Genetic Report for FGFR3 Gene-

PHYSICIAN	SPECIMEN		PATIENT	
Clinical Oncologist	Date Sample taken:	N/A	Name:	Danny TRAINOR
	Date received:	27/09/2024	Date of birth:	23/08/1958
	Report date:	08/11/2024	Patient ID:	N/A
	Hospital:	EQA Hospital	Gender:	Female
	Specimen #:	03.55171.034	Accession #:	AF24-009

Sample type: Section of artificial FFPE tumour, > 50 % neoplastic cells; no micro dissection performed.

Reason for request / clinical indication: Danny has unresectable metastatic urothelial cancer. She has been diagnosed with disease progression following treatment with chemotherapy and PD-L1 inhibitor therapy. Tissue from a biopsy has been provided for testing to determine suitability for treatment with FGFR inhibitor therapy.

Specimen Adequacy:

Percentage of neoplastic cells in the tissue section: > 50 %

Material used in the test:

DNA extracted from FFPE was tested.

Methodology:

Illumina MiSeq Next Generation Sequencing (NGS) platform

Reagent:

AmpliSeq for Illumina Focus Panel (Illumina, Inc.)-DNA part

NCBI Reference Sequence (GRCh37, hg19):

FGFR3: NM_ 000142.5

Detection Range:

DNA – Hotspot genes: AKT1, ALK, AR, BRAF, CDK4, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET, ROS1, SMO

DNA – CNV: ALK, AR, BRAF, CCND1, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, KIT, KRAS, MET, MYC, MYCN, PDGFRA, PIK3CA

RNA – Fusion drivers: ABL1, ALK, AKT3, AXL, BRAF, EGFR, ERBB2, ERG, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, MET, NTRK1, NTRK2, NTRK3, PDGFRA, PPARG, RAF1, RET, ROS1

Results:

PATHOGENIC VARIANT DETECTED

No.	Gene	Exon	Nucleotide Change	Amino Acid Change	Consequence	Classification
1.	FGFR3	7	c.746C>G	p.(Ser249Cys)	Missense_variant	Pathogenic

Quality Control Status (Percent On-Target Aligned Reads > 80 %) :

PASS

Interpretation:

Patient is likely to benefit from FGFR inhibitor therapy.

Recommendation:

The patient should be referred to clinical genetics and a physician to determine the appropriate therapeutic strategy.

OOOOOO INSTITUTE OF PATHOLOGY
Ampliseq for Illumina Focus Panel for Illumina NGS Test Report

Lab ID: 1095

-Molecular Genetic Report for FGFR3 Gene-

PHYSICIAN	SPECIMEN		PATIENT	
Clinical Oncologist	Date Sample taken:	N/A	Name:	Danny TRAINOR
	Date received:	27/09/2024	Date of birth:	23/08/1958
	Report date:	08/11/2024	Patient ID:	N/A
	Hospital:	EQA Hospital	Gender:	Female
	Specimen #:	03.55171.034	Accession #:	AF24-009

Limitation of the test:

1. Variant Allele Frequency (VAF) ≥ 5 %
2. Fusion Score cutoff ≥ 4
3. Copy Number ≥ 3

Notes:

The AmpliSeq for Illumina Focus Panel was developed for biomarker analysis in both DNA and RNA concurrently. The panel enables highly accurate mutation detection in 52 genes with known relevance to solid tumors, including lung, colon, breast, ovarian, melanoma, and prostate. This panel is designed for detection of target gene fusion, single nucleotide variation (SNVs), small insertions and deletions (small In/Dels, < 25 bp), and copy number variation (CNVs). All detected mutations in introns, untranslated regions, benign/likely benign variants, variants of uncertain significance, and polymorphisms are excluded, thus not reported in the results.

This test, performed in our laboratory, is accredited by Taiwan Accreditation Foundation (ISO15189).

Analysis Information and classification system:

The results were analyzed using the DNA Amplicon Workflow Version 3.24.1.14+develop. Relevant data sources for variant evidence are the Molecular Health Dataome (Molecular Health GmbH) and external databases listed below, e.g. GnomAD, COSMIC, dbSNP, and ClinVar. The variant classification according to the American College of Medical Genetics and Genomics (ACMG)[1], AMP/ASCO/CAP somatic validation guidelines[2], and CanVIG-UK Consensus Specification for Cancer Susceptibility Genes (CSGs) of ACGS Best Practice Guidelines for Variant Classification[3].

[1] Richards et al. (2015) Genetics in Medicine. 17(5): 405-24. (PMID: 25741868)
[2] Li, M. M et al. (2017) The Journal of Molecular Diagnostics. 19(1): 4-23. (PMID: 27993330)
[3] https://www.cangene-canvaruk.org/_files/ugd/ed948a_4a53f28bfddf4fe39fe9d0214bda9ad9.pdf

Technician: Pathologist:

病解專醫字第 000364 號

Results:

PATHOGENIC VARIANT DETECTED

No.	Gene	Exon	Nucleotide Change	Amino Acid Change	Consequence	Classification
1.	FGFR3	7	c.746C>G	p.(Ser249Cys)	Missense_variant	Pathogenic

Quality Control Status (Percent On-Target Aligned Reads > 80 %) :

PASS

Interpretation:

Patient is likely to benefit from FGFR inhibitor therapy.

Recommendation:

The patient should be referred to clinical genetics and a physician to determine the appropriate therapeutic strategy.

Taipei Institute of Pathology 台北病理中心

Patient name	VL23-004	Age	64
Patient ID	VL23-004	Ethnicity	CLM
Date of birth	20 Mar 1959	Sex	Female
		Diagnosis	NSCLC

Sample collection date	29 Mar 2023	Accession ID	TIP
Primary tumor site	Left lower lobe	Lab test name	VCF Archer VariantPlex CTL 31 genes TIP
Sample type	FFPE	Report date	10 May 2023

LAB TEST DESCRIPTION

Archer VariantPlex Comprehensive Thyroid & Lung Panel

The Archer VariantPlex Comprehensive Thyroid & Lung panel is a targeted next-generation sequencing (NGS) product to detect CNVs, SNVs and indels in 31 thyroid and lung cancer-implicated genes from DNA.

Gene targets:

AKT1, ALK, BRAF, CCND1, CTNNB1, DDR2, EGFR, EIF1AX, ERBB2, FGFR1, FGFR2, FGFR3, GNAS, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MDM2, MET, NRAS, PEGFRA, PIK3CA, PTEN, RET, ROS1, STK11, TERT, TP53, TSHR

RESULTS

This section provides the counts of clinically significant variants found in the patient sample. A variant is counted as positive if it has AMP score of IA to IID as the first criteria. As a second criteria variants with AMP score below IID and CVI score of 4 - 7 are considered as positive.

Clinically significant variants: **Positive**
Number of significant variants: 1

DETECTED VARIANTS

This section provides details on all detected variants matching the filter criteria. VAF = variant allele frequency.

SMALL VARIANTS

Gene	HGVS DNA	HGVS Protein	AMP score	Total reads	VAF
EGFR	c.2573T>G	p.L858R	Tier IA	234	31.20%
EGFR	c.2326C>T	p.R776C	—	97	23.71%

SUMMARY

Overview of potential treatment impacts

17 Effective | 0 Ineffective | 0 Safety

Overview of prognostic and diagnostic findings

0 Prognostic | 0 Diagnostic

Clinical trials found

172 Trials

Treatment	Potential impact	Drug approval	Trials	Biomarker	Biomarker score		VAF
Erlotinib Ramucirumab	Effective	Approved Approved	9	EGFR p.L858R (SNV)	AMP Tier I A	7 Clinically Approved	31.20%
Osimertinib	Effective	Approved	48	EGFR p.L858R (SNV)	AMP Tier I A	7 Clinically Approved	31.20%
Gefitinib	Effective	Approved	4	EGFR p.L858R (SNV)	AMP Tier I A	7 Clinically Approved	31.20%
Afatinib	Effective	Approved	4	EGFR p.L858R (SNV)	AMP Tier I A	7 Clinically Approved	31.20%
Erlotinib	Effective	Approved	4	EGFR p.L858R (SNV)	AMP Tier I A	7 Clinically Approved	31.20%
Dacomitinib	Effective	Approved	1	EGFR p.L858R (SNV)	AMP Tier I A	7 Clinically Approved	31.20%
Alflutinib	Effective	Investigational	12	EGFR p.L858R (SNV)	AMP Tier I B	6 Clinical	31.20%
Almonertinib	Effective	Investigational	7	EGFR p.L858R (SNV)	AMP Tier I B	6 Clinical	31.20%
Icotinib	Effective	Other	9	EGFR p.L858R (SNV)	AMP Tier I B	6 Clinical	31.20%
Nazartinib	Effective	Other	0	EGFR p.L858R (SNV)	AMP Tier II D	5 Clinical	31.20%
Met306	Effective	Other	0	EGFR p.L858R (SNV)	AMP Tier II D	5 Clinical	31.20%
Zorifertinib	Effective	Other	1	EGFR p.L858R (SNV)	AMP Tier II D	4 Clinical	31.20%

Taipei Institute of Pathology 台北病理中心

Patient name VL23-004
Patient ID VL23-004
Date of birth 20 Mar 1959

Age 64
Ethnicity CLM
Sex Female
Diagnosis NSCLC

Treatment	Potential impact	Drug approval	Trials	Biomarker	Biomarker score		VAF
Mobocertinib	Effective	Approved*	1	EGFR p.L858R (SNV)	AMP Tier II D	3 Preclinical	31.20%
Lazertinib	Effective	Investigational	7	EGFR p.L858R (SNV)	AMP Tier II D	3 Preclinical	31.20%
Sunvozertinib	Effective	Investigational	2	EGFR p.L858R (SNV)	AMP Tier II D	3 Preclinical	31.20%
Zipalertinib	Effective	Investigational	0	EGFR p.L858R (SNV)	AMP Tier II D	3 Preclinical	31.20%
Avitinib	Effective	Other	1	EGFR p.L858R (SNV)	AMP Tier II D	3 Preclinical	31.20%

* the drug is approved for the cancer type but either none of the currently approved biomarkers for this drug were identified, or an approved resistance biomarker for the drug was identified in this patient. Therefore, the drug label may not cover the analyzed patient; VAF = Variant allele frequency

Biomarker score: AMP score and CVI score. **Clinically approved:** Approved biomarker (by the FDA, EMA, or NCCN) to predict a specific effect in the patient's disease. **Clinical:** Not yet approved biomarker for the patient's disease. Observed in clinical studies as a potential biomarker to predict a specific effect of the drug. **Preclinical:** This biomarker has not yet been observed/tested in patients to predict a specific effect of the drug. It is supported by preclinical evidence or translational data.

You can find more details on the biomarker score (AMP and CVI score) in the glossary.

Taipei Institute of Pathology 台北病理中心

Patient name	VL23-004	**Age**	64
Patient ID	VL23-004	**Ethnicity**	CLM
Date of birth	20 Mar 1959	**Sex**	Female
		Diagnosis	NSCLC

DESCRIPTION KEY

- Potentially effective treatments. These treatment recommendations are based solely on tumor biology and do not override your oncologist's clinical treatment plan.
- Potentially ineffective treatments. These treatments, in combination with the biomarkers identified in the patient tumor, have been reported to predict lack of effectiveness. Treatment of a patient with any of these reported drugs may lead to disease progression.
- Treatments with potential to cause an adverse reaction. These treatments, in combination with the biomarkers identified in the patient tumor, have been reported to predict safety issues. Treatment of a patient with any of these reported drugs may lead to serious drug-related toxicities.
- Biomarkers identified in the patient tumor that have been reported to have a prognostic relevance.
- Biomarkers identified in the patient tumor that have been reported to have a diagnostic relevance.
- ⚠ The report contains conflicting evidence about the potential effect of the treatment.

Potential impact	Treatment	Drug approval	Biomarker score
Effective	Gefitinib	Approved	AMP Tier I A · 7 Clinically Approved
Effective	Afatinib	Approved	AMP Tier I A · 7 Clinically Approved
Effective	Erlotinib	Approved	AMP Tier I A · 7 Clinically Approved
Effective	Dacomitinib	Approved	AMP Tier I A · 7 Clinically Approved
Effective	Osimertinib	Approved	AMP Tier I A · 7 Clinically Approved
Effective	Erlotinib + Ramucirumab	Approved, Approved	AMP Tier I A · 7 Clinically Approved
Effective	Alflutinib	Investigational	AMP Tier I B · 6 Clinical
Effective	Almonertinib	Investigational	AMP Tier I B · 6 Clinical

AMP score:
Displays the classification of a biomarker according to the recommendations of the Association for Molecular Pathology (AMP). Source: Marilyn M. Li, Michael Datto, Eric J. Duncavage, Shashikant Kulkarni, Neal I. Lindeman, Somak Roy, Apostolia M. Tsimberidou, Cindy L. Vnencak-Jones, Daynna J. Wolff, Anas Younes, and Marina N. Nikiforova "Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer," Journal of Molecular Diagnostics, vol. 19, no. 1, pp. 4-23 ,2017, doi: 10.1016/j.jmoldx.2016.10.002.

- Tier IA: Variants of strong clinical significance. FDA-approved therapy or biomarkers included in professional guidelines.
- Tier IB: Variants of strong clinical significance. Well-powered studies with consensus from experts in the field.
- Tier IIC: Variants of potential clinical significance. FDA-approved therapies for different cancer types or investigational therapies. Multiple small published studies with some consensus.
- Tier IID: Variants of potential clinical significance. Preclinical trials or a few case reports without consensus.
- Tier III: Variants of unknown clinical significance.
- Tier IV: Benign or likely benign variants.

Taipei Institute of Pathology 台北病理中心

Patient name	VL23-004	Age	64
Patient ID	VL23-004	Ethnicity	CLM
Date of birth	20 Mar 1959	Sex	Female
		Diagnosis	NSCLC

MEDICAL GUIDELINES

Relevant treatment information from medical guidelines and potential impact based on detected biomarkers

Potential impact	Treatment	Disease	Disease details	Guideline	Evidence Level
Effective	Afatinib	Non-Small Cell Lung Cancer	Squamous cell carcinoma, Adenocarcinoma (with mixed subtypes), Large cell carcinoma	NCCN	1 for first-line therapy if EGFR mutation discovered prior to first-line systemic therapy 2A for all others 2B for locoregional recurrence or symptomatic local disease (excluding mediastinal lymph node recurrence with prior radiation therapy) with no evidence of disseminated disease

Recommended use:
Single-agent therapy for EGFR exon 19 deletion or exon 21 L858R recurrent, advanced, or metastatic disease as

- first-line therapy
- continuation of therapy following disease progression on afatinib for asymptomatic disease, symptomatic brain lesions, or symptomatic systemic limited progression (if T790M negative)

Effective	Afatinib	Non-Small Cell Lung Cancer	Squamous cell carcinoma,	NCCN	2A for all others

Taipei Institute of Pathology 台北病理中心

Patient name	VL23-004	Age	64
Patient ID	VL23-004	Ethnicity	CLM
Date of birth	20 Mar 1959	Sex	Female
		Diagnosis	NSCLC

CLINICAL TRIALS

The following trials are potentially best suited for your patient's indication, considering all reported treatment recommendations. See https://clinicaltrials.gov (clinical trials from NCT) or https://trialsearch.who.int (clinical trials from other registries) for more information.

Clinical trials in total: 172 **Trial country:** AU, CN, DE, ES, FR, GB, HK, IT, JP, KR, SG, TW, US

Title	Phase and ID	Intervention	Disease	Age and sex
A Study of Amivantamab and Lazertinib in People With Non-Small Cell Lung Cancer (NSCLC)	Phase 2; NCT04965090	Lazertinib	Carcinoma, Non-Small-Cell Lung	
Status: Recruiting **Location: US**-7 locations **Matching trial biomarker(s): Inclusion:** EGFR activating mutation **Stratification:** EGFR sensitizing mutation **Patient biomarkers:** EGFR p.L858R, EGFR p.R776C				
Osimertinib Alone or With Chemotherapy for EGFR-Mutant Lung Cancers	Phase 2; NCT04410796	Osimertinib	Carcinoma, Non-Small-Cell Lung	
Status: Recruiting **Location: US**-13 locations **Matching trial biomarker(s): Inclusion:** EGFR activating mutation **Stratification:** EGFR exon_19.X, EGFR p.L858R **Patient biomarkers:** EGFR p.L858R, EGFR p.R776C				
Study of Osimertinib in Patients With a Lung Cancer With Brain or Leptomeningeal Metastases With EGFR Mutation (ORBITAL)	Phase 2; NCT04233021	Osimertinib	Carcinoma, Non-Small-Cell Lung	
Status: Recruiting **Location: FR**-34 locations **Matching trial biomarker(s): Inclusion:** EGFR p.L858R **Stratification:** EGFR any mutation **Patient biomarkers:** EGFR p.L858R, EGFR p.R776C				

CVI score:

The clinical variant interpretation (CVI) scores 7-1 indicate the reliability of a biomarker to predict a specific patient outcome. This can include predictive treatment effects; in this case, the scores 7-1 apply for biomarkers associated with a single drug or drug combination.

The CVI scores are defined as follows:

7, Clinically approved: The biomarker has been approved by a regulatory agency such as the FDA to predict a specific effect (i.e., response, resistance, or toxicity) in the patient's disease or cancer type.

6, Clinical: Patient's disease: The biomarker has not yet been approved by a regulatory agency for the patient's disease. However, the biomarker has been observed in at least one large cohort study to predict a specific effect of the drug (i.e., to be effective, resistance) in the patient's disease. Other diseases: The biomarker has been approved by a regulatory agency to predict a specific effect of the drug (response, resistance) with other diseases or conditions. This CVI will be available for matching with the less-specific disease Neoplasms in CVIs. Biomarkers predicting toxicity: For all disease matches, this score indicates that there is evidence from a randomized controlled trial or its meta-analysis for biomarkers predicting a drug to be toxic.

5, Clinical: The biomarker has not yet been approved by a regulatory agency for the patient's disease. However, this biomarker has been observed to predict a specific effect of the drug (i.e., response, resistance) on patients with other diseases or conditions. For biomarkers predicting a drug to be effective or resistant, there is evidence from some patients in several cohort studies and additional preclinical evidence. For biomarkers predicting a drug to be toxic, there is evidence from >1 prospective studies or meta-analyses from prospective and/or retrospective studies.

4, Clinical: The biomarker has not yet been approved by a regulatory agency for the patient's disease. However, this biomarker has been observed to predict a specific effect of the drug (i.e., response, resistance) on patients with other diseases or conditions. For biomarkers predicting a drug to be effective or resistant, there is evidence from a few clinical case reports and additional preclinical evidence. For biomarkers predicting a drug to be toxic, there is evidence from a prospective study, >1 retrospective studies, or >1 cohort studies.

3, Preclinical: The biomarker has not yet been observed/tested in patients to predict a specific effect. The biomarker has been observed in preclinical experiments. There is experimental evidence from cell lines or mouse models, for example.

2, Preclinical: The biomarker has not yet been observed/tested in patients or preclinical models to predict a specific effect. However, this effect can be inferred when drug-sensitivity data are available for another variant. This applies only if the two variants have the identical functional impact on the same downstream pathway.

1, Preclinical: The biomarker has not yet been observed/tested in patients or preclinical models to predict a specific effect. However, this effect can be inferred when drug-sensitivity data are available for another variant. This applies only if both variants have the identical functional impact on the protein.

Drug approval:
The development stage of the treatment for the patient's indication in the patient's country.

- **Approved** - This drug is launched for the primary or a secondary patient disease.
- **Off-label** - This drug is launched for a disease other than the primary or secondary patient diseases.
- **Investigational** - This drug is currently under clinical development in the patient disease.
- **Other** - None of the other stages are applicable. The drug is, for example, suspended, discontinued, or withdrawn. Other is also used for the drug approval stage of drug classes.

Drug-drug interactions:
A drug-drug interaction is a situation in which a substance (usually another drug) affects the activity of one or both drugs when both are administered together. In the MH Guide report, drug-drug interactions are reported where a drug is predicted to affect the activity of the agent(s) in the treatment option.

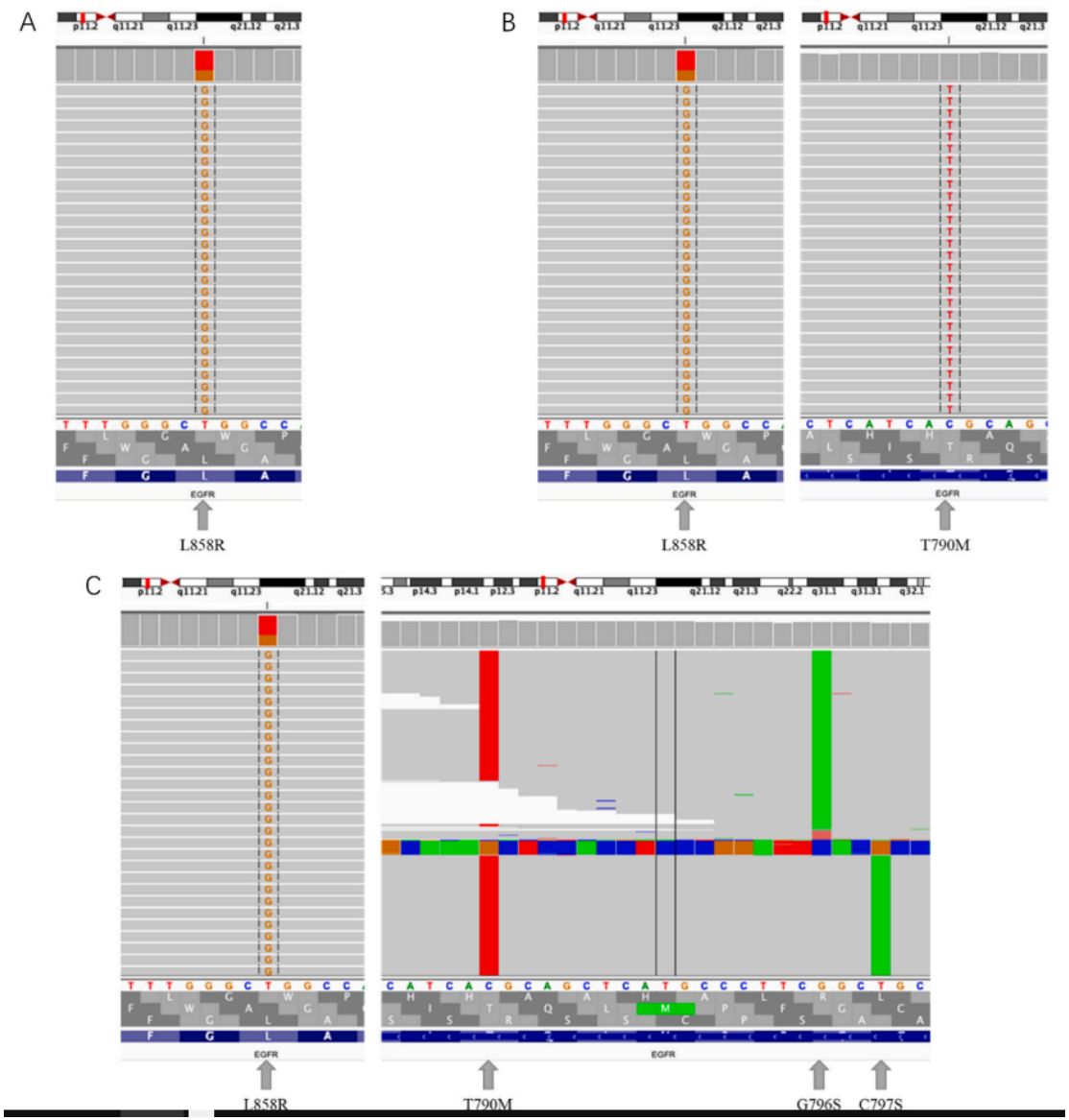
Medications with potential for adverse reaction or ineffectiveness.:
Medications with potential for adverse reaction or ineffectiveness refers to Molecular Health's ability to identify treatments that are predicted to be associated with negative physiological responses to a drug therapy (i.e., drug resistance and toxicity).

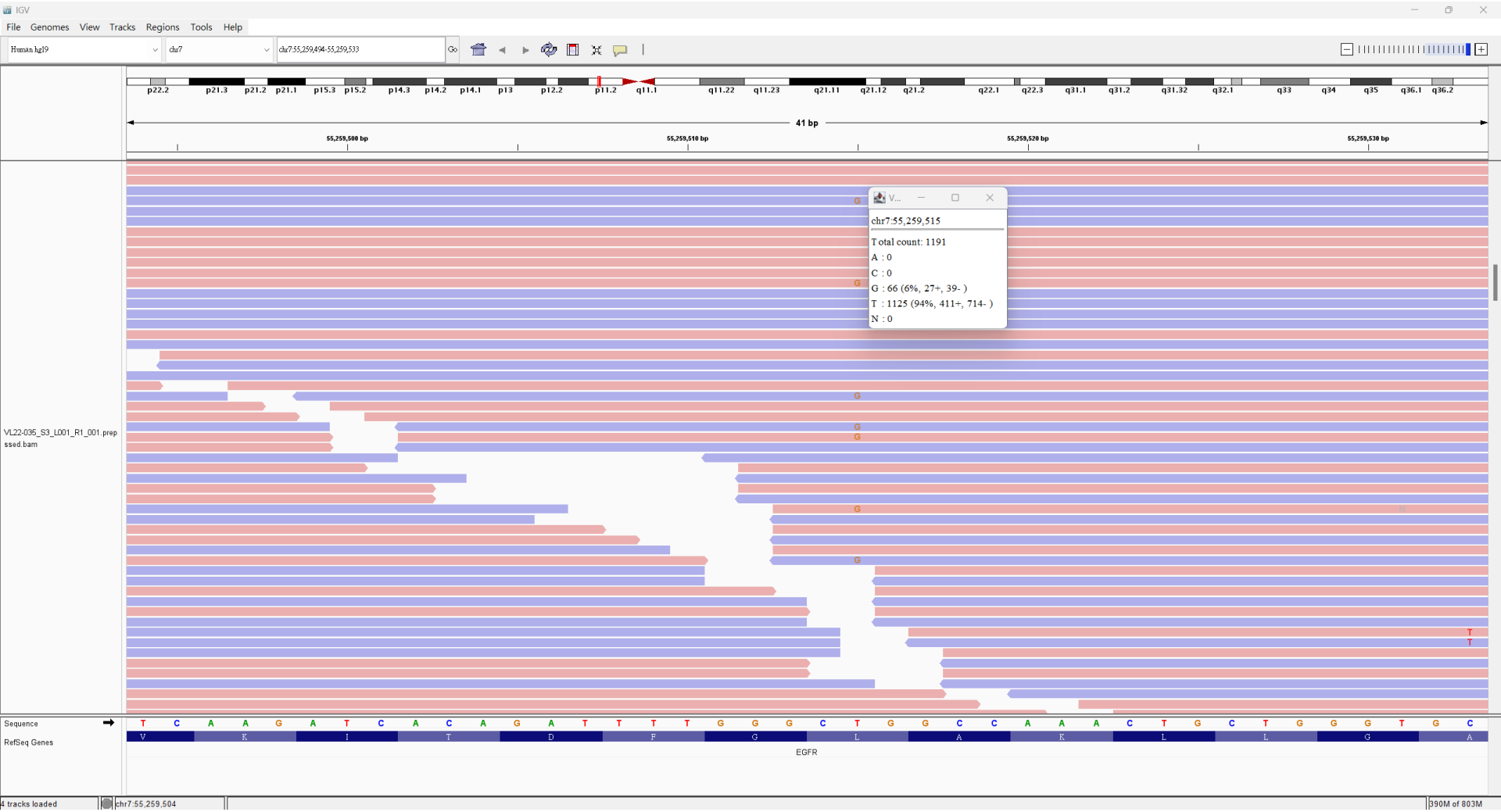
Open trials:
Clinical trials that are currently recruiting patients with specific disease indication(s) to assess the clinical efficacy and safety of the listed treatment.

Potential impact:
The specific drug effect predicted by the identified mutation (i.e., response, resistance, or toxicity).

PubMed ID:
A PubMed identifier is a unique number assigned to each PubMed record - also termed PMID. A PMID can be used to retrieve a specific publication from the PubMed database by entering the PMID in the search box on the PubMed site at http://www.ncbi.nlm.nih.gov/pubmed.

Treatment:
The generic name of the therapeutic agent listed on the report.


A
B
C
EGFR
L858R
T790M
G796S
C797S


IGV
File Genomes View Tracks Regions Tools Help
Human hg19
chr7
chr7:55,259,494-55,259,533
Go
p22.2 p21.3 p21.2 p21.1 p15.3 p15.2 p14.3 p14.2 p14.1 p13 p12.2 p11.2 q11.1 q11.22 q11.23 q21.11 q21.12 q21.2 q22.1 q22.3 q31.1 q31.2 q31.32 q32.1 q33 q34 q35 q36.1 q36.2
41 bp
55,259,500 bp
55,259,510 bp
55,259,520 bp
55,259,530 bp
chr7:55,259,515
Total count: 1191
A : 0
C : 0
G : 66 (6%, 27+, 39-)
T : 1125 (94%, 411+, 714-)
N : 0
VL22-035_S3_L001_R1_001.prepssed.bam
Sequence
RefSeq Genes
EGFR
4 tracks loaded
chr7:55,259,504
390M of 803M


IGV
File Genomes View Tracks Regions Tools Help
Human hg19
chr7
chr7:55,259,494-55,259,533
p22.2 p21.3 p21.2 p21.1 p15.3 p15.2 p14.3 p14.2 p14.1 p13 p12.2 p11.2 q11.1 q11.22 q11.23 q21.11 q21.12 q21.2 q22.1 q22.3 q31.1 q31.2 q31.32 q32.1 q33 q34 q35 q36.1 q36.2
41 bp
55,259,500 bp
55,259,510 bp
55,259,520 bp
55,259,530 bp
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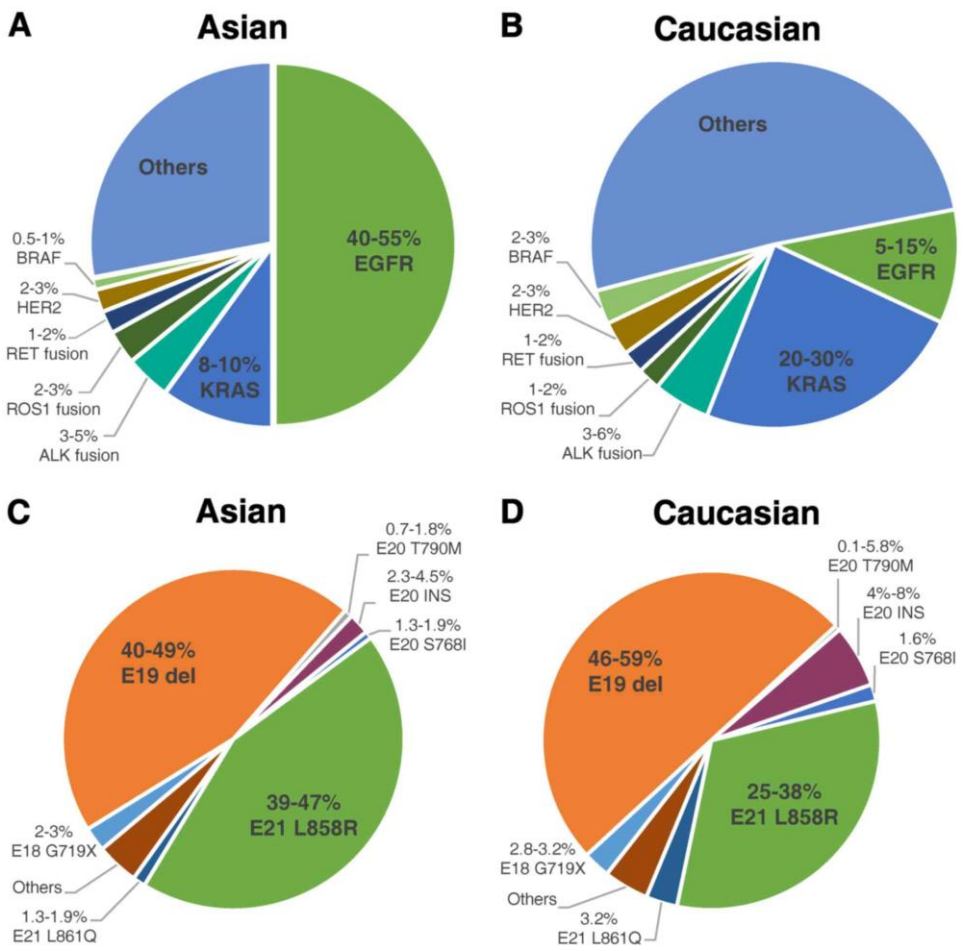
Total count: 1191
A : 0
C : 0
G : 66 (6%, 27+, 39-)
T : 1125 (94%, 411+, 714-)
N : 0
VL22-035_S3_L001_R1_001.prepssed.bam
Sequence
RefSeq Genes
EGFR

諮詢、抱怨的受理與解決

EGFR mutation frequency and distribution in Asian and Caucasian populations

- Around 50 (40-55)% of Asian lung adenocarcinoma patients.
  - Exon 19 deletion: around 45 (40-49)% of EGFR mutations.

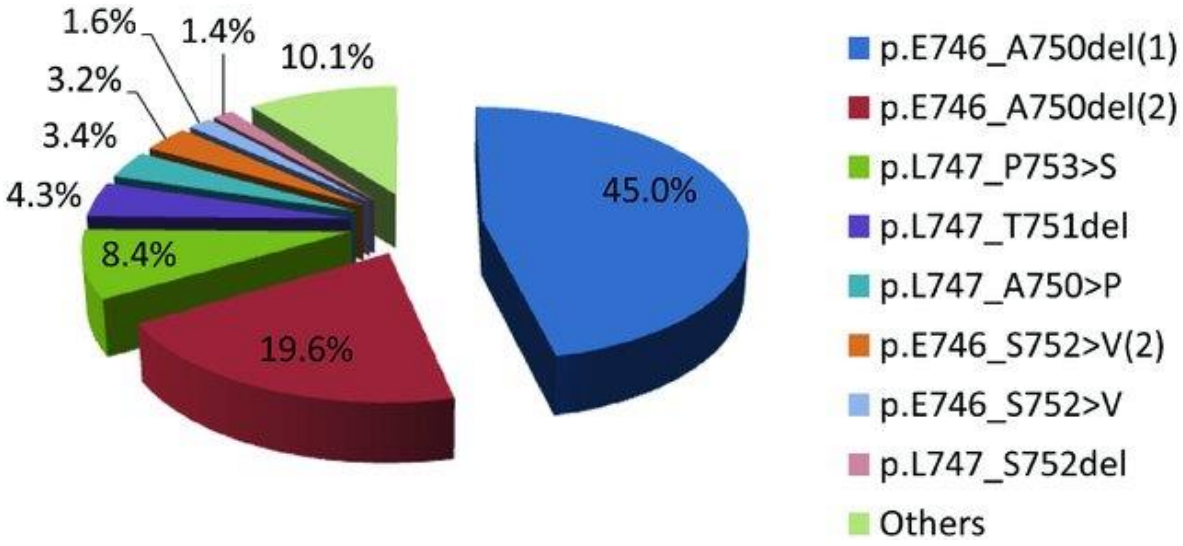
- 50% X 45% = 22.5%
  - 每 100 個亞洲肺腺癌病人中，約 23 人有 EGFR exon 19 deletion 突變。



ONCOLOGY REPORTS 37: 1347-1358, 2017

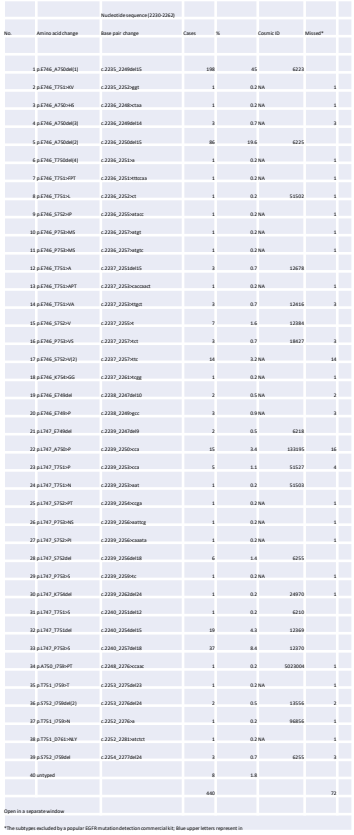
EGFR exon 19 deletion subtypes

- Exon 19 deletion: more than 39 subtypes report.



Oncotarget, 2017, Vol. 8, (No. 67), pp: 111246-111257

EGFR mutation test and Exon 19 deletion



		Nucleotide sequence (2230-2262)				
No.	Amino acid change	Base pair change	Cases	%	Cosmic ID	Missed*
1	p.E746_A750del(1)	c.2235_2249del15	198	45	6223	
2	p.E746_T751>V	c.2235_2252>gt	1	0.2	NA	1
3	p.E746_A750>G	c.2236_2248>tcca	1	0.2	NA	1
4	p.E746_A750del(2)	c.2236_2248del14	3	0.7	NA	3
5	p.E746_A750del(2)	c.2236_2250del15	86	19.6	6225	
6	p.E746_T751del(4)	c.2236_2251>a	1	0.2	NA	1
7	p.E746_T751>VPT	c.2236_2251>ttccaa	1	0.2	NA	1
8	p.E746_T751>L	c.2236_2252>t	1	0.2	51502	1
9	p.E746_S752>P	c.2236_2255>atacc	1	0.2	NA	1
10	p.E746_P753>MS	c.2236_2257>atgt	1	0.2	NA	1
11	p.E746_P753>MS	c.2236_2257>atgcc	1	0.2	NA	1
12	p.E746_T751>A	c.2237_2251del15	3	0.7	12678	
13	p.E746_T751>APT	c.2237_2252>accaact	1	0.2	NA	1
14	p.E746_T751>VA	c.2237_2252>tgct	3	0.7	12416	3
15	p.E746_S752>V	c.2237_2255>t	7	1.6	12384	
16	p.E746_P753>VS	c.2237_2257>tct	3	0.7	18627	3
17	p.E746_S752>I(2)	c.2237_2257>tc	14	3.2	NA	14
18	p.E746_K754>GG	c.2237_2261>cgg	1	0.2	NA	1
19	p.E746_E749del	c.2238_2247del10	2	0.5	NA	2
20	p.E746_E749>P	c.2238_2249>gcc	3	0.9	NA	3
21	p.L747_E749del	c.2239_2247del9	2	0.5	6218	
22	p.L747_A750>P	c.2239_2250>cca	15	3.4	133195	15
23	p.L747_T751>P	c.2239_2252>cca	5	1.1	53127	4
24	p.L747_T751>N	c.2239_2252>aac	1	0.2	51503	
25	p.L747_S752>PT	c.2239_2256>ccga	1	0.2	NA	1
26	p.L747_P753>MS	c.2239_2256>atttg	1	0.2	NA	1
27	p.L747_S752>N	c.2239_2256>caaata	1	0.2	NA	1
28	p.L747_S752del	c.2239_2256del18	6	1.4	6255	
29	p.L747_P753>S	c.2239_2259>c	1	0.2	NA	1
30	p.L747_K754del	c.2239_2262del24	1	0.2	24970	1
31	p.L747_T751>S	c.2240_2251del12	1	0.2	6210	
32	p.L747_T751del	c.2240_2254del15	19	4.3	12369	
33	p.L747_P753>S	c.2240_2257del18	37	8.4	12370	
34	p.A750_P753>PT	c.2248_2276>ccaac	1	0.2	5023008	1
35	p.T751_I759>T	c.2253_2275del23	1	0.2	NA	1
36	p.S752_I759del(2)	c.2253_2276del24	2	0.5	13556	2
37	p.T751_I759>N	c.2252_2276>a	1	0.2	96856	1
38	p.T751_D761>MEY	c.2252_2281>atctct	1	0.2	NA	1
39	p.S752_I759del	c.2254_2277del24	3	0.7	6255	3
40	untyped		8	1.8		
			440			72

Open in a separate window

*The subtypes excluded by a popular EGFR mutation detection commercial kit; Blue upper letters represent in

這篇文獻440個 Exon 19 deletion cases 中，才看到一個 exon 19 T751-I759 deletion，屬於罕見突變

Nucleotide sequence (2230-2262)								
No.	**Amino acid change**	**Base pair change**	**atc aag gaa tta aga gaa gca aca tct ccg aaa gcc aac aag gaa atc ctc gat gaa gcc**	**Cases**	**%**	**Cosmic ID**	**Missed***
			I K E L R E A T S P K A N K E I L D E A				
37	p.T751_I759>N	c.2252_2276>a	atc aag gaa tta aga gaa gca aca tct ccg aaa gcc aac aag gaa atAc ctc gat gaa gcc	1	0.2	96856	1

而這個突變位點，在我們常用的 ClinVAR 資料庫中還查不到。
目前只有這篇文獻報告過一個case，實屬於罕見突變

Oncotarget, 2017, Vol. 8, (No. 67), pp: 111246-111257

EGFR mutation test and Exon 19 deletion

- 現行之IVD KIT，羅氏與 Idylla 兩家，偵測範圍中包含 29 種 exon 19 Deletion；Qiagen偵測範圍中包含 18 種 exon 19 Deletion。
- 以上三家 IVD KIT 都未包含 exon 19 T751-I759 deletion。

- 參考文獻*在 440 個 Exon 19 deletion cases 中，才看到一個 (0.2%) exon 19 T751-I759 deletion，屬於罕見突變。

- 50% X 45% = 22.5%
  - 每 100 個亞洲肺腺癌病人中，約 23 人有 EGFR exon 19 deletion 突變。
  - 22.5% X 0.2% = 0.045%，約 1 萬個亞洲肺腺癌病人中，才有約5 個 exon 19 T751-I759 deletion。非常罕見。
- 因為 exon 19 T751-I759 deletion 為非常罕見的突變，因此通常不會在 PCR-based test 試劑的設計中。除非使用 NGS，否則不可能被發現。

*Oncotarget, 2017, Vol. 8, (No. 67), pp: 111246-111257

EGFR exon 19 deletion subtypes

- 敝中心使用之現行羅氏IVD kit，有 29 種 exon 19 Del；有IVD mark 及 clinical trial，具有臨床意義。其偵測範圍(見右表)
- Exon 19 T751_I759 del 沒有在羅氏 IVD kit 偵測範圍內。
- 查其他 TFDA許可之 IVD kit: QIAGEN (18 種 19Del偵測範圍)，及 Idylla (29 種 19Del 偵測範圍)，也都沒有T751_I759 del。
- 因為 exon 19 T751-I759 deletion 為非常罕見的突變，因此通常不會在PCR-based test 的試劑設計中。
- 罕見突變位點除非使用 NGS，否則不可能被發現。

Table 3 The cobas EGFR Test is designed to detect the following mutations

Exon	EGFR Mutation Group	EGFR Nucleic Acid Sequence	HGVS* Protein Nomenclature	HGVS* Nucleotide Nomenclature	COSMIC ID[14]
Exon 18	G719X	2156G>C	LRG_304p1:p.(Gly719Ala)	LRG_304t1:c.2156G>C	6239
		2155G>A	LRG_304p1:p.(Gly719Ser)	LRG_304t1:c.2155G>A	6252
		2155G>T	LRG_304p1:p.(Gly719Cys)	LRG_304t1:c.2155G>T	6253
Exon 19	Ex19Del	2240_2251del12	LRG_304p1:p.(Leu747_Thr751delinsSer)	LRG_304t1:c.2240_2251delTAAGAGAAGCAA	6210
		2239_2247del9	LRG_304p1:p.(Leu747_Glu749del)	LRG_304t1:c.2239_2247delTTAAGAGAA	6218
		2238_2255del18	LRG_304p1:p.(Glu746_Ser752delinsAsp)	LRG_304t1:c.2238_2255delATTAAGAGAAGCAACATC	6220
		2235_2249del15	LRG_304p1:p.(Glu746_Ala750del)	LRG_304t1:c.2235_2249delGGAATTAAGAGAAGC	6223
		2236_2250del15	LRG_304p1:p.(Glu746_Ala750del)	LRG_304t1:c.2236_2250delGAATTAAGAGAAGCA	6225
		2239_2253del15	LRG_304p1:p.(Leu747_Thr751del)	LRG_304t1:c.2240_2254delTAAGAGAAGCAACAT	6254
		2239_2256del18	LRG_304p1:p.(Leu747_Ser752del)	LRG_304t1:c.2239_2256delTTAAGAGAAGCAACATCT	6255
		2237_2254del18	LRG_304p1:p.(Glu746_Ser752delinsAla)	LRG_304t1:c.2237_2254delAATTAAGAGAAGCAACAT	12367
		2240_2254del15	LRG_304p1:p.(Leu747_Thr751del)	LRG_304t1:c.2240_2254delTAAGAGAAGCAACAT	12369
		2240_2257del18	LRG_304p1:p.(Leu747_Pro753delinsSer)	LRG_304t1:c.2240_2257delTAAGAGAAGCAACATCTC	12370
		2239_2248TTAAGAGAAG>C	LRG_304p1:p.(Leu747_Ala750delinsPro)	LRG_304t1:c.2239_2248delinsC	12382
		2239_2251>C	LRG_304p1:p.(Leu747_Thr751delinsPro)	LRG_304t1:c.2239_2251delinsC	12383
		2237_2255>T	LRG_304p1:p.(Glu746_Ser752delinsVal)	LRG_304t1:c.2237_2255delinsT	12384
		2235_2255>AAT	LRG_304p1:p.(Glu746_Ser752delinsIle)	LRG_304t1:c.2235_2255delinsAAT	12385
		2237_2252>T	LRG_304p1:p.(Glu746_Thr751delinsVal)	LRG_304t1:c.2237_2252delinsT	12386
		2239_2258>CA	LRG_304p1:p.(Leu747_Pro753delinsGln)	LRG_304t1:c.2239_2258delinsCA	12387
		2239_2256>CAA	LRG_304p1:p.(Leu747_Ser752delinsGln)	LRG_304t1:c.2239_2256delinsCAA	12403
		2237_2253>TTGCT	LRG_304p1:p.(Glu746_Thr751delinsValAla)	LRG_304t1:c.2237_2253delinsTTGCT	12416
		2238_2252>GCA	LRG_304p1:p.(Leu747_Thr751delinsGln)	LRG_304t1:c.2238_2252delinsGCA	12419
		2238_2248>GC	LRG_304p1:p.(Leu747_Ala750delinsPro)	LRG_304t1:c.2238_2248delinsGC	12422
		2237_2251del15	LRG_304p1:p.(Glu746_Thr751delinsAla)	LRG_304t1:c.2237_2251delAATTAAGAGAAGCAA	12678
		2236_2253del18	LRG_304p1:p.(Glu746_Thr751del)	LRG_304t1:c.2236_2253delGAATTAAGAGAAGCAACA	12728
		2235_2248>AATTC	LRG_304p1:p.(Glu746_Ala750delinsIlePro)	LRG_304t1:c.2235_2248delinsAATTC	13550
		2235_2252>AAT	LRG_304p1:p.(Glu746_Thr751delinsIle)	LRG_304t1:c.2235_2252delinsAAT	13551
		2235_2251>AATTC	LRG_304p1:p.(Glu746_Thr751delinsIlePro)	LRG_304t1:c.2235_2251delinsAATTC	13552
		2253_2276del24	LRG_304p1:p.(Ser752_Ile759del)	LRG_304t1:c.2252_2276delATCTCCGAAAGCCAACAAGGAAAT	13556
		2237_2257>TCT	LRG_304p1:p.(Glu746_Pro753delinsValSer)	LRG_304t1:c.2237_2257delinsTCT	18427
		2238_2252del15	LRG_304p1:p.(Leu747_Thr751del)	LRG_304t1:c.2240_2254delTAAGAGAAGCAACAT	23571
		2233_2247del15	LRG_304p1:p.(Lys745_Glu749del)	LRG_304t1:c.2233_2247delAAGGAATTAAGAGAA	26039
Exon 20	S768I	2303G>T	LRG_304p1:p.(Ser768Ile)	LRG_304t1:c.2303G>T	6241
	T790M	2369C>T	LRG_304p1:p.(Thr790Met)	LRG_304t1:c.2369C>T	6240
	Ex20Ins	2307_2308ins9 GCCAGCGTG	LRG_304p1:p.(Ala767_Val769dup)	LRG_304t1:c.2300_2308dupCCAGCGTGG	12376
		2319_2320insCAC	LRG_304p1:p.(His773dup)	LRG_304t1:c.2317_2319dupCAC	12377
		2310_2311insGGT	LRG_304p1:p.(Asp770_Asn771insGly)	LRG_304t1:c.2310_2311insGGT	12378
		2311_2312ins9 GCGTGGACA	LRG_304p1:p.(Ser768_Asp770dup)	LRG_304t1:c.2303_2311dupGCGTGGACA	13428
		2309_2310AC>CCAGCGTGGAT	LRG_304p1:p.(Ala767_Val769dup)	LRG_304t1:c.2309_2310delinsCCAGCGTGGAT	13558
Exon 21	L858R	2573T>G	LRG_304p1:p.(Leu858Arg)	LRG_304t1:c.2573T>G	6224
		2573_2574TG>GT	LRG_304p1:p.(Leu858Arg)	LRG_304t1:c.2573_2574delinsGT	12429
	L861Q	2582T>A	LRG_304p1:p.(Leu861Gln)	LRG_304t1:c.2582T>A	6213

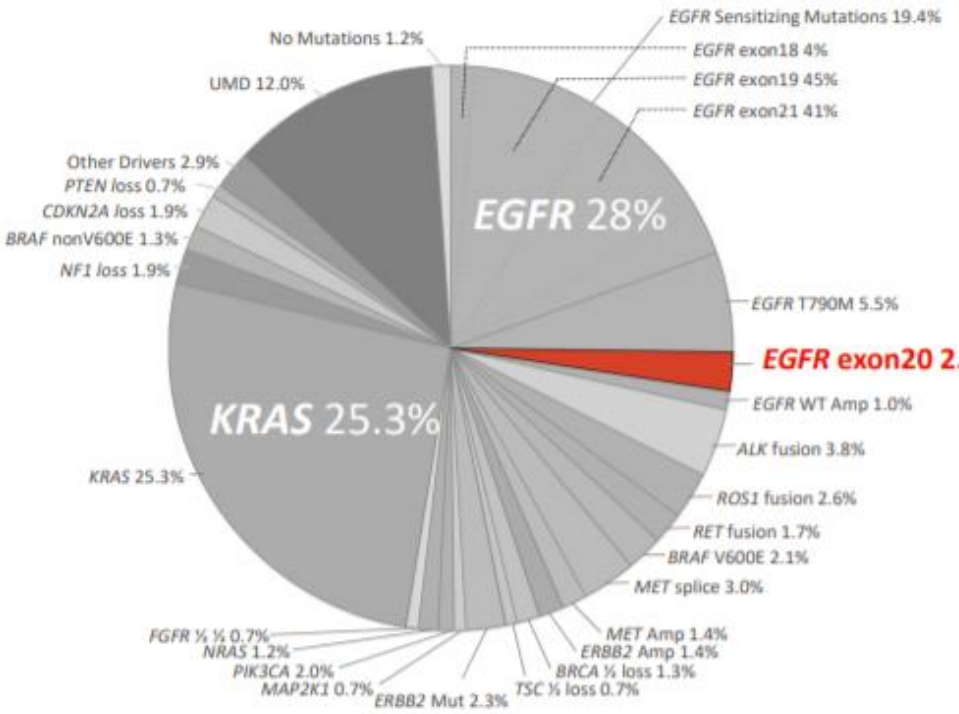
* HGVS – Human Genome Variation Society

EXON 20 insertion 在EGFR突變中非常少，而且過去的EGFR TKI對它的效果也很差，所以不太被重視

EXON 20 INSERTIONS ARE A RARE SUBSET OF EGFR MUTANT NSCLC

Takeda

Non-Sq NSCLC
200,000 pts/yr[1]



EGFR Exon 20 insertions
2,000 pts/yr[2]

Insertion variants	
1. V769_D770insASV	(≈20%)
2. D770_N771insSVD	(≈19%)
3. H773_V774insH	(≈8%)
4. A763_Y764insFQEA	(≈7%)
5. H773_v774insPH	(≈5%)
6. H773_V774insNPH	(≈4%)
7. N771_P772insN	(≈3%)
8. H773_V774insAH	(≈3%)
9. Other	(≈31%)

Sources: Leduc C et al., Ann Oncol 2017; Jorge S et al. Braz J Med Biol Res 2014; Kobayashi Y & Mitsudomi T. Cancer Sci 2016; Arcila M et al. Mol Cancer Ther 2013; Oxnard G et al. J Thorac Oncol 2013

1. Estimated US annual incidence of non-squamous NSCLC
2. Represents annual incidence of the US addressable patient population

EXON 20 insertion 在EGFR突變中非常少，而且過去的EGFR TKI對它的效果也很差，所以不太被重視

Seminars in Cancer Biology 61 (2020) 167–179

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journal homepage: www.elsevier.com/locate/semcancer

Review

Rare epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer

Peter T. Harrison[1], Simon Vyse[1], Paul H. Huang*

Division of Molecular Pathology, The Institute of Cancer Research, London, SW3 6JB, UK

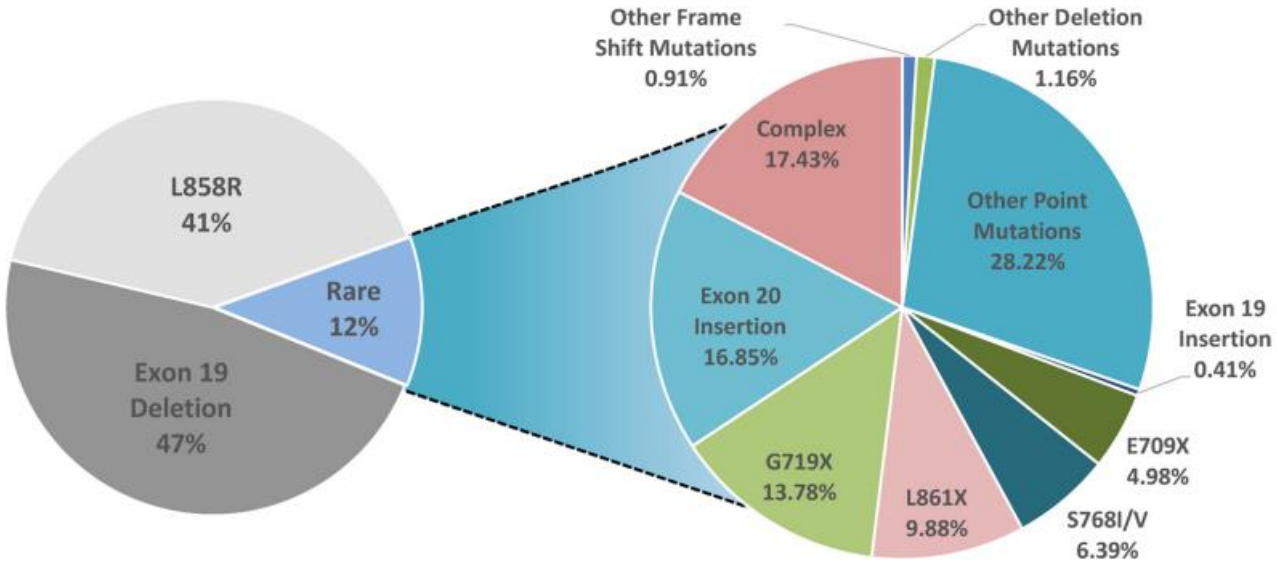
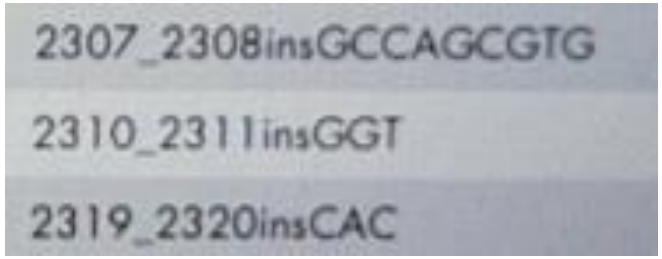


Fig. 1. Pie chart showing the frequencies of EGFR mutations in NSCLC. Data was acquired from COSMIC databases. Data was filtered to contain only mutations from adenocarcinoma. The common resistance mutations T790M and C797S were filtered out.

國內常用之IVD試劑，Exon 20看的到的突變種類

Qiagen 看三種



LRG_304p1:p.(Ala767_Val769dup)

LRG_304p1:p.(Asp770_Asn771insGly)

LRG_304p1:p.(His773dup)

ROCHE 看五種

Exon 20	Ex20Ins	2307_2308ins9 GCCAGCGTG	LRG_304p1:p.(Ala767_Val769dup)	LRG_304t1:c.2300_2308dupCCAGCGTGG	12376
		2319_2320insCAC	LRG_304p1:p.(His773dup)	LRG_304t1:c.2317_2319dupCAC	12377
		2310_2311insGGT	LRG_304p1:p.(Asp770_Asn771insGly)	LRG_304t1:c.2310_2311insGGT	12378
		2311_2312ins9 GCGTGGACA	LRG_304p1:p.(Ser768_Asp770dup)	LRG_304t1:c.2303_2311dupGCGTGGACA	13428
		2309_2310AC> CCAGCGTGGAT	LRG_304p1:p.(Ala767_Val769dup)	LRG_304t1:c.2309_2310delinsCCAGCGTGGAT	13558

Idylla 看五種，與羅氏相同

Exon 20	insG	c.2310_2311insGGT
	insASV9	c.2308_2309insGCCAGCGTG
	insASV11	c.2308_2311delinsCCAGCGTGGAT
	insSVD	c.2311_2312insGCGTGGACA
	insH	c.2319_2320insCAC

這位病人NGS結果: Exon 20 : N771_P772insT mutation，都不在這幾個commercial IVD試劑的設計中

Exon 20 insertion 再細分下去，每一種突變的比例

Insertion variants	
1. V769_D770insASV	(≈20%)
2. D770_N771insSVD	(≈19%)
3. H773_V774insH	(≈8%)
4. A763_Y764insFQEA	(≈7%)
5. H773_v774insPH	(≈5%)
6. H773_V774insNPH	(≈4%)
7. N771_P772insN	(≈3%)
8. H773_V774insAH	(≈3%)
9. Other	(≈31%)

這位病人NGS結果: Exon 20 : N771_P772insT mutation，在這張表裡，是第 9 others，少見的EGFR突變(exon 20)中的罕見核酸序列突變
假設EGFR exon 20 佔全部EGFR突變的2%，而這個病人的突變是佔 EGFR exon 20 的3%以下，則這個突變可能佔 EGFR突變的萬分之六以下，可能佔全部肺腺癌病人的萬分之三以下。

而這個 Exon20 Asn771_Pro772insThr 突變在常用的資料庫都沒有登錄，真的很罕見

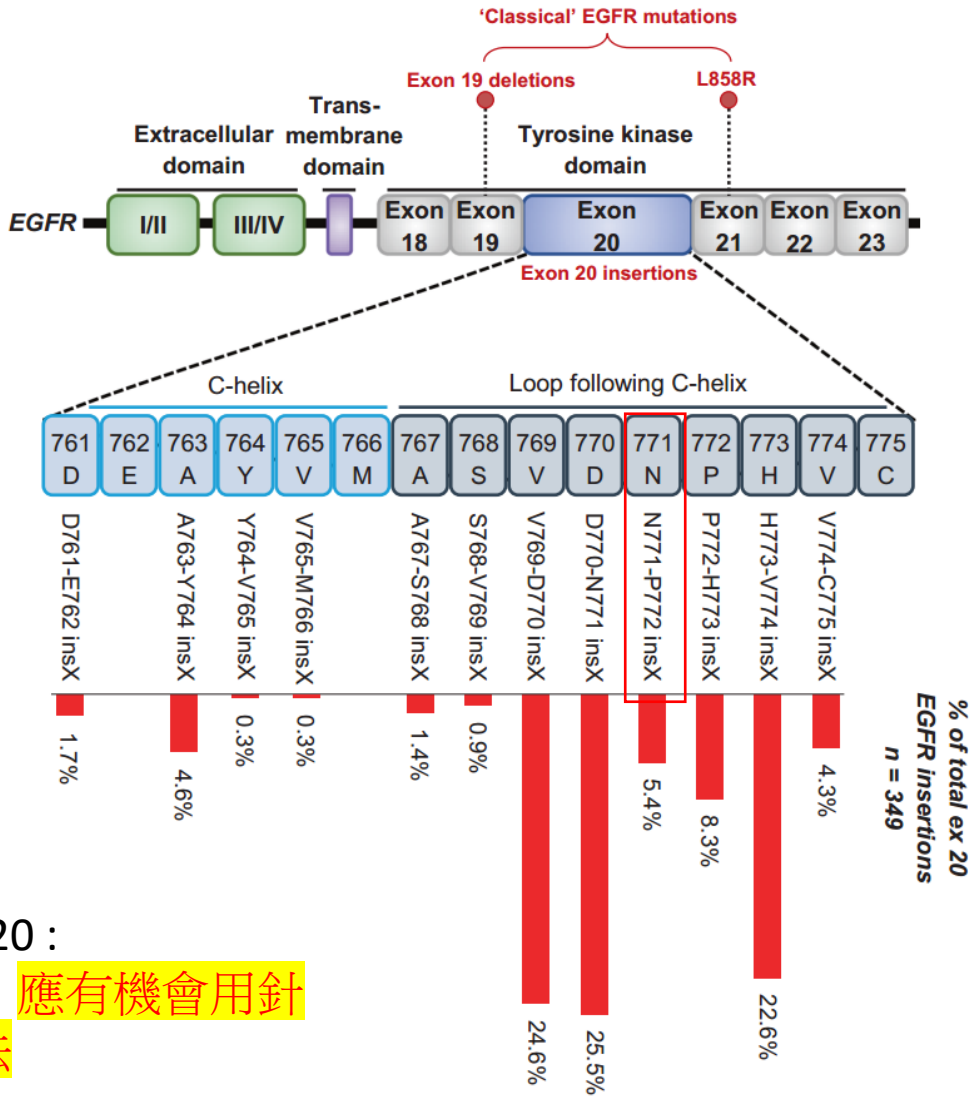
7	g.55181322C>T	CA455165084	EGFR,EGFR-AS1	c.2313C>T (p.Asn771=) c.*28+8394C>T (n.*28+8394C>T) c.2178C>T (p.Asn726=) n.1249G>A c.2154C>T (p.Asn718=) c.1512C>T (p.Asn504=)	
7	g.55181326del	CA891842016	EGFR,EGFR-AS1	c.2317del (p.His773ThrfsTer?) c.*28+8398del (n.*28+8398del) c.2182del (p.His728ThrfsTer?) n.1249del c.2158del (p.His720ThrfsTer?) c.1516del (p.His506ThrfsTer?)	
7	g.55181322_55181330dup	CA135860	EGFR,EGFR-AS1	c.2313_2321dup (p.Val774_Cys775insProHisVal) c.*28+8394_*28+8402dup (n.*28+8394_*28+8402dup) c.2178_2186dup (p.Val729_Cys730insProHisVal) n.1241_1249dup c.2154_2162dup (p.Val721_Cys722insProHisVal) c.1512_1520dup (p.Val507_Cys508insProHisVal)	ClinVar dbSNP
7	g.55181322_55181323insACA	CA891842017	EGFR,EGFR-AS1	c.2313_2314insACA (p.Asn771_Pro772insThr) c.*28+8394_*28+8395insACA (n.*28+8394_*28+8395insACA) c.2178_2179insACA (p.Asn726_Pro727insThr) n.1248_1249insTGT c.2154_2155insACA (p.Asn718_Pro719insThr) c.1512_1513insACA (p.Asn504_Pro505insThr)	
7	g.55181322_55181323insTTA	CA2573102929	EGFR,EGFR-AS1	c.2313_2314insTTA (p.Asn771_Pro772insLeu) c.*28+8394_*28+8395insTTA (n.*28+8394_*28+8395insTTA) c.2178_2179insTTA (p.Asn726_Pro727insLeu) n.1248_1249insTAA c.2154_2155insTTA (p.Asn718_Pro719insLeu) c.1512_1513insTTA (p.Asn504_Pro505insLeu)	
7	g.55181323C>A	CA367578678	EGFR,EGFR-AS1	c.2314C>A (p.Pro772Thr) c.*28+8395C>A (n.*28+8395C>A) c.2179C>A (p.Pro727Thr) n.1248G>T c.2155C>A (p.Pro719Thr) c.1513C>A (p.Pro505Thr)	

Signal Transduction and Targeted Therapy www.nature.com/sigtrans

REVIEW ARTICLE OPEN

Targeting *EGFR* exon 20 insertion mutations in non-small cell lung cancer

Simon Vyse[1] and Paul H. Huang[1]



這位病人NGS結果: Exon 20 : N771_P772insT mutation，應有機會用針對Exon 20突變的治療方法

Molecular Oncology

Variability of *EGFR* exon 20 insertions in 24 468 Chinese lung cancer patients and their divergent responses to EGFR inhibitors

YanRu Qin[1], Hong Jian[2], Xiaoling Tong[3], Xue Wu[3], Fufeng Wang[4], Yang W. Shao[4,5] and Xinmin Zhao[6,7]

Table 2. Treatment effects of TKIs in different EGFR e20ins. 1st-gen TKIs include gefitinib, erlotinib, and icotinib. PD, progressive disease.

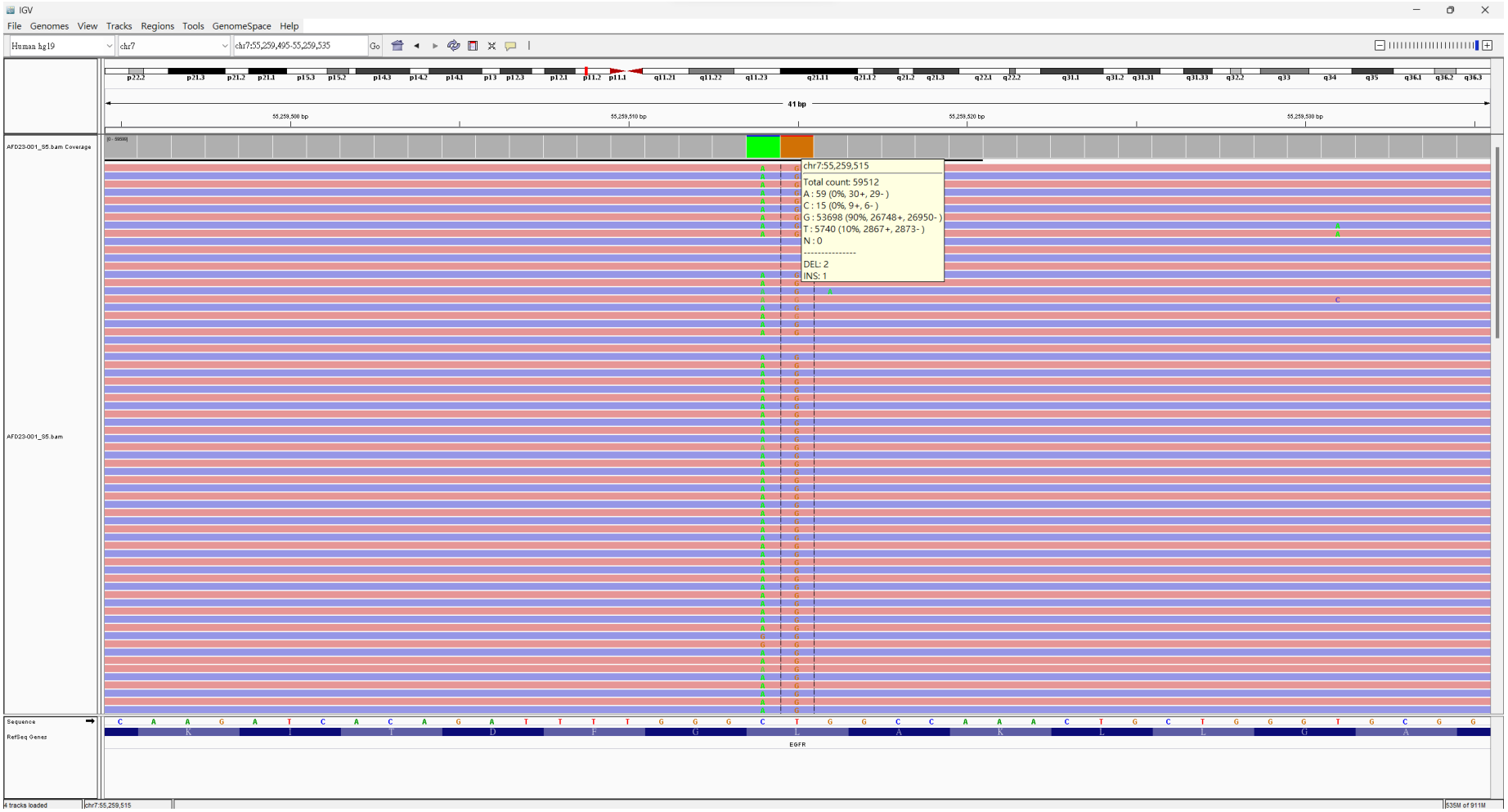
EGFR e20ins (No. of TKI-treated patients)	1st-gen TKIs	Afatinib	Osimertinib	Poziotinib
A767_V769dup (*n* = 17)	2 PR, 3 SD, 7 PD		1 PR, 2 PD	1 SD, 3 PD
S768_D770dup (*n* = 14)	3 SD, 8 PD	1 SD, 3 PD	1 SD, 1 PD, 1 PD	2 SD, 1 PR, 1 PD
N771_H773dup (*n* = 5)	1 SD, 2 PD	1 SD	1 SD, 1 PD	
A763_Y764insFQEA (*n* = 5)	3 PR, 3 SD		1 PR, 1 PD	
P772_H773dup (*n* = 3)	2 PD		1 PD	
H773_V774dup (*n* = 3)	1 PD	1 SD, 1 PD	1 PD	1 PD
H773dup (*n* = 3)	1 PD	1 SD		1 PD
D770delinsGY (*n* = 2)	3 PD		1 PD	
D770_N771insG (*n* = 2)	1 PR, 1 SD			
D770_N771insGL (*n* = 1)			1 PD	
D770_N771insY (*n* = 1)	1 PD		1 PD	
N771_P772insT (*n* = 1)				1 PR
H773_V774insAH (*n* = 1)			1 PR	
N771_P772insHN (*n* = 1)	1 PD		1 SD	
N771_P772insL (*n* = 1)			1 SD	
N771delinsTH (*n* = 1)	1 PD			
P772_V774dup (*n* = 1)	1 PD			
V769_D770insGTV (*n* = 1)	1 PD			
V769_D770insGVV (*n* = 1)		1 PR		1 PD
V769_D770insP (*n* = 1)	1 PD			
ORR	6/47 (12.8%)	1/9 (11.1%)	3/18 (16.7%)	2/12 (16.7%)

Rare mutation pattern of EGFR L858R

- L858R mutation 有好幾種形式
- c. 2573T>G
- c. 2573_2574delinsGT
- c. 2573_2574delinsGA
- 以上幾種較常見

- 這位病人結果是
- c. 2572_2573inv (非常罕見)

NGS顯示這位病人結果是
c. 2572_2573inv (非常罕見)



NGS顯示這位病人結果是
c. 2572_2573inv

EGFR	NM_005228.4	21/28	c.2572_2573inv	p.(Leu858Arg)	missense_variant	Heterozygous	100	0.89770377	26897	29962

7:55259514:55259515:AG	7	55259514	CT	AG

Roche: 顯示您的病人是陰性；因為它只認的出兩種L858R的突變；沒有設計probe 去看這種罕見的 L858R 的

Exon 21	L858R	2573T>G	LRG_304p1:p.(Leu858Arg)	LRG_304t1:c.2573T>G	6224
		2573_2574TG>GT	LRG_304p1:p.(Leu858Arg)	LRG_304t1:c.2573_2574delinsGT	12429
	L861Q	2582T>A	LRG_304p1:p.(Leu861Gln)	LRG_304t1:c.2582T>A	6213

* HGVS – Human Genome Variation Society

Idylla: 顯示這位病人是陰性；它有比羅氏的KIT多設計了一個 L858R的突變型態的probe (三種)；
但是它也沒有設計probe 去看這種罕見的 L858R 的

21	L858R	p.Leu858Arg	c.2573T>G	L858R
			c.2573_2574delinsGT	
			c.2573_2574delinsGA	
	L861Q	p.Leu861Gln	c.2582T>A	L861Q

NM_005228.5(EGFR):c.2572_2573inv (p.Leu858Arg)

Cite this record

Interpretation: Likely pathogenic

Review status: ☆☆☆☆ no assertion criteria provided
Submissions: 1
First in ClinVar: Mar 8, 2017
Most recent Submission: Mar 8, 2017
Last evaluated: Jul 14, 2015
Accession: VCV000376280.1
Variation ID: 376280
Description: 2bp inversion

Variant details
Conditions
Gene(s)

NM_005228.5(EGFR):c.2572_2573inv (p.Leu858Arg)

Allele ID: 363159
Variant type: Inversion
Variant length: 2 bp
Cytogenetic location: 7p11.2
Genomic location: 7: 55191821-55191822 (GRCh38) GRCh38 UCSC
7: 55259514-55259515 (GRCh37) GRCh37 UCSC

HGVS:

Nucleotide	Protein	Molecular consequence
NM_005228.5:c.2572_2573inv MANE SELECT	NP_005219.2:p.Leu858Arg	missense
NM_001346897.2:c.2437_2438inv	NP_001333826.1:p.Leu813Arg	missense
NM_001346898.2:c.2572_2573inv	NP_001333827.1:p.Leu858Arg	missense

... more HGVS

Protein change: L805R, L813R, L591R, L858R
Other names: -
Canonical SPDI: NC_000007.14:55191820:CT:AG
Functional consequence: -
Global minor allele frequency (GMAF): -
Allele frequency: -
Links: ClinGen: CA16602728
VarSome

https://www.ncbi.nlm.nih.gov/clinvar/variation/376280/?new_evidence=true

COSMIC
Catalogue Of Somatic Mutations In Cancer

GRCh 37 Archive

Projects | Data | Tools | News | Help | About | Genome Version | Search COSMIC... SEARCH | Login

Mutation
COSV51801258

GRCh37 · COSMIC v97

- Overview
- Tissue distribution
- Samples
- Pathways affected
- References

Reset page

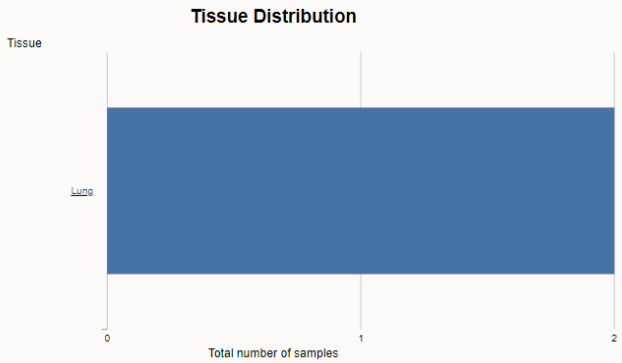
Overview

This section shows a general overview of the selected mutation. It describes the source of the mutation i.e gene name/sample name/tissue name with unique ID, and also shows the mutation syntax at the amino acid and nucleotide sequence level. You can see more information on our help pages.

Genomic Mutation ID	COSV51801258
Legacy Identifier	COSM13553
Gene name	EGFR
AA mutation	p.L858R (Substitution - Missense, position 858, L→R)
CDS mutation	c.2572_2573inv (Complex - compound substitution)
Nucleotides inserted	AG
Genomic coordinates	GRCh37, 7:55259514..55259515, view Ensembl contig
CDD	n/a
HomoloGene	n/a
Ever confirmed somatic?	No
Remark	n/a
Recurrent	n/a
Drug resistance	n/a
Alternative Ids	59605847{EGFR_ENST00000455089}, 58510821{EGFR_ENST00000442591}, 58888573{EGFR_ENST00000454757}

Tissue distribution

This section displays the distribution of mutated samples and tissue types (top 5). You can see more information on our help pages.



這個 c. 2572_2573inv有多罕見?
COSMIC資料庫只有登錄過兩例肺癌的!

https://cancer.sanger.ac.uk/cosmic/mutation/overview?id=23383354

PHARMGKB

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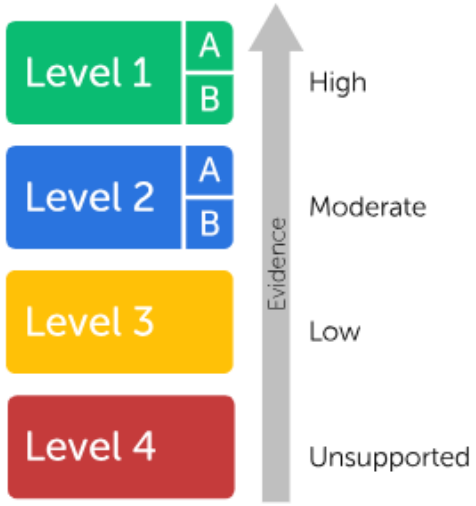
Menu Focus

Clinical Annotation Levels of Evidence



The assignment of clinical annotation levels of evidence (LOE) is primarily informed by the PharmGKB annotation scoring system for clinical annotations and variant annotations. Descriptions and scoring ranges for each level are given in the table below. Information about how PharmGKB assigns rare variant status can be found here.

LEVEL OF EVIDENCE	STANDARD SCORING RANGE	RARE VARIANT SCORING RANGE	DESCRIPTION
1A	≥80	≥80	Level 1A clinical annotations describe variant-drug combinations that have variant-specific prescribing guidance available in a current clinical guideline annotation or an FDA-approved drug label annotation. Annotations of drug labels or clinical guidelines must give prescribing guidance for specific variants (e.g. CYP2C9*3, HLA-B*57:01) or provide mapping from defined allele functions to diplotypes and phenotypes to be used as supporting

Evidence Level	Defining Characteristics	CIViC	OncoKB	JAX-CKB	CGI	MMatch	PMKB
Level A	*Evidence from professional guidelines or FDA-approved therapies relating to a biomarker and disease.*	Level A	Level 1 / 2A /R1	Guideline / FDA Approved	Clinical Practice	Level 1A	Tier 1
Level B	*Evidence from clinical trials or other well-powered studies in clinical populations, with expert consensus.*	Level B	Level 3A	Phase III	Clinical Trials III-IV	Level 1B	
Level C	*Evidence for therapeutic predictive markers from case studies, or other biomarkers from several small studies. Also evidence for biomarker therapeutic predictions for established drugs for different indications.*	Predictive Level C	Level 2B, Level 3B	Clinical Study/ Phase I / Phase II	Clinical Trials I-II, Case Reports	Level 2C	Tier 2
Level D	*Preclinical findings or case studies of prognostic or diagnostic biomarkers. Also includes indirect findings.*	Non-predictive Level C / Level D / Level E	Level 4	Phase 0, Pre-clinical	Pre-clinical Data	Level 2D	Tier 3

Evidence Level Classification

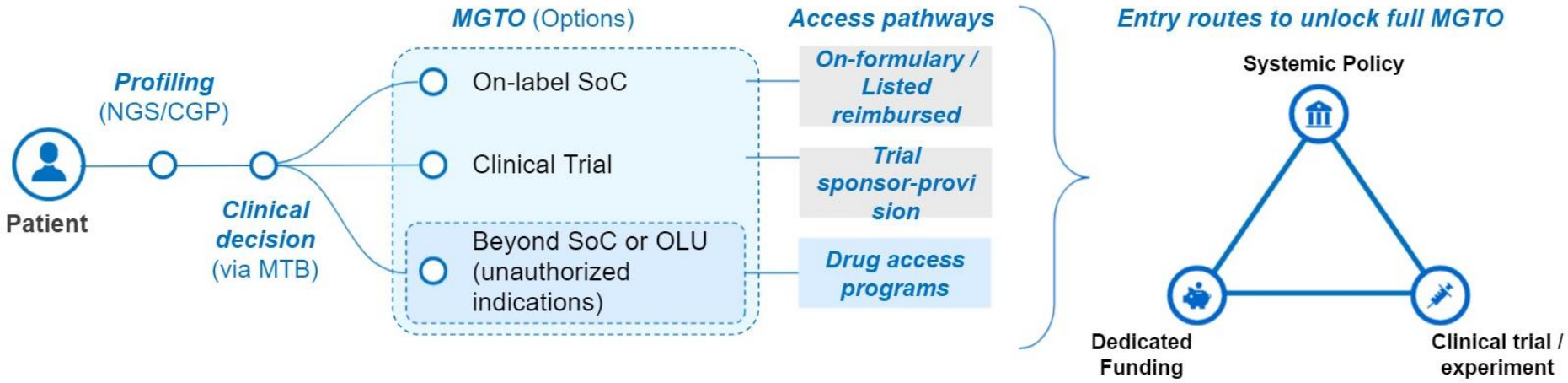
Molecular Tumor Board

實驗室(病理醫師)+ 分子生物+ 臨床腫瘤學專家的專業技術知識

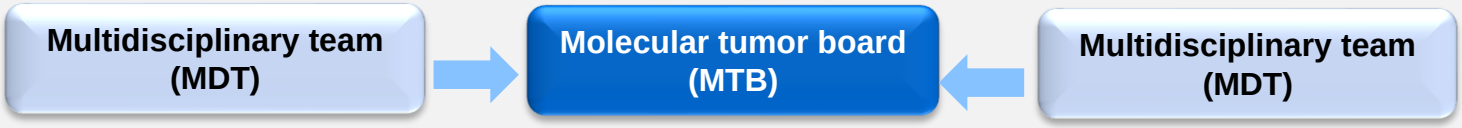
Molecular Tumor Board: Aims

- The aim of MTBs is to identify and discuss all potential therapeutic strategies, based on genetic analysis, for patients who are not responding to standard-of-care systemic therapies
- MTB recommendations should be derived from a multidisciplinary discussion, including not only specific molecular alterations but all features concerning the patient (e.g., performance status, comorbidities)

Trends in Cancer, September 2020, Vol. 6, No. 9



Clinical decision making entities are evolving as comprehensive genomic profiling capabilities & expertise mature



High Level Description	Used to discuss treatment decisions but without incorporating CGP results	Used to discuss treatment decisions incorporating CGP results	In this story (2004)
Oncologist	✓	✓	✓
Radiologist	✓	✓	✓ **(nuclear medicine)**
Pathologist	✓	✓	✓
Surgeon	✓	✓	✓
Clinical Trial Coordinator	✓	✓	✓ **(the patient's surgeon)**
Clinical Geneticist		✓	✓ **(the pathologist)**
Molecular Biologist / Genomicist		✓	✓ **(the pathologist)**
Bioinformaticist		✓	✓ **(the pathologist)**
Genomic expertise required	None	High	✓ **(the pathologist)**
Social worker/financial			✓ **(找人抖内)**

Other types of expertise may also be involved in focused genomic analysis and molecular tumor boards, e.g. structural biologists

醫療機構的責任-提供病人有效安全的檢查

For tests to be used clinically, evidence must be provided to illustrate the clinical value.

I just won the Biotechnology Innovation Award...isn't it good enough?

FIGURE 23.2 The ACCE model: an analytical process for evaluating scientific data on emerging genetic tests. Source: *From Haddow JE, Palomaki GE. ACCE: a model process for evaluating data on emerging genetic tests. In: Khoury M, Little J, Burke W, editors. Human genome epidemiology: a scientific foundation for using genetic information to improve health and prevent disease. Oxford University Press; 2003. p. 217–233.*

特定醫療技術檢查檢驗醫療儀器施行或使用管理辦法(特管辦法)

特管法審查 Clinical Utility

醫事司 Premarket Approval 行政審查:

人員資格(23個學會，專科醫師)+認證項目(5認證機構)+臨床用途(及費用)…

食藥署: 接受多元認證系統 (analytical validation/clinical validation)

我國現況與法規

衛生福利部 特管法 110年2月9日上路

精準醫療時代需要新的檢驗工具

新的檢驗工具進步太快需要管理

提供生技產業與醫療體系合作機會

管理的範圍

附表四

項目名稱
一、抗癌瘤藥物之伴隨檢測
二、癌症篩檢、診斷、治療及預後之基因檢測
三、產前及新生兒染色體與基因變異檢測
四、藥物不良反應或藥物代謝之基因檢測
五、遺傳代謝與罕見疾病之基因檢測
六、病原體鑑定、毒力及抗藥性基因檢測
七、其他藥物伴隨基因檢測。（於藥物仿單中，明載於用藥前應執行檢測）

儘可能以基因檢測為主的範圍…

特定醫療技術檢查檢驗醫療儀器施行或使用管理辦法(特管辦法)

特定醫療技術檢查檢驗醫療儀器施行或使用管理辦法(特管辦法)

醫事司 特管法 申請核准登記

Thanks for your Attention