肝癌與膽管癌腫瘤樣本應用於NGS之經驗與成果分享

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2020.12.15

Disclosure

- I am an employee of ACT Genomics

ACT Genomics: Footprint and staff

Founded in July, 2014 in Taipei, Taiwan

196 Staff

Total Staff Numbers

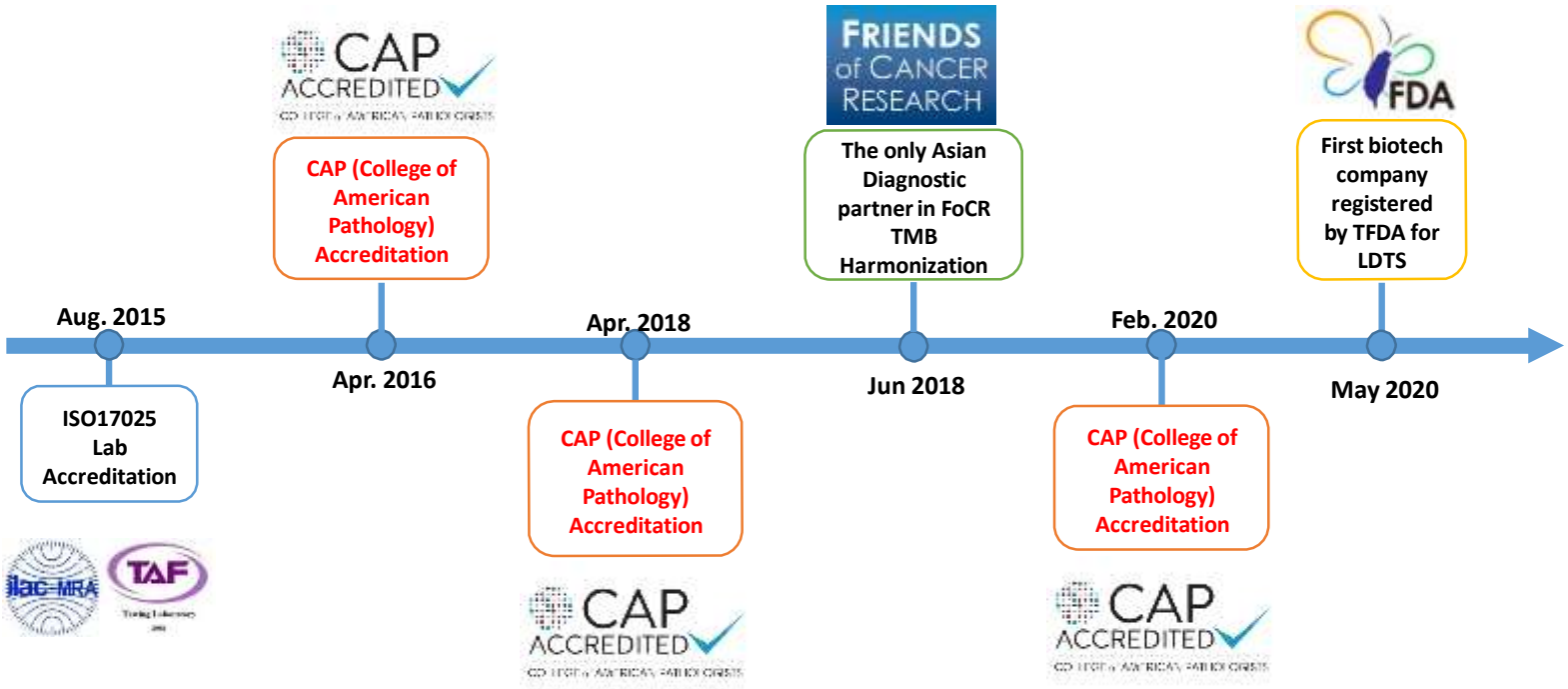
34 PhD 92 Master

54 Staff

Bioinformatics Group

20 PhD 24 Master

Lab Accreditations

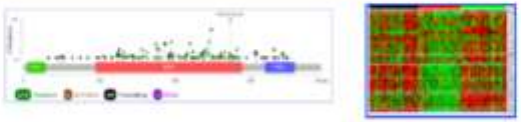


ACTG: Domain Knowledge in Cancer Genomics and Biomarker

Knowledge in clinical sample

Higher success rate even with small core needle biopsy

Knowledge in biomarker



Better biomarker selection to suit different needs

Knowledge in detection technology

Assay with better sensitivity and specificity

Knowledge in clinical needs

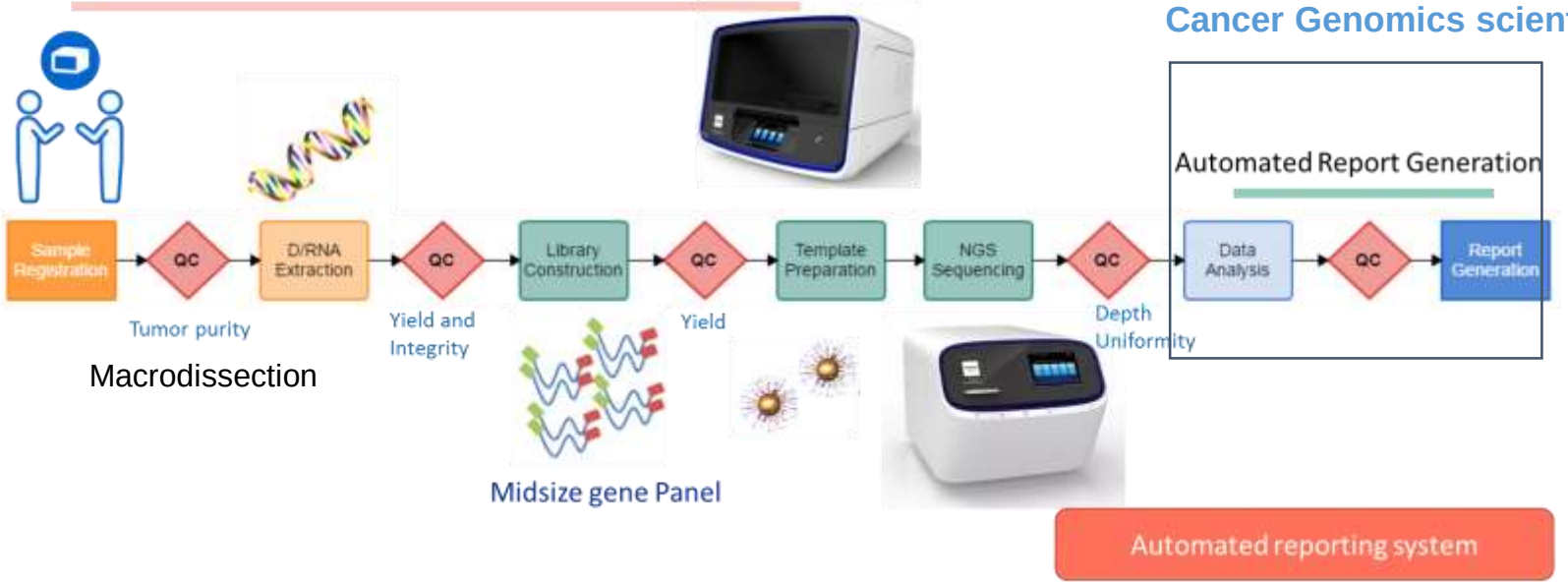
Better test design to manage different disease stage

Next Generation Sequencing (NGS) Clinical Workflow

LIMS (Laboratory Information Management System)

- Workflow management
- Sample tracking
- Test quality

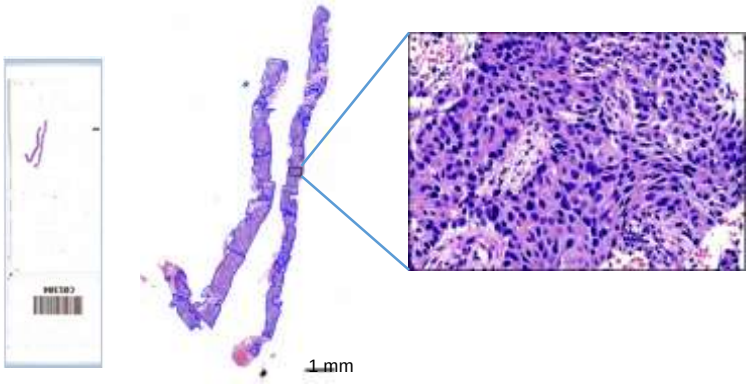
Bioinformatics
Medical Informatics
Cancer Genomics scientists



Example of clinical sequencing data

Cancer type: Lung adenocarcinoma
Sample type: FFPE
Collect site: Lung

Sex: Female
Tumor stage : T4N3M1b
Age : 45 y/o
Treatment : Afatinib Tx for 5 months

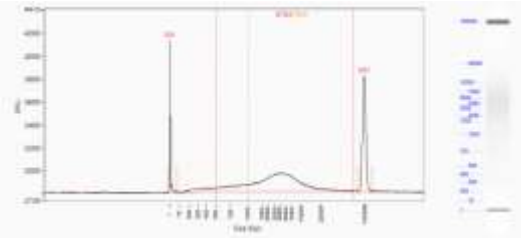


Tumor purity : 90%

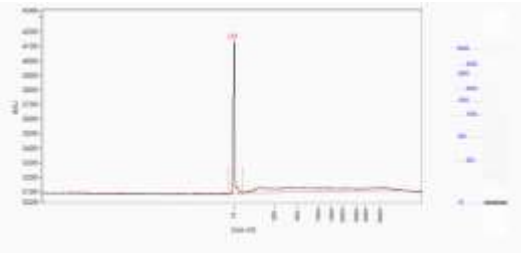
ACTOnco Sequencing QC
- **Depth: 1128x**
- **Uniformity: 93%**

ACTFusion Sequencing QC
- **Number of mapped reads: 182,630**

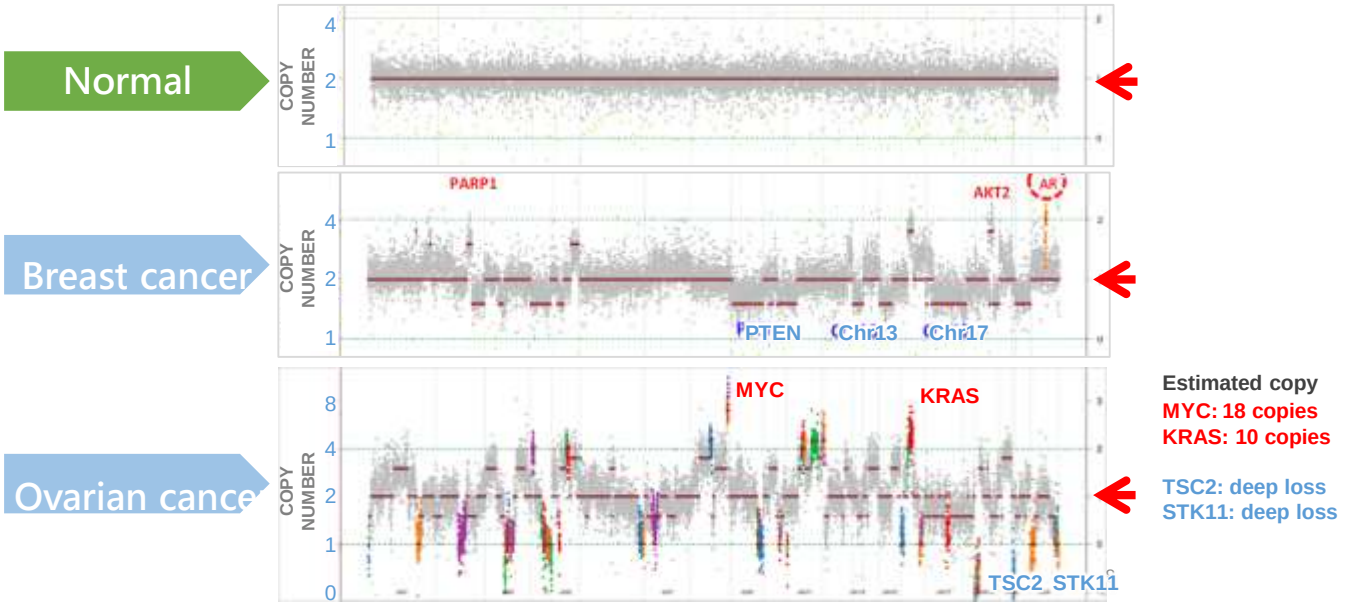
 # section extracted: 7
DNA yield: 1705 ng
>500bp: 88%



 # section extracted: 3
RNA yield: 307 ng
>500bp: 47%



NGS Data: copy number variants (CNV)



Clinical Samples used for Cancer Biomarker

Solid Tissues:

- Frozen tissues
- FFPE samples
- Core needle biopsy

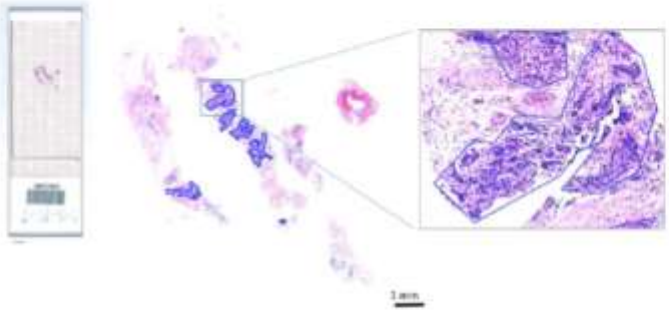
Liquid Biopsy:

- Blood
- CSF

Others:

- Purified genomic DNA
- Cells

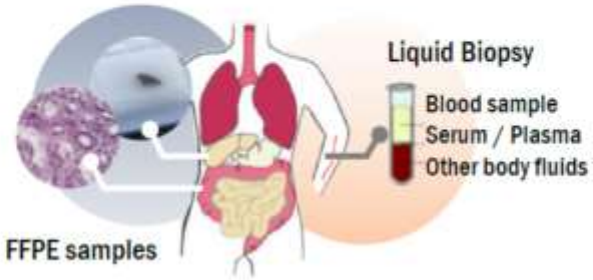
Macro-dissection of tumor regions



FFPE success rate

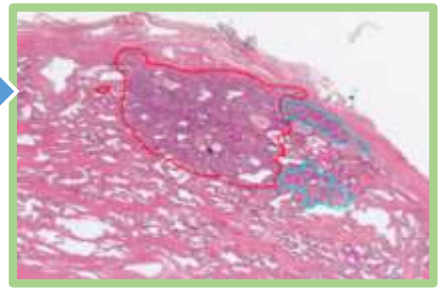
ACTG: 97.5%

Industry average: 60-80%



Complementary information from tissue biopsy and liquid biopsy

Tissue biopsy



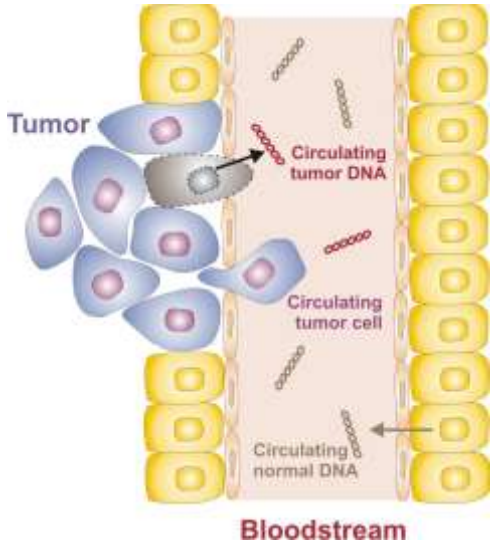
- Standard pathological specimen
- Provides comprehensive information of genetic alterations
- Sample can be stored over longer periods of time
- Provides sufficient amount of DNA/RNA to conduct molecular analyses

Liquid biopsy

- Non-invasive sampling
- Enables repeated sampling
- Reflects tumor heterogeneity
- Can be used for early cancer screening
- Dynamic monitoring of disease progress, drug resistance development and metastasis

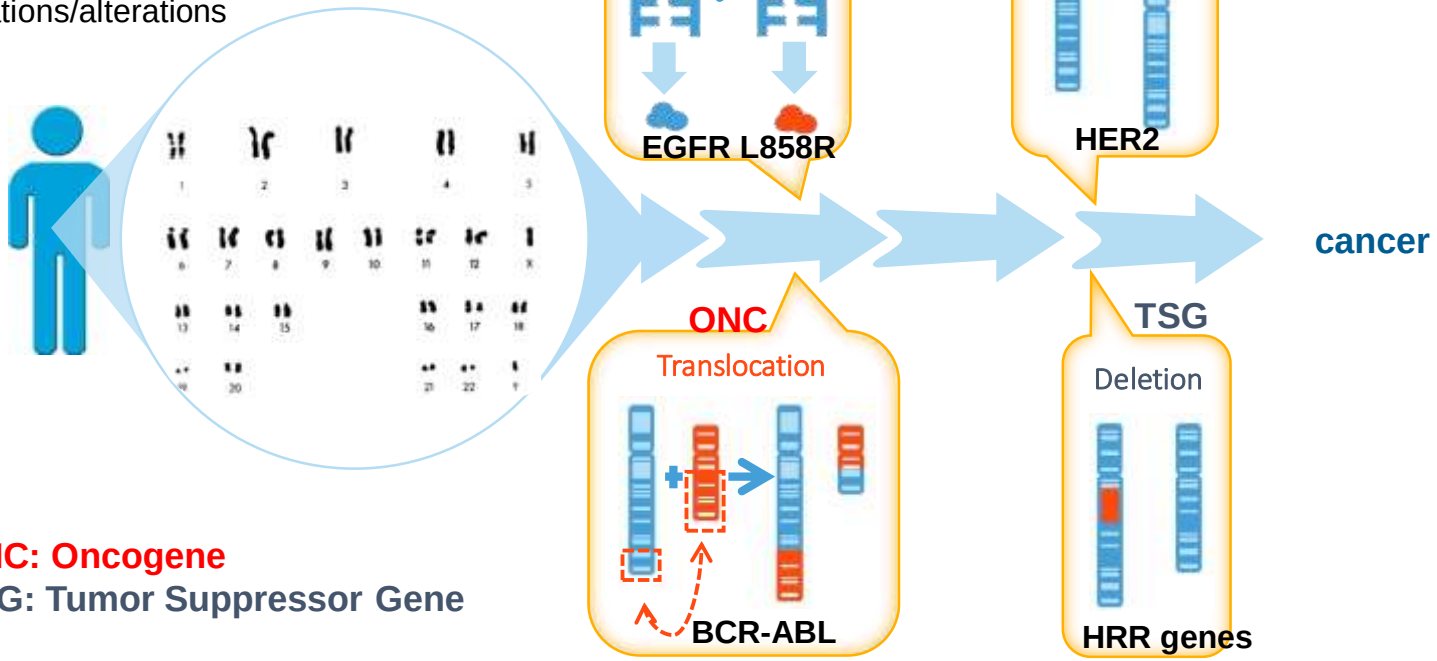
Molecular features of ctDNA

- **Size:** 120 ~ 180 bp
- **Sources:** from necrotic or apoptotic tumor cells
- **Abundant in blood:** 0.01% ~ 50% of total cfDNA
- **Clearance:** through kidney to urine, T1/2 = 2 hr
- **Genetic alterations:** mutation, rearrangement, copy number gain or loss, methylation
- **ctDNA ≠ CTC cells:** ctDNA is more abundant than CTC cells in blood circulation



Cancer is a Genetic Disease

- Genetic mutations turn normal cells into tumor cells
- All tumor cells harbor some genetic mutations/alterations

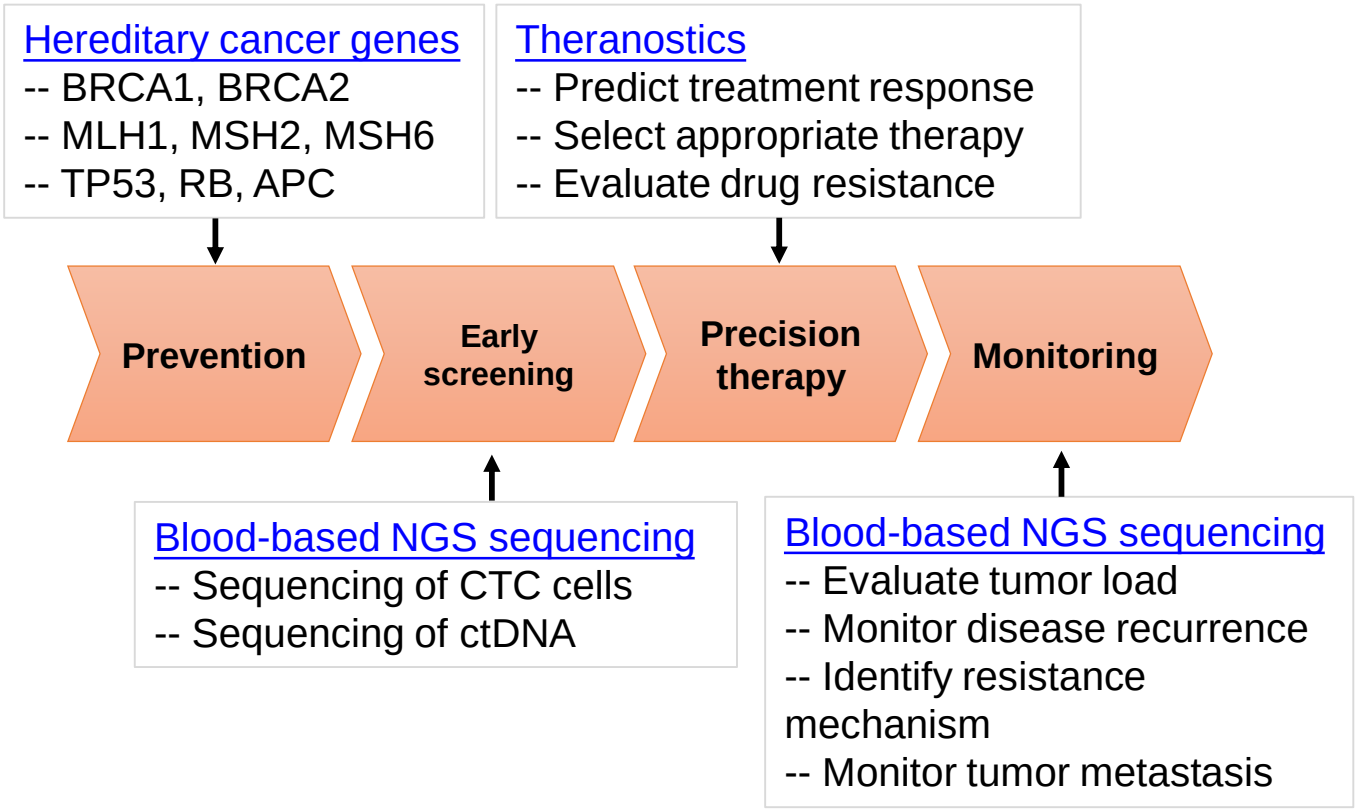


ONC: Oncogene
TSG: Tumor Suppressor Gene

Genomic Markers for Precision Medicine

- **Prevention**
  Germline mutation in critical genes is associated with increased risk of certain cancer types – BRCA1/2
- **Diagnosis**
  Tumor-associated mutations would serve as diagnostic markers with high specificity.
- **Therapeutics**
  Tumor-associated mutations could provide important guidance for selecting treatment strategy.
- **Prognosis**
  Tumor-associated mutations may offer good predictive values for clinical outcome.
- **Disease monitor**
  Tumor-associated mutations can be used to monitor residual disease as well as disease recurrence.

Applications of NGS in Cancer Management



About the Study - 1

Cancer type studied

- Hepatocellular (HCC):
- Cholangiocarcinoma (CCA)
- Mixed hepatocellular and intrahepatic cholangiocellular carcinoma (HCC-CC)

Study aim

以HCC、CCA以及HCC-CC檢體進行次世代定序技術以及多重定量PCR，嘗試瞭解該疾病在台灣族群的基因突變狀態以及基因表達譜，以深入剖析癌症基因資訊，並使用分析後數據與預後連接，統計不同基因突變對於預後的影響。我們預計收集CCA, HCC, 以及 HCC-CC的檢體分別分析mutational spectrum與gene expression profiling進行組間統計比較。以及癌腫間基因變異與預後的關聯性。

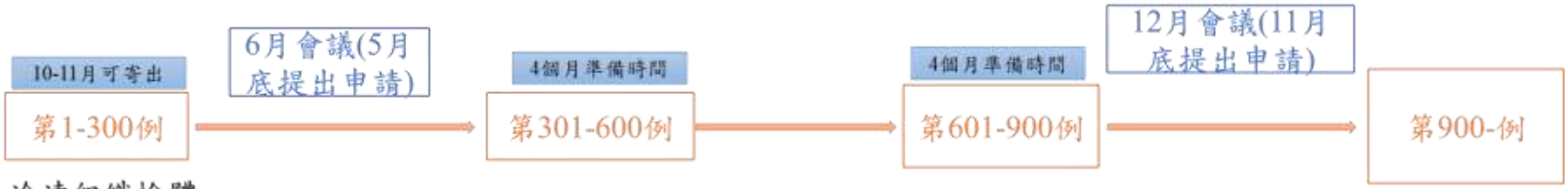
About the Study - 2

- 預計收案數目: 1800 cases
- 第一批申請數目: 300 cases
- 檢體寄送種類:
  DNA & RNA (各5ug)(提供 tumor purity ≥30%檢體)

- 隨檢體提供資訊

1. Pathology report (Tumor purity, cancer type, cancer staging)
2. Gender & Age
3. Outcome (PFS、OS)

整體實驗排程



冷凍組織檢體
DNA&RNA QC 需經過確認
預計於先行確定100例檢體狀況
(約收到檢體後兩個月)
再確認後續申請檢體

Type	Number of 1st batch	DNA&RNA QC
HCC	200	50
CCA	50	25
HCC-CC	50	25

- 時程規劃

1. IRB以每年審核為主，預計每年進行展延，預計於2022年6月完成研究報告。
2. 每次與BioBank申請300例，每次申請後約4-5個月獲得檢體。
3. 尚未結案前，IRB每年會詢問檢測進度與分析狀況(不需提供數據)，預計有份結案報告即可

啟動計畫/檢體申請流程

	2019.5	2019.6	2019.7	2019.8	2019.9	2019.10	2019.11	2019.12	2020.1
理想預期	提出申請 提出IRB申請	IRB審查完成 申請檢體委員會審查會議	付款，檢體準備			檢體寄送完成			
可能逾期狀況	提出申請 提出IRB申請	IRB審查完成 申請檢體委員會審查		檢體委員會審查會議通過	付款，檢體準備			檢體寄送完成	

Research Status

Cancer type	# of sample proposed	# of sample received (1st batch)	# of sample assayed (1st batch)
HCC	1500	200	200
CCA	150	50	50
HCC-CC	150	50	50

Clinical Information Received

HCC

- Gender
- Age
- Smoking history
- Drinking history
- 是否達酗酒標準
- Tumor size(cm)
- Grading
- AFP
- 腫瘤型態(by pathology)
- Vascular invasion
- Pathology stage
- Cirrhosis
- Viral status
- Treatment before surgery
- Metastasis
- Metastasis site

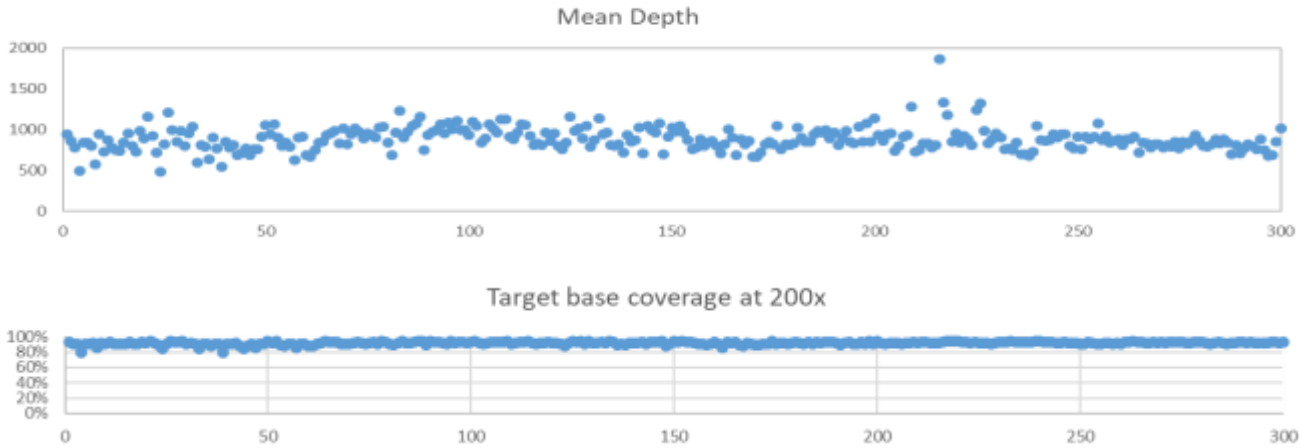
Study Progress

ACTOnco

	No.	%
# sample received	300	100.0
Pathol_QC1	300	100.0
DNA_QC2	283	94.3
Lib_QC3	300	100.0
Seq_QC4	300	100.0
Data_QC5	300	100.0

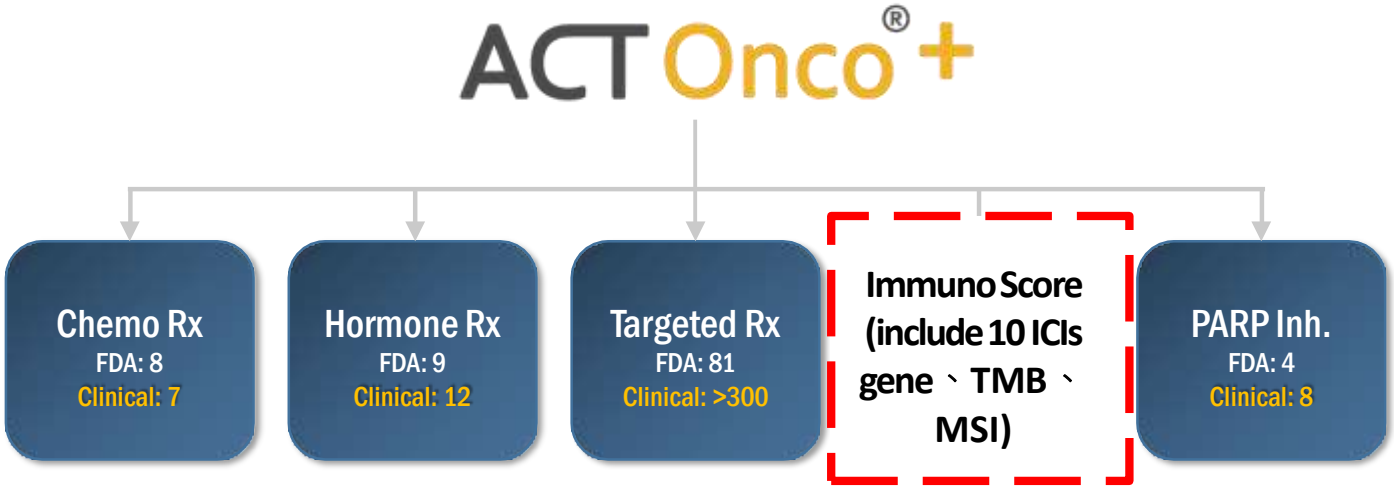
ACTTME

	No.	%
# sample received	300	100.0
Pathol_QC1	300	100.0
RNA_QC2	300	100.0



No contamination and deamination issues

One Assay to Cover All Types of Cancer Drugs



- A comprehensive cancer panel used to guide treatment selection for all major solid tumors, lymphomas and sarcomas
- Targeting > 100 therapies in cancer treatment: chemotherapy, hormone therapy, targeted therapy, PARP inhibitors and immunotherapy
- Used to predict treatment response for additional ~ 350 agents currently under development

ACT TME™

Tumor Microenvironment (TME) gene expression profiling

Category	ACT TME™ assay specification
Detection mechanism	TaqMan RT-qPCR
Target number	112 gene targets
Sample type	RNA from FFPE & cell samples
Sample amount	25 - 100 ng of total RNA
Throughput	Up to 192 samples per day/operator/12K instrument
Run time	~2.5 hours
Sensitivity	~100 copies
Dynamic range	>5 logs
QC criteria	DV300 ≥ 2% for FFPE & Detectable gene number > N/A

Genomic Information from clinical samples

- Landscape of genomic alterations

  (1) Single nucleotide variants (SNVs

  (2) Small insertions and deletions (Indels)

  (3) Copy number variants (CNVs)

- Genomic signatures (30% – 40% tumor purity)

  (1) Tumor mutational burden (TMB)

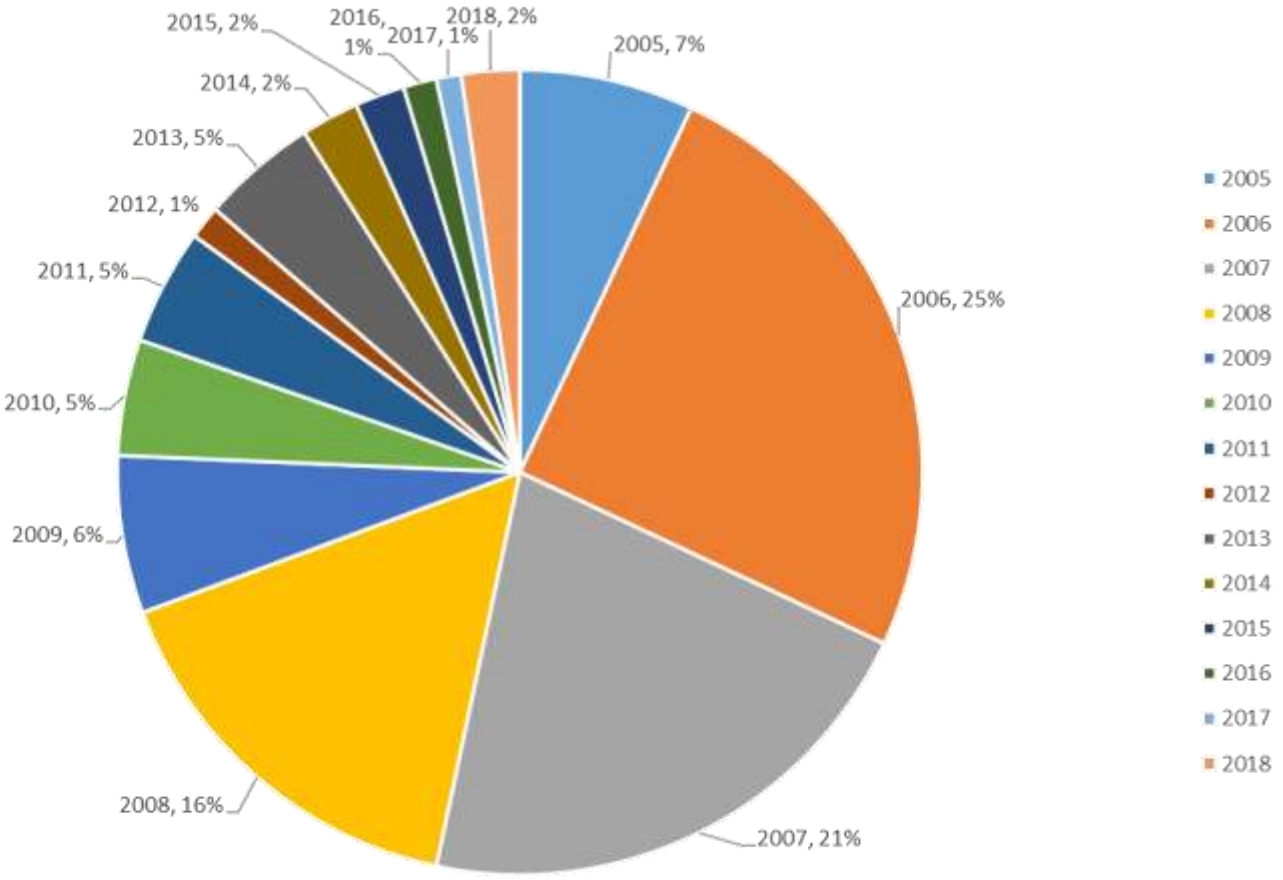
  (2) Microsatellite instability (MSI) status

  (3) LOH score

- Tumor microenvironment study

- Fusion Genes

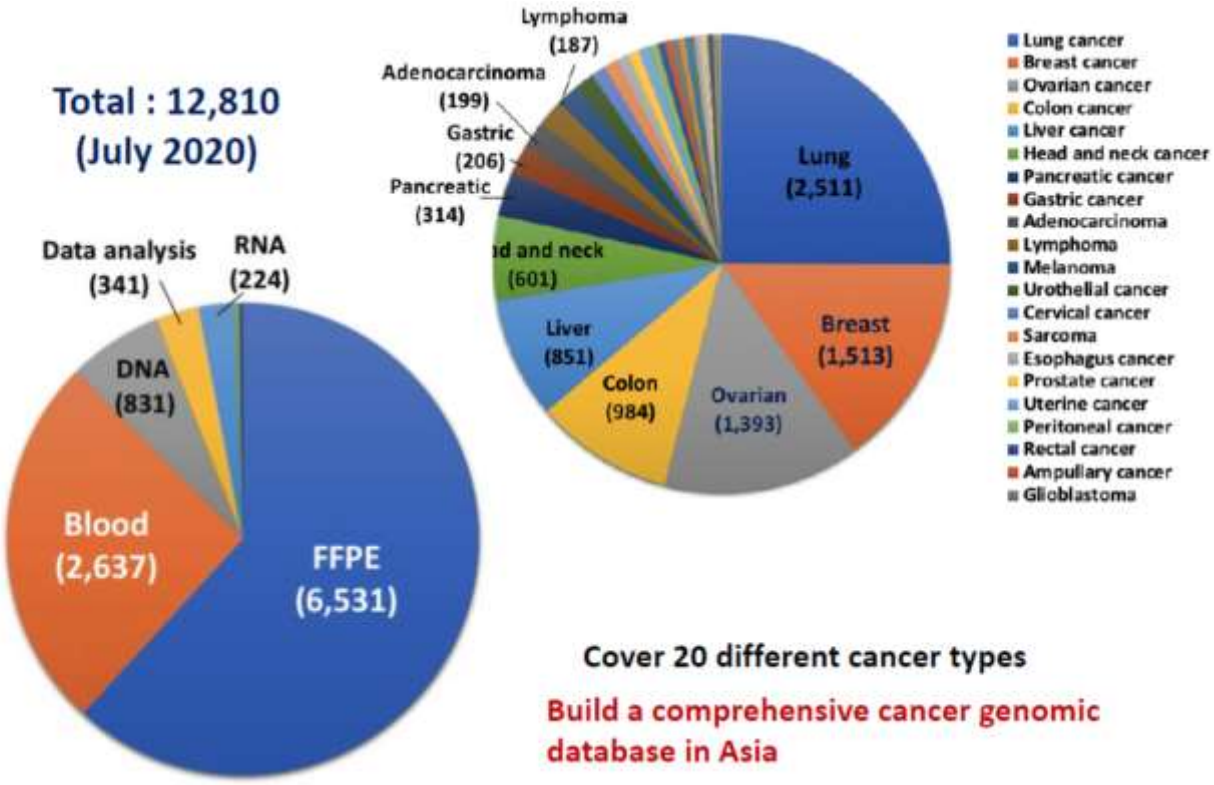
Sample collection year (%)



Four tissue-agnostic drugs approved by the US FDA for solid tumors

Tissue-agnostic Cancer Therapeutic	Indication	US FDA Approval Date
Pembrolizumab (Keytruda)	Adult and pediatric patients with unresectable or metastatic, microsatellite instability-high **(MSI-H)** or mismatch repair deficient **(dMMR)** solid tumors	30 May 2017
Larotrectinib (Vitrakvi)	Adults and children whose tumor is positive for the marker **neurotrophic receptor tyrosine kinase (NTRK) gene fusion**	26 November 2018
Entrectinib (Rozlytrek)	Adult and adolescent patients whose cancers have the specific genetic defect **neurotrophic tyrosine receptor kinase (NTRK) gene fusion**	15 August 2019
Pembrolizumab (Keytruda)	Adult and pediatric patients with unresectable or metastatic solid tumors with **high TMB**	16 June 2020

More Than 1,20000 clinical Samples Sequenced



Cover 20 different cancer types

Build a comprehensive cancer genomic database in Asia

Thank you