孟超肝胆医院
MENGCHAO HEPATOBILIARY HOSPITAL

循环肿瘤DNA(ctDNA)检测策略及临床意义

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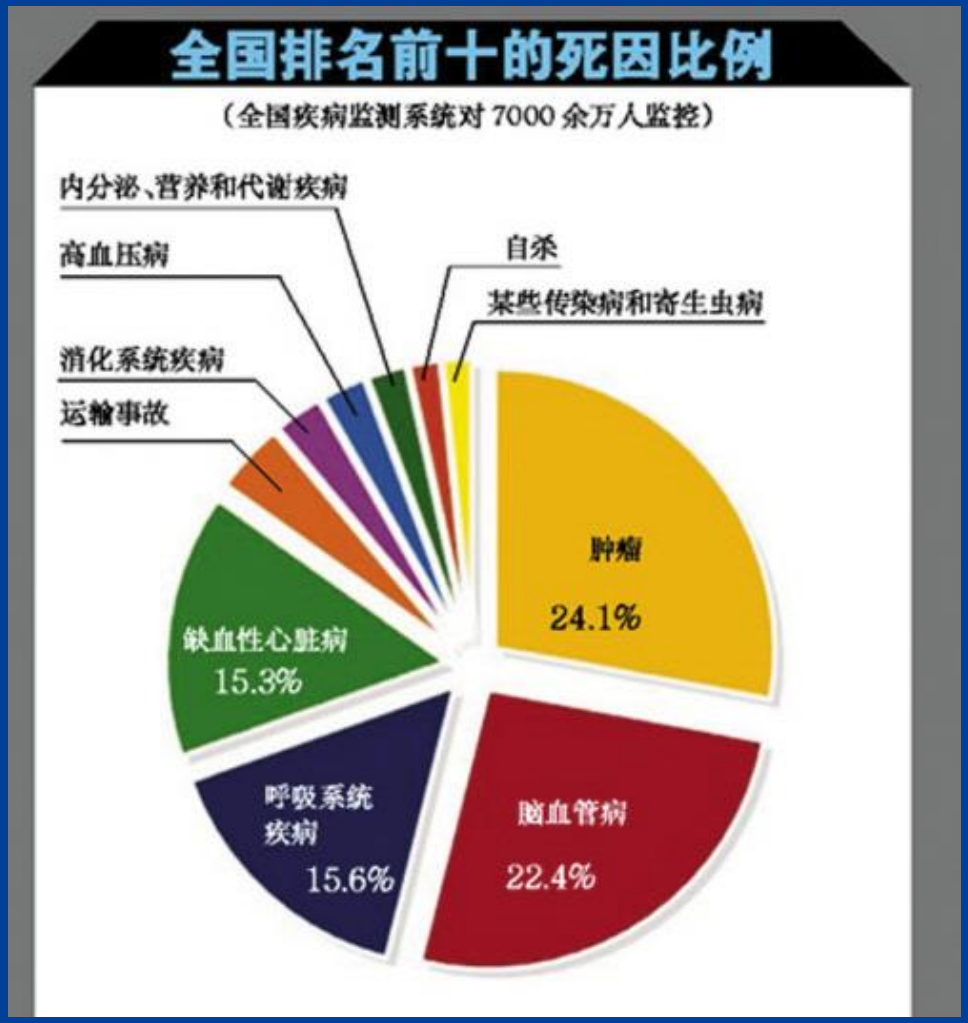
2015.06.12



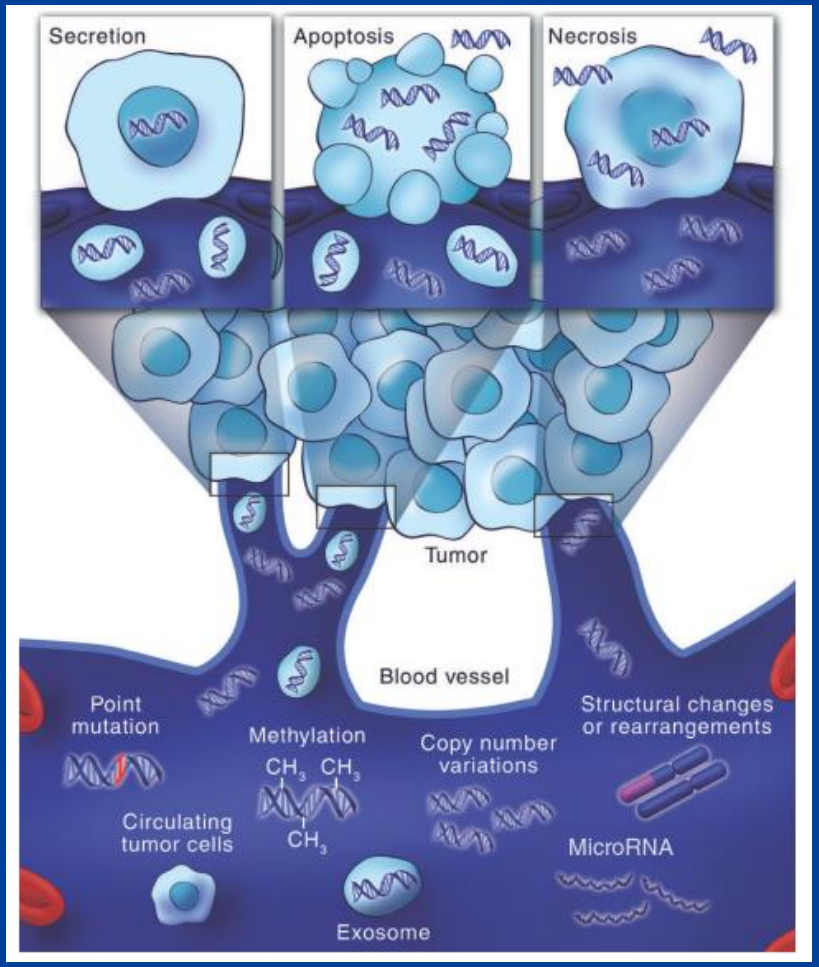
- 肿瘤诊疗的困境：早期诊断困难，错过最佳治疗时机；易复发转移，预后差；放化疗易产生耐受；缺乏有效的治疗靶点等。

- 早诊早治，实时监控、靶向用药及个体化诊疗是提高肿瘤远期生存率、降低死亡率的关键。

肿瘤诊断方法

- 血清标志物：蛋白类（癌胚抗原，糖类抗原，甲胎蛋白等），核酸类（microRNA, LncRNA）
- 影像学诊断(超声，CT，MRI，PET，PET-CT)
- 循环肿瘤细胞（circulating tumor cell , CTC）
- 循环肿瘤DNA（ circulating tumor DNA，ctDNA）

循环肿瘤DNA(ctDNA)

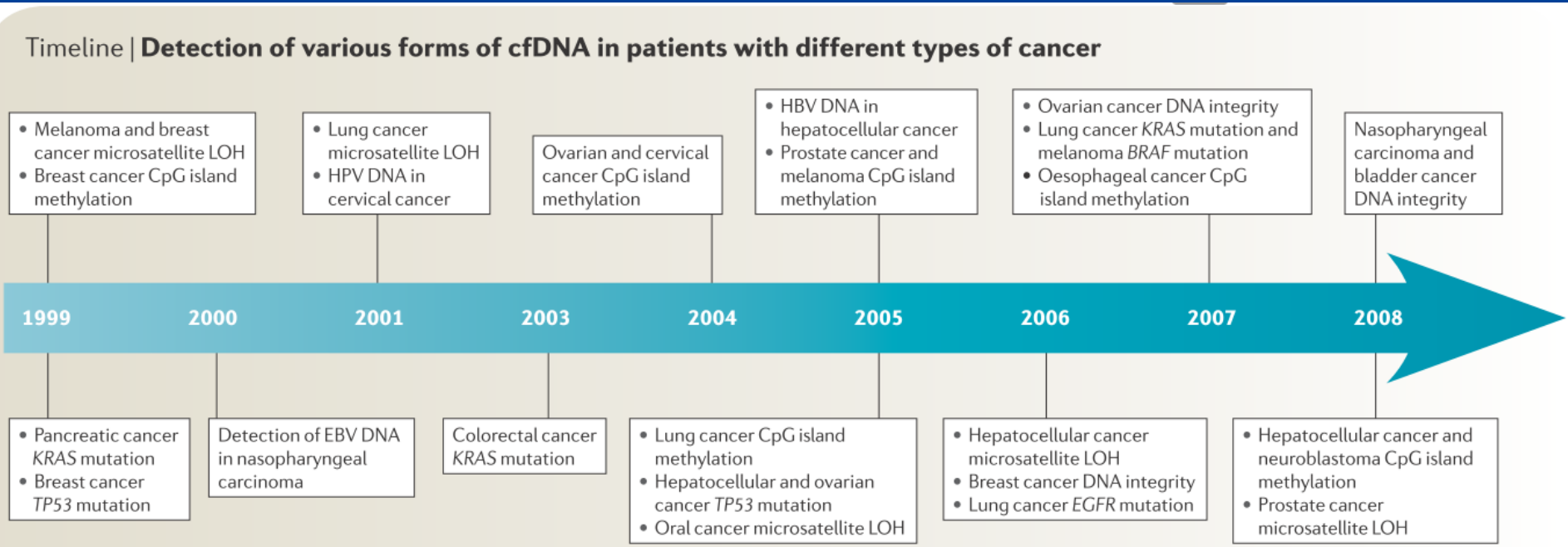


- 人体血液循环系统中不断流动的携带一定特征（包括突变，缺少，插入，重排，拷贝数异常，甲基化等）来自肿瘤基因组的DNA片段；
- 来源：1、来自于坏死的肿瘤细胞；2、来自于凋亡的肿瘤细胞；3、循环肿瘤细胞；4、来自于肿瘤细胞分泌的外排体；
- 含量低，约占整个循环DNA的1%，甚至只有0.01%。

ctDNA发展简史

- 1948年首次发现人体血液中存在着循环DNA，癌症患者的循环肿瘤DNA则发现于1977年。
- 1994年发现这些DNA含有癌症的标志性突变，证明其来自于肿瘤。
- 鉴于肿瘤DNA会流入血液，香港中文大学的卢煜明（Dennis Lo）教授推测胎儿DNA应该也能进入血液。1997年证明孕妇血液中携带着男性胎儿的Y染色体。该发现允许医生们在怀孕初期通过无创方式检测胎儿的性别，甚至筛查唐氏综合症等发育疾病。这是产前诊断领域的一次革命。

ctDNA发展简史

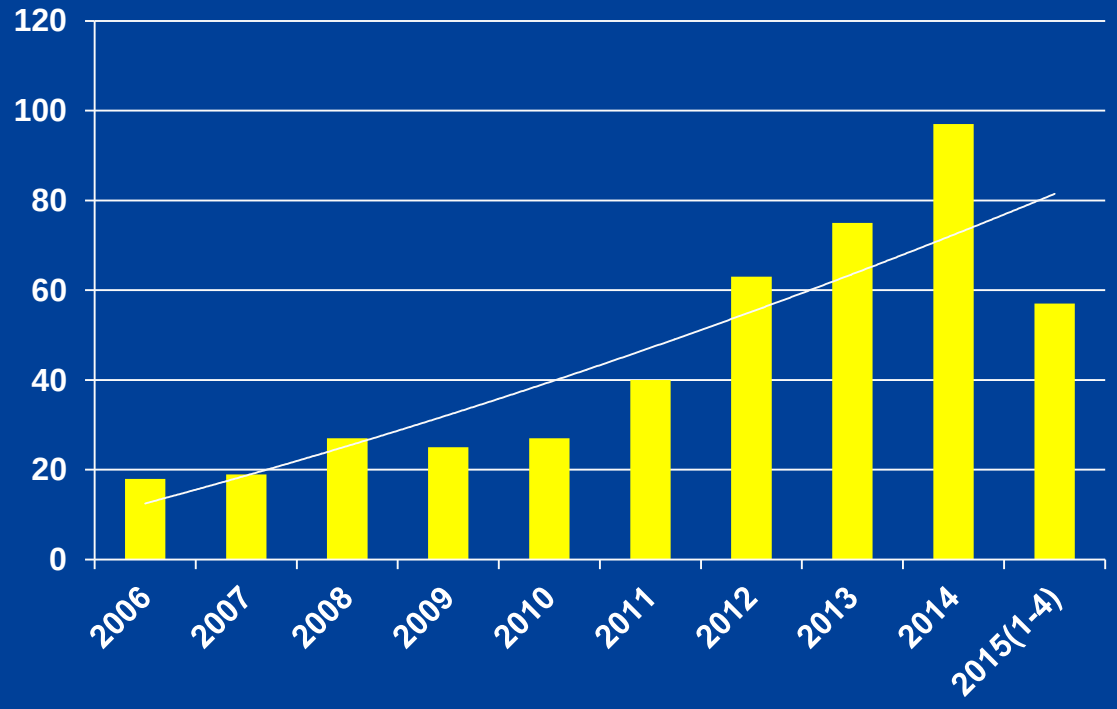


The development of the detection of genetic and epigenetic alterations, as well as the measurement of DNA integrity and viral DNA, in blood from patients with different tumour types over the past decade is shown. We show only significant, prognostic findings from >40 patients with serum, plasma or bodily fluid detection of cell-free DNA (cfDNA) from individual cancers. This timeline is not meant to be comprehensive and is based on our own personal view of what have been important clinical translational events. EBV, Epstein–Barr virus; EGFR, epidermal growth factor receptor; HBV, hepatitis B virus; HPV, human papilloma virus; LOH, loss of heterozygosity.

Nature Reviews Cancer **11**, 426-437 (June 2011) | doi:10.1038/nrc3066
Cell-free nucleic acids as biomarkers in cancer patients

研究热点

近10年ctDNA相关发表文章数（pubmed数据库）



NEJM：4篇

PNAS：7篇

JAMA：1篇

Sci Transl Med：6篇

Cancer research:12篇

Clin Cancer Res:28篇

ctDNA优势

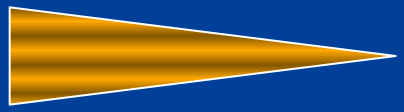
一类具备广泛应用前景的肿瘤标志物，可无创的用于肿瘤早期诊断、发展过程监测、预后判断、以及个性化用药指导。

- 无创、无损、实时、多次；
- 当不能获得肿瘤组织信息时，可以开展“液体活检”；
- 假阳性率低，灵敏度高，准确度高，可用于肿瘤的早期诊断；
- 能够对肿瘤的演化和适应性改变进行监控；
- 能够对肿瘤病人的治疗效果进行实时监控（尤其在追踪肿瘤转归、转移和复发等方面），并进行个性化的治疗方案指导（如个性化靶向药物用药，个性化化疗等）。

ctDNA检测技术

Technique	Sensitivity	Optimal Application
Sanger sequencing	> 10%	Tumor tissue
Pyrosequencing	10%	Tumor tissue
Next-generation seqeuncing	2%	Tumor tissue
Quantative PCR	1%	Tumor tissue
ARMS	0.10%	Tumor tissue
BEAMing, PAP, Digital PCR, TAM-Seq	0.01% or lower	ctDNA, rare variants in tumor tissue

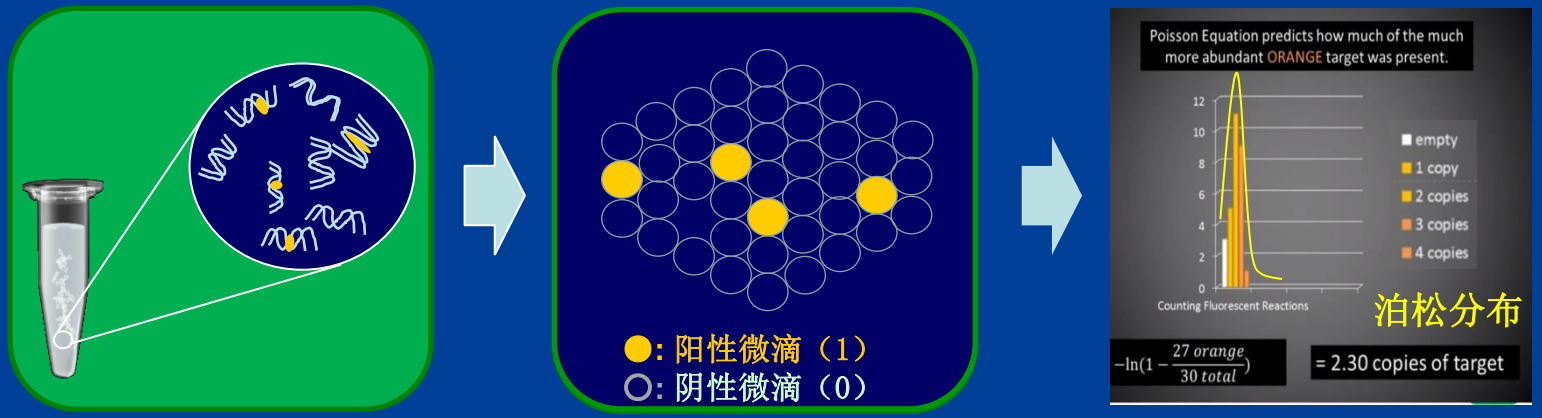
已知突变位点检测（突变率< 2%）	未知突变位点检测（突变率> 2%）
●数字PCR	●全基因组测序
●BEAMing技术	●全外显子测序
●标记扩增深度测序（TAM-Seq）	●RNA末端平行分析法
●癌症个体化深度测序（CAPP-Seq）	●全基因组甲基化测序



敏感度

数字PCR

- 将一个标准PCR反应分配到大量微小的反应器中，在每个反应器中包含或不包含一个或多个拷贝的目标分子(DNA模板)，实现“单分子模板PCR扩增”，扩增结束后，通过阳性反应器的数目“数出”目标序列的拷贝数。



有限稀释　　PCR扩增　　泊松分布

一个
待分析的PCR反应体系

成千上万个
独立的PCR反应体系

阴性微滴比例推算
起始靶分子的绝对量

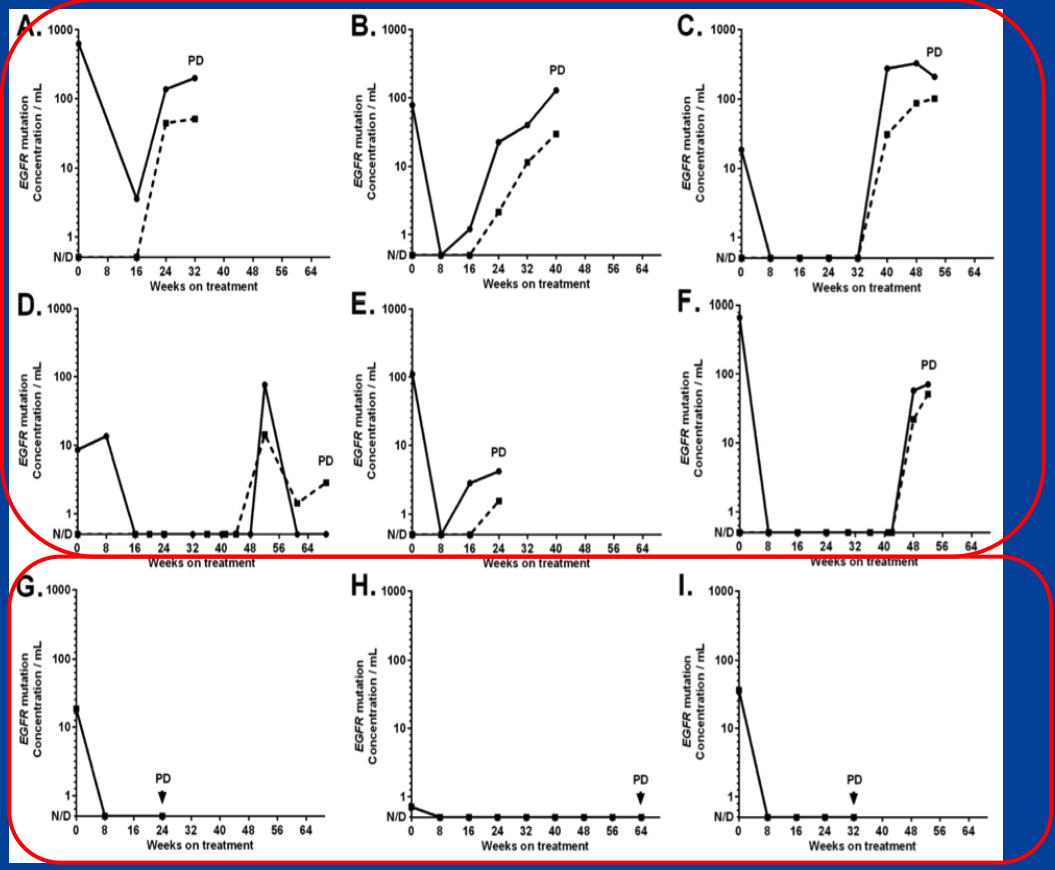
优点：可绝对定量，灵敏度可达单个核酸分子，检测限低至0.001%；

缺点：只能检测已知的突变位点，通量低；

Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA

Geoffrey R Oxnard, Cloud P Paweletz, Yanan Kuang, et al.

Clin Cancer Res Published OnlineFirst January 15, 2014.

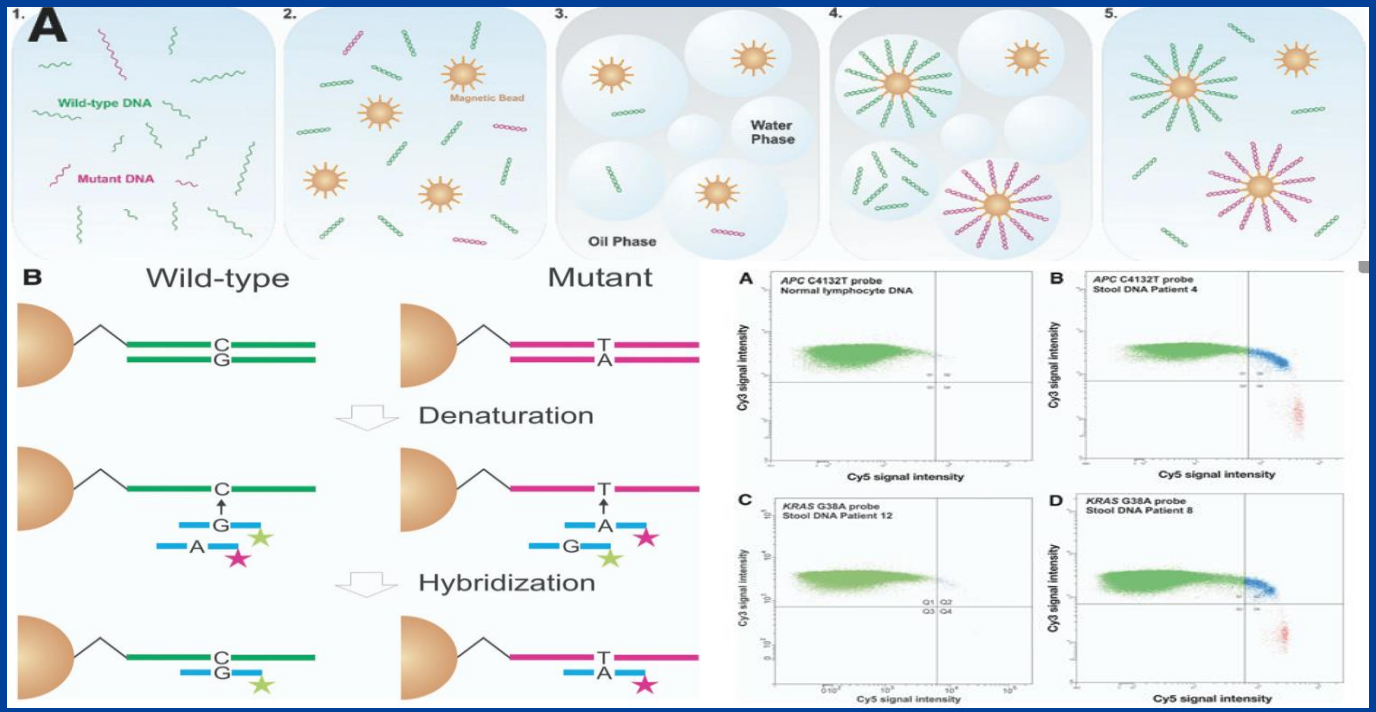


- 利用数字PCR监测肺癌病人中第一次服用埃罗替尼抗肿瘤药物后的血浆EGFR突变情况。
- 经过治疗后，所有病人的EGFR突变都出现下降，其中有8个病人突变完全消失。
- 有6个病人的EGFR突变在后续出现上升，提前揭示疾病进展。另外3个病人的EGFR突变没有变化，病情无明显进展。

数字PCR可以用于评估和监测血浆中ctDNA的特异突变

BEAMing技术

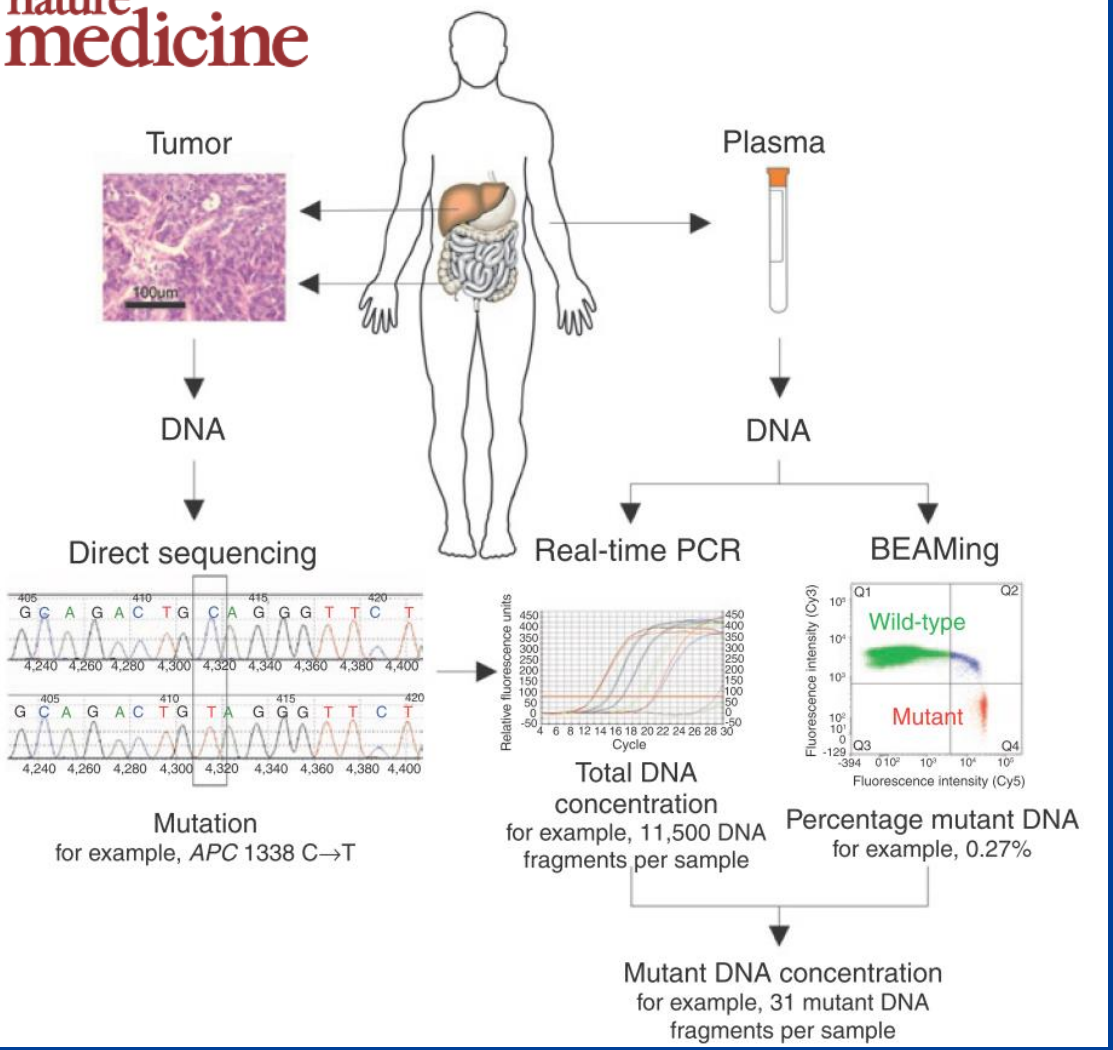
- 结合数字PCR与流式技术，利用特异性PCR引物扩增目标突变区后，与磁珠（磁珠上固定有特异的PCR 引物）混合进行油包水单分子扩增反应。反乳化作用后，利用不同颜色的荧光探针结合磁珠上的PCR产物，发出红色或绿色荧光。使用流式细胞仪分析磁珠颜色来确定突变情况。



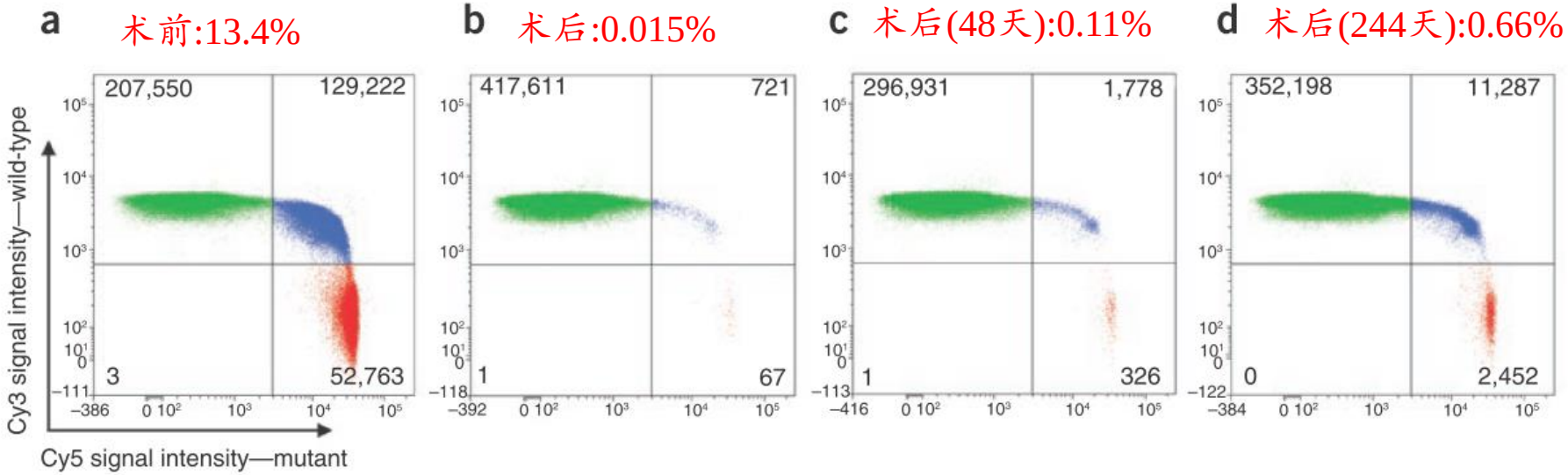
Circulating mutant DNA to assess tumor dynamics

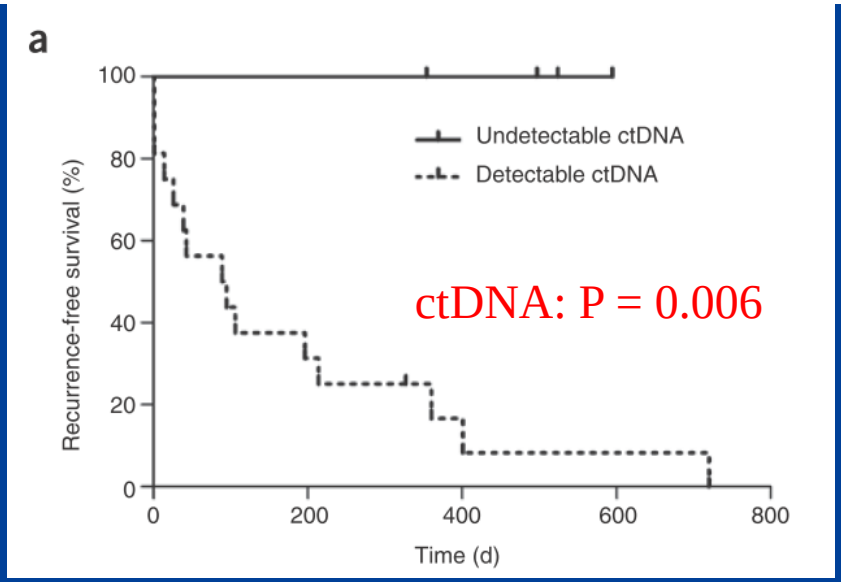
Frank Diehl[1,5], Kerstin Schmidt[1,5], Michael A Choti[2], Katharine Romans[1], Steven Goodman[3], Meng Li[1], Katherine Thornton[1], Nishant Agrawal[1], Lori Sokoll[4], Steve A Szabo[1], Kenneth W Kinzler[1], Bert Vogelstein[1] & Luis A Diaz Jr[1]

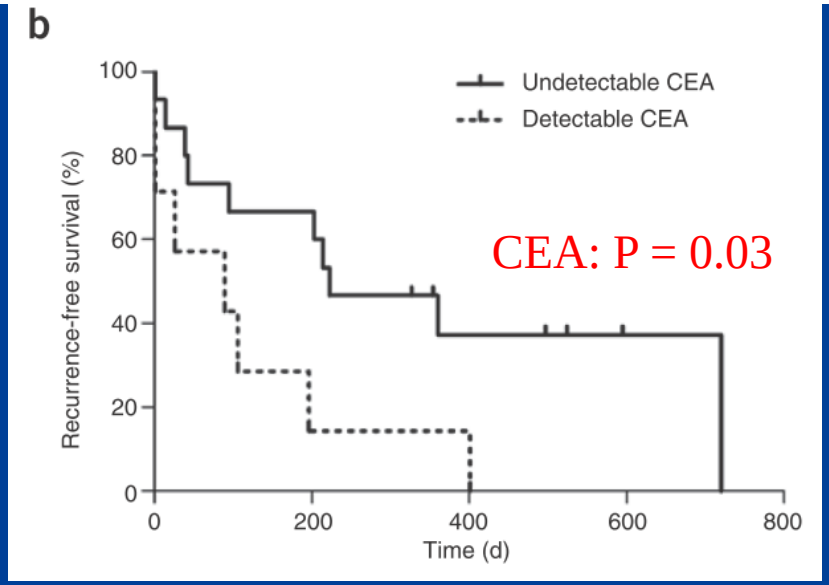
nature medicine



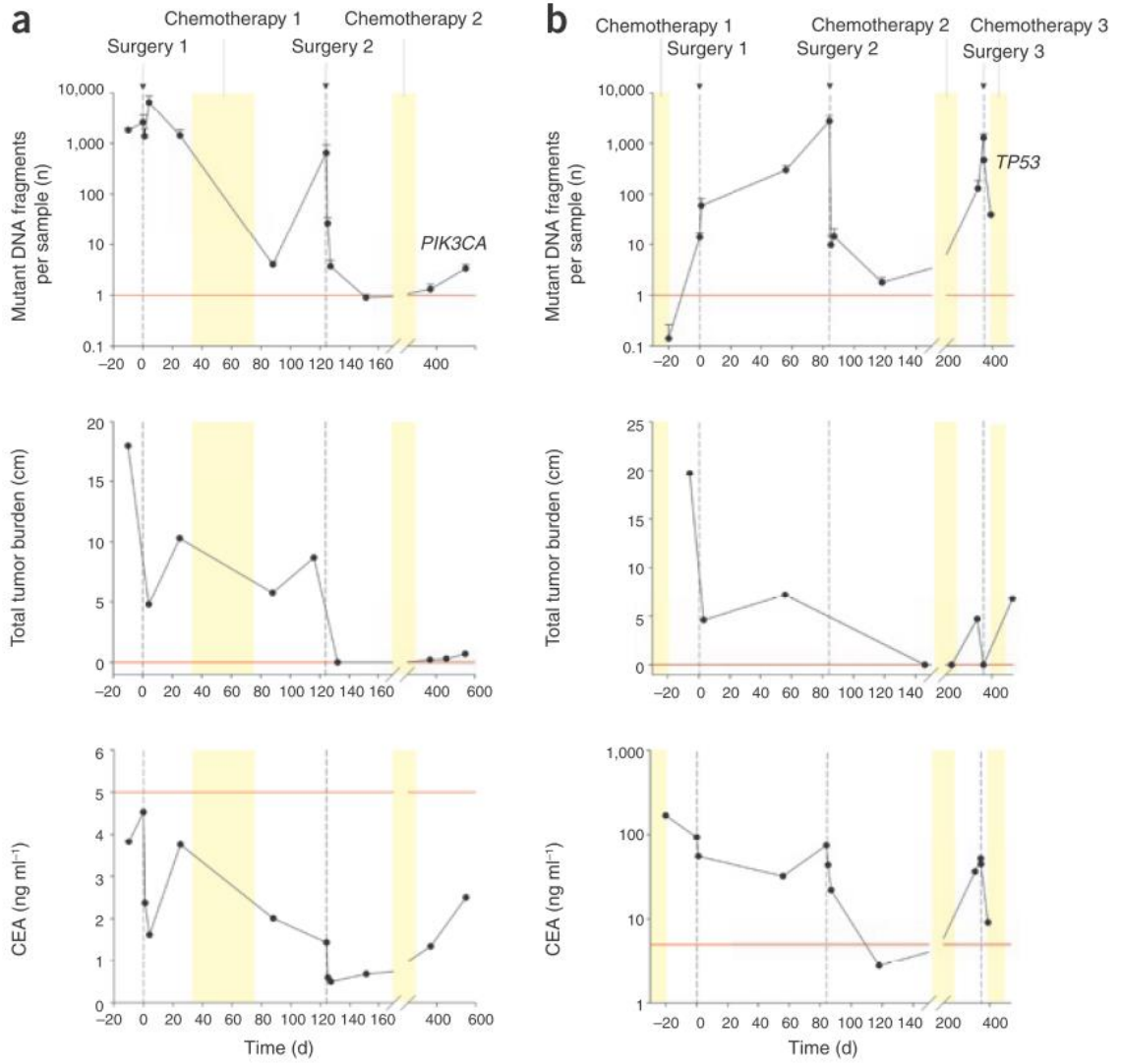
- 利用肿瘤组织进行sanger测序定义肿瘤组织的基因突变；
- 再利用Beaming技术检测18例癌症病人中192份血浆样本中ctDNA的突变情况，检测灵敏度可达到0.005%。


a
术前:13.4%
b
术后:0.015%
c
术后(48天):0.11%
d
术后(244天):0.66%
207,550
129,222
3
52,763
417,611
721
1
67
296,931
1,778
1
326
352,198
11,287
0
2,452
Cy3 signal intensity—wild-type
Cy5 signal intensity—mutant


a
Undetectable ctDNA
Detectable ctDNA
ctDNA: P = 0.006
Recurrence-free survival (%)
Time (d)

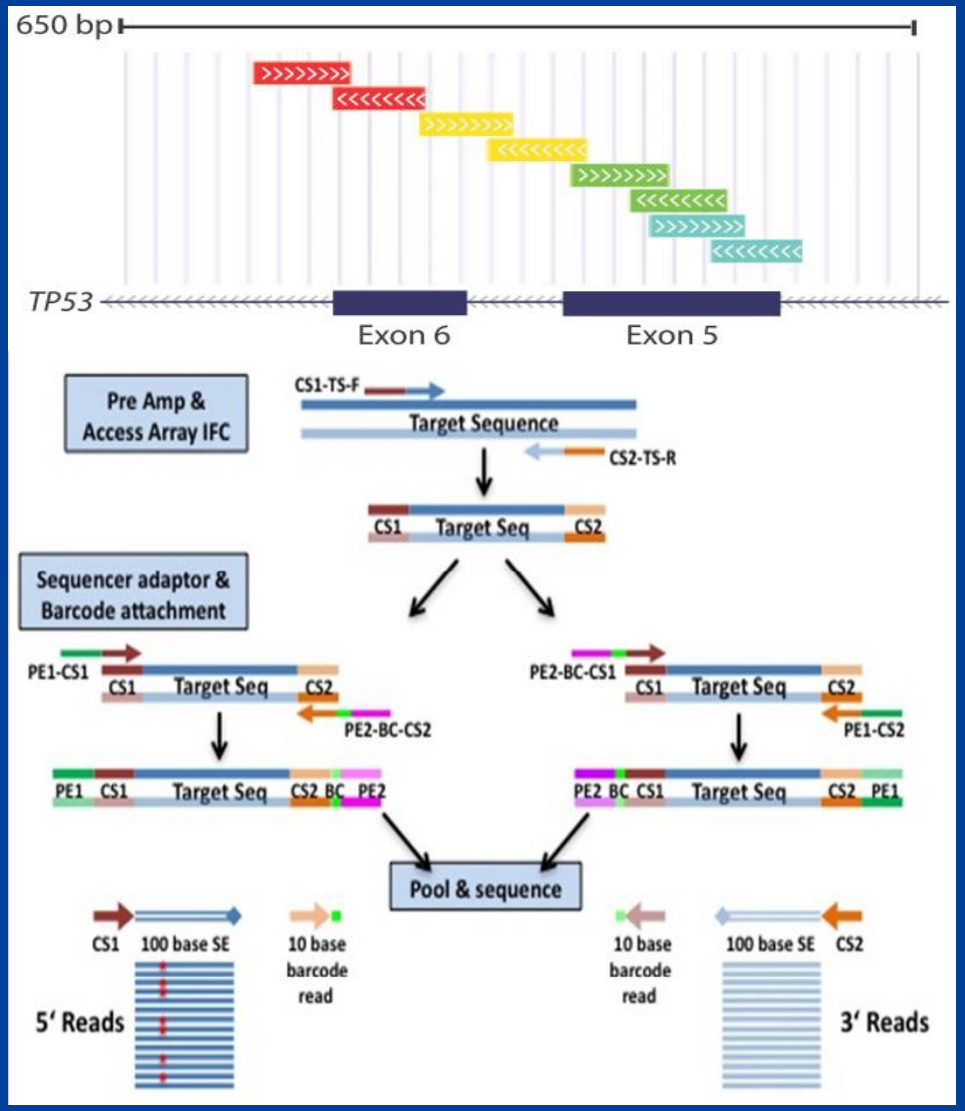

b
Undetectable CEA
Detectable CEA
CEA: P = 0.03
Recurrence-free survival (%)
Time (d)

Comparison of ctDNA, CEA and imaging dynamics in individual study subjects



- 病人都经过多次手术和化疗
- 第一次手术进行局部切除时，ctDNA含量并没有降低；第二次手术完全切除时，ctDNA含量大幅下降。
- ctDNA能实时显示病人体内肿瘤负荷的动态信息，以及治疗的效果（手术及化疗）。

标记扩增深度测序（TAM-Seq）



- 二步扩增：

预扩增：利用特异性引物进行15循环的目标区域扩增

标签扩增：利用通用不同特性的接头标签进行二次扩增

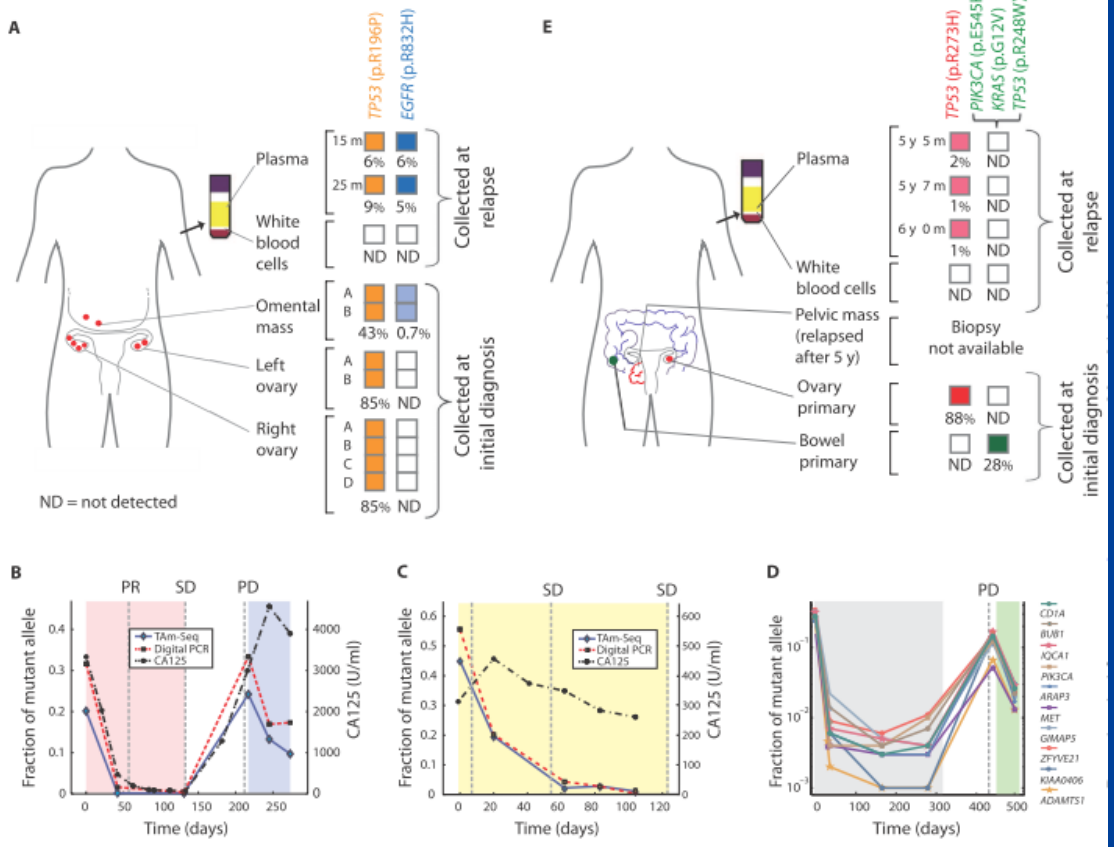
- 双向重复测序：提高测序精度
- 突变频率检出率可达2%
- 敏感性和特异性高于97%
- 成本较低，利于临床应用

Science Translational Medicine AAAS

Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA

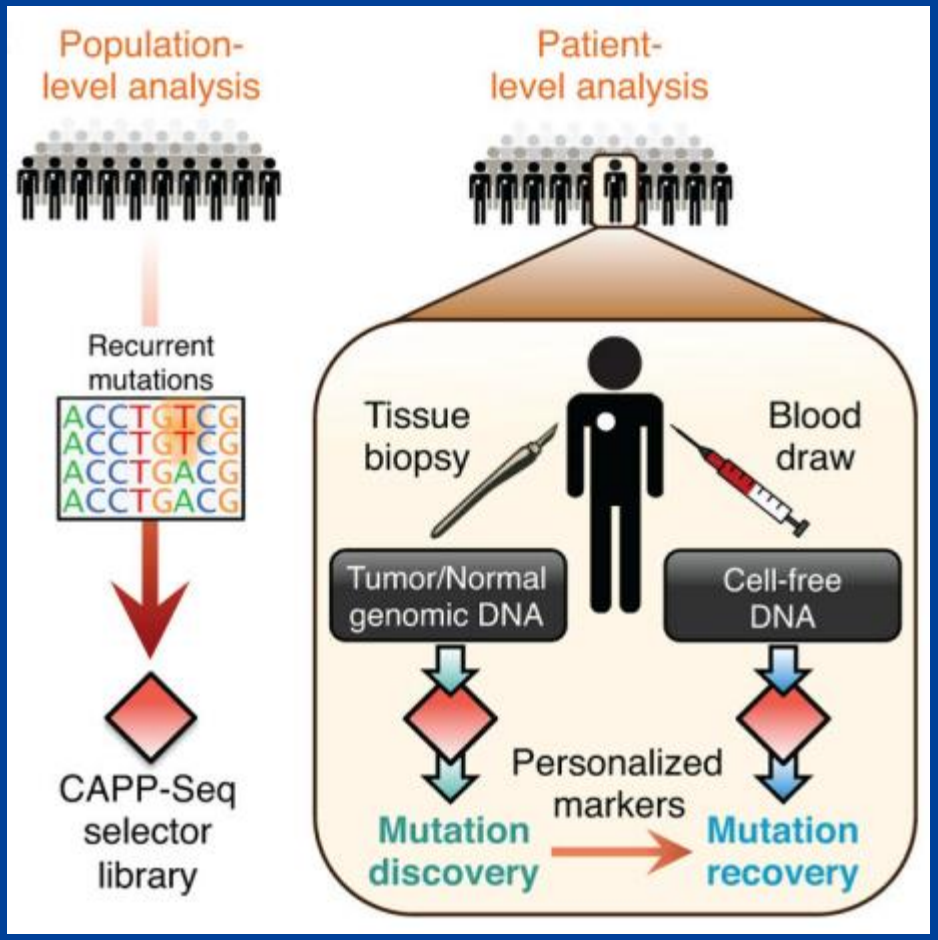
Tim Forshew *et al.*

Sci Transl Med **4**, 136ra68 (2012);
DOI: 10.1126/scitranslmed.3003726



- 设计了48对引物去扩增TP53，EGFR，BARF，KRAS等癌症相关基因的5995bp突变区域
- 同时可对10个特异的癌症突变位点进行监控
- 用于确定多发癌症患者的复发病灶
- 用于监控癌症患者的肿瘤动态发展

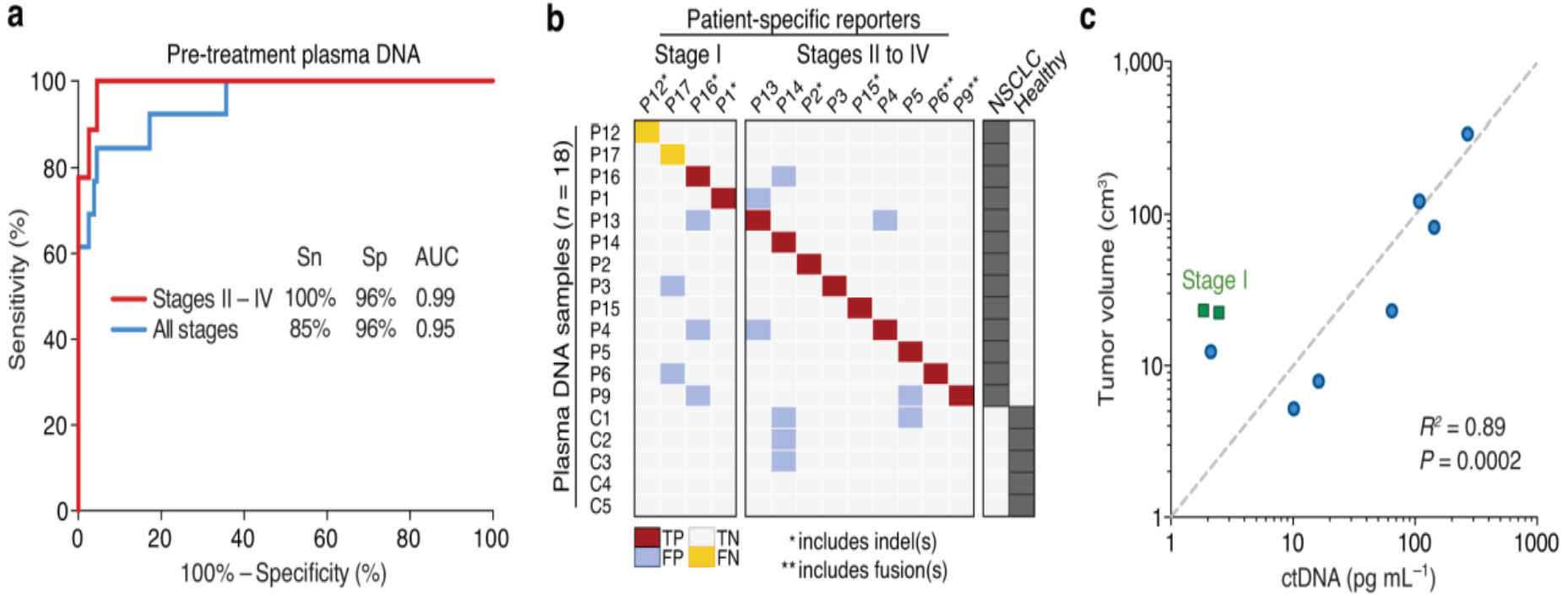
癌症个体化深度测序 (CAPP-Seq)



- 该方法利用定制化的突变位点库作为”筛选器”， 对样本进行靶向捕获后再进行超深度测序（可达到10000X），其对肿瘤的ctDNA检测灵敏度更高，特异性更强，与全外显子测序等相比经济可行。

An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage

Nat Med. 2014 May ; 20(5): 548–554.



- Selector 区域：139个基因的521个外显子和13个内含子，约125K，占人基因组的0.004%
- 测序覆盖深度10000X

- Stage I 检出率50%
- Stage II-IV 检出率100%
- 特异性可达到96%
- 突变检出率0.02%

ctDNA的临床应用

- 肿瘤的早期诊断
- 动态监测肿瘤的发生发展及疗效
- 评估肿瘤的异质性
- 靶向检测耐药突变，跟踪获得性耐药的发展，进行个性化用药指导
- 评估肿瘤复发转移风险

ctDNA的分析指标

1. cfDNA含量
2. cfDNA完整性
3. ctDNA突变频率
4. ctDNA甲基化频率
5. 微卫星杂合性

ctDNA的分析指标

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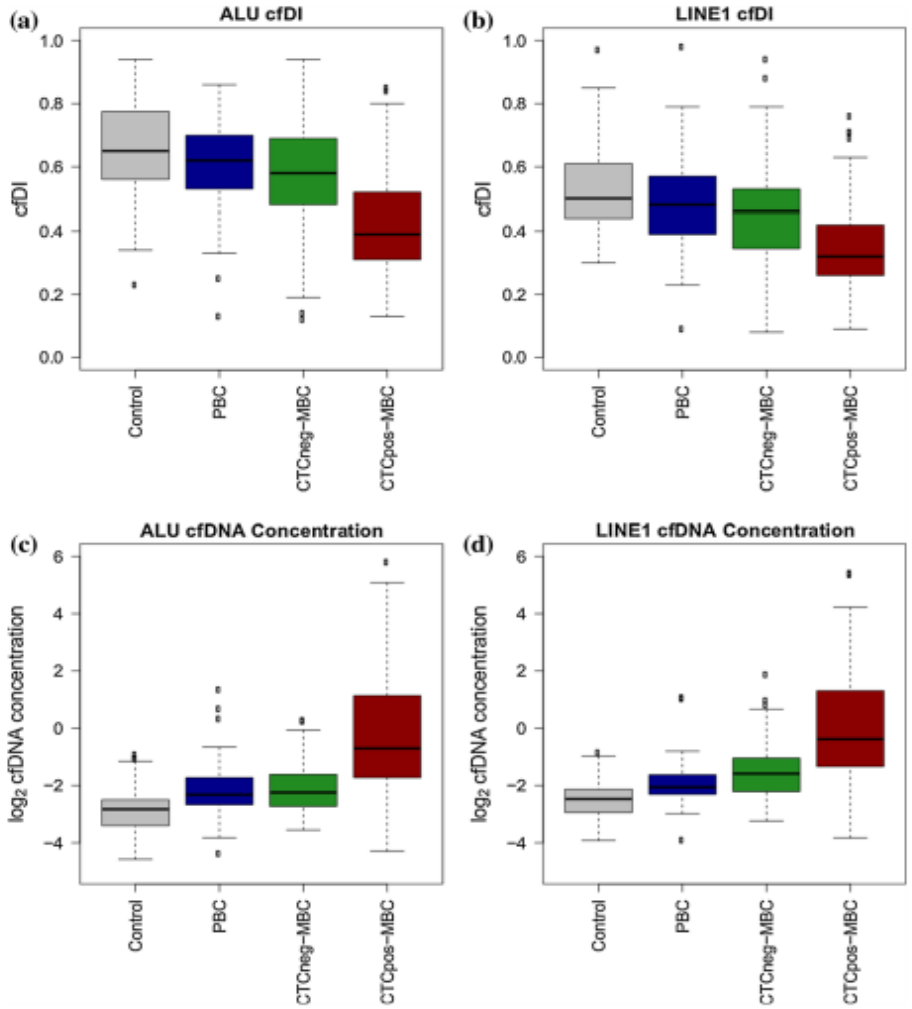
5. 微卫星杂合性

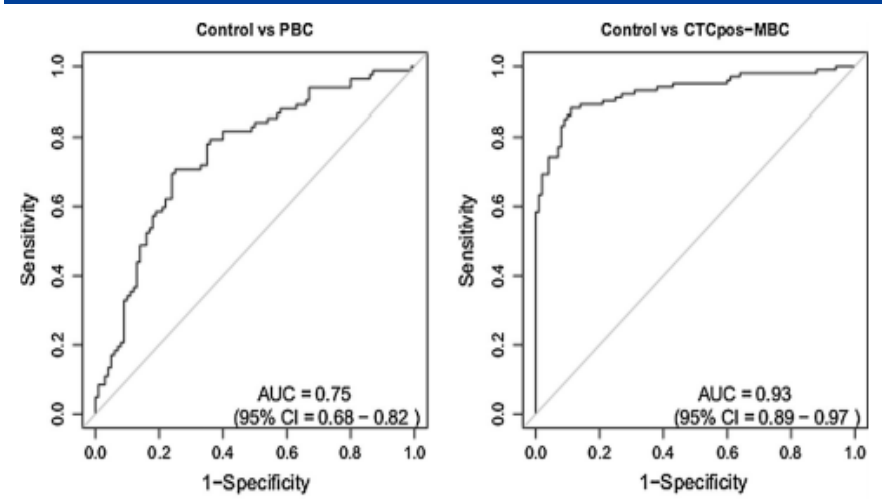
Breast Cancer Res Treat (2014) 146:163–174
DOI 10.1007/s10549-014-2946-2

EPIDEMIOLOGY

Plasma DNA integrity as a biomarker for primary and metastatic breast cancer and potential marker for early diagnosis



- 乳腺癌病人（尤其在转移性乳腺癌）的血浆cfDNA的完整性显著下调，而血浆cfDNA的浓度显著上调，可作为早期诊断的标志物，具有较好的敏感度和特异性。



Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients

Peiyong Jiang[a,b,1], Carol W. M. Chan[a,b,1], K. C. Allen Chan[a,b,c,1], Suk Hang Cheng[a,b], John Wong[d], Vincent Wai-Sun Wong[a,e,f], Grace L. H. Wong[a,e,f], Stephen L. Chan[c,g], Tony S. K. Mok[c,g], Henry L. Y. Chan[a,e,f], Paul B. S. Lai[c,d], Rossa W. K. Chiu[a,b], and Y. M. Dennis Lo[a,b,c,2]

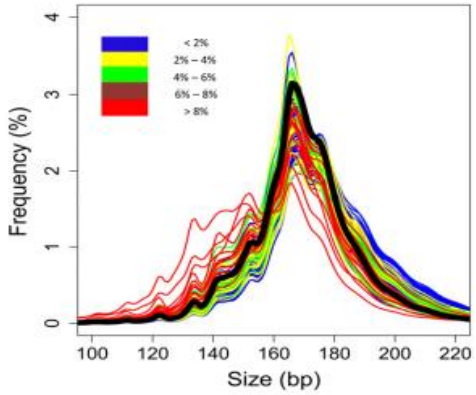
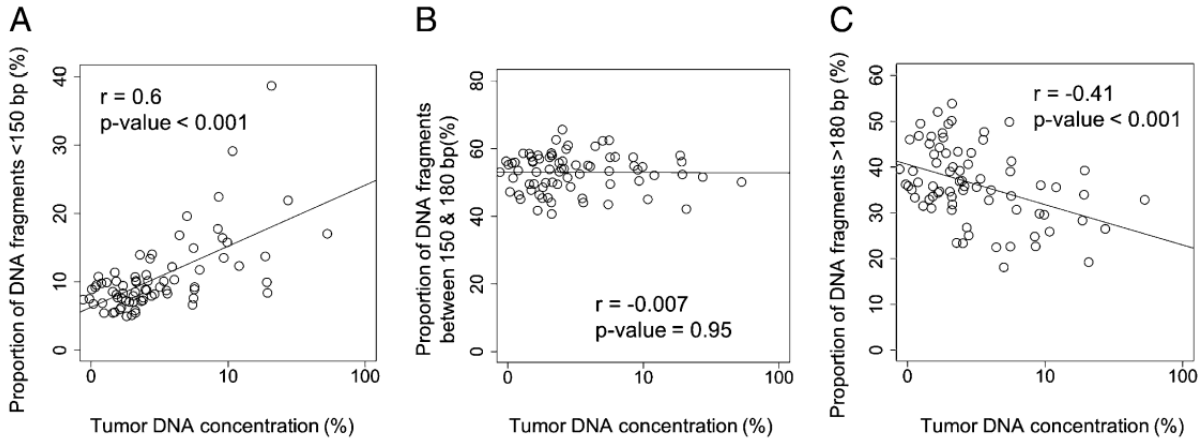
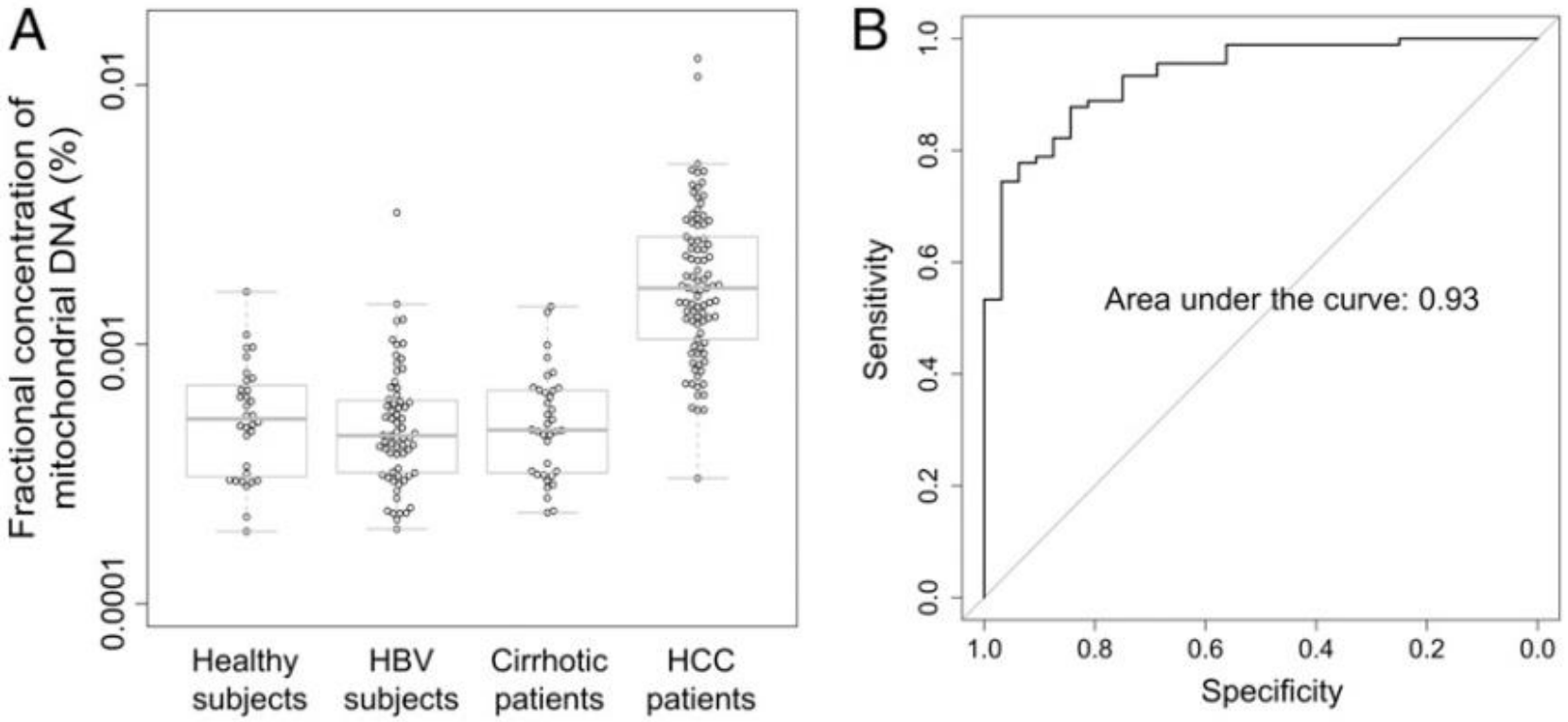


Fig. 4. Size distributions of plasma DNA fragments in the HCC patients w different fractional concentrations of tumor-derived DNA in plasma. The med size distribution profile for the 32 healthy subjects is shown as a thick black li



- 肝癌病人的血浆中存在两种不同长度的DNA分子：短链DNA分子（<150bp）和长链DNA分子（ >180bp ）；
- 短链DNA分子与ctDNA突变率及拷贝数异常相关，且与ctDNA浓度正相关；
- 长链DNA分子不携带肿瘤相关ctDNA特征，且与ctDNA浓度负相关。

线粒体DNA可用于肝癌的诊断



Proc Natl Acad Sci U S A. 2015 Mar 17;112(11):E1317-25. doi: 10.1073/pnas.1500076112. Epub 2015 Feb 2.
Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients.

- 肝癌病人血浆中线粒体DNA的含量显著高于健康人、乙肝及肝硬化患者，对肝癌的诊断具有较高的灵敏性和特异性，分别达80%和94%，可作为肝癌诊断的有效分子标记。

ANTICANCER RESEARCH *30*: 2785-2790 (2010)

Cell-free Circulating DNA: Diagnostic Value in Patients with Renal Cell Cancer

ANTICANCER RESEARCH *32*: 3119-3124 (2012)

Cell-free Serum DNA in Patients with Bladder Cancer: Results of a Prospective Multicenter Study

J Clin Pathol 2013;**66**:775-778

Circulating cell-free DNA in serum as a biomarker of colorectal cancer

American Journal of Clinical Pathology 2015; 143(1):18-24.

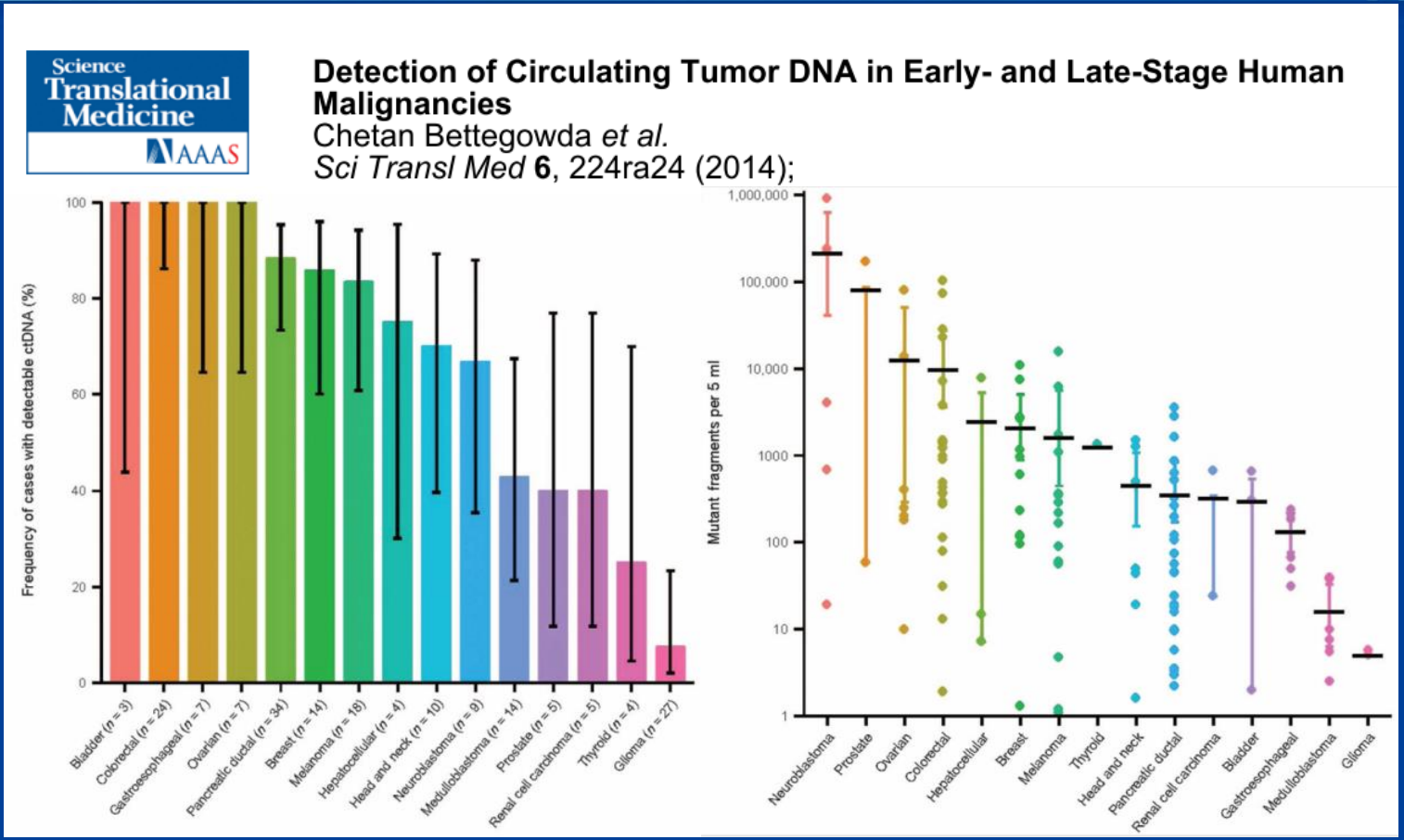
Measurement of Circulating Cell-Free DNA Levels by a Simple Fluorescent Test in Patients With Breast Cancer

PLoS One. 2015; 10(4): e0108247.

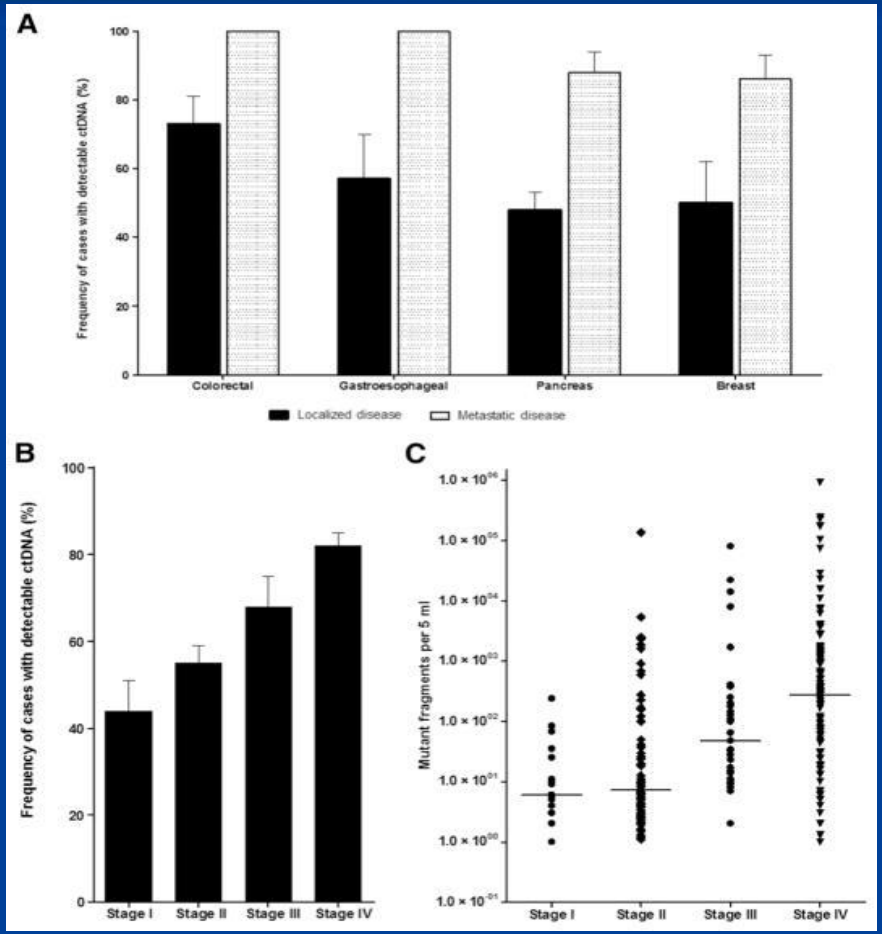
Circulating Free DNA as Biomarker and Source for Mutation Detection in Metastatic Colorectal Cancer

ctDNA的分析指标

1. cfDNA含量
2. cfDNA完整性
3. **ctDNA突变频率**
4. ctDNA甲基化频率
5. 微卫星杂合性



- 利用数字PCR检测640例不同癌症病人的血浆ctDNA，在超过75%的病人血液中检测到ctDNA，原发癌检出率约为50%。
- 在结肠癌，胃癌，胰腺癌，乳腺癌中，ctDNA检出率分别为73%, 57%, 48%, 50% 。



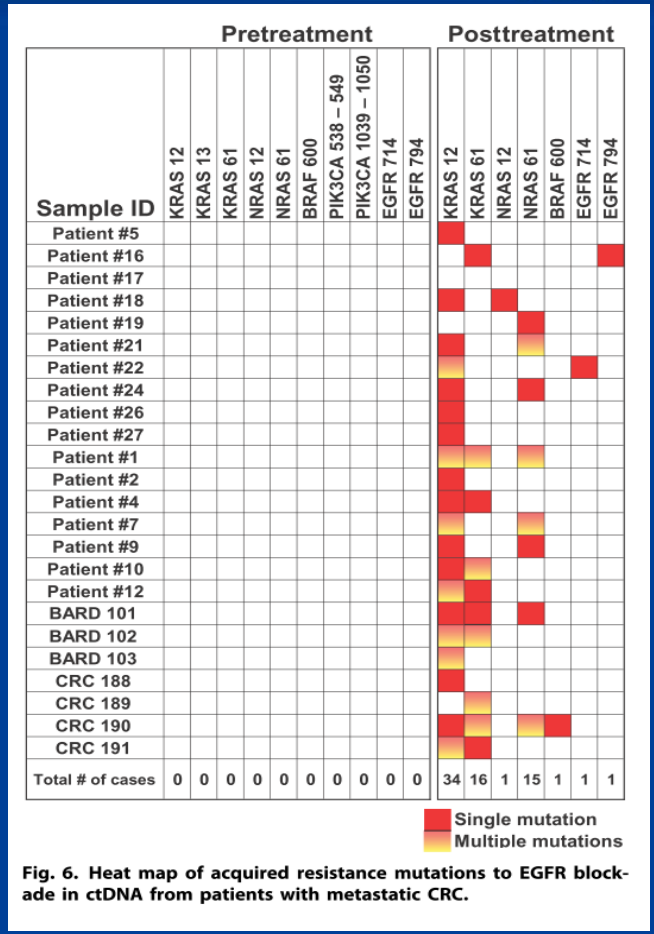
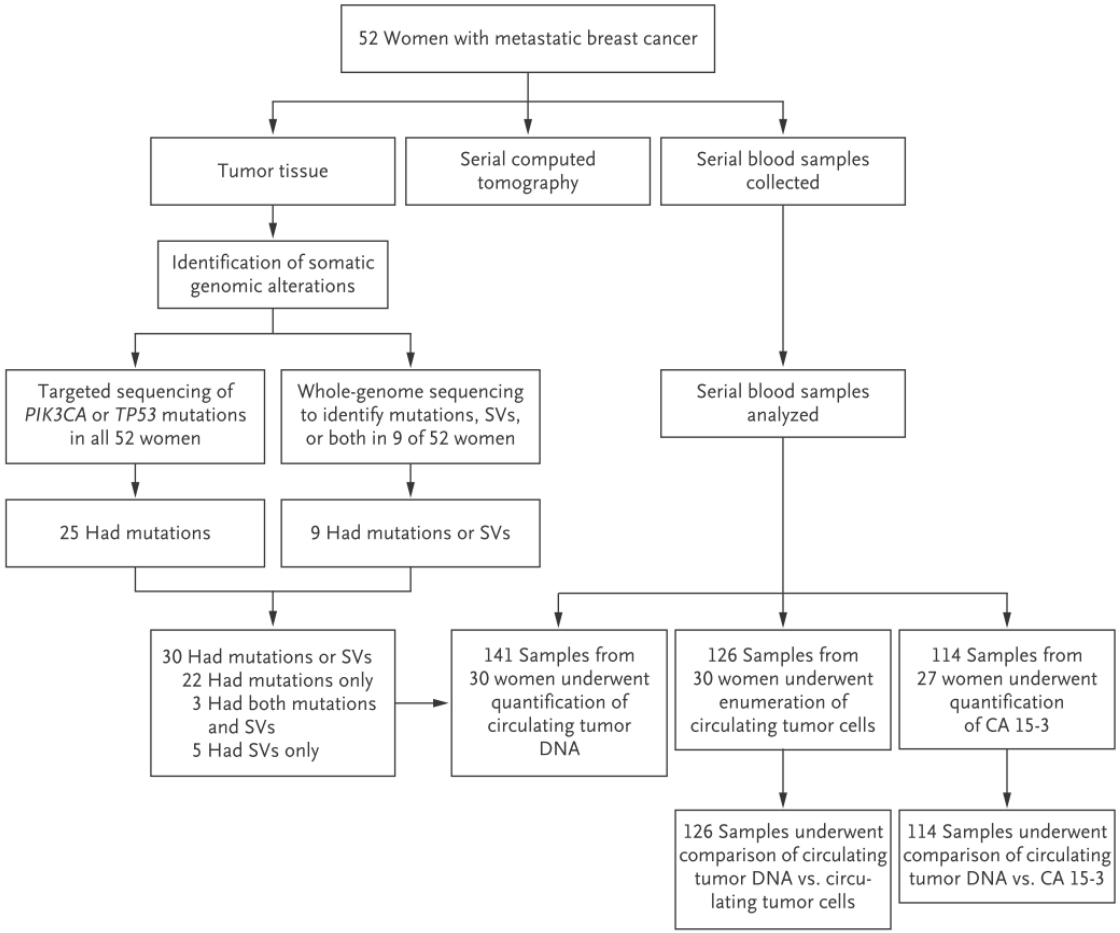


Fig. 6. Heat map of acquired resistance mutations to EGFR blockade in ctDNA from patients with metastatic CRC.

- 相对于原发癌，ctDNA在转移癌中检出率更高，且分期越高，ctDNA检出率也更高；
- 可用于耐药突变的检测；
- ctDNA是普遍适用的、敏感和特异的标志物,可用于不同类型癌症患者的临床监测。

Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer

N Engl J Med 2013; 368:1199-1209

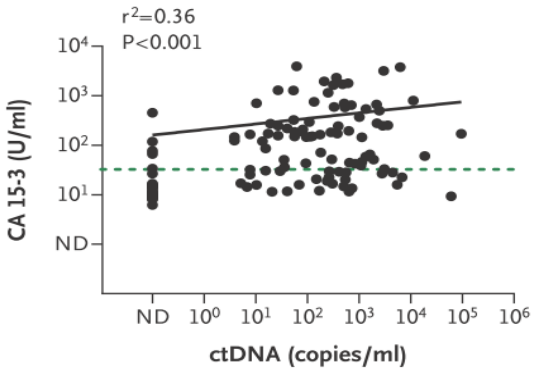


- 在30例转移性乳腺癌患者接受系统性治疗的不同时间点测定血液中的ctDNA，CA 15-3，CTC细胞的含量，比较三者之间的敏感度。

Comparison of Circulating Tumor DNA, CA 15-3, and Circulating Tumor Cells as Blood-Based Biomarkers

A CA 15-3 vs. ctDNA

CA 15-3	ctDNA		
	Detected	Not detected	Total
Patients			
Elevated (>32.4 U/ml)	21	0	21
Not elevated (≤32.4 U/ml)	5	1	6
Total	26	1	27
ctDNA sensitivity, 26/27 (96%) CA 15-3 sensitivity, 21/27 (78%)			
Samples			
Elevated (>32.4 U/ml)	67	4	71
Not elevated (≤32.4 U/ml)	27	16	43
Total	94	20	114
ctDNA sensitivity, 94/114 (82%) CA 15-3 sensitivity, 71/114 (62%)			



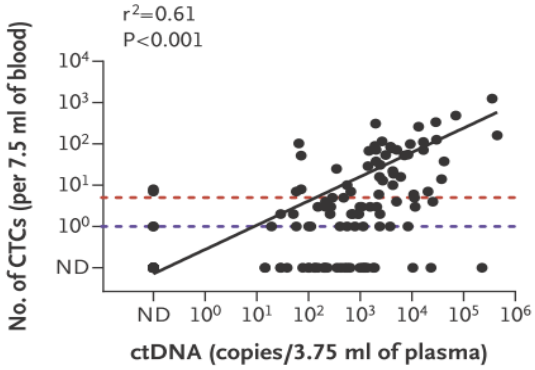
灵敏度：

循环肿瘤DNA：85%

CA 15-3：59%

B CTC vs. ctDNA

CTC	ctDNA		
	Detected	Not detected	Total
Patients			
Elevated (≥5)	18	0	18
Detected (1–4)	7	1	8
Not detected (0)	4	0	4
Total	29	1	30
ctDNA sensitivity, 29/30 (97%) CTC sensitivity (detected, >0), 26/30 (87%) CTC sensitivity (elevated, ≥5), 18/30 (60%)			
Samples			
Elevated (≥5)	45	1	46
Detected (1–4)	28	2	30
Not detected (0)	33	17	50
Total	106	20	126
ctDNA sensitivity, 106/126 (84%) CTC sensitivity (detected, >0), 76/126 (60%) CTC sensitivity (elevated, ≥5), 46/126 (37%)			
Median ratio of ctDNA copy numbers (per 3.75 ml of plasma) to number of CTCs (per 7.5 ml of whole blood)=133 (interquartile range, 51–528)			

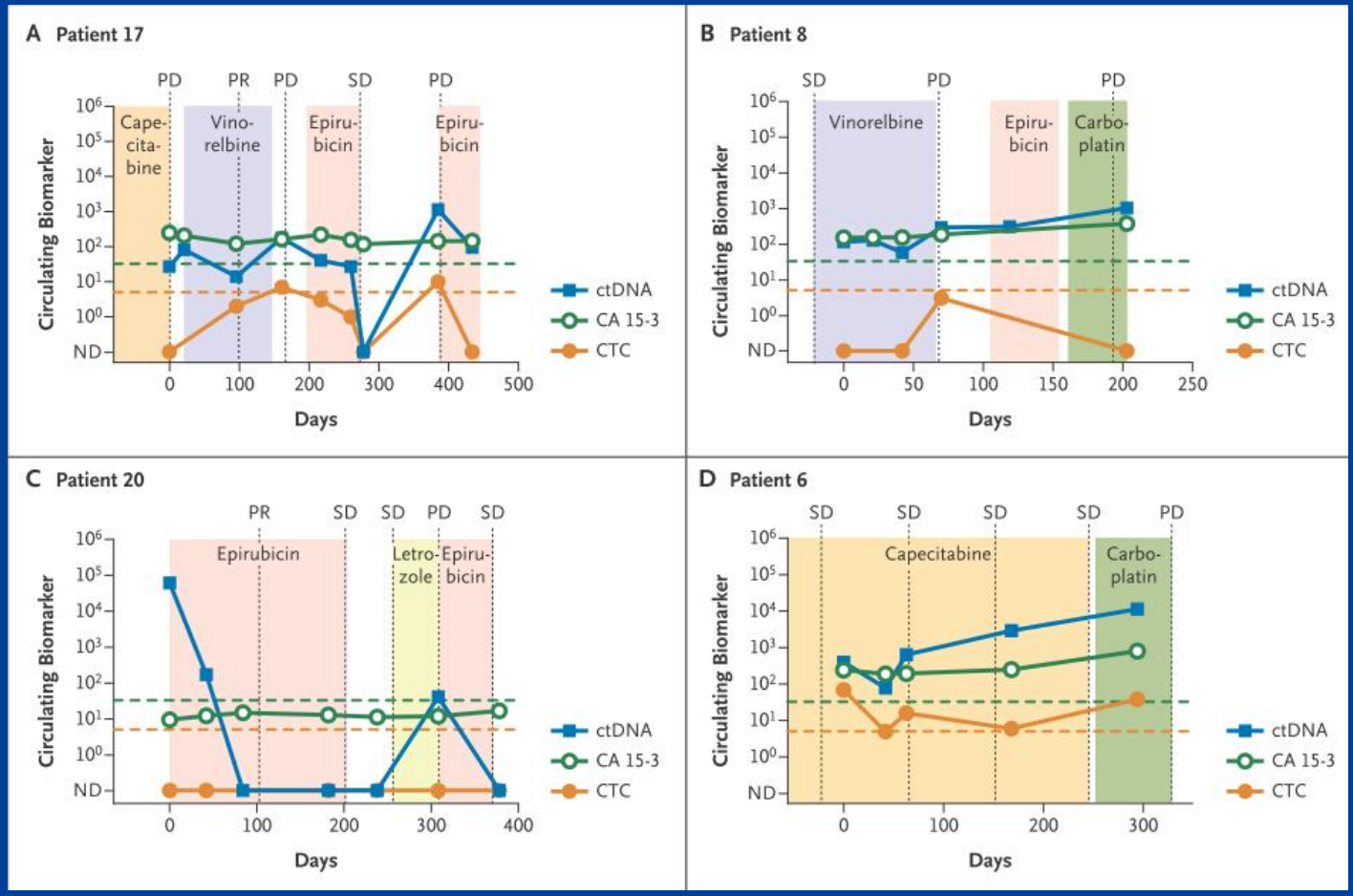


灵敏度：

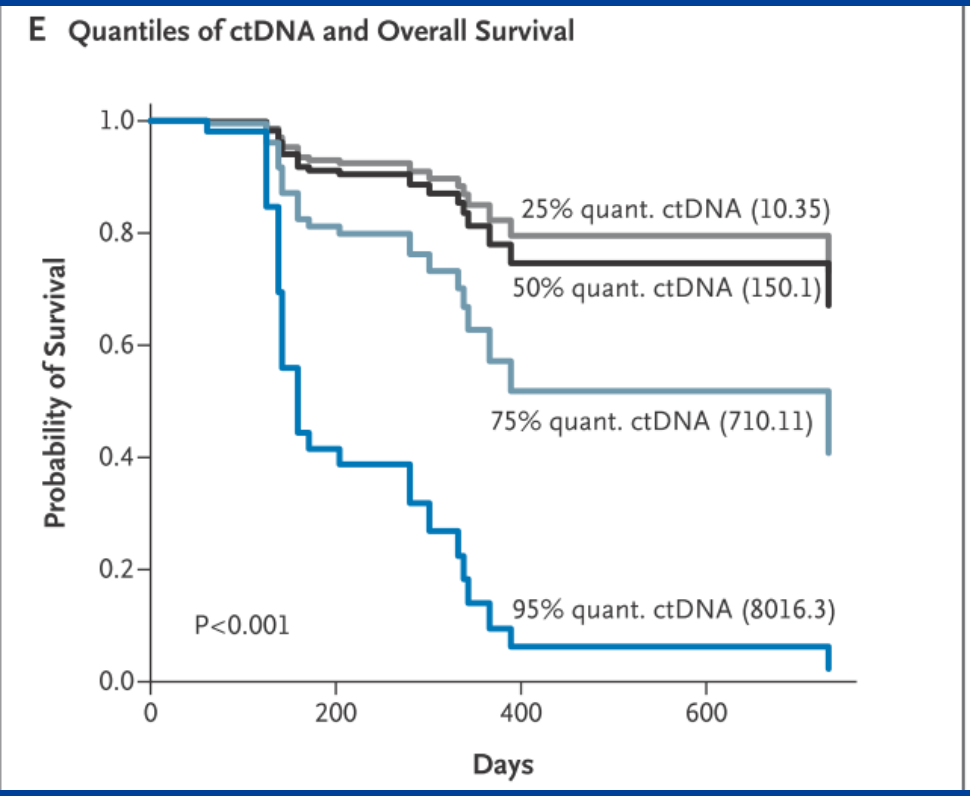
循环肿瘤DNA：90%

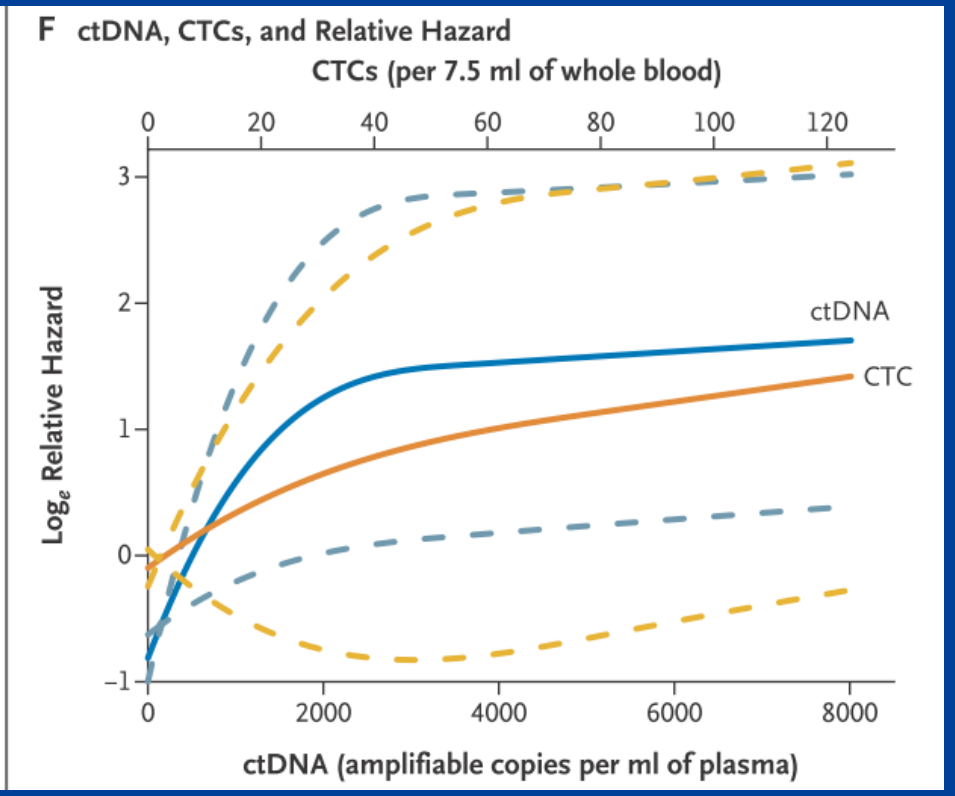
循环肿瘤细胞：67%

Comparison of Circulating Biomarkers to Monitor Tumor Dynamics and Predict Survival.



- 结论：相对于蛋白类诊断标志物CA15-3及血液中的循环肿瘤细胞，ctDNA在监测肿瘤的动态发展中具有更高的灵敏度。



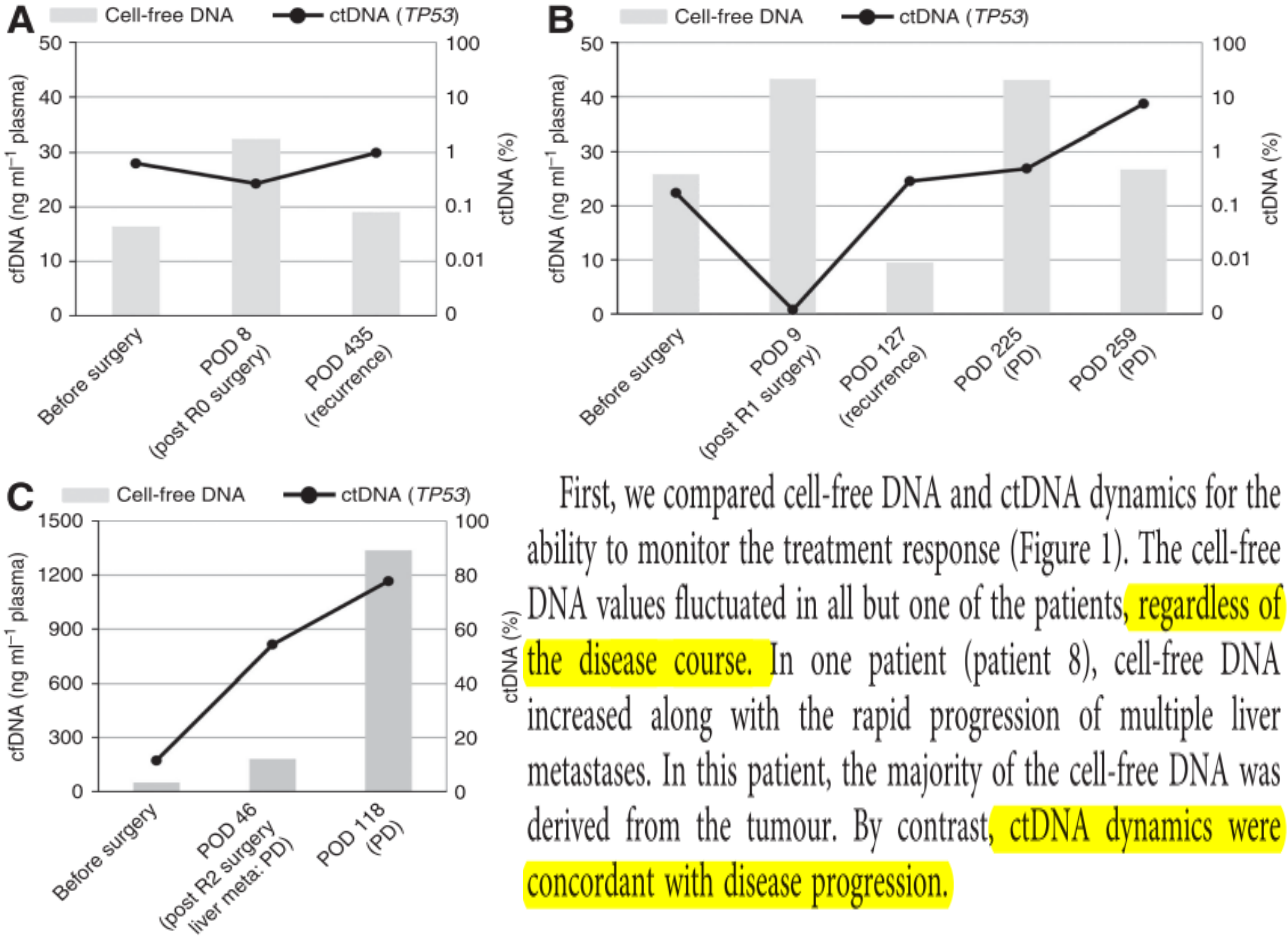


- 血浆中ctDNA的含量与预后密切相关，含量越高，病人的5年生存率越低（P<0.001）。
- ctDNA含量的增高及CTC数目的增加，病人的预后显著变差，而CA15-3没有预后判断功能。

Monitoring gastric cancer progression with circulating tumour DNA

British Journal of Cancer (2015) 112, 352–356

T Hamakawa[1,2], Y Kukita[2], Y Kurokawa[1], Y Miyazaki[1], T Takahashi[1], M Yamasaki[1], H Miyata[1], K Nakajima[1], K Taniguchi[2], S Takiguchi[1], M Mori[1], Y Doki[*,1] and K Kato[*,2]

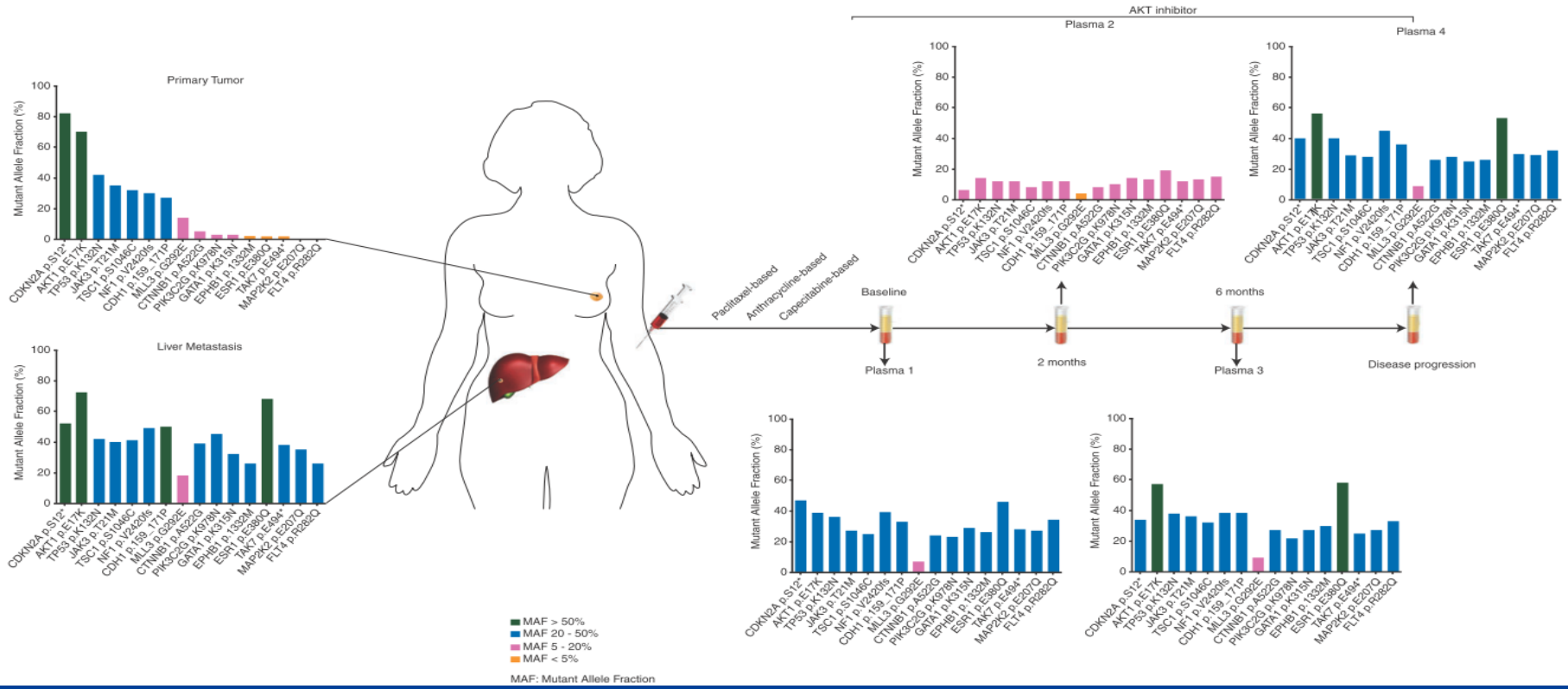


First, we compared cell-free DNA and ctDNA dynamics for the ability to monitor the treatment response (Figure 1). The cell-free DNA values fluctuated in all but one of the patients, regardless of the disease course. In one patient (patient 8), cell-free DNA increased along with the rapid progression of multiple liver metastases. In this patient, the majority of the cell-free DNA was derived from the tumour. By contrast, ctDNA dynamics were concordant with disease progression.

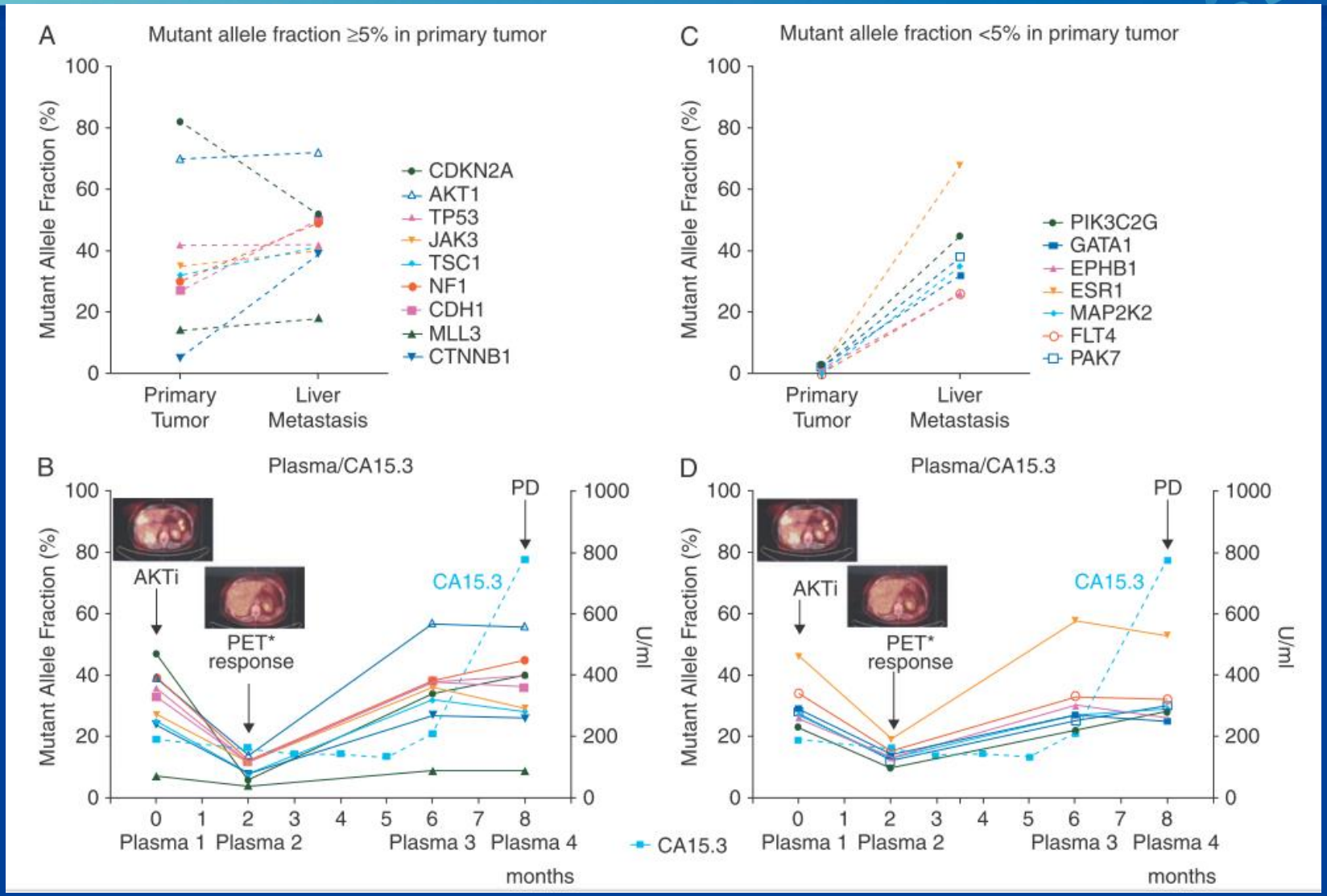
- 监测ctDNA的突变率比监测cfDNA浓度，对胃癌动态发展的监控灵敏度更高。

Capturing intra-tumor genetic heterogeneity by *de novo* mutation profiling of circulating cell-free tumor DNA: a proof-of-principle

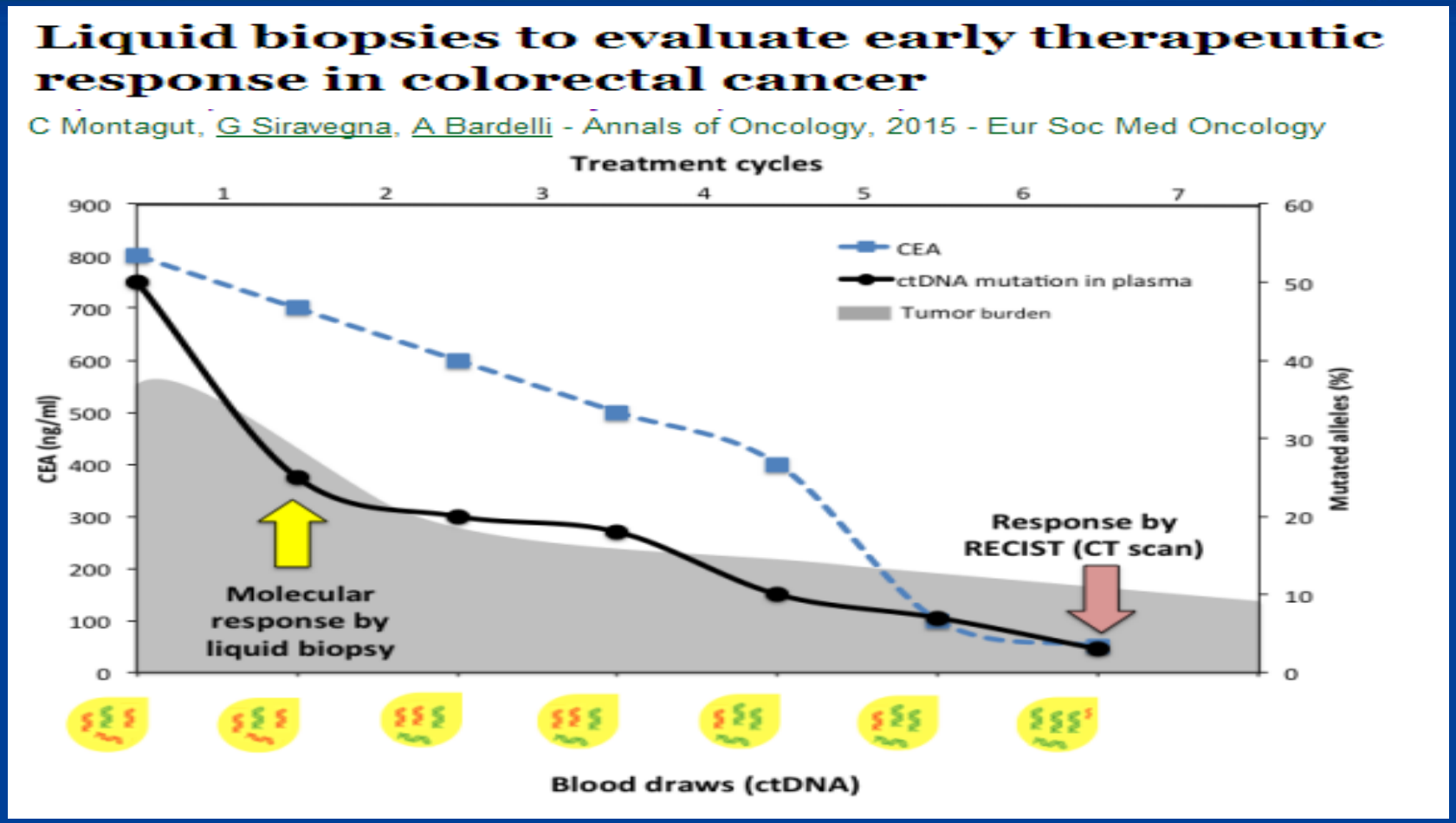
Annals of Oncology 25: 1729–1735, 2014



- 利用区域捕获测序技术检测乳腺癌原发瘤及肝脏转移瘤的基因突变情况，并监控血液中ctDNA在治疗过程中的动态变化。



- ctDNA在监测肿瘤的动态发展中具有更高的灵敏度和特异性；
- ctDNA能比蛋白类标志物CA15.3更早的预测肿瘤动态发展情况。



- 相对于传统的影像学及蛋白类血清标志物，通过评估ctDNA的突变频率能更早、更准确的预测结肠癌患者肿瘤经治疗后的动态发展情况。

ctDNA的分析指标

1. cfDNA含量
2. cfDNA完整性
3. ctDNA突变频率
4. ctDNA甲基化频率
5. 微卫星杂合性

PNAS | November 19, 2013 | vol. 110 | no. 47

Noninvasive detection of cancer-associated genome-wide hypomethylation and copy number aberrations by plasma DNA bisulfite sequencing

K. C. Allen Chan[a,b,c,1], Peiyong Jiang[a,b,1], Carol W. M. Chan[a,b,1], Kun Sun[a,b], John Wong[d], Edwin P. Hui[c,e], Stephen L. Chan[c,e], Wing Cheong Chan[f], David S. C. Hui[g], Simon S. M. Ng[c,d], Henry L. Y. Chan[a,g], Cesar S. C. Wong[h], Brigette B. Y. Ma[c,e], Anthony T. C. Chan[c,e], Paul B. S. Lai[c,d], Hao Sun[a,b], Rossa W. K. Chiu[a,b], and Y. M. Dennis Lo[a,b,c,2]

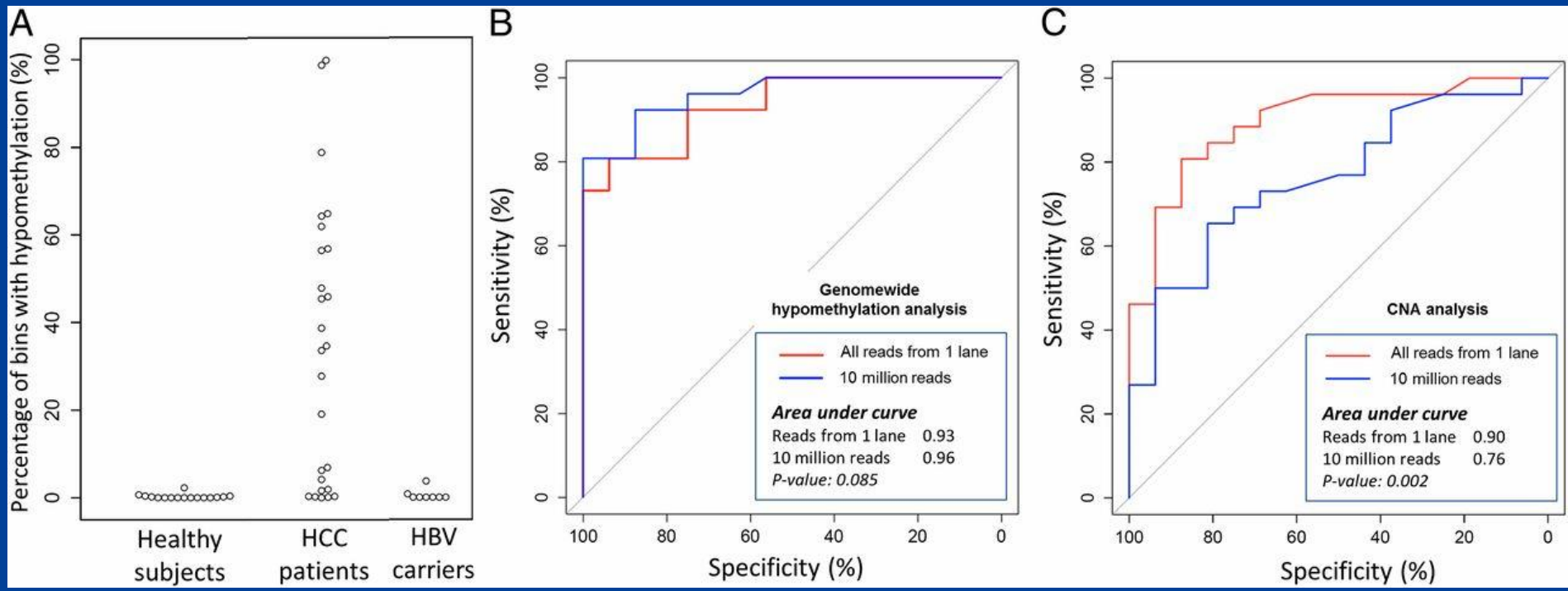
Table 4. Overall diagnostic performance for plasma-based detection of the 38 nonmetastatic cancers

No. of sequenced reads	Algorithm	Sensitivity, %	Specificity, %
Mean of 93 million	Hypomethylation alone	74	94
	CNA alone	71	88
	Hypomethylation OR CNA	87	88
	Hypomethylation AND CNA	58	94
10 million	Hypomethylation alone	68	94
	CNA alone	39	94
	Hypomethylation OR CNA	74	94
	Hypomethylation AND CNA	34	94

- 利用亚硫酸氢盐平行测序检测不同肿瘤患者（肝癌，乳腺癌，肺癌，鼻咽癌等）血浆DNA甲基化发生情况及血浆DNA拷贝数异常情况，用于肿瘤的诊断及肿瘤负荷的监测；

- 血浆中甲基化癌症诊断灵敏度为74%，特异性为94%，具有较好的癌症诊断应用价值。

Percentage of bins showing hypomethylation in HCC patients and chronic hepatitis B virus (HBV) carriers with cirrhosis



- 血浆DNA的甲基化程度在肝癌患者显著上升，可用于肝癌的诊断；灵敏度为81%，特异性为94%；
- 血浆DNA拷贝数异常用于肝癌诊断，灵敏度为81%，特异性为88%。

Clin Cancer Res July 1, 2004 *10;* 4420

The Acquisition of *hMLH1* Methylation in Plasma DNA after Chemotherapy Predicts Poor Survival for Ovarian Cancer Patients

Journal of Thoracic Oncology: 2011 *6* :1632-1638

SHOX2 DNA Methylation Is a Biomarker for the Diagnosis of Lung Cancer in Plasma

World J Gastroenterol. 2011 Nov 28; 17(44): 4917–4921.

Plasma DNA methylation of Wnt antagonists predicts recurrence of esophageal squamous cell carcinoma

Int. J. Cancer: 131, 1153–1157 (2012)

DNA methylation patterns in blood of patients with colorectal cancer and adenomatous colorectal polyps

ONCOLOGY REPORTS 32: 505-512, 2014

Differential promoter methylation of kinesin family member 1a in plasma is associated with breast cancer and DNA repair capacity

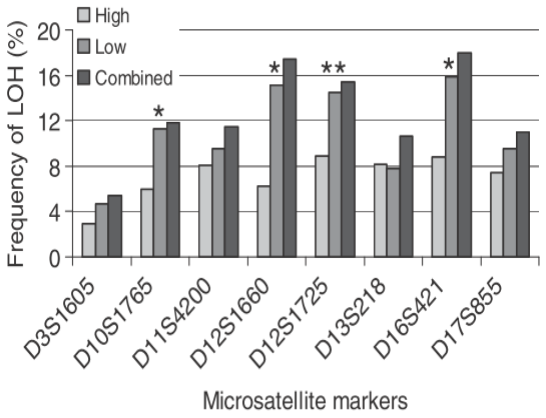
ctDNA的分析指标

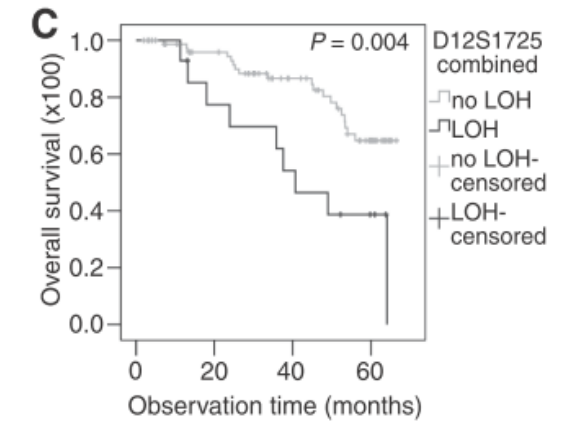
1. cfDNA含量
2. cfDNA完整性
3. ctDNA突变频率
4. ctDNA甲基化频率
5. 微卫星杂合性

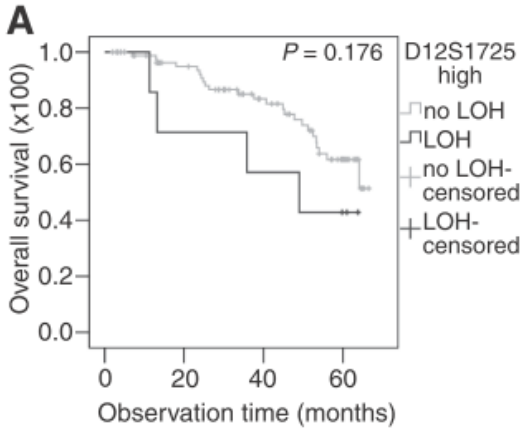
Loss of Heterozygosity at Tumor Suppressor Genes Detectable on Fractionated Circulating Cell-Free Tumor DNA as Indicator of Breast Cancer Progression

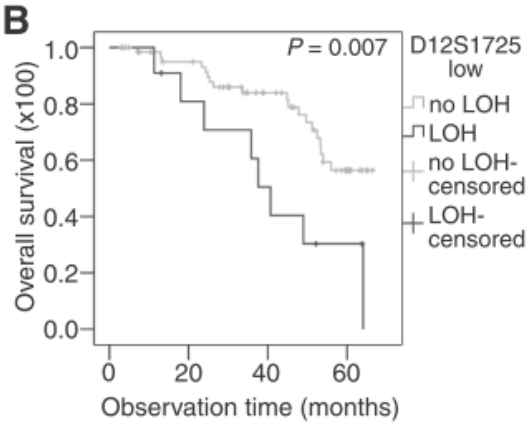
Heidi Schwarzenbach, Corinna Eichelser, Jolanthe Kropidlowski, et al.

Clin Cancer Res 2012;18:5719-5730. Published OnlineFirst September 25, 2012.





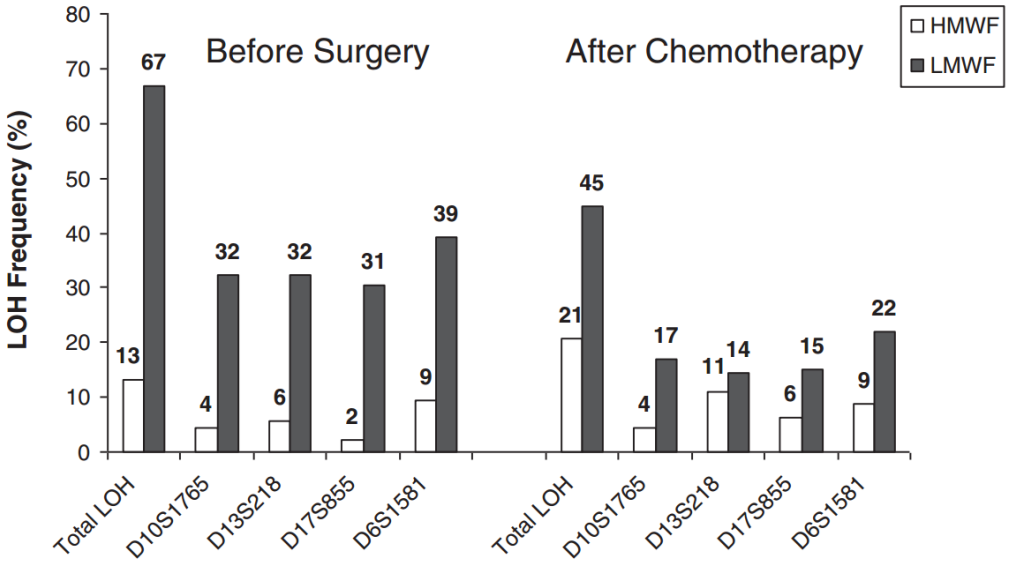


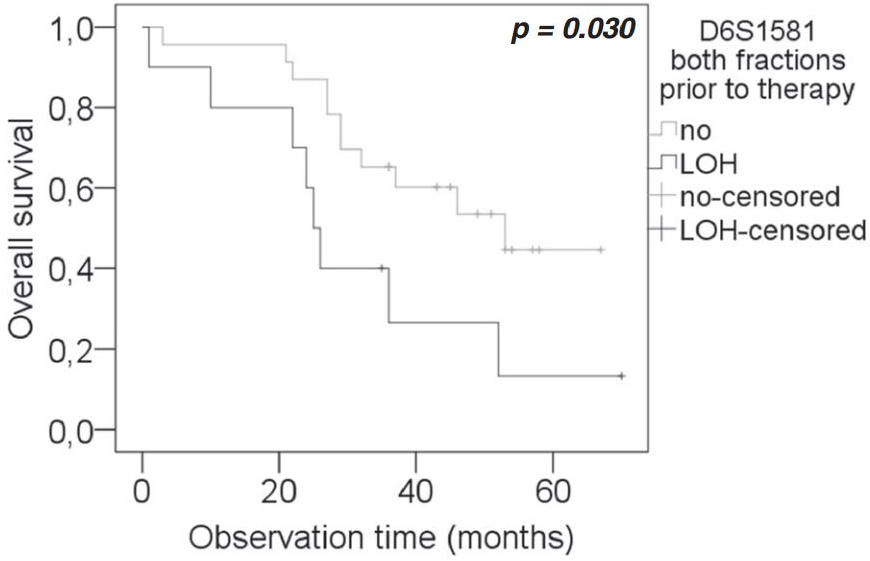


- 血浆中短链DNA分子发生杂合性丧失的频率显著高于长链DNA分子。
- 血浆中短链DNA的 cyclin D2 基因（D12S1725）的微卫星杂合性缺失，患者的预后显著变差，可作为乳腺癌患者重要的预后标志物。

LOH at 6q and 10q in fractionated circulating DNA of ovarian cancer patients is predictive for tumor cell spread and overall survival

Jan Dominik Kuhlmann[1*], Heidi Schwarzenbach[2], Pauline Wimberger[1], Micaela Poetsch[3], Rainer Kimmig[1] and Sabine Kasimir-Bauer[1]





- 在卵巢癌患者中，短链DNA微卫星杂合性缺失的频率达到67%, 化疗后下调达到45%。
- 血浆中 M6P/IGF2R (D6S1581) 的杂合性缺失与卵巢癌患者的生存率相关，有杂合性缺失的病人预后较差 (p = 0.030)。

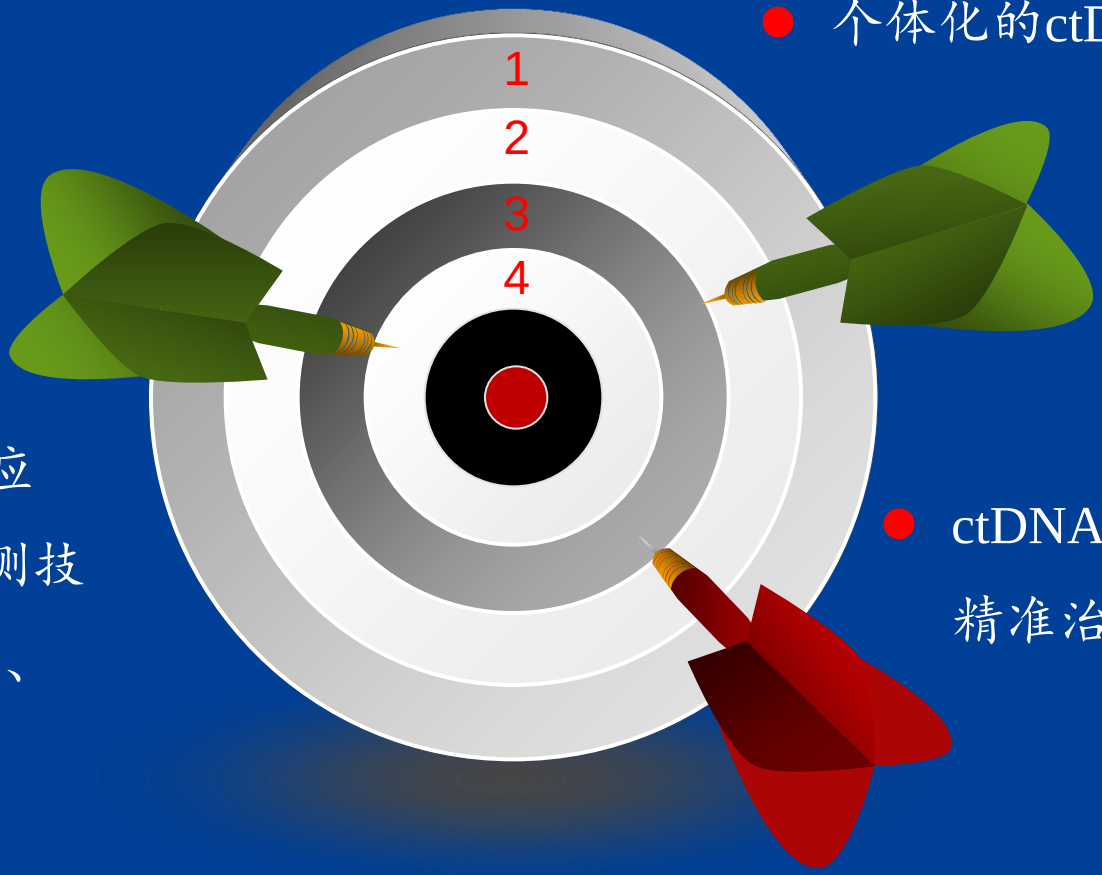
ctDNA在肿瘤诊断与预后领域的应用

Cancer	cfDNA	Diagnostic	Prognostic	Refs
Bladder	DNA integrity	✓	✓	123
	Methylation	✓		124
	Microsatellite alterations	✓		125
Breast	Methylation	✓	✓	126–130
	Microsatellite alterations	✓	✓	33–35
	DNA integrity		✓	30,131
	Mutation		✓	34
	Mitochondrial	✓		132
Cervical	Methylation	✓	✓	133,134
	Viral DNA	✓		135
Colorectal	Mutation	✓	✓	47,136–139
	DNA integrity	✓		31
	Methylation	✓	✓	136,140–143
Hepatocellular carcinoma	Methylation	✓	✓	144–146
	Microsatellite alterations		✓	147
	Mutation	✓	✓	148,149
	DNA integrity	✓	✓	29
	Viral DNA	✓		150
Lung	Mutation		✓	48,53,151,152
	Methylation	✓	✓	153–157
	Microsatellite alterations	✓	✓	36,37
Non-Hodgkin's lymphoma	Mutation		✓	158
	Viral DNA	✓	✓	159–161
	Methylation	✓		162
	DNA integrity	✓		162
Melanoma	Mutation	✓	✓	49,163,164
	Methylation		✓	111,115
	Microsatellite alterations	✓	✓	165–168
Ovarian	Methylation	✓	✓	169,170
	DNA integrity	✓		59
	Mutation		✓	171
	Mitochondrial	✓		172
Pancreatic	Methylation	✓		173,174
	DNA integrity	✓		31
	Mutation	✓	✓	46
Prostate	Methylation	✓	✓	38,175–179
	Microsatellite alterations	✓		13,38
	DNA integrity	✓	✓	180
	Mitochondrial		✓	26,181

*This table represents different forms of cell-free nucleic acid (cfNA) that have been detected in patients with the most prevalent cancers in both males and females[182]. This table is not meant to be comprehensive and is based on our own view of studies that offer substantial clinical insight. cfDNA, cell-free DNA.

ctDNA临床应用面临的挑战

- 个体化的ctDNA标志物
- 发展适合临床应用的ctDNA检测技术(快速、精准、低成本)
- ctDNA在肿瘤个性化精准治疗领域的应用



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谢谢！

THANKS

2015.06.12

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