Cancer Screening & Gut Microbiome

Dr. Cornelia Man

Liquid Biopsies & Gut Microbiome *Mar 7, 2023*

1. Liquid Biopsies 液态活檢 (CTC, CtDNA/CfDNA)
2. Molecular Microbiology分子微生物學: Close look at our Gut Microbiome

Wellness Profiling *FEB 21, 2023*

1. Biological/Cellular Aging
2. Anti-oxidants 抗氧化劑 & Micronutrients 微量元素 Profiling
3. Heavy Metal Toxicity

TUMOUR DNA & CANCER CELLS DETECTION IN LIQUID BIOPSIES

Conventional Cancer Screening Tests/Imaging & their Limitations

Cancer Biomarkers (blood tests)

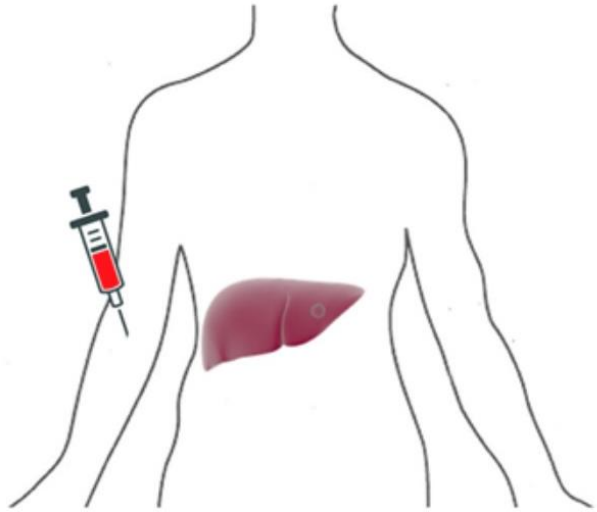
Occult Blood screening of CA Colorectal

Mammogram screening for CA Breast

A New Technological Advance: liquid biopsy Tests

Non-invasive comparing to traditional tissue biopsy, which involves removing a piece of tissue or a sample of cells from your body.

A sample of peripheral blood (10-20ml)



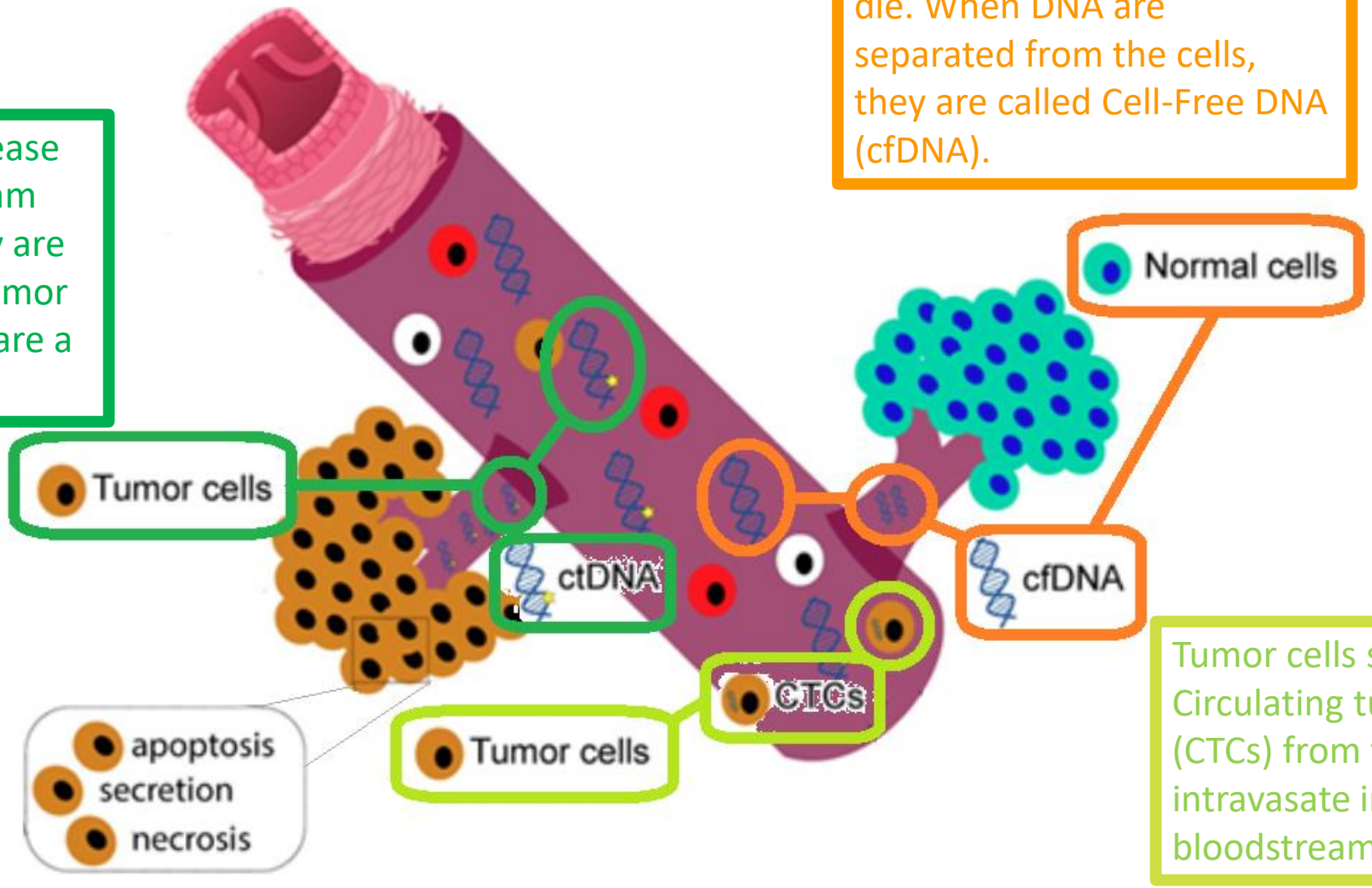
Applications:

- To look for tumor cells or tumour DNA
- Allow screening , early diagnosis and monitoring of cancer
- Identify therapeutic targets/Target Drugs
- Adjust therapeutic plan when drug resistance emerge

Inside a Liquid biopsy

Normal cells release DNA into bloodstream when they die. When DNA are separated from the cells, they are called Cell-Free DNA (cfDNA).

Tumor cells also release DNA into bloodstream when they die. They are called Circulating-Tumor DNA (ctDNA). They are a subtype of cfDNA.



Tumor cells shed Circulating tumor cells (CTCs) from the tumor and intravasate into the bloodstream.

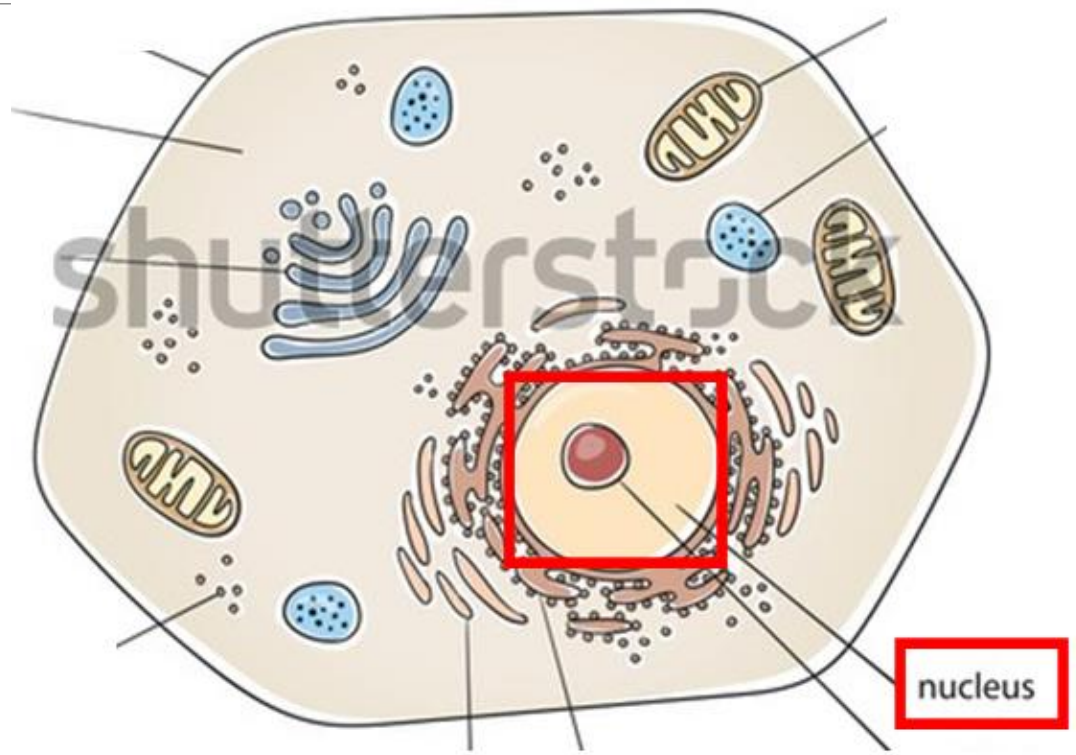
Circulating Tumour DNA

DNA (Deoxyribo Nucleic Acid)

DNA is the **hereditary material** in human.

Most DNA is located in the **nucleus**

Nearly every cell in a person's body has the **same DNA**



The information in DNA is **stored as codes**

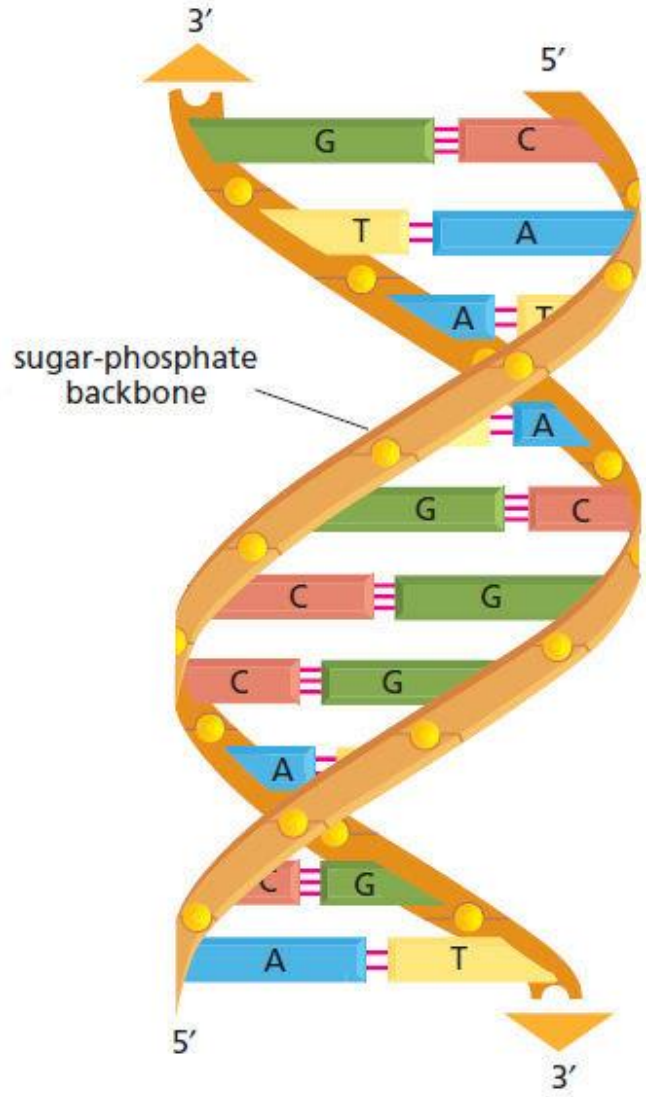
Made up of four chemical bases:

- Adenine (A)
- Guanine (G)
- Cytosine (C)
- Thymine (T)

The order/sequence of these bases determines the information available for building and maintaining an organism

DNA can replicate = make copies of itself.

This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell



Cancer genomics

Cancer Starts When ……. Certain DNA in healthy cell changes and become abnormal over time

- This change is called a **Genetic Mutation**

Cancer is a Dynamic Disease…….During the course of disease, cancer cells keep mutating and become more **heterogeneous** that is, diverse in DNA composition

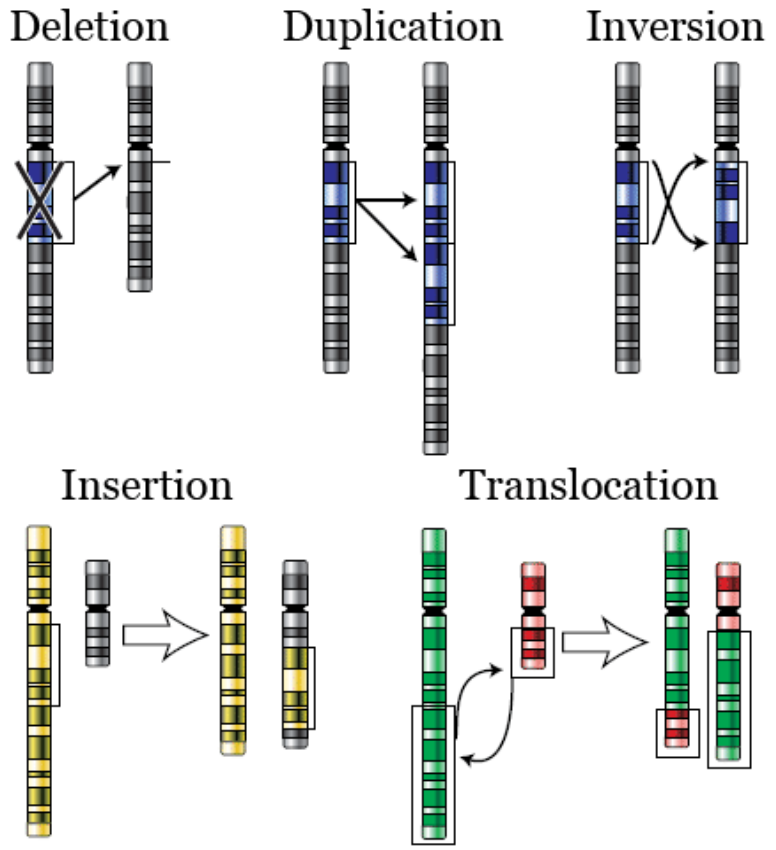
Drug Resistance……. Due to tumour heterogeneity, a tumor mass can include a diverse collection of cells harboring distinct genetic signatures with different levels of sensitivity to treatment.

Solution: An accurate assessment of tumor DNA composition is essential for the identification of effective therapies.

Types of mutations

Chromosomal

- chromosome structure is changed



Point Mutations

Change in A Single Nucleotide in DNA

Type of Mutation	Description	Example	Effect
Silent	codes for the same amino acid	CGA (Arginine) → CGC (Arginine)	None
Missense	codes for a different amino acid	AAA (Lysine) → AAC (Asparagine)	Variable
Nonsense	Codes for a stop codon	GAA (Glutamate) → UAA (stop)	serious

Frameshift mutation

A deletion or insertion of one or more nucleotides that change the reading frame

- The reading frame is changed plus all the codons that follow
  This will have a drastic effect on the protein products

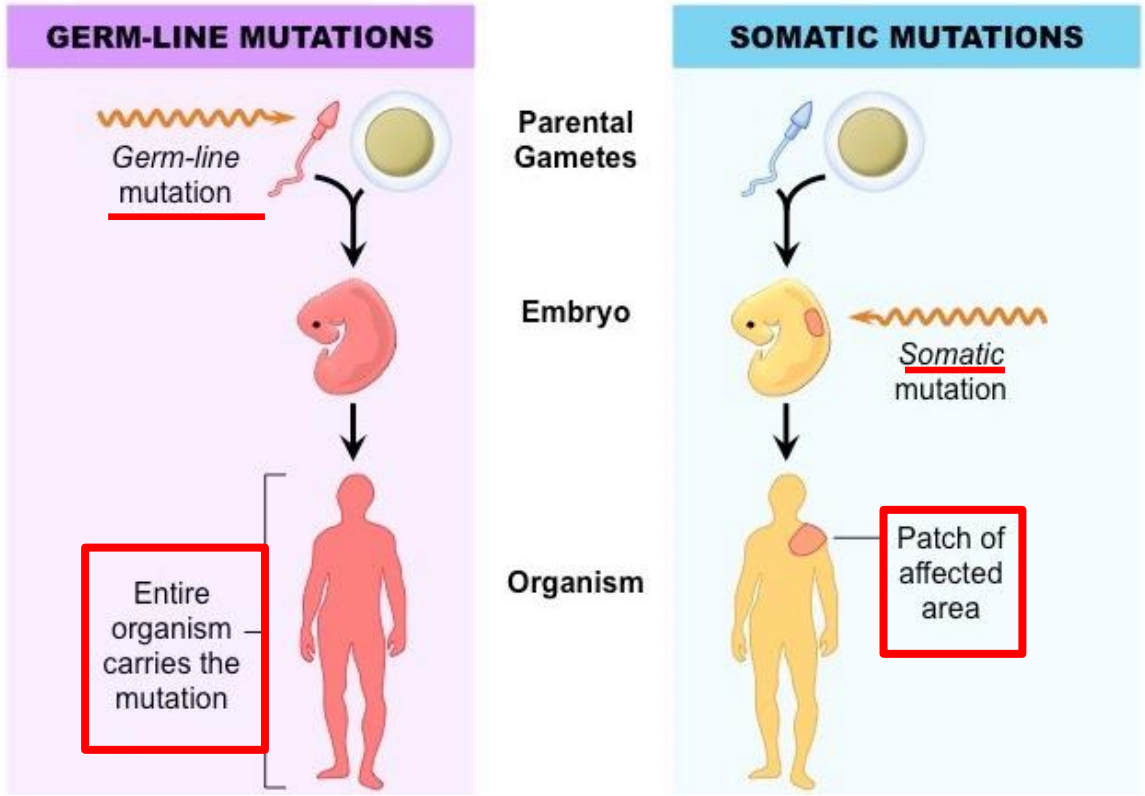
- Original sequence:
  AUG-CCC-CAG-GAA-AAA = start-Proline-Glutamine-Glutamate-Lysine

- an insertion of nucleotide A occurs:
  AUG-**A**CC-CCA-GGA-AAA-A = start-Threonine-Proline-Glycine-Lysine

Germline & Somatic Mutations

Germline Mutations

Changes to the DNA that is inherited from the egg or the sperm cells during conception

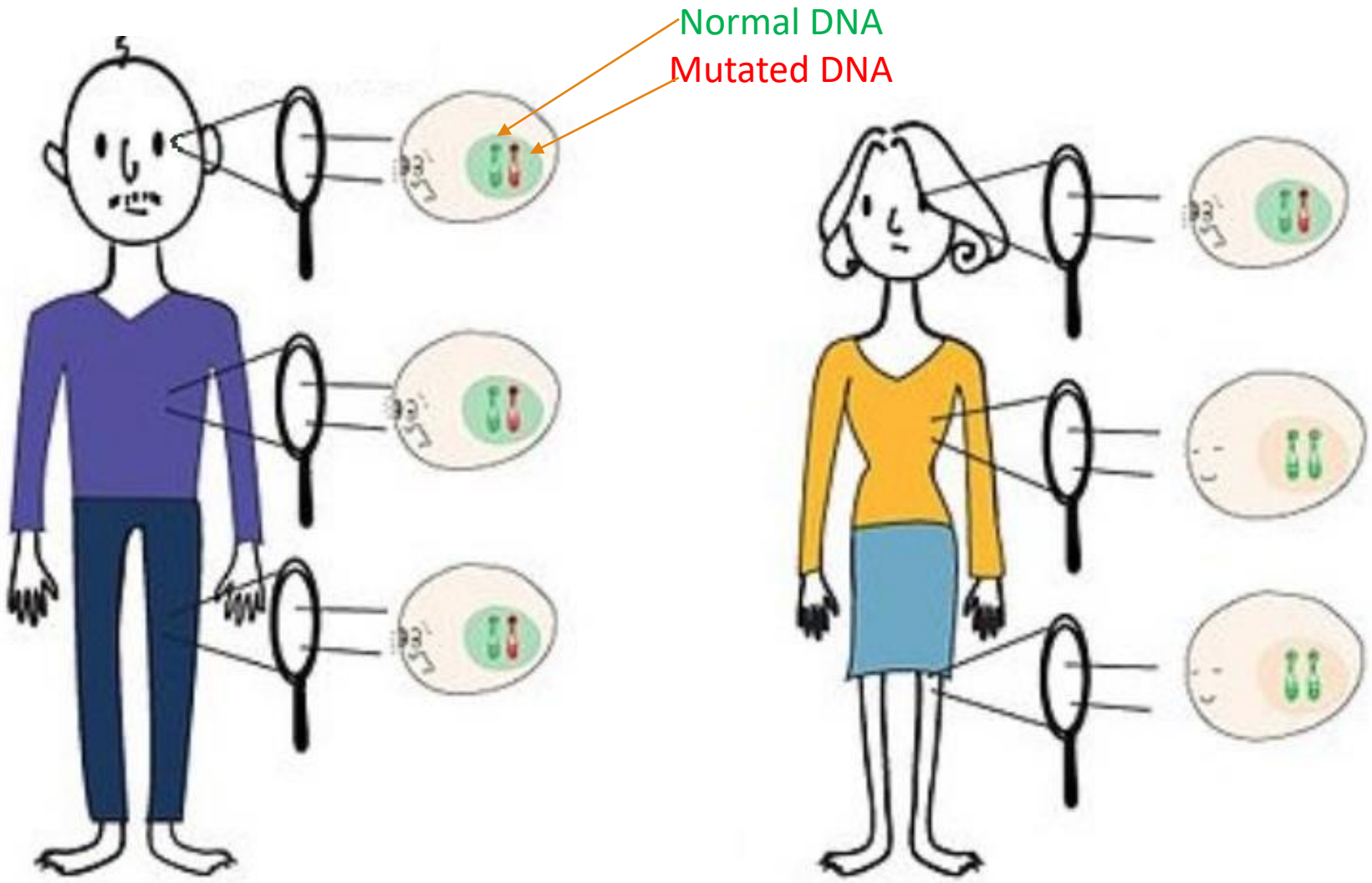


Somatic Mutations

Changes to the DNA that happened after conception to cells other than the eggs or sperms

Germline mutations

At least half of the total DNA contain the mutation



Somatic mutations

Only a small percentage of the total DNA contain the mutation

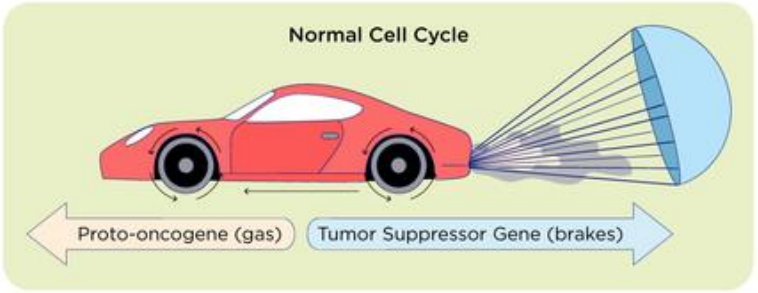
Two major classes of genes causing cancer: Oncogene and Tumor Suppressor Gene

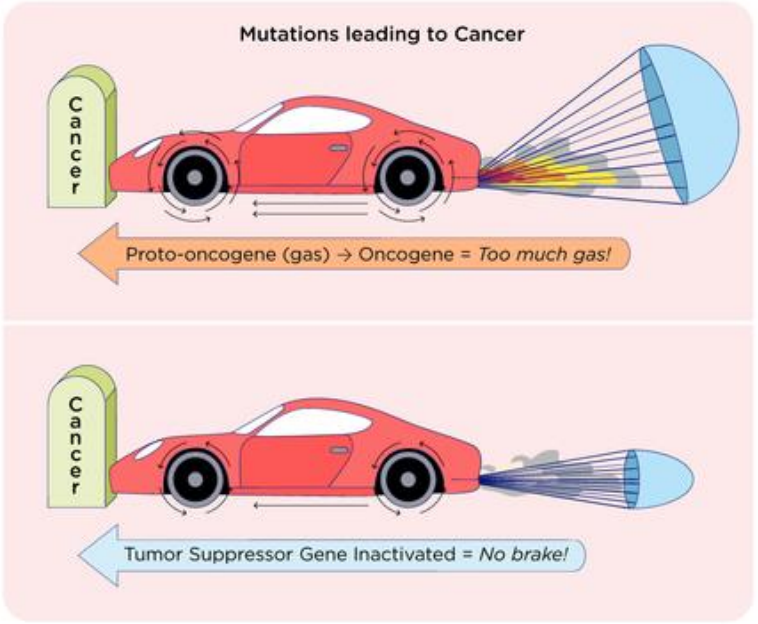
Most cancers are caused by mutations in two basic classes of genes – oncogenes and tumor suppressor genes.

An oncogene is a gene that has the potential to cause cancer.

- Proto-oncogenes is the precursor of oncogenes. It codes for proteins that stimulate the cell cycle and promote cell growth and proliferation.
- When a proto-oncogene is mutated, it becomes a cancer-causing oncogene.

Tumor suppressor genes code for proteins that repress cell cycle progression and promote apoptosis, as their normal function to prevent cancer.





Jack Westin

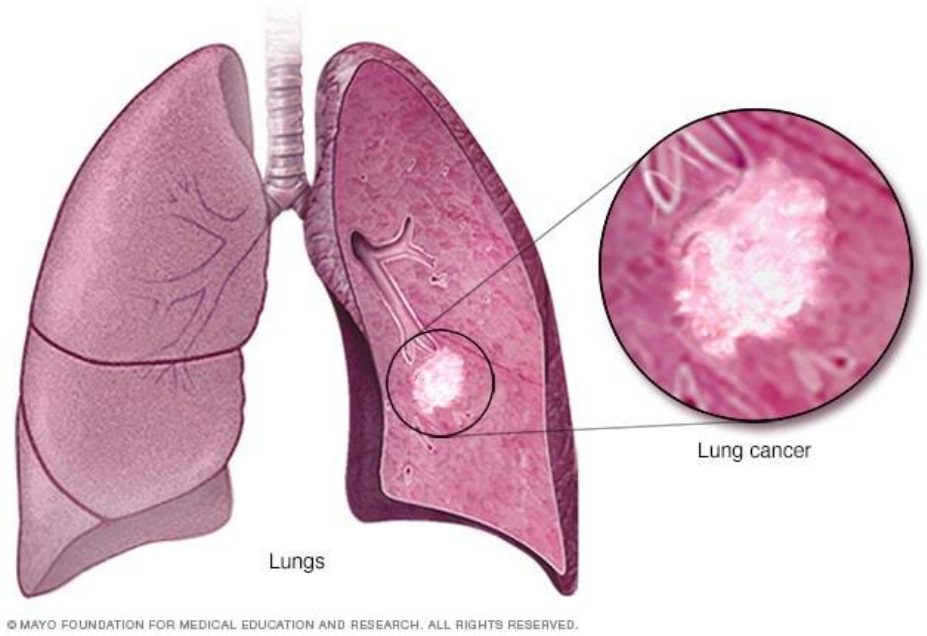
What is target therapy

Targeted therapy is a type of cancer treatment. It uses drugs to target specific genes and proteins that help cancer cells survive and grow.

To prescribe target therapies, Oncologists need to identify the specific genetic changes that promote tumour growth

Unlike chemo therapies, target therapies will not lead to death of normal epithelial cells

An example of how to match the cancer with Target Drug

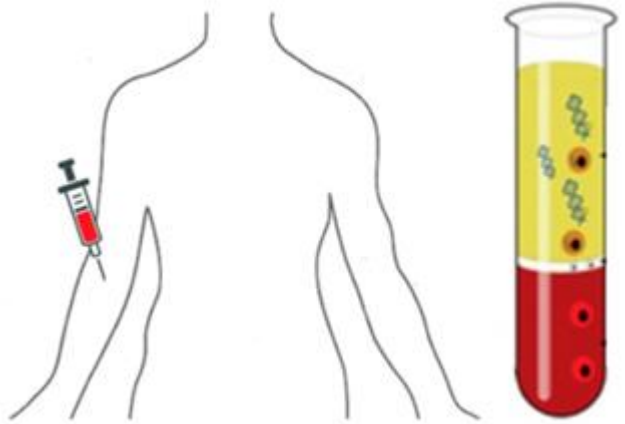


You need to know what the DNA mutation is !!!

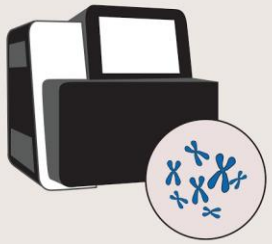
List of available Targeted therapies for Lung Cancer:

- *EGFR* inhibitors
- Drugs targeting *EGFR* Exon 20 insertion mutations
- *ALK* inhibitors
- Drugs targeting the *ROS1* fusion
- Drugs targeting *KRAS* G12C mutations
- Drugs targeting *NTRK* fusion
- Drugs targeting *BRAF* V600E mutations
- Drugs targeting *MET* exon 14 skipping
- Drugs targeting *RET* fusion

Which Targeted therapy should be use??



NEXT GENERATION SEQUENCING



EGFR Exon 21 L858R mutation

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This patient is suitable for *EGFR* inhibitors

Importance of Disease Monitoring

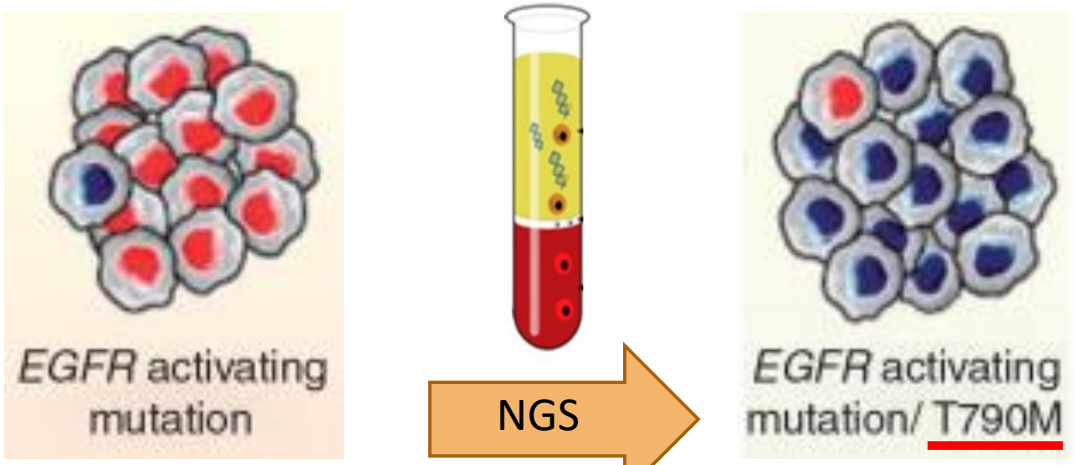
After matching the disease with a target drug, does it mean the treatment journey is ended?

The cancer cells may develop Drug Resistance

may relapse

Disease Monitoring is necessary

How to monitor the disease: An example



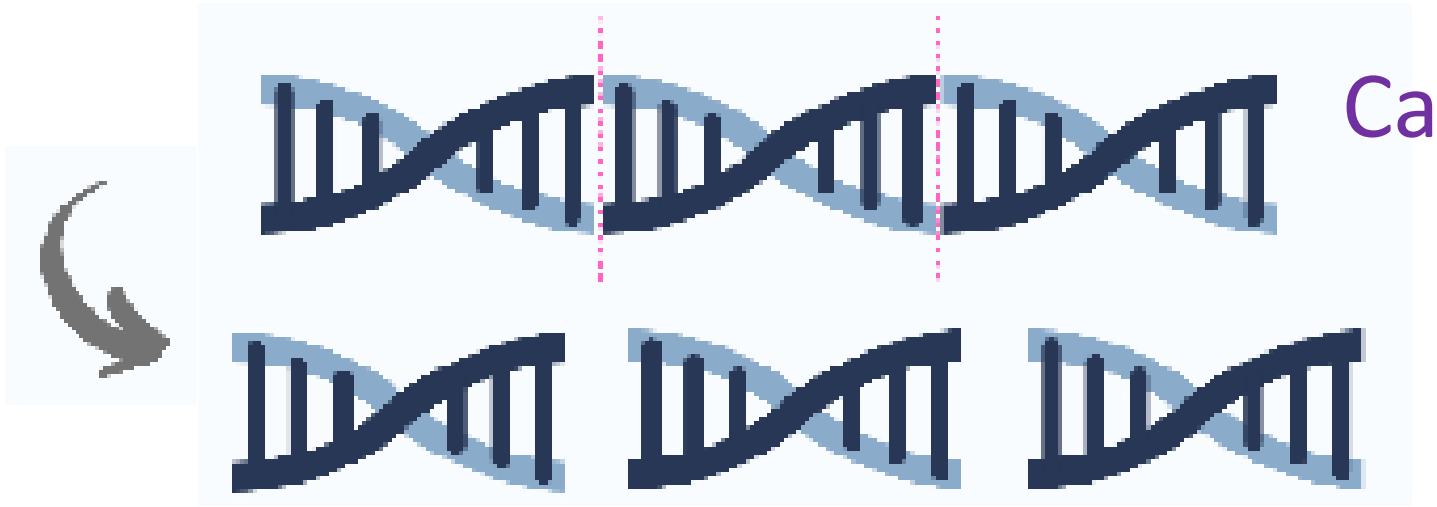
1st/2nd Generation EGFR-TKIs

3rd Generation EGFR-TKIs

Next generation sequencing (NGS)

The Lab Technique called is used in Liquid Biopsy CtDNA tests

CtDNA are short DNA fragments came from the cancer genome
NGS enables sequencing of these short DNA fragments



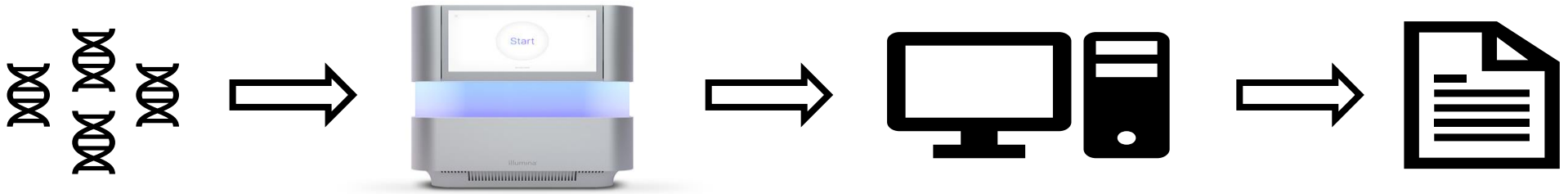
Why is bioinformatics/AI needed for NGS?

NGS simultaneously sequences multiple ctDNA targets or whole gene to detect different types of mutations

More than one patient sample can be tested at the same time

As such, large amount of data is generated from each NGS Sequencing run

bioinformatic analysis is required to turn NGS data into comprehensible report format for clinical use.



Samples | NGS Sequencing | Bioinformatic analysis | Clinical reports

Bioinformatics ?

YouSeq: https://youtu.be/RkttaYc8hfw

What does Sequencing Depth mean?

Sequencing depth = number of times (reads) that the target ctDNA has been sequenced

- Example:
  - Position A is sequenced 5 times, represented by 5 separate reads covering it. The sequencing depth of position A is 5X
  - Position B is sequenced 2 times. The sequencing depth of position B is 2X

How much sequencing depth is required?

- depends on the application of the test

- To detect low frequency mutation (1% or lower) in ctDNA of cancer patients, in general at least 10,000X is recommended.

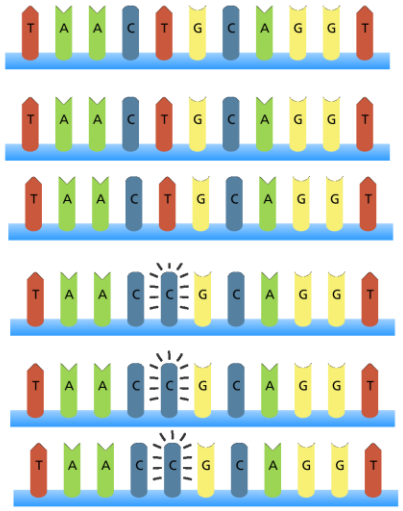
  - The filtering is done during bioinformatic analysis.

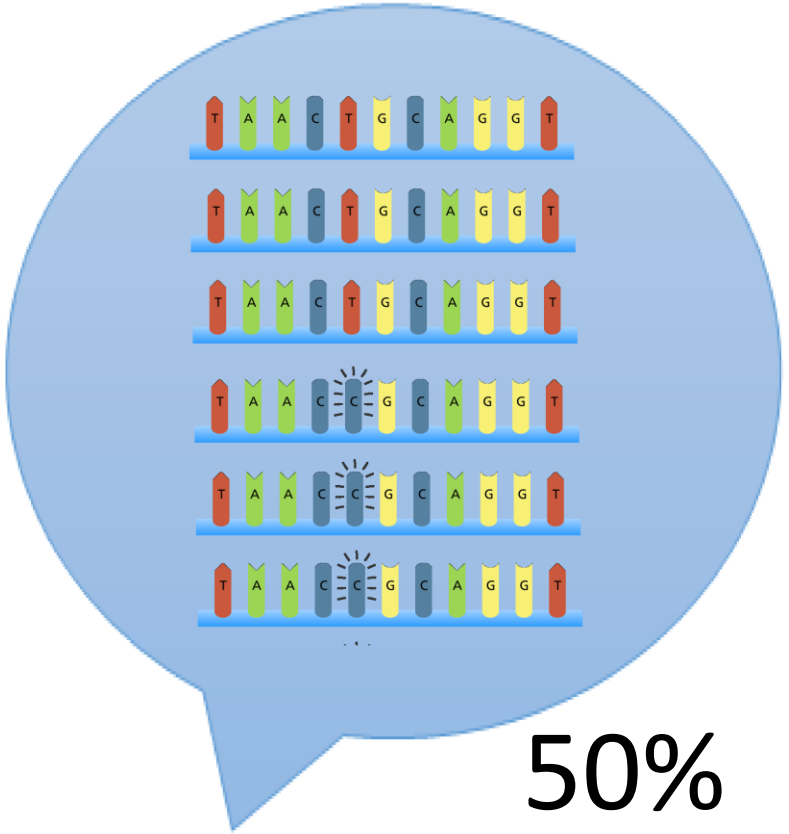
NGS detection of Germline & Somatic mutations

germline mutations

frequency ~50% if heterozygous or ~100% if homozygous.

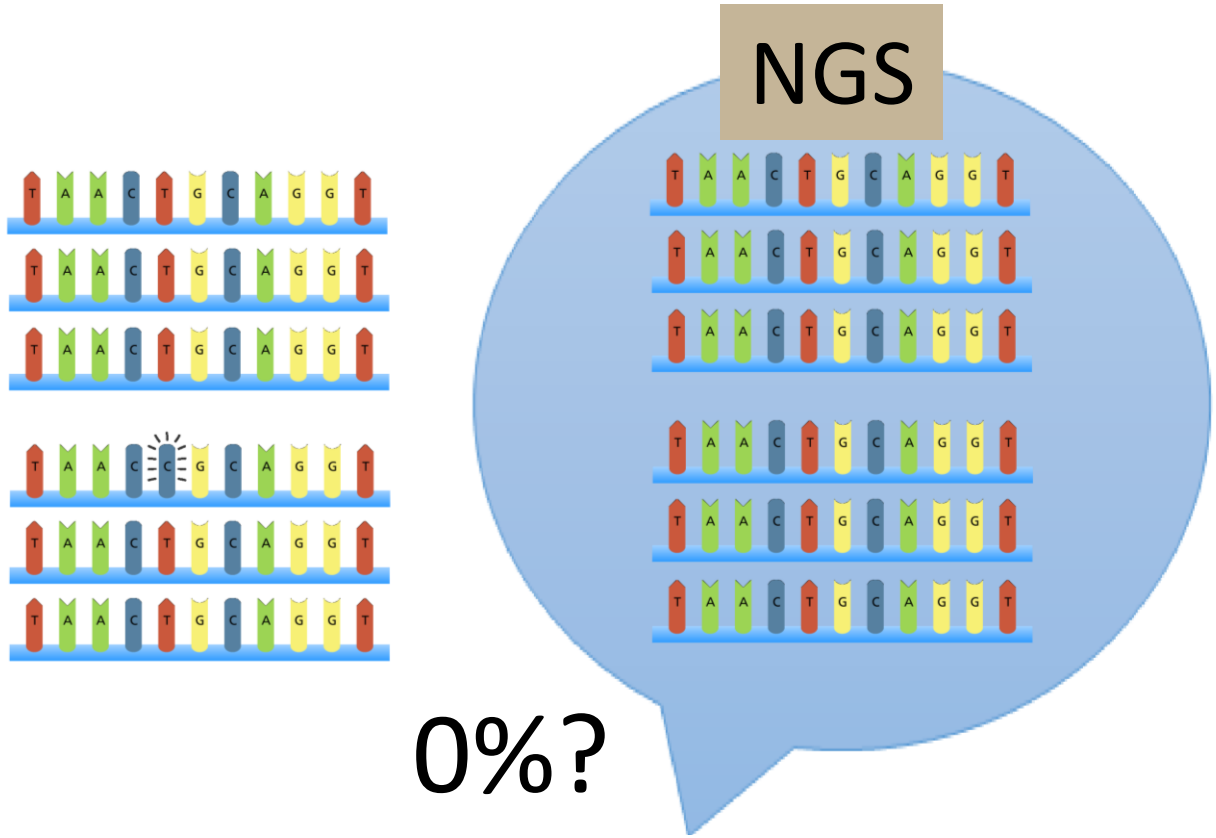
Germline Mutation detected
Even only reading 50% DNA



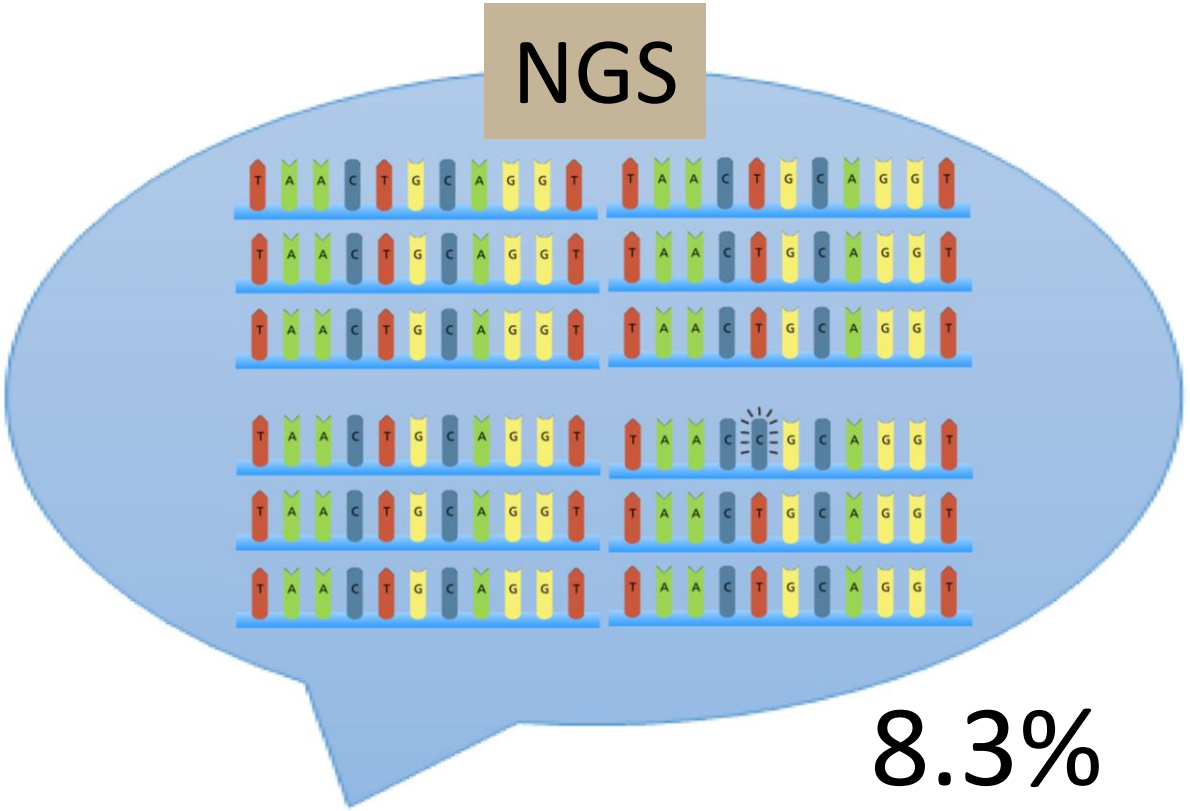


50%

Somatic mutations present at low frequency can be challenging to detect.



Mutation missed as only
sequenced 6 out of 12 DNA fragments



Mutation detected as sequenced all
DNA fragments
1 out of 12 reads got the mutation

Therefore, Germline mutations detection by NGS generally does not require Deep sequencing.

specificity and sensitivity of ctDNA are high

NGS is an advanced sequencing technology with accuracy as high as 99.9%.

- i.e. 99.9% of the times the nucleotides are correctly called

The accuracy is further safeguarded by high sequencing depth

Therefore, the sensitivity and the specificity of ctDNA tests are very high (>99%), even for cancer mutations with very low frequency.

Sensitivity:
ability of a test to correctly identify samples **with mutations**

Specificity:
ability of a test to correctly identify samples **without mutations**

Sample Report

CA Colorectal

ctDNA - SUMMARY OF FINDINGS

ALTERATION† VAF / Fold Change		FDA-APPROVED THERAPIES (This Indication)	OTHER THERAPIES	CLINICAL TRIALS
BRAF V600E Exon 15 SNV	27.45%	SENSITIVE Cetuximab + Encorafenib, Dabrafenib + Trametinib	SENSITIVE Encorafenib + Panitumumab (Guidelines), Binimetinib + Cetuximab + Encorafenib (FDA Breakthrough) SENSITIVE (Other Indications) Atezolizumab + Cobimetinib + Vemurafenib (FDA Approval), Binimetinib + Encorafenib (Guidelines, FDA Approval), Dabrafenib (Guidelines, FDA Approval), Dabrafenib + Trametinib (Guidelines, FDA Approval), Vemurafenib (Guidelines, FDA Approval), Vemurafenib + Cobimetinib (Guidelines, FDA Approval), Cobimetinib (Guidelines), Selumetinib (Guidelines), Trametinib (Guidelines) RESISTANT (Other Indications) Osimertinib (Compelling Clinical Evidence)	Yes
KRAS & NRAS Absence of Resistance Mutation		SENSITIVE Cetuximab, Panitumumab	None	N.A.
PIK3CA P449T	18.37%	None	SENSITIVE (Other Indications) Alpelisib + Fulvestrant (FDA Approval)	Yes

Sample Report

CA Lung

ctDNA - SUMMARY OF FINDINGS

ALTERATION† VAF / Fold Change		FDA-APPROVED THERAPIES (This Indication)	OTHER THERAPIES	CLINICAL TRIALS
EGFR L858R Exon 21 SNV	9.93%	SENSITIVE Afatinib, Dacomitinib, Erlotinib, Erlotinib + Ramucirumab, Gefitinib, Osimertinib	SENSITIVE Bevacizumab + Erlotinib (Guidelines), Icotinib (Guidelines), Patritumab Deruxtecan (FDA Breakthrough)	Yes
AKAP13-RET Fusion (Exon 7|Intron 11) Fusion	0.19%	SENSITIVE Pralsetinib, Selpercatinib	SENSITIVE Cabozantinib (Guidelines) SENSITIVE (Other Indications) Pralsetinib (Guidelines, FDA Approval), Selpercatinib (Guidelines, FDA Approval)	Yes
IDH2 R140Q Exon 4 SNV	6.17%	None	SENSITIVE (Other Indications) Enasidenib (FDA Approval), Bevacizumab (Guidelines)	No
TP53 R248Q Exon 7 SNV	0.32%	None	SENSITIVE (Other Indications) Eprenetapopt (FDA Breakthrough)	Yes

- Microsatellite Status: MS Stable
- No ctDNA alterations were found in the following treatment-relevant genes: *ALK, BRAF, ERBB2, KRAS, MET, NTRK1, NTRK2, NTRK3, ROS1*
- FDA-approved therapies are available in Lung Carcinoma associated with these Genomic Findings. See Section on Genomic Findings With Clinical Actionability (ctDNA) for more details.

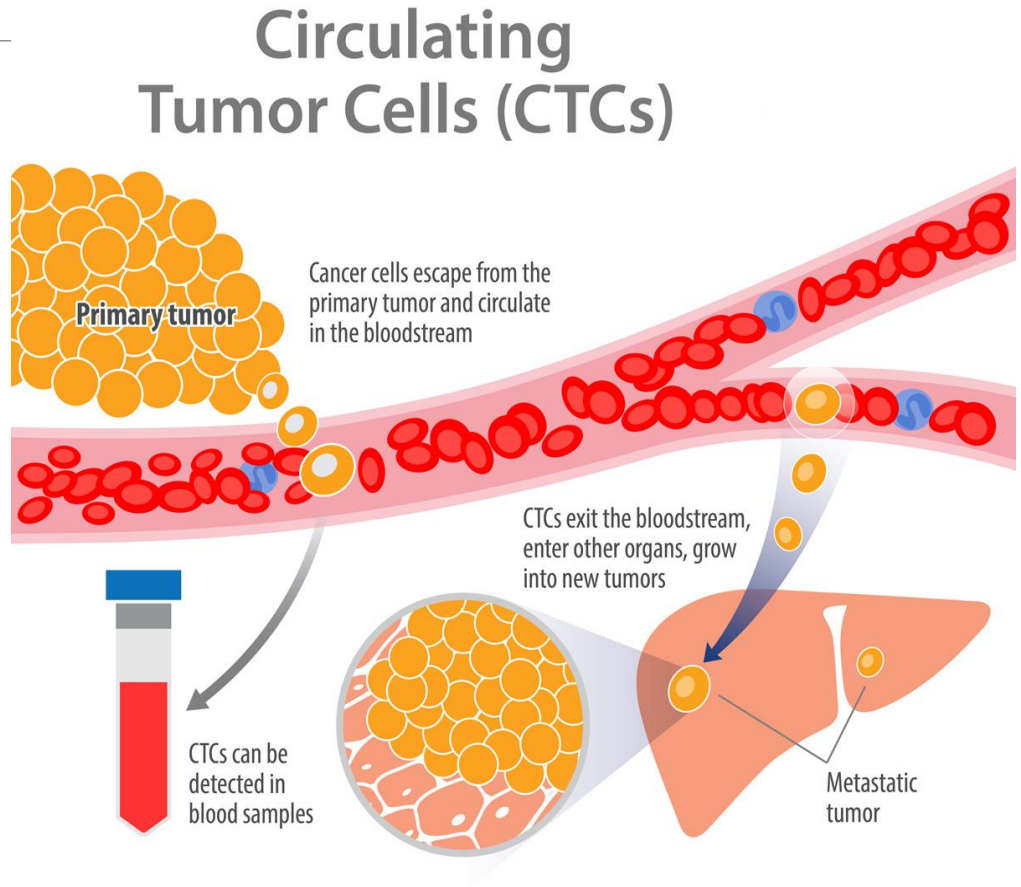
Circulating tumor cells (CTCs)

Circulating Tumor Cells (CTCs) 循環腫瘤細胞

Circulating tumor cells (CTCs) are tumor cells that shed from the primary tumor and intravasate into the peripheral blood circulation system responsible for metastasis.

10-100 CTC per 10 mL of whole blood in cancer patients with metastatic disease.

Reliable surface biomarkers



https://blog.crownbio.com/ctc-metastasis-mouse-models

Circulating Tumor Cells (CTCs) surface biomarkers
循環腫瘤細胞分子標記物

CTC markers mainly includes:

- Epithelial markers
- Mesenchymal markers
- Cancer specific CTC markers

EpCAM (Epithelial Cell Adhesion Molecule):

- a “universal” marker of cancers

CD45：

- A marker of all hematopoietic cells (blood cells)
- CTCs: CD45 negative

Cancer types	Epithelial markers	Mesenchymal markers	Specific markers
Breast cancer	EpCAM/CK8,18,19[234,237,265,266,267,268,269,270,271,272,273,274,275,276,277,278,279,280]	Vimentin[280,281,282,283]	HER2[37,38,39,40,41,42,43,44,45,46]
	CK 5/7/8/18/19[9]	Twist[253,282,284]	ER[39,48,49,50]
	E-Cadherin[9,280,281]	Fibronectin[9,280]	AR[285]
		N-Cadherin[9,280,286]	MRP[48]
		SERPINE1/PAI1[9]	
		β-catenin[281]	
Prostate cancer	EpCAM/CK8,18,19[287,288]	Vimentin[102,289,290,291]	PSMA[51,52,53]
		Twist[290,291]	PSA[239]
			EGFR[51]
			ARV7[256,292,293,294]
			PIM1[295]
			AR ^{v567es}[294]
Kidney cancer	EpCAM[240]	-	CD147[296]
Bladder cancer	EpCAM/CK8,18,19[297,298,299,300]	-	-
Colorectal cancer	EpCAM/CK8,18,19[301,302]	Vimentin[252,303,304,305]	PI3K α[306]
		Twist[252,303,305]	CEA[307,308,309]
		SNAI1[303,305]	PRL3[252]
		AKT2[303,305,306]	
		LOXL3[310]	
		Plastin3[311]	
Non-small-cell lung cancer	CK7/8/18/19[312,313]	Vimentin[109,313,314]	Folate receptor[54,55,56]
	EpCAM/CK8,18,19[109,313,314,315,316,317,318,319]	Twist[313]	Telomerase activity[320]
		N-Cadherin[314]	
		AXL[313]	

Lin et al., Signal Transduction and Targeted Therapy. 2021, 404

Clinical use of Circulating Tumor Cells (CTCs) blood test

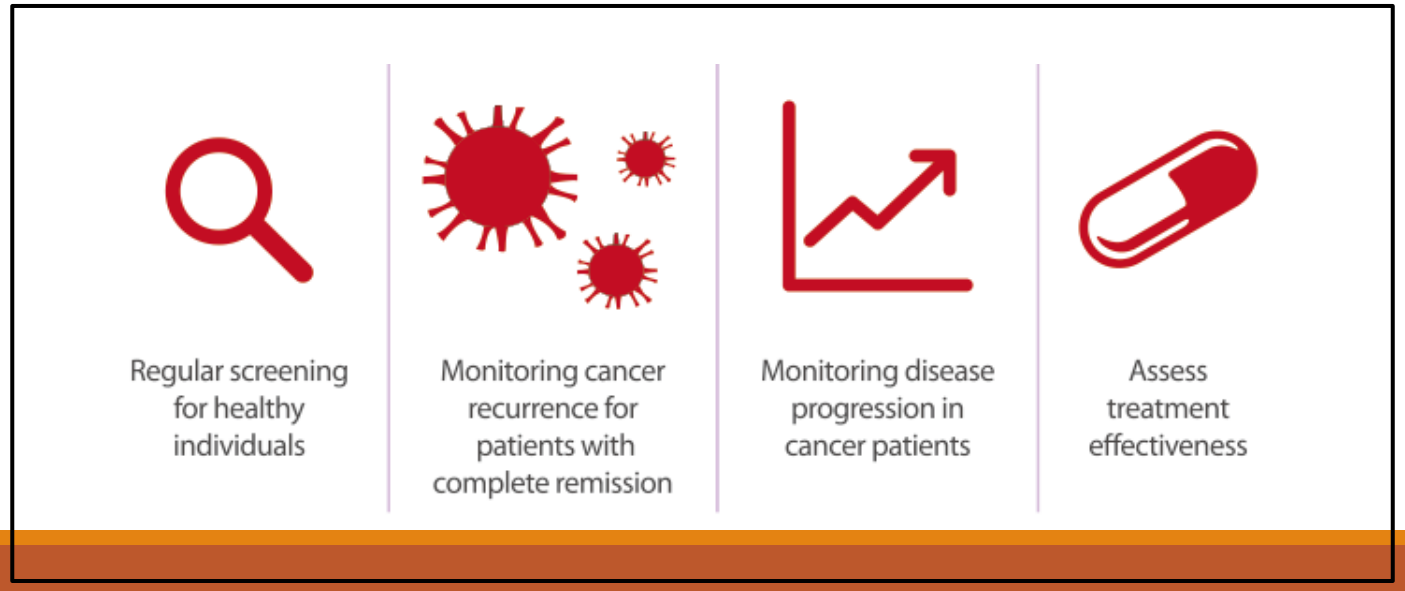
循環腫瘤細胞血液檢測的臨床應用

Regular screening for healthy individuals 健康人群定期篩查

Monitoring cancer recurrence for patients with complete remission 監測癌症復發

Monitoring disease progression in cancer patients 監測癌症病人病程進展

Assess treatment effectiveness
評估藥物或病人治療療效



Parameters Superior to Tissue biopsy

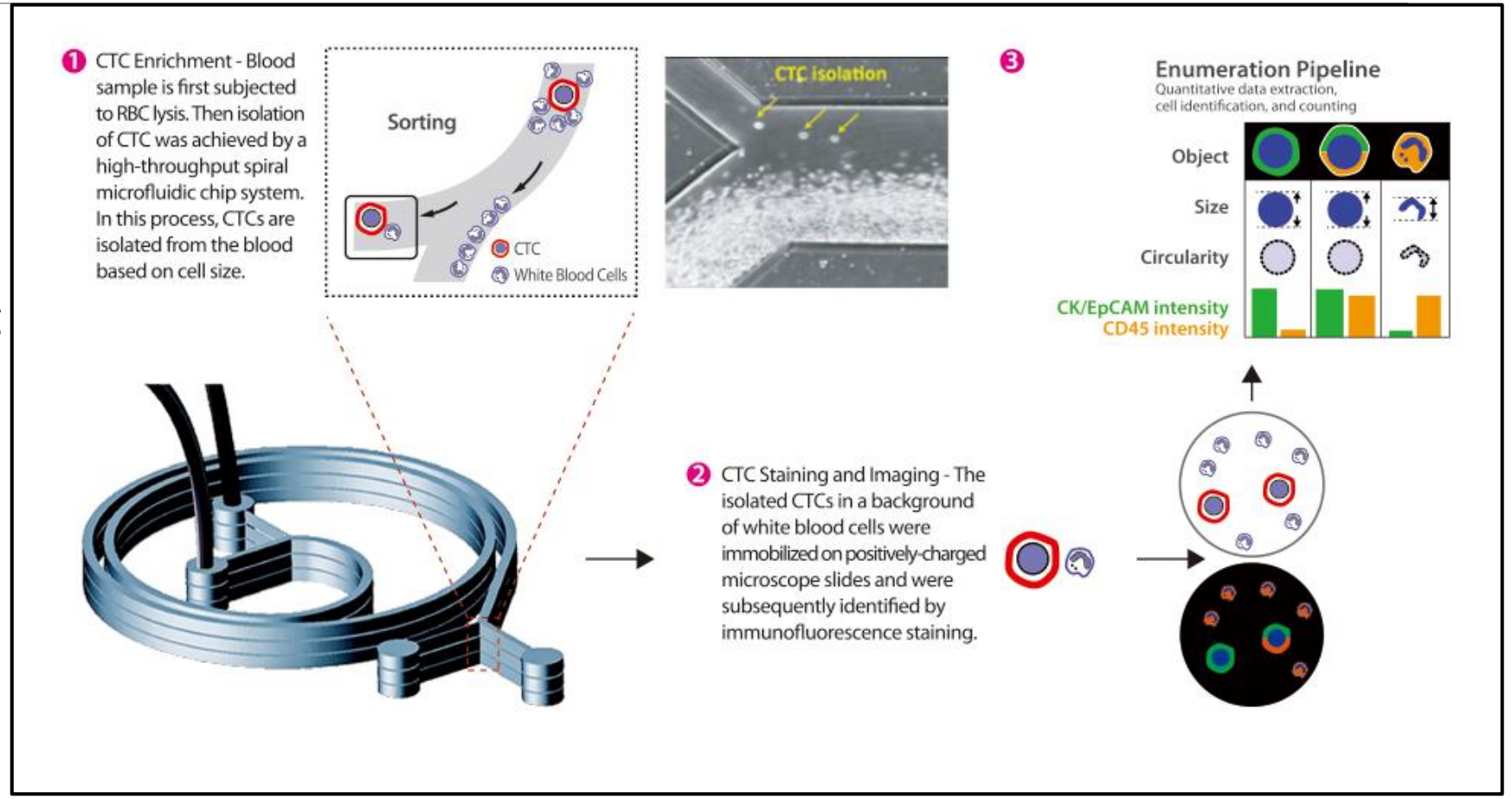
CTC Blood Test	Tissue Biopsy
Non-invasive ✓	Suffered and invasive
Real-time detection Early diagnosis ✓	Not real-time detection, some tumors, e.g. lung tumor, are not accessible for biopsy
Responds to surgical and therapeutic effect ✓ Reflects real-time status of the patients	Information provided by biopsy is static, and it becomes inaccurate with cancer progression
More sensitive ✓	Less sensitive (limited by tumor size)

Workflow for Circulating Tumor Cells (CTCs) blood test

循環腫瘤細胞血液檢測流程

CTCs Blood test:

1. CTCs enrichment
   循環腫瘤細胞富集
2. CTC staining and imaging
   循環腫瘤細胞染色和成像
3. CTC enumeration
   循環腫瘤細胞數目計算



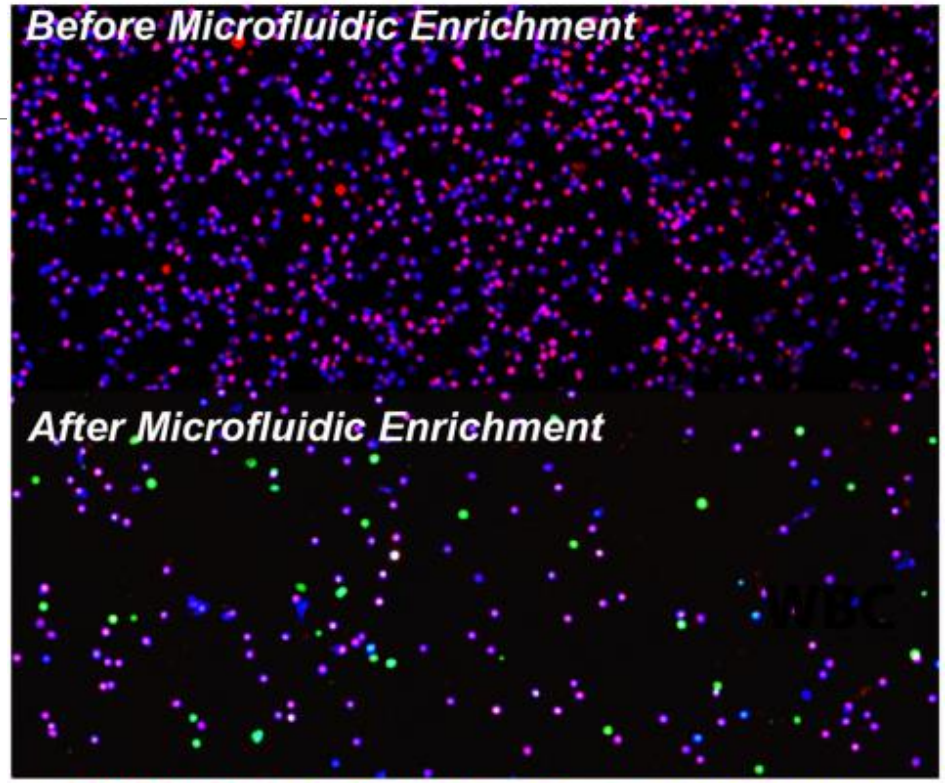
Wong et al., Plos One. 2017 May 8

Immunofluorescence staining of blood sample
血液樣本的免疫熒光染色

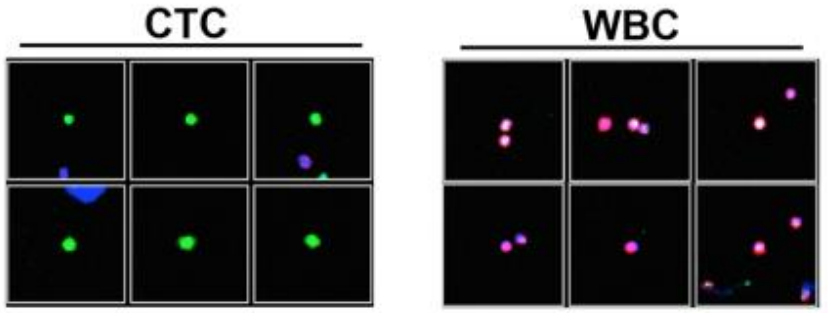
Markers:

- CK/EpCAM: CTCs
- CD45: blood cell
- DAPI: Nucleus

CTCs: CK/EpCAM+; CD45- and DAPI+



CD45 in Red; CK/EpCAM in Green; DAPI in blue



Wong et al., Plos One. 2017 May 8

Sample report of Circulating Tumor Cells (CTCs) blood test

循環腫瘤細胞血液檢測樣品報告

Tumor Monitoring Blood Test Report
腫瘤監測血液測試報告

Referral: 轉介:	Advanced Therapy Center Limited	Reference No.: 參考編號:	003255
Examinee Name: 受測者姓名:	[redacted]	Specimen No.: 樣本編號:	AC9021C0
ID No./Passport No.: 身份証明文件號碼:	[redacted]	Specimen Type: 樣本類別:	Blood
D.O.B: 出生日期:	[redacted]	Specimen Collection Date: 樣本抽取日期:	28 July 2021
Sex: 性別:	Male	Specimen Received Date: 樣本接收日期:	28 July 2021
Race: 種族:	Chinese	Reporting Date: 報告日期:	5 August 2021
Clinical History/Referral Reason: 臨床病歷 / 轉介原因:	History of CA colorectal Dm2	Report No.: 報告編號:	CTM-3HHL1C

Test Items 檢驗項目 | **Results 結果**

Circulating Tumor Cell (CTC) Counting

***Result:** **Unfavorable**

50 CTCs / 7.5 mL of blood

** Unfavorable result if CTC number is ≥ 10. Favorable result if CTC number is ≤ 9.*

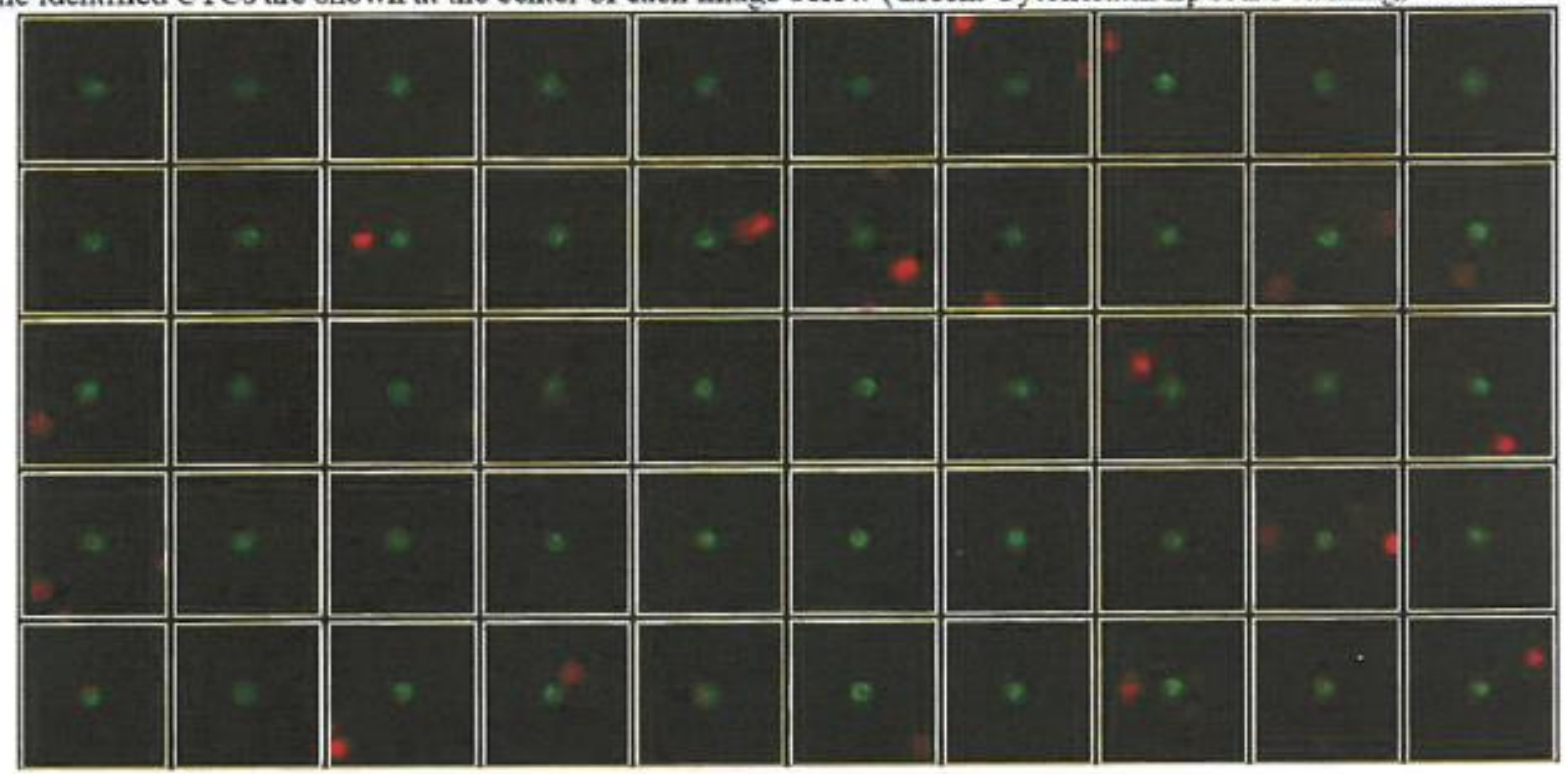
CTC Definition:
- Cell size ≥ 15 μm
- Positive immunofluorescence staining for Cytokeratin/EpCAM biomarkers
- Negative immunofluorescence staining for CD45 biomarkers
- Positive for fluorescent DNA staining

Result Details:
- _3055_ cells, with the size ≥ 15 μm, were found in 7.5 mL of blood.
- _50_ of _3055_ cells were positive for Cytokeratin/EpCAM and DNA staining and negative for CD45 staining. Therefore, 50 circulating tumor cell were detected based on the aforementioned criteria. See the table in the next pages for the analytical results.

Quality Control: Result for positive control done in parallel: Passed, detected _86_ cancer cells
Result for negative control done in parallel: Passed, detected _0_ cancer cells

The identified CTCs are shown at the center of each image below:
以下每幅影像的中心位置顯示檢測出來的血液循環腫瘤細胞:

The identified CTCs are shown at the center of each image below (Green: Cytokeratin/EpCAM staining, Red: CD45)



WBCs are shown below as a reference (Green: Cytokeratin/EpCAM staining, Red: CD45)

CTCs:
CK/EpCAM+; CD45-

Possible false negative/positive results on marker-based CTCs test
可能的假陰性/假陽性結果

False negative:

- Cancer cells undergo frequent changes in protein expression and have the potential to lose surface markers
- Tumour mass is not close to vasculature, may not circulate into the blood stream

False positive

- Many tumor-markers can be expressed on non-cancercells