Correlation of Genomic Alterations Between Tumor Tissue and Circulating Tumor DNA by Next-generation Sequencing

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Introduction of Circulating tumor DNA (ctDNA), 1/2

1. Recent studies have shown that genomic alterations in solid cancers can be characterized by sequencing circulating tumor DNA (ctDNA).

2. This technique is effectively a form of “liquid biopsy”, and such examinations are more widely available and easier to process than standard tumor biopsies.

3. DNA fragments are released into the bloodstream from apoptotic or necrotic cells. In patients with solid tumors, ctDNA is also released via necrosis, autophagy, apoptosis, and other physiological events induced by microenvironmental stress as well as the effects of treatment.

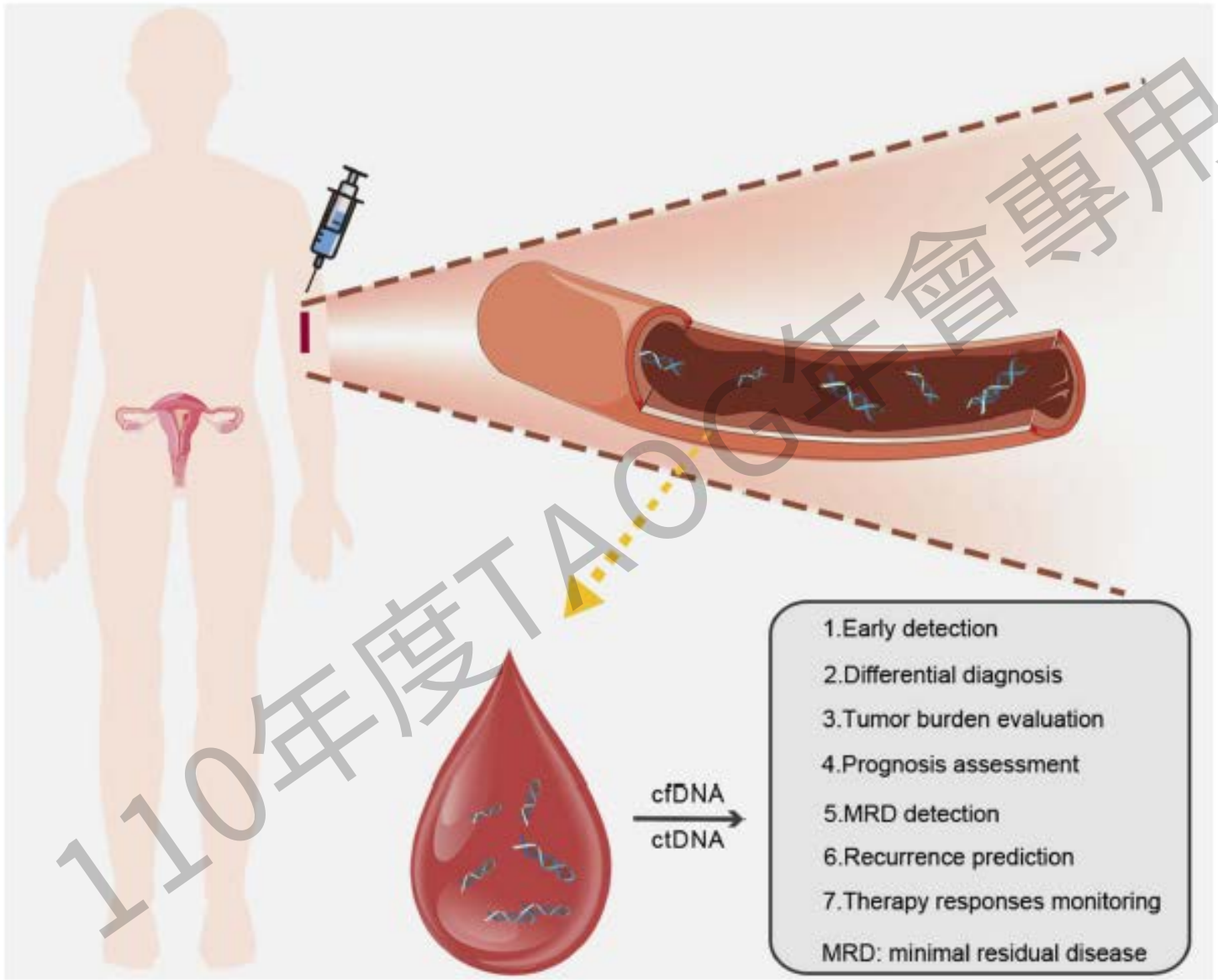
Introduction of Circulating tumor DNA (ctDNA), 2/2

4. The analysis of ctDNA could provide a comprehensive description of tumor genome, overcome the heterogeneity of tissue biopsy, and supplement the missing mutations in tissue samples. Furthermore, ctDNA could be used as a target of liquid biopsy.

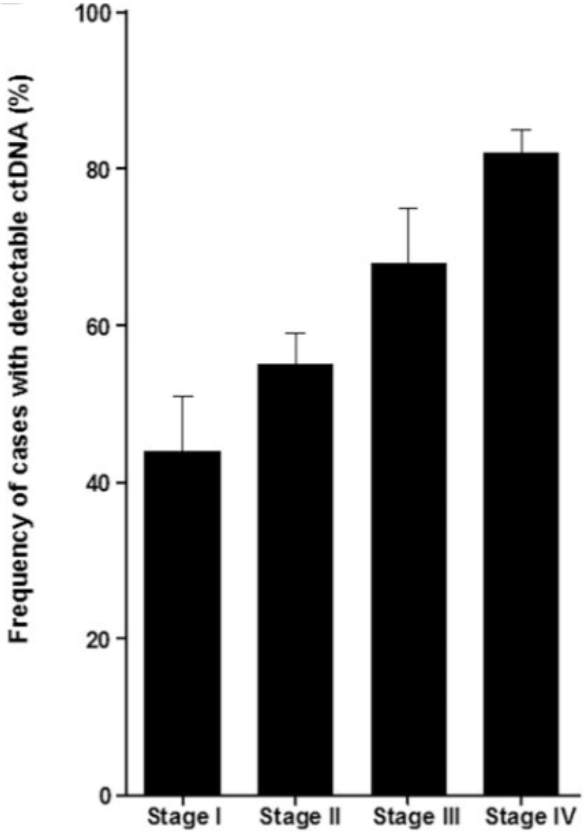
5. Recent improvements in PCR-based assays for analyzing blood samples for ctDNA have provided rapid, cost-effective, and non-invasive alternatives to tumor biopsies. These methods provide information about molecular alterations due to point mutations, including tumor-specific mutations, and have been used as diagnostic, prognostic, and therapeutic decision-making tools.

6. As a new tumor marker, ctDNA promises better personalized therapy and precision medicine.

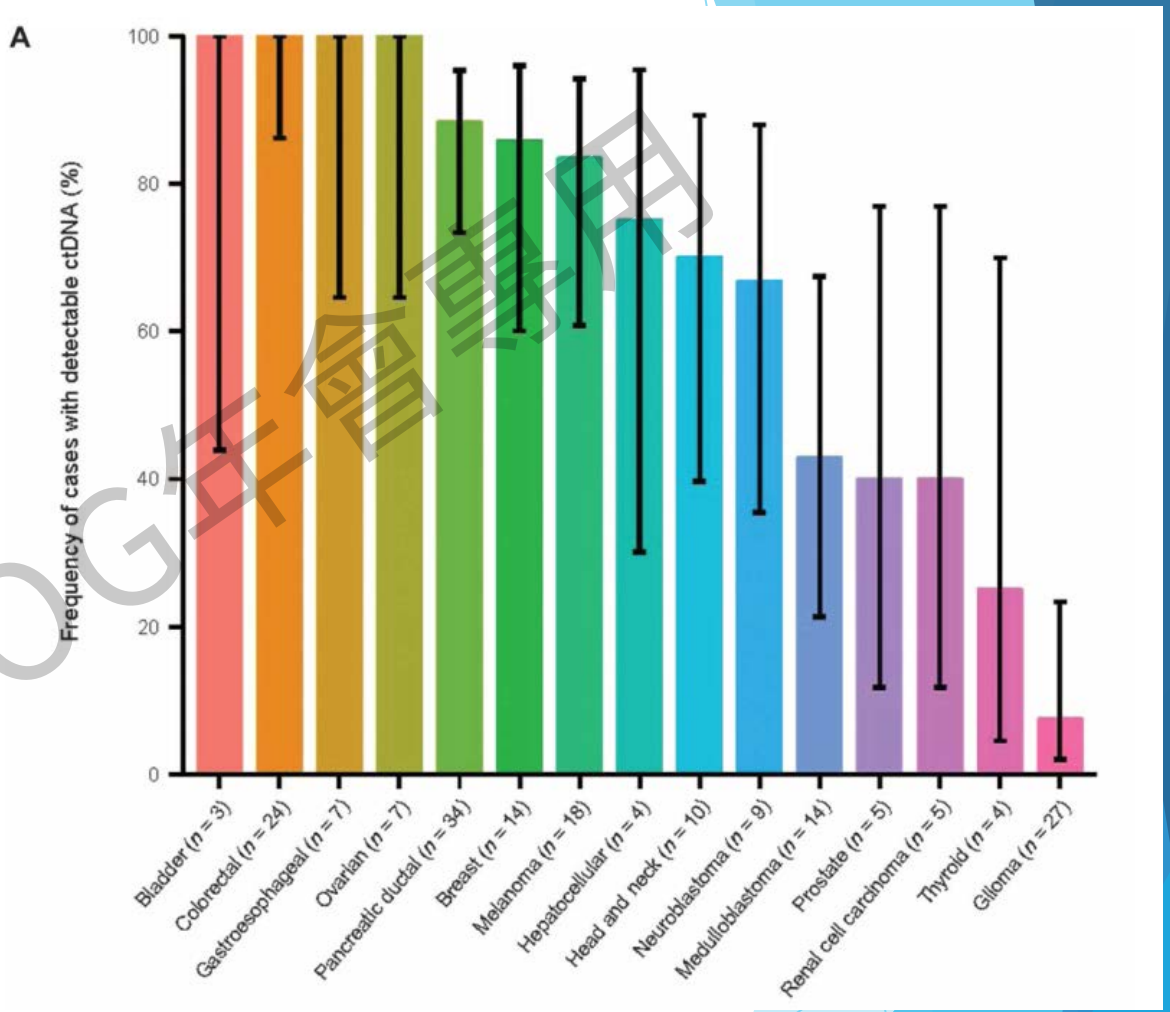
Figure. Applications of cfDNA/ctDNA in ovarian or endometrial cancer patients.



Q chen et al, Circulating Cell-Free DNA or Circulating Tumor DNA in the Management of Ovarian and Endometrial Cancer; Onco Targets Ther. 2019; 12: 11517–11530.



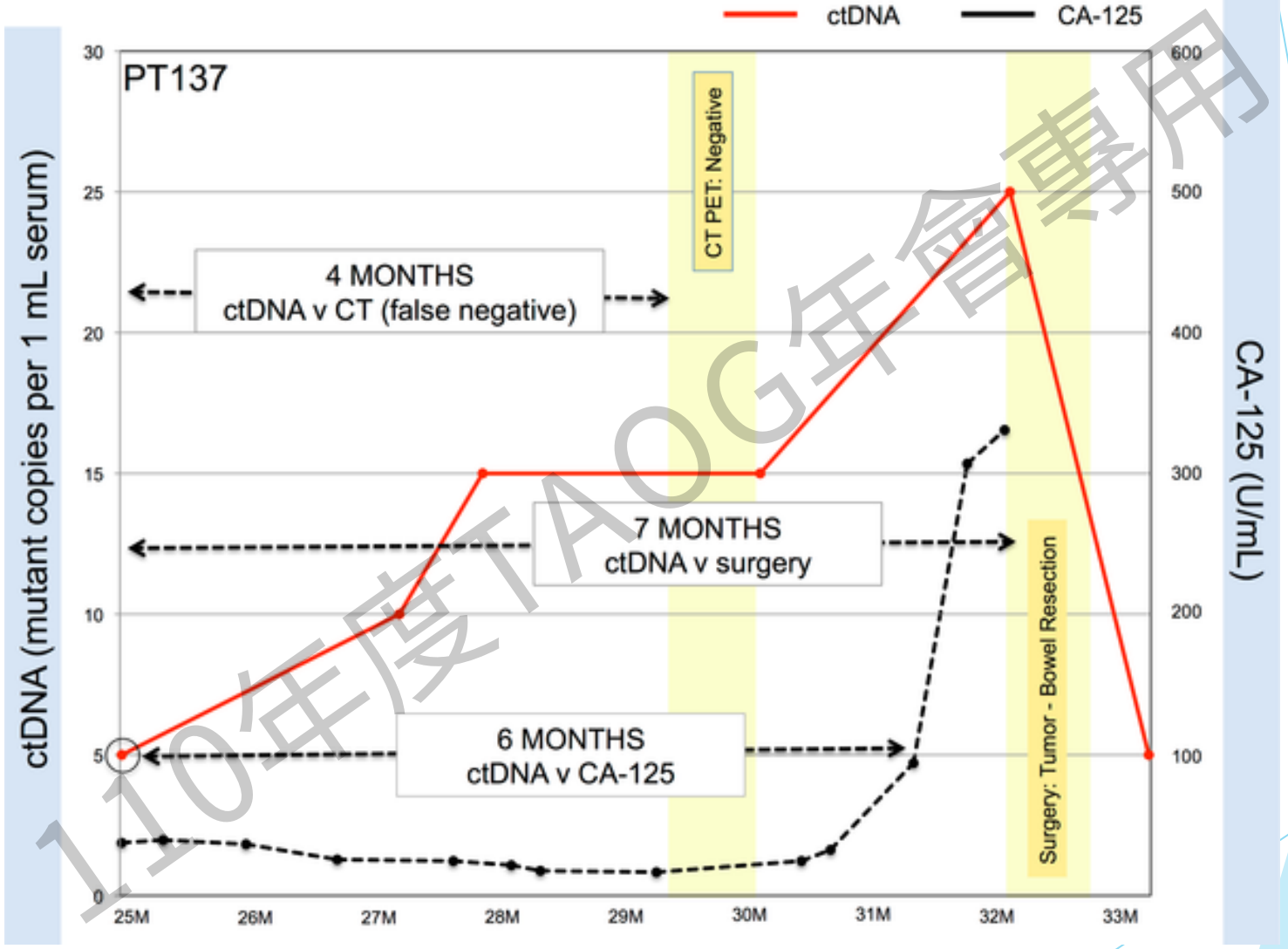
ctDNA in localized and nonlocalized malignancies: Differences in the fraction of patients with detectable levels of ctDNA also correlated with stage: **47%** of patients with stage I cancers of any type had detectable ctDNA, whereas the fraction of patients with detectable ctDNA was **55, 69, and 82%** for patients with stage II, III, and IV cancers, respectively



ctDNA in advanced malignancies: Fraction of patients with detectable ctDNA

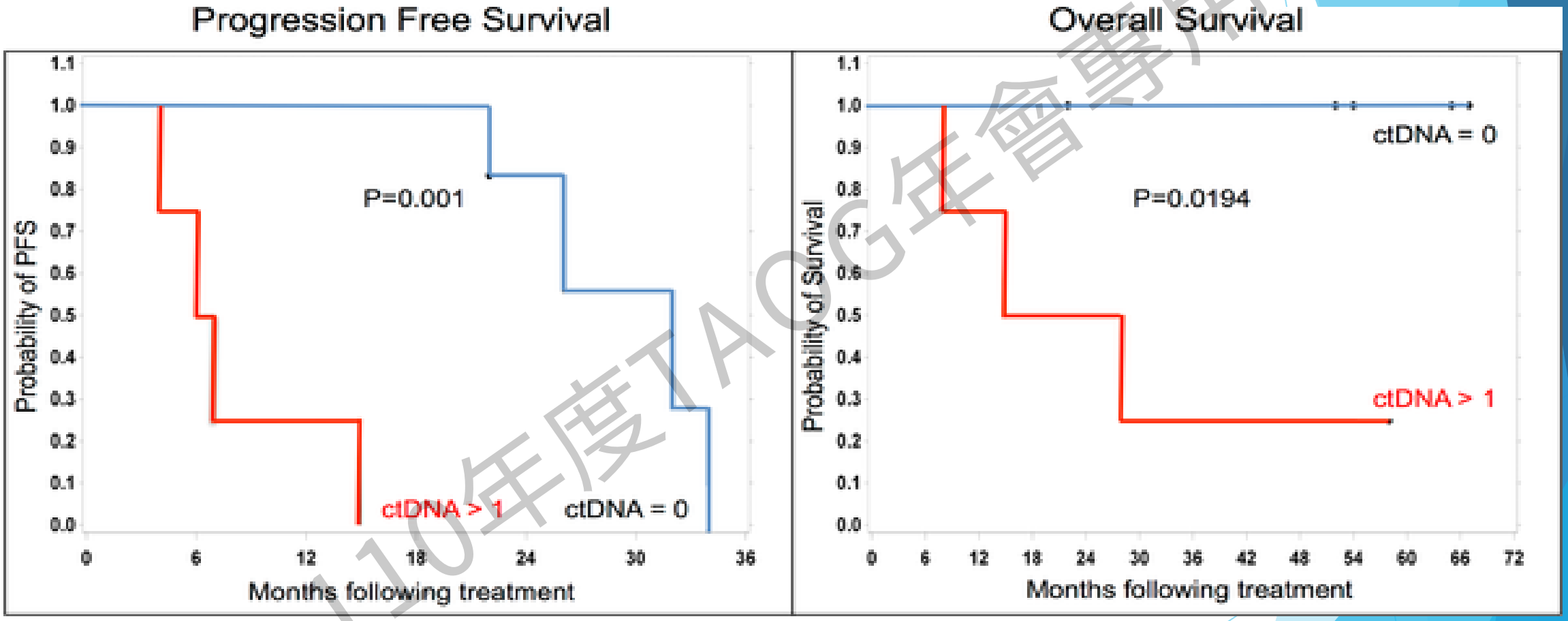
C. Bettegowda, et al. Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies; *Sci Transl Med*. 2014 February 19; 6(224): 224ra24

Circulating tumor DNA can detect relapse earlier than CA125 and CT scan imaging



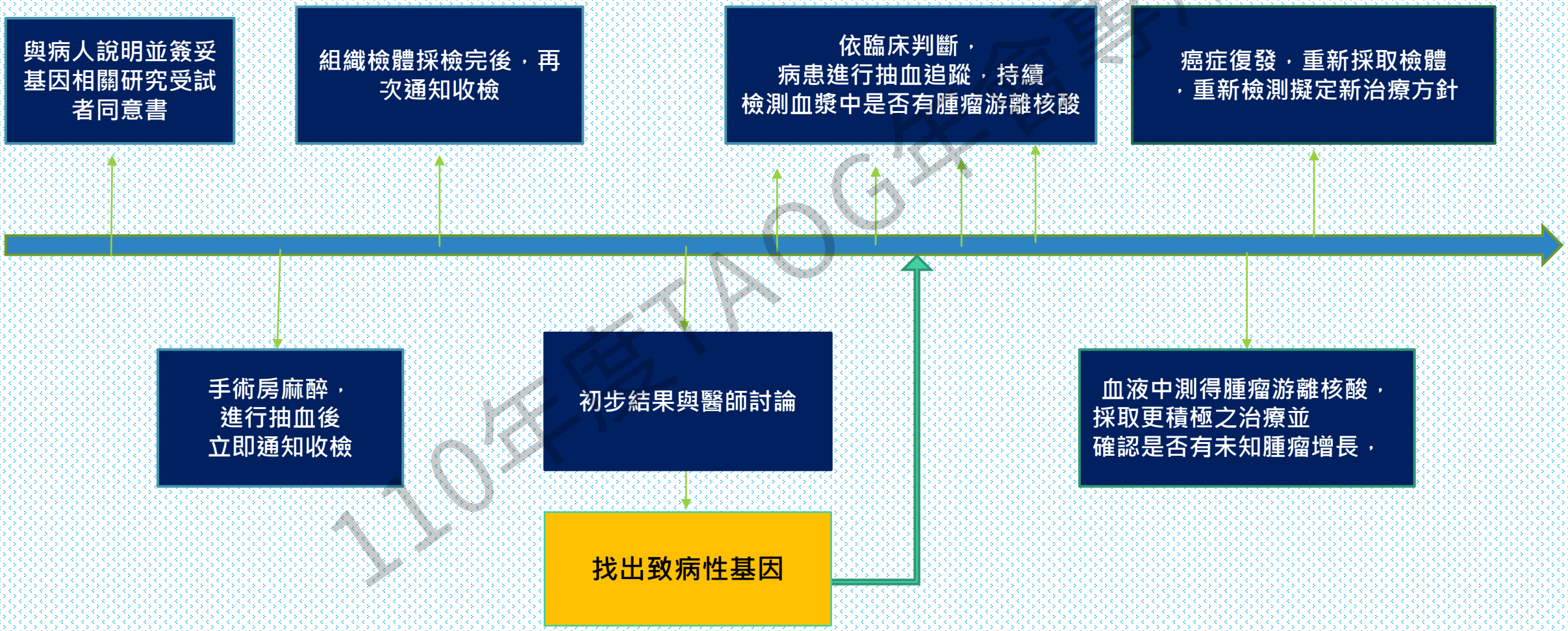
Pereira E, Camacho-Vanegas O, Anand S, Sebra R, Catalina Camacho S, et al. (2015) Personalized Circulating Tumor DNA Biomarkers Dynamically Predict Treatment Response and Survival In Gynecologic Cancers. **PLOS ONE 2015 Dec 30. 10(12): e0145754.** https://doi.org/10.1371/journal.pone.0145754
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0145754

Undetectable levels of ctDNA following initial treatment are associated with improved survival



Pereira E, Camacho-Vanegas O, Anand S, Sebra R, Catalina Camacho S, et al. (2015) Personalized Circulating Tumor DNA Biomarkers Dynamically Predict Treatment Response and Survival In Gynecologic Cancers. **PLOS ONE 2015 Dec 30. 10(12): e0145754.** https://doi.org/10.1371/journal.pone.0145754
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0145754

精準醫學-癌基因體相關檢驗


與病人說明並簽妥基因相關研究受試者同意書
組織檢體採檢完後，再次通知收檢
依臨床判斷，病患進行抽血追蹤，持續檢測血漿中是否有腫瘤游離核酸
癌症復發，重新採取檢體，重新檢測擬定新治療方針
手術房麻醉，進行抽血後立即通知收檢
初步結果與醫師討論
找出致病性基因
血液中測得腫瘤游離核酸，採取更積極之治療並確認是否有未知腫瘤增長，

Genomic profiling requires a tumour biopsy (1/2)

Solid vs liquid biopsy

Solid biopsy

e.g. surgical biopsy / excision or fine needle aspirate[1]

\+ ***Considered the "gold standard" for cancer diagnosis and allows both morphological and molecular assessment[1-3]***

- **Involves a relatively invasive procedure[1,2]**
- **May not be feasible for some tumours, especially when not amenable or when highly necrotic[1-4,7,8]**
- **May not provide sufficient sample for all necessary pathological workup[1,3]**
- **Requires more surgical infrastructure and has longer turn-around time than liquid biopsy[9,5]**
- **Is not suitable for longitudinal monitoring[2]**
- **Single site biopsy may not represent tumour heterogeneity[10]**

1. Francis, G. & Stein, S. (2015) *Int J Mol Sci* 16:14122-42; 2. De Rubis, G., et al. (2019) *Trends Pharmacol Sci* 40:172-86; 3. Chouaid, C., et al., (2014) *Lung Cancer* 86:170-3; 4. Bardelli, A., et al. (2017) *Cell* 31:172–9; 5. Wan, J.C.M., et al., (2017) *Nat Rev Cancer* 17:223-38; 6. Mattox, A.K. (2019) *Sci Transl Med* 11:eeay1984; 7. Kato, S., et al. (2017) *Cancer Res* 77:4238-46; 8. Stevenson, M., et al. (2014) *Cancer Invest* 32:291–8; 9. Temilola , D.O., et al. (2019) *Cells* , 8, 862; doi:10.3390/cells8080862; 10. Scherer, F. (2020) in *Recent Results in Cancer Research*: *Tumor Liquid Biopsies*. Springer.

Genomic profiling requires a tumour biopsy (2/2)

Solid vs liquid biopsy

Liquid biopsy

e.g. blood, urine, saliva or cerebrospinal fluid[1,2,4]

Not yet comparable to solid biopsy with respect to evidence for clinical utility and applicability in initial cancer diagnosis and management[2,5,6]

- **Less invasive than solid biopsy[1,2]**
- **May be used when tissue biopsies cannot be performed due to inaccessibility[1,4]**
- **Provides an option when tissue samples are limited or exhausted[1]**
- **Requires less surgical infrastructure and has shorter turn-around time than tissue biopsy[9,5]**
- **Is suitable for repeat sampling during longitudinal monitoring[2,5]**
- **Can capture the genomic heterogeneity of all cancerous lesions[10]**

1. Francis, G. & Stein, S. (2015) *Int J Mol Sci* 16:14122-42; 2. De Rubis, G., et al. (2019) *Trends Pharmacol Sci* 40:172-86; 3. Chouaid, C., et al., (2014) *Lung Cancer* 86:170-3; 4. Bardelli, A., et al. (2017) *Cell* 31:172–9; 5. Wan, J.C.M., et al., (2017) *Nat Rev Cancer* 17:223-38; 6. Mattox, A.K. (2019) *Sci Transl Med* 11:eeay1984; 7. Kato, S., et al. (2017) *Cancer Res* 77:4238-46; 8. Stevenson, M., et al. (2014) *Cancer Invest* 32:291–8; 9. Temilola , D.O., et al. (2019) *Cells* , 8, 862; doi:10.3390/cells8080862; 10. Scherer, F. (2020) in *Recent Results in Cancer Research*: *Tumor Liquid Biopsies*. Springer.

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https://doi.org/10.1007/s00432-018-2747-9

ORIGINAL ARTICLE – CANCER RESEARCH

Correlation of genomic alterations between tumor tissue and circulating tumor DNA by next-generation sequencing

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Abstract

Purpose Analysis of circulating tumor DNA (ctDNA) offers an unbiased and noninvasive way to assess the genetic profiles of tumors. This study aimed to analyze mutations in ctDNA and their correlation with tissue mutations in patients with a variety of cancers.

循環癌細胞、循環癌DNA檢驗

收檢方式-

- 共計抽四支採血管，**每管至少抽滿8ml。**
- **先將採血管標記編號，並依照順序抽取。**

(勿用針筒抽完再打進採血管中，

請使用真空採血針或靜脈留置針進行採血。)

- 採集完成後，立即上下輕輕混合5次。
- 以室溫存放，並立即通知收檢。

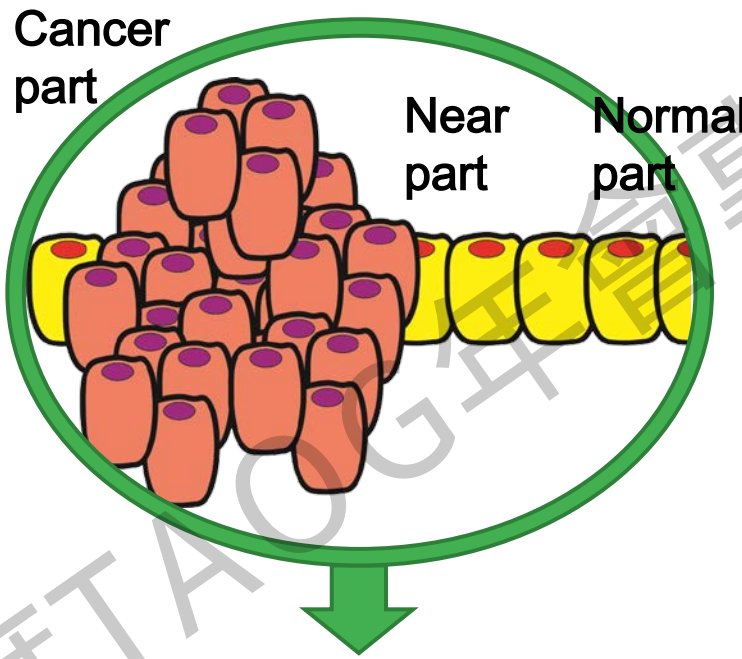
姓名: 病歷號: 1 第一管 1	姓名: 病歷號: 2 第二管 2	姓名: 病歷號: 3 第三管 3	姓名: 病歷號: 4 第四管 4

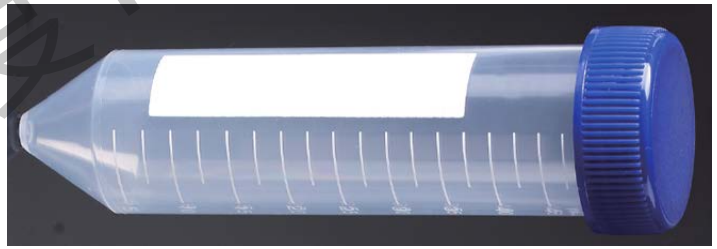
癌基因體套組檢驗

(組織)收檢方式-

- 大塊腫瘤組織採檢，最佳狀況應**包含腫瘤部位、正常組織部位。**將其放至於含保存液之50ml尖底離心管。
- 其餘部位(小)腫瘤組織塊，**僅採集腫瘤部位**，採集後放至於4ml血清管中，可採集1至數個於不同管中。
- 確定容器蓋子鎖緊，避免保存液外漏。
- 檢體保存於室溫中，並通知收檢。

cross section(50 ml tube)



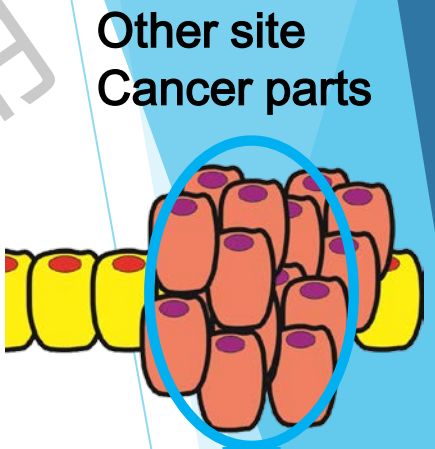


姓名:

病歷號:

組織部位:

Harvest in 4 ml tube



姓名:

病歷號:

組織部位:

姓名:

病歷號:

組織部位:

Table 1

Characteristics of patients with both tissue and ctDNA NGS testing.

Parameters	Total number of patients, $N=21$
Gender	
Women	13 (61.9%)
Men	8 (38.1%)
Age (median, range)	64 years (40–73)
Tumor origin	
Endometrial	7 (33.33%)
Colorectal	5 (23.81%)
Esophageal	5 (23.81%)
Lung	4 (19.05%)
Stage	
Endometrial	
IA	4
IB	1
II	1
IIIC	1
Colorectal	
IIIB	3
IVA	2
Esophageal	
IA	1
IIB	3
Unknown	1
Lung	
IA	2
IIA	1
Unknown	1
Metastasis	
No	15
Yes	4
Unknown	2
Grade	
1	3
2	9
3	7
Unknown	2

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Table 2 Mutations detected in tissue but missed in liquid biopsy and detected in both

Patient ID	Mutation location (hg.19)	Gene	Actionable	Reference allele	Alternative allele	tVAF (%, tissue)	bVAF (%, plasma)	Detection in plasma (Fisher's exact test p-value)
UCEC-01	10:89692905	***PTEN***	No	G	A	35/54 64.81	0/78	Not detected
	11:108115681	***ATM***	No	G	T	32/92 34.78	0/91	Not detected
	5:67588951	***PIK3R1***	No	C	T	45/98 45.92	0/13	Not detected
UCEC-02	3:178916936	***PIK3CA***	Yes	G	A	70/671 10.43	0/98	Not detected
	3:178952085	***PIK3CA***	Yes	A	G	66/586 11.26	0/211	Not detected
UCEC-03	3:178917478	***PIK3CA***	Yes	G	A	69/109 63.30	0/57	Not detected
	3:41266113	***CTNNB1***	Yes	C	G	51/255 20.00	0/239	Not detected
	1:27022913	***ARID1A***	No	CCCGCCGC-CGCCAGC AGCCTGG GCAA		106/167 63.47	0/22	Not detected
UCEC-04	14:105246551	***AKT1***	Yes	C	T	232/240 96.67	2/87 2.30	Detected (high confidence, p<0.05)
UCEC-05	3:178936094	***PIK3CA***	Yes	C	A	145/338 42.90	0/182	Not detected
	3:41266113	***CTNNB1***	Yes	C	T	169/369 45.80	0/188	Not detected
UCEC-06	No mutation identified in tissue							
UCEC-07	10:89717672	***PTEN***	No	C	T	30/84 35.71	1/71 1.41	Detected (high confidence, p<0.05)
	17:63532585	***AXIN2***	No	C	-	15/103 14.56	0/83	Not detected
	10:89717770	***PTEN***	No	A	-	61/123 49.59	1/143 0.7	Detected (high confidence, p<0.05)

tVAF: variant allele frequency in tissue, bVAF: variant allele frequency in blood

Ya-Sian Chang, Yao-Ching Hung, Jan-Gowth Chang, et. al. J Cancer Research and Clin. Oncol. (2018) 144:2167–2175

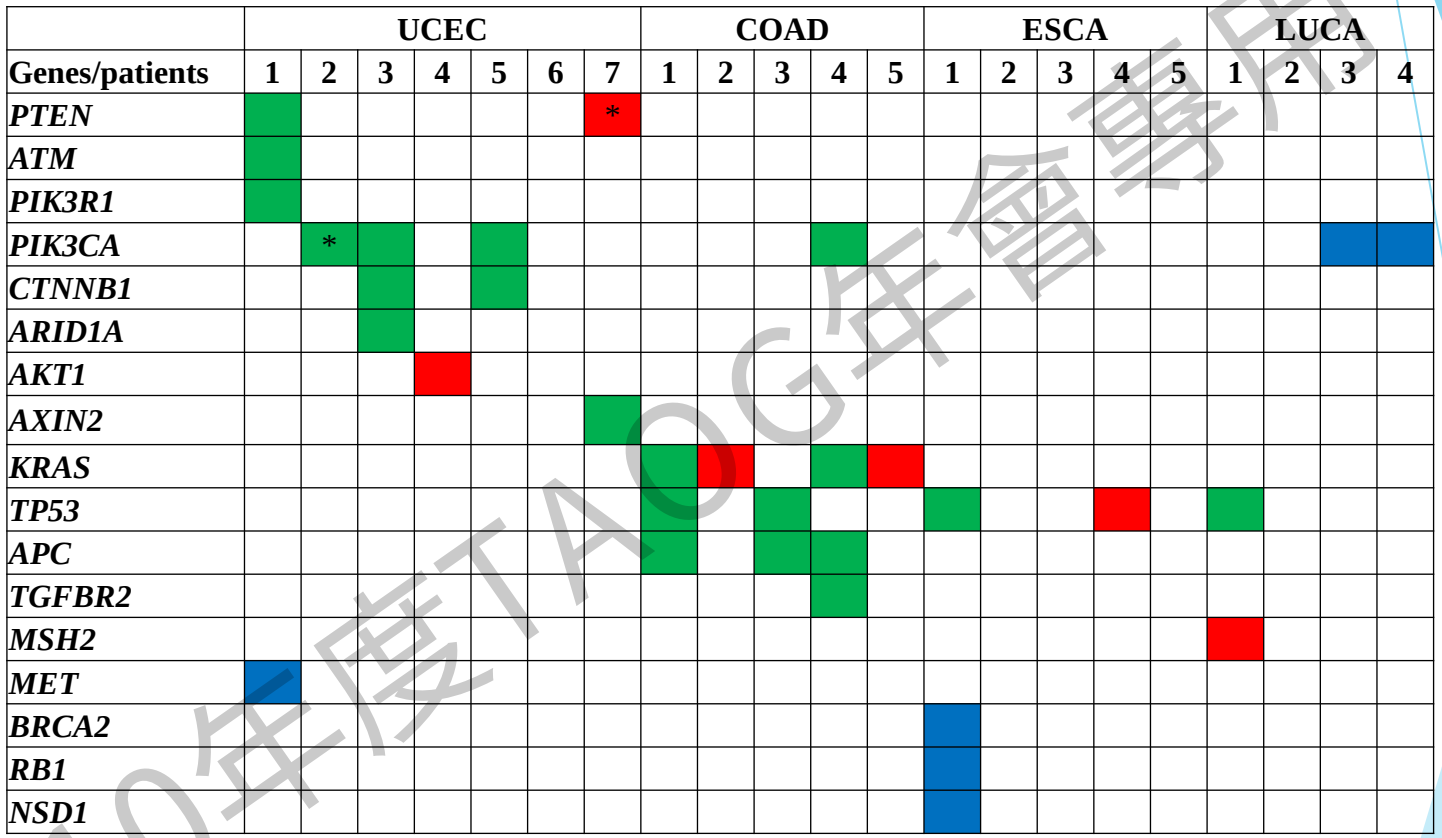
Table 3 Mutations detected in liquid biopsy but missed in tissue.

Patient ID	Mutation location (hg.19)	Gene	Actionable	Ref allele	Alt allele	tVAF (%, tissue)	bVAF (%, plasma)	p-value (Fisher's exact test)
UCEC-01	7:116417457	***MET***	Yes	G	A	0/66	5/22 22.73	0.0007

tVAF: variant allele frequency in tissue, bVAF: variant allele frequency in blood

Ya-Sian Chang, Yao-Ching Hung, Jan-Gowth Chang, et. al. J Cancer Research and Clin. Oncol. (2018) 144:2167–2175

Fig. 1 Heat map of detected mutations and their concordance in tissue and plasma. A total of 21 patients were tested for both NGS assays.



Green: tissue mutation
Blue: plasma mutation
Red: concordant plasma and tissue mutation
White: no mutation present
*** 2 mutations**

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Table 4 Sensitivity, specificity and diagnostic accuracy across six genes.

		Tissue mutations		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
ctDNA mutations		(+)	(-)					
PIK3CA	(+)	0	2					
	(-)	4	15	0	88.24	0	78.95	71.43
CTNNB1	(+)	0	0					
	(-)	2	19	0	100	0	90.48	90.48
AKT1	(+)	1	0					
	(-)	0	20	100	100	100	100	100
KRAS	(+)	2	0					
	(-)	2	17	50	100	100	89.47	90.48
TP53	(+)	1	0					
	(-)	4	16	20	100	100	80	80.95
MET	(+)	0	1					
	(-)	0	20	0	95.24	0	100	95.24
Total positive		4	3					
Total negative		12	89					
Total (positive+negative)		16	92	25	96.74	57.14	88.12	86.11

PPV: positive predictive value, NPV: negative predictive value

Ya-Sian Chang, Yao-Ching Hung, Jan-Gowth Chang, et. al.
J Cancer Research and Clin. Oncol. (2018) 144:2167–2175

Table. Overall concordance between ctDNA and tissue-based DNA by tissue biopsy site (primary or metastatic) (N=78, Gynecologic cancer patients)

Patients who had both ctDNA and tissue DNA sequencing (N=78)*					
		Tissue DNA (+)	Tissue DNA (-)	Overall concordance	Kappa ** (SE)
TP53	ctDNA (+)	35	6	75.6%	0.51 (0.10)
	ctDNA (-)	13	24		
PIK3CA	ctDNA (+)	11	6	78.2%	0.42 (0.12)
	ctDNA (-)	11	50		
KRAS	ctDNA (+)	9	3	88.5%	0.60 (0.12)
	ctDNA (-)	6	60		

L. M. Charo et al. Molecular Oncology, 2020

It is not only about technology, but also about biology: Why is the tumour profile not 100% concordant with liquid biopsy?

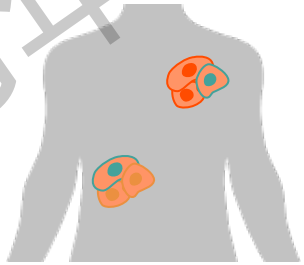
Tissue biopsy may not capture the genomic landscape of a patient's entire tumour burden

Intratumour heterogeneity

The genomic landscape **within a single tumour manifestation** may not be uniform

Tissue biopsy may not capture subclonal populations of tumour cells with distinct alterations

Intrapatient heterogeneity



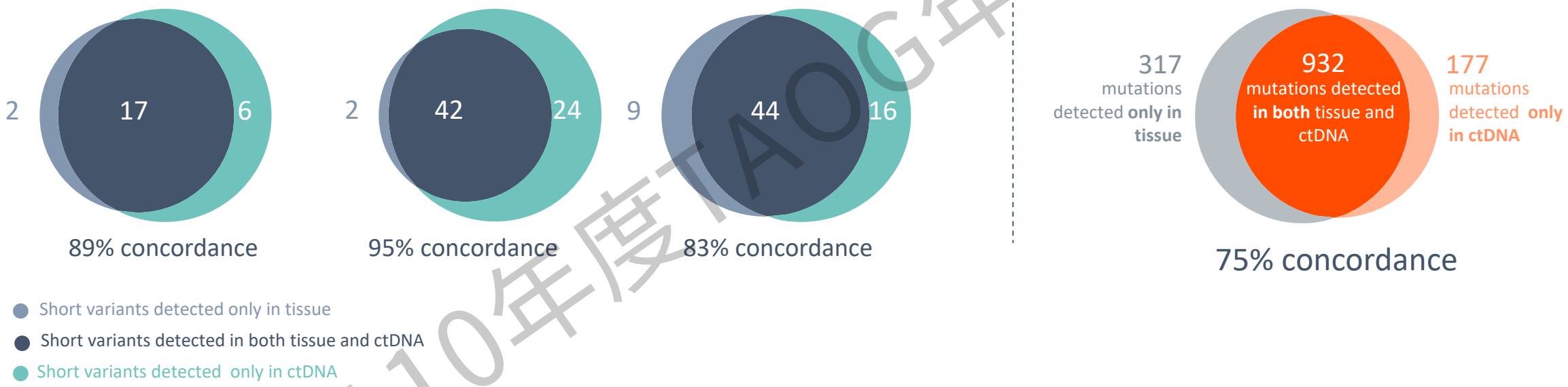
The genomic landscape may **differ between tumour sites** within a patient

Tissue biopsy from a single lesion will miss alterations unique to other lesions

Keep in mind: *The genomic landscape of a cancer evolves over time, hence archival tissue may not fully represent the tumour genotype at progression*

Scherer, F. (2020) in *Recent Results in Cancer Research*: *Tumor Liquid Biopsies*. Springer.

Alterations detected in ctDNA are generally concordant with those in temporally-matched tissue



Concordance on positives between tissue and ctDNA was generally high for short variants*.
However, some alterations were only identified in ctDNA, suggesting that liquid biopsy may capture tumour heterogeneity[1-4]

*** Point mutations and short insertions and deletions.**
ctDNA: circulating tumour DANN; GI: gastrointestinal.
1. Chung, J. H., et al. (2017) *Ann Oncol* 28:2866-73; 2. Schrock, A.B., et al. (2018) *Clin Cancer Res* 24(8):1881-90;
3. Clark, T.A., et al. (2018) *J Mol Diagn* 20(5):686-702; 4. Shu, Y., et al. (2017) *Sci Rep* 7:583.

Conclusion

Added clinical value through capturing genomic heterogeneity

Discordance between mutation profiles from solid and liquid biopsies is generally common because…

Liquid biopsy

…can capture the genomic heterogeneity of all cancerous lesions, thus, ctDNA can detect mutations that may not be identified in one single biopsy

…but may not detect mutations identified in tissue samples due to a low content of ctDNA in cfDNA (e.g. in cancers with low tumour burden)

*Thus combining all high-confidence somatic mutations present either in **solid or liquid biopsy** samples for the generation of a final report is recommended*

cfDNA: cell-free DNA; ctDNA: circulating tumour DNA.

Shu, Y., et al. (2017) *Sci Rep* 7:583.

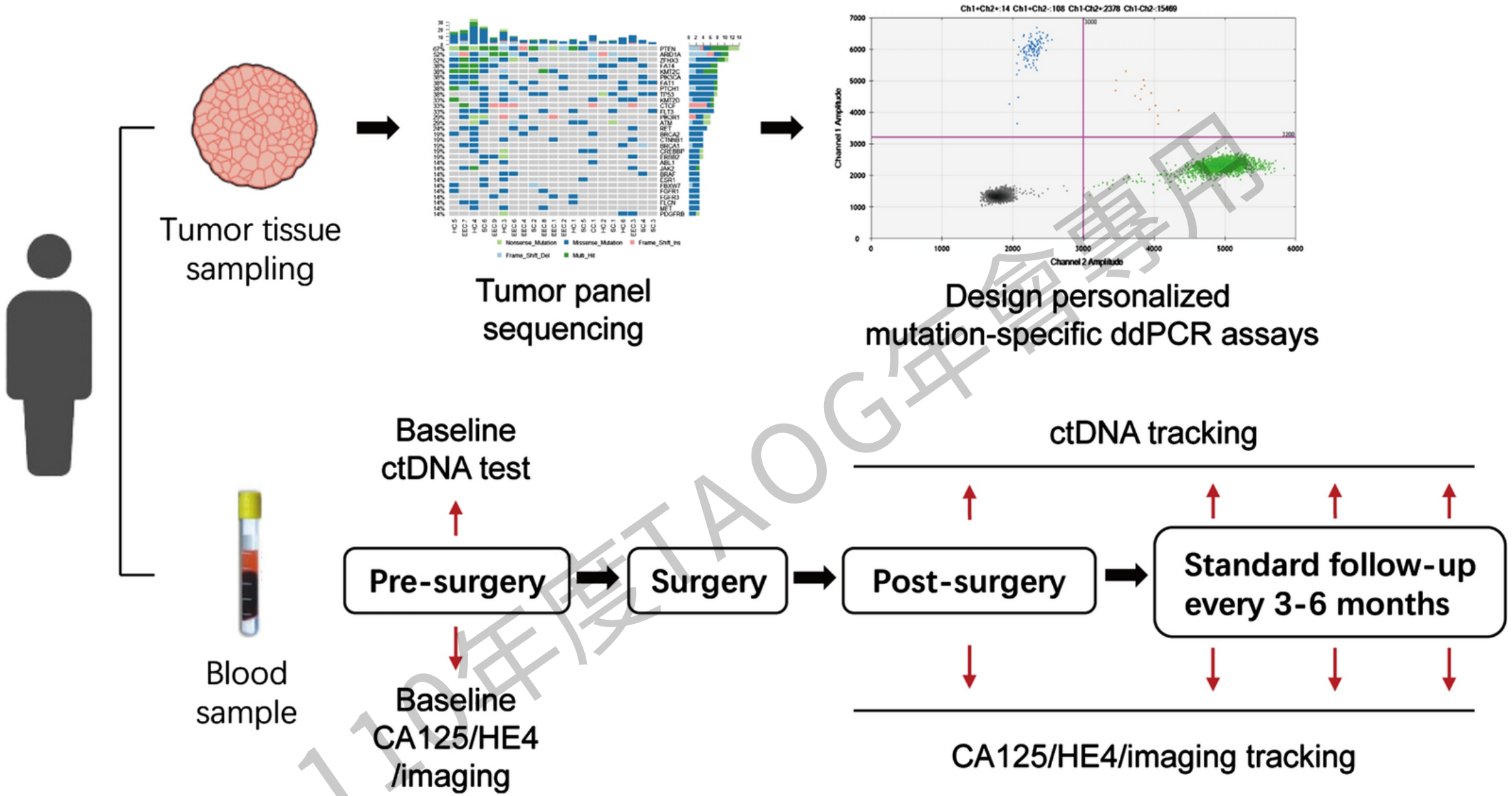


Fig. Personalized ddPCR assays for mutation tracking of ctDNA in plasma of patients with high-risk endometrial cancer.

W. Feng et al; J of Translational Medicine. 2021: 19 (51)

Conclusion

- 1. Our results demonstrated that ultradeep targeted sequencing of cfDNA in the plasma of patients with a diverse range of cancer types is a feasible, reliable, and minimally invasive approach to interrogating cancer genetics.

- 2. It emerges as a promising tool for revealing clinically useful biomarkers and may advance our understanding of drug resistance, enhance our ability to quantify minimal residual disease, and assist in the development of novel therapeutic targets.