Korean Society for Health Promotion and Disease Prevention

2020년 대한임상건강증진학회 동계학술대회

2020. 12. 6 (일)

액체 생검의 임상적 의의와 최신 경향

김 경 철 (강남메이저병원)

김경철, 가정의학 전문의

연세의대 졸업 (MD)
연세대 보건대학 석사 (MPH)
연세대 노화과학 박사 (PhD)
보스턴 Tufts 대학 영양 유전학 연수

전) 차의과학대학 차움 병원
임상 유전체 센터, 교수
전) 테라젠이텍스 바이오 연구소 부사장

현) 강남 메이저 (구, 강남 미즈메디)
경영원장 (2019.3~)
현) 국가생명윤리위원회 유전자 전문위원
현) EDGC 유전체 회사 자문의사

헬시에이징 강남메이저

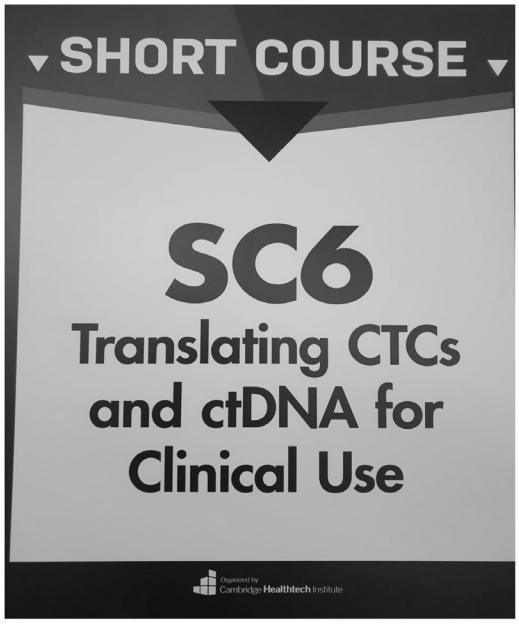
미래의학의 주요 아젠다

- **Companion diagnostics/ Target therapy**
- **Beyond Genomics : Epigenetics**
- **Cancer immunotherapy**
- **Liquid Biopsy : circulating cell free DNA/ circulating tumor cell/ Exosome, DNA methylation, microRNA etc.**
- **Microbiome NGS**
- **Mobile Health Technologies/ AI based health care**
- **Point-of-Care Diagnostics**
- **Digital Medicine**

Tri-conference 2015~2020

헬시에이징 강남메이저

Tri-Conference, San Francisco. CA 2020 March 1-4



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Good Condition for Cancer Biomarker

1) Early detection (Before tumor diagnosed via conventional methods)
2) Prognostic marker (After treatment change), Reversible
3) Good Sensitivity, Specificity, PPV, NPV
4) Non-invasive, Good assesibility
5) High through output / more Genes/ less prices

민감도 (Sensitivity) : 병이 있을 때 병이 있다고 말해야 함, 위음성(false negative)를 최소화해야 함. 암 조기 진단 시 중요한 지표

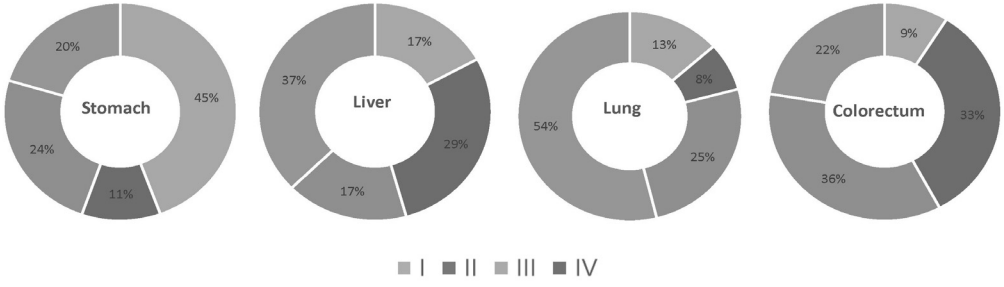
특이도 (Specificity) : 병이 없을 때 병이 없다고 말해야 함, 위양성 (false positive)를 최소화해야 함. 암 재발 추적 시 중요한 지표

헬시에이징 강남메이저

암 조기 진단의 필요성

● 4대 장기(위/간/폐/대장)별 암 진단 당시 10중 2명이 4기로 암 진단에서 조기진단에 대한 필요성이 높아질 것으로 예상 됨

Stage at diagnosis: ~ 50% are in stage III/IV



Source: Severance cancer center

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단백질 방식의 Tumor marker의 한계

Tumor Markers

Marker	Cancer	Sensitivity	Specificity
Prostate-specific antigen (PSA)	Prostate	57–93	55–68
Carcinoembryonic antigen (CEA)	Colorectal	40–47	90
Alpha-fetoprotein (AFP)	Hepatocellular	98	65
Cancer antigen 19-9	Pancreatic	78–90	95
Cancer antigen 125	Ovarian		
Beta-HCG	Testicular, Choriocarcinoma		
NSE	Small cell lung Neuroblastoma		

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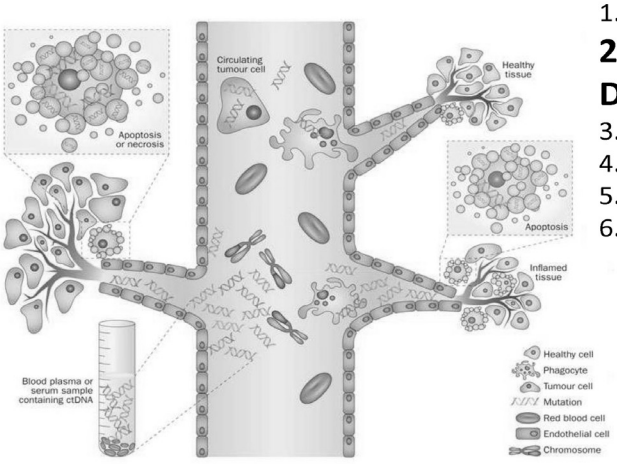
액체 생검 : Liquid Biopsy

- NGS 기술 발달로 혈액 내 미량 존재하는 Circulating tumor DNA(ctDNA)의 다양한 변이를 한번에 검출 가능



Crowley, Nature Reviews Clinical Oncology, 2013

1.Circulating tumor Cell (CTC)
2.Circulating cell fee DNA (CfDNA) mutation
3. cfDNA methylation
4. Exosome
5. microRNA
6. Oncogenic Viral DNA

MIT Technology Review 2015 10 Breakthrough Technologies

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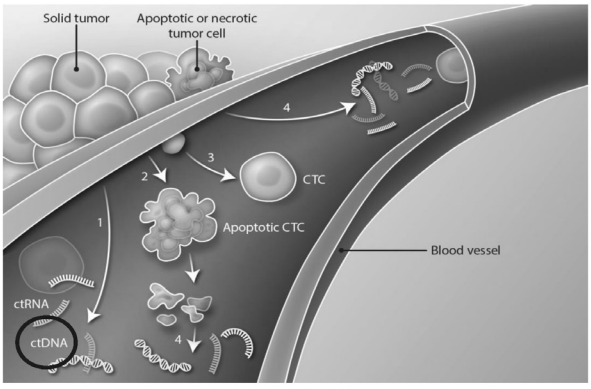
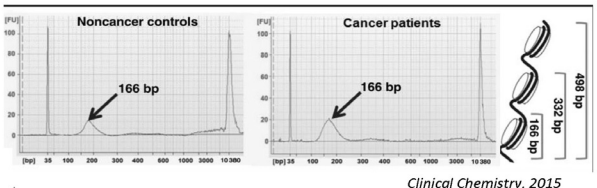
Circulating Cell free DNA (cfDNA)란?

- dsDNA
- highly fragmented DNA (140 ~ 170 base pairs) in plasma and serum with half life of ~2hours
- Sources of DNA release: necrosis, apoptosis
- Not cancer specific
  - Exercises, trauma, surgery
- Cancer specific cell free DNA: **ctDNA (circulating tumor DNA)** can be present in 0.01% ~ >90% of the total cfDNA



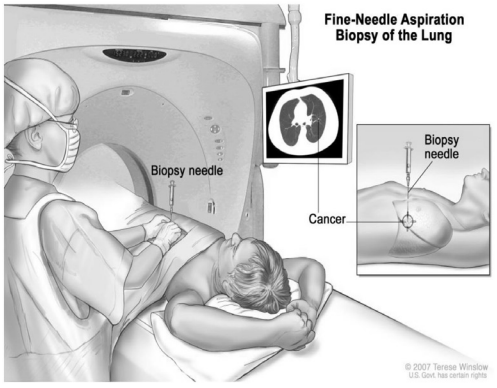


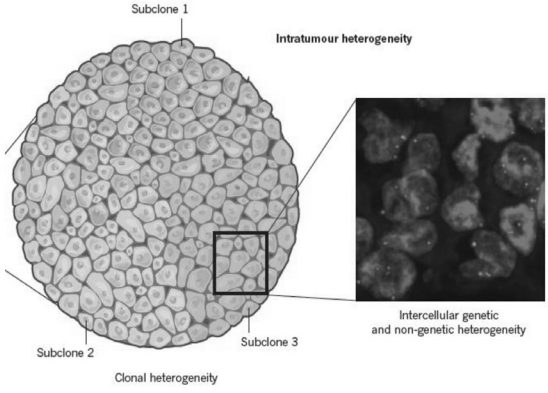
Clinical Chemistry, 2015

헬시에이징 강남메이저

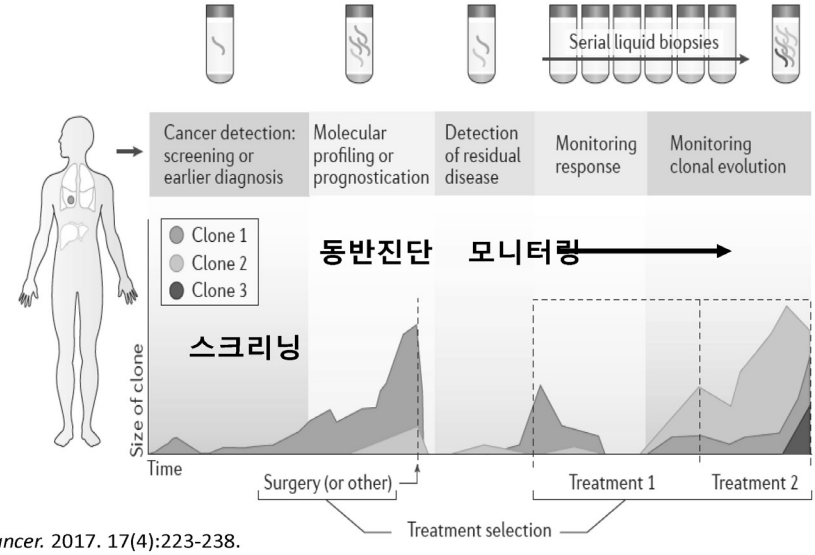
Liquid biopsy vs. Tissue biopsy

- Risk of complication and patient safety (조직검사의 위험)
- Tumor heterogeneity (암 조직의 다양성)





액체생검 (Liquid Biopsy)의 임상적 유용성



Nat Rev Cancer. 2017. 17(4):223-238.

Clinical application of cfDNA (cell free DNA)

Objective	cfDNA amount	Mutation 종류
1. Early detection of cancer before diagnosis (Stage 0~1)	scanty << 10ng → need **sensitivity↑↑**	unknown → cover gene>100
2. Early detection of recurrence after definitive treatment (Stage 1~3)	10 ~ 50 ng → need **sensitivity↑**	known **<< unknown** → cover gene>50
3. Treatment monitoring during chemotherapy (Stage 4)	10 ~ 1000 ng → need **accurate mea sure** of copies	known >> unknown → cover 8~10 genes

Oncogenesis

small size
no info of genomic mutation

After treatment

small size
info of genomic mutation+
→ new clone would recur

Metastatic setting

large size
info of genomic mutation+
→ new clone↑ as existing clone↓

1

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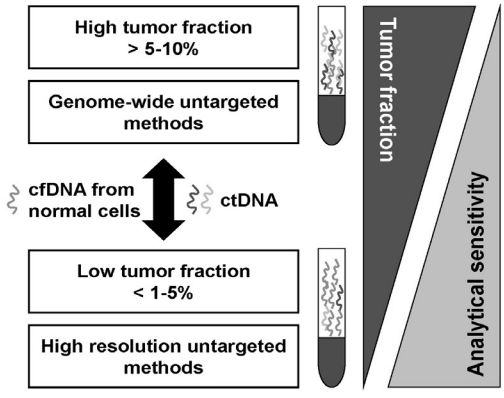
Technology for ctDNA analysis

Increasing genomic coverage (and cost)

PCR → Targeted seq. → WGS



- **WGS (LOD 5-10%)**
- **Whole methylome (LOD 5-10%)**
- **Plasma-Seq (LOD 5-10%)**
- **WES (LOD 5%)**
- **Tam-Seq (LOD 2%)**
- **CAPP-Seq (LOD <0.1%)**
- **Safe-SeqS (LOD <0.1%)**
- **BEAMing (LOD <0.01%)**
- **dPCR (LOD <0.01%)**

LOD = Limit of Detection

13

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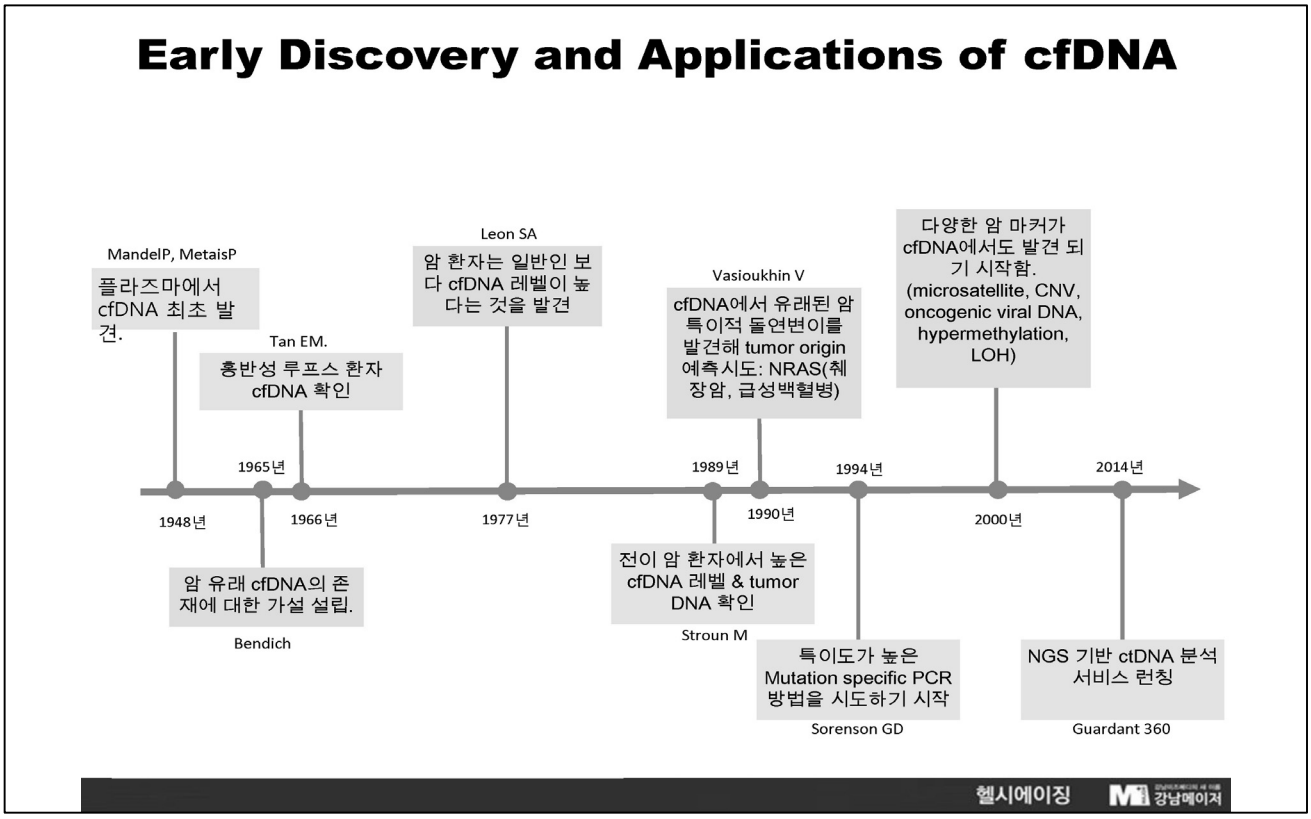
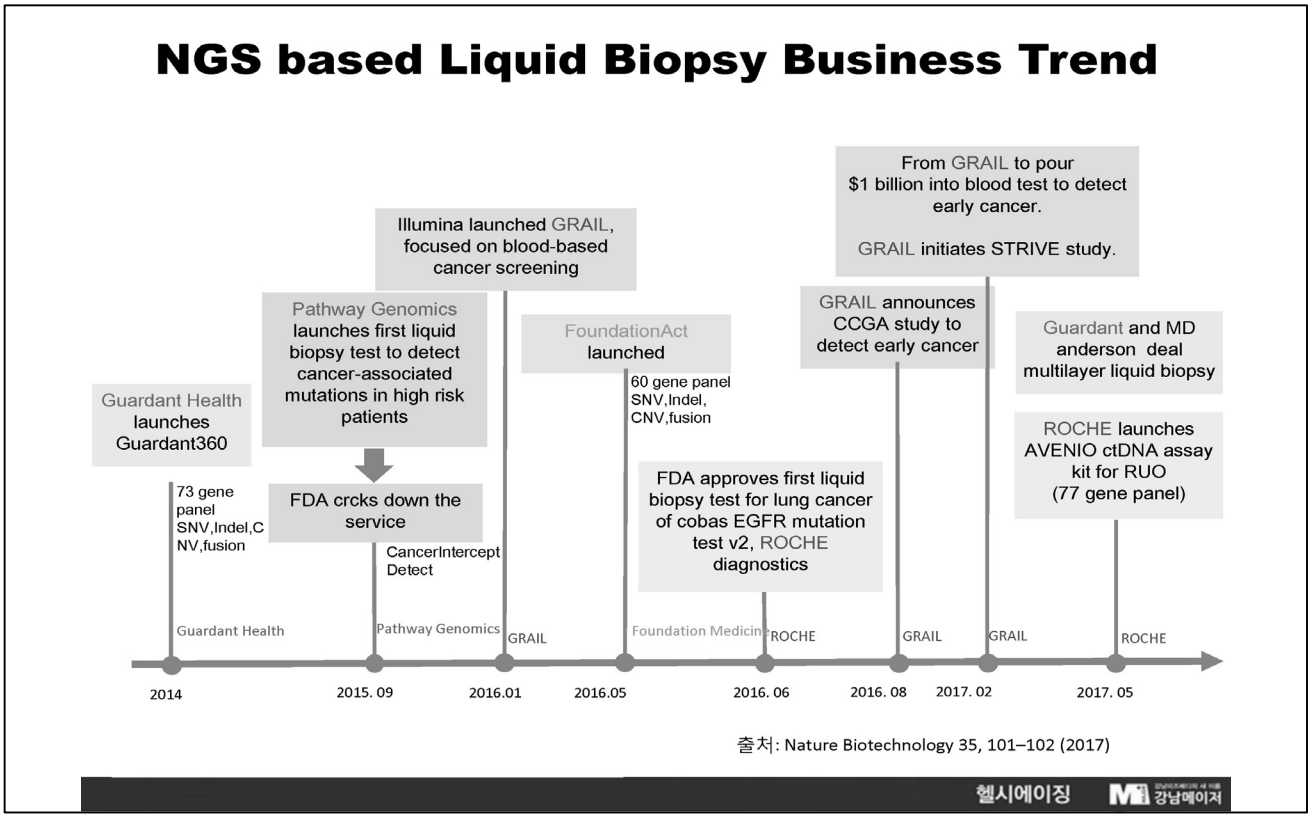

Early Discovery and Applications of cfDNA
MandelP, MetaisP
플라즈마에서 cfDNA 최초 발견.
1948년
Tan EM.
홍반성 루프스 환자 cfDNA 확인
1965년
1966년
암 유래 cfDNA의 존재에 대한 가설 설립.
Bendich
Leon SA
암 환자는 일반인 보다 cfDNA 레벨이 높다는 것을 발견
1977년
Vasioukhin V
cfDNA에서 유래된 암 특이적 돌연변이를 발견해 tumor origin 예측시도: NRAS(췌장암, 급성백혈병)
1989년
1990년
전이 암 환자에서 높은 cfDNA 레벨 & tumor DNA 확인
Stroun M
1994년
특이도가 높은 Mutation specific PCR 방법을 시도하기 시작
Sorenson GD
다양한 암 마커가 cfDNA에서도 발견 되기 시작함. (microsatellite, CNV, oncogenic viral DNA, hypermethylation, LOH)
2000년
2014년
NGS 기반 ctDNA 분석 서비스 런칭
Guardant 360
헬시에이징
강남메이저


NGS based Liquid Biopsy Business Trend
Guardant Health launches Guardant360
73 gene panel SNV,Indel,CNV,fusion
Guardant Health
2014
Pathway Genomics launches first liquid biopsy test to detect cancer-associated mutations in high risk patients
FDA crcks down the service
CancerIntercept Detect
Pathway Genomics
2015. 09
Illumina launched GRAIL, focused on blood-based cancer screening
GRAIL
2016.01
FoundationAct launched
60 gene panel SNV,Indel, CNV,fusion
Foundation Medicine
2016.05
FDA approves first liquid biopsy test for lung cancer of cobas EGFR mutation test v2, ROCHE diagnostics
ROCHE
2016. 06
GRAIL announces CCGA study to detect early cancer
GRAIL
2016. 08
From GRAIL to pour $1 billion into blood test to detect early cancer.
GRAIL initiates STRIVE study.
GRAIL
2017. 02
Guardant and MD anderson deal multilayer liquid biopsy
ROCHE launches AVENIO ctDNA assay kit for RUO (77 gene panel)
ROCHE
2017. 05
출처: Nature Biotechnology 35, 101–102 (2017)
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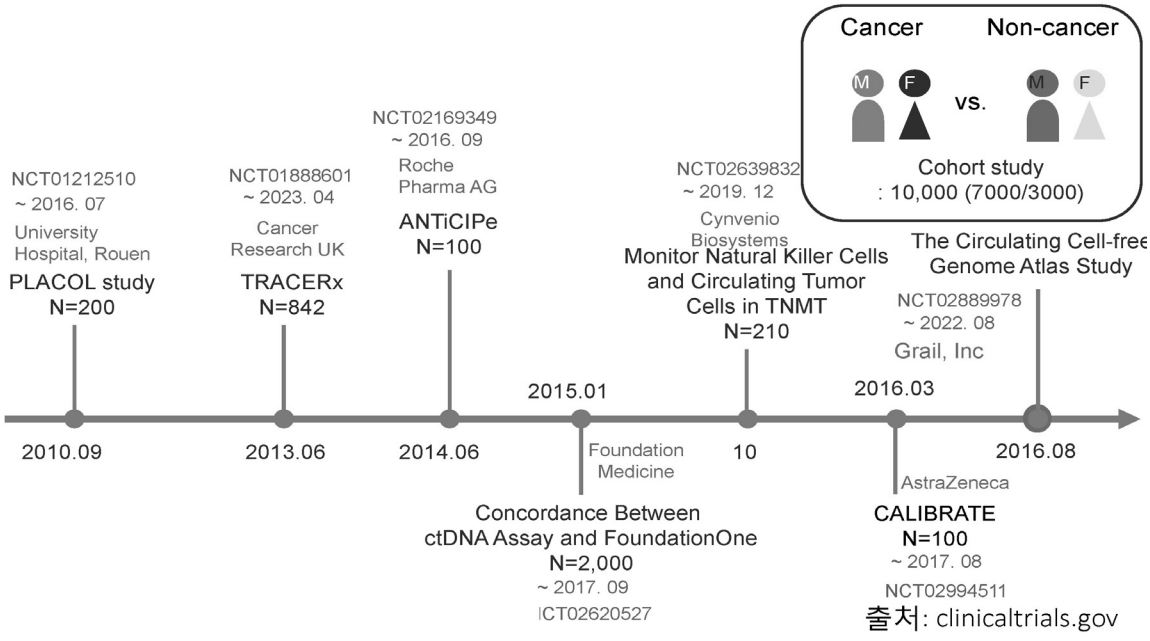
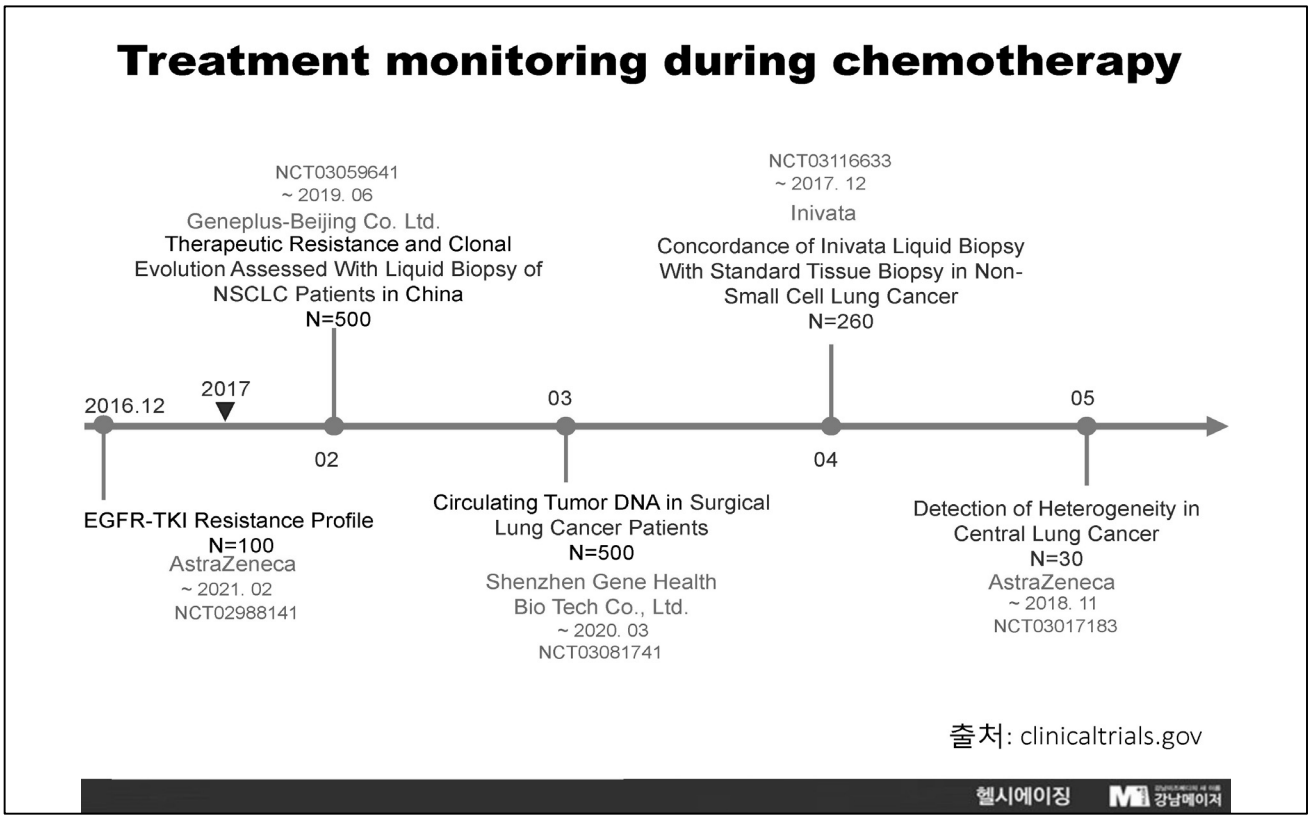
Selected liquid biopsy platforms in clinic use

- 전 세계적으로 38개 이상의 회사가 액체생검 서비스 및 기기 개발에 집중하고 있음.

기관명	상품명	관련기술	내 용	가 격
Biocept	Target Selector	Sequencing /FISH	ctDNA 및 CTC 를 이용하여 13개 유전 변이 확인	-
Grail	-	Sequencing (Deep seq)	Illumina 기반 진단기술 개발 중	약 1,000달러 (2019년 예상)
Guardant	Guardant360	Sequencing (Digital seq)	70개 유전자 변이 확인	약 5,800달러
Oncocyte	Oncocyte DX test	Sequencing	폐, 유방, 방광암 진단	약 4,000달러
Genomic Health	Ocotype SEQ	Sequencing	17개 유전자 변이 확인	-
Myriad	myRisk	sequencing	28개 암 유전자 Panel	약4,000~4,500달러
Foundation medicine	FoundationACT™	Sequencing (Deep seq)	ctDNA를 이용하여 deep seq 수행 (총 61개 유전자)	약 5,800달러
Qiagen	REPLI-g Single Cell Kit 등	PCR	ctDNA, miRNA, RNA등 분리 KIT 개발	약 700~900달러
Trovagene	Trover Test	Sequencing	Urine을 이용하여 암 유전자 변이 모니터링(EGRF,KRAS,BRA)	

Treatment monitoring during chemotherapy




Treatment monitoring during chemotherapy
NCT03059641
~ 2019. 06
Geneplus-Beijing Co. Ltd.
Therapeutic Resistance and Clonal Evolution Assessed With Liquid Biopsy of NSCLC Patients in China
N=500
NCT03116633
~ 2017. 12
Inivata
Concordance of Inivata Liquid Biopsy With Standard Tissue Biopsy in Non-Small Cell Lung Cancer
N=260
2016.12
2017
02
03
04
05
EGFR-TKI Resistance Profile
N=100
AstraZeneca
~ 2021. 02
NCT02988141
Circulating Tumor DNA in Surgical Lung Cancer Patients
N=500
Shenzhen Gene Health Bio Tech Co., Ltd.
~ 2020. 03
NCT03081741
Detection of Heterogeneity in Central Lung Cancer
N=30
AstraZeneca
~ 2018. 11
NCT03017183
출처: clinicaltrials.gov
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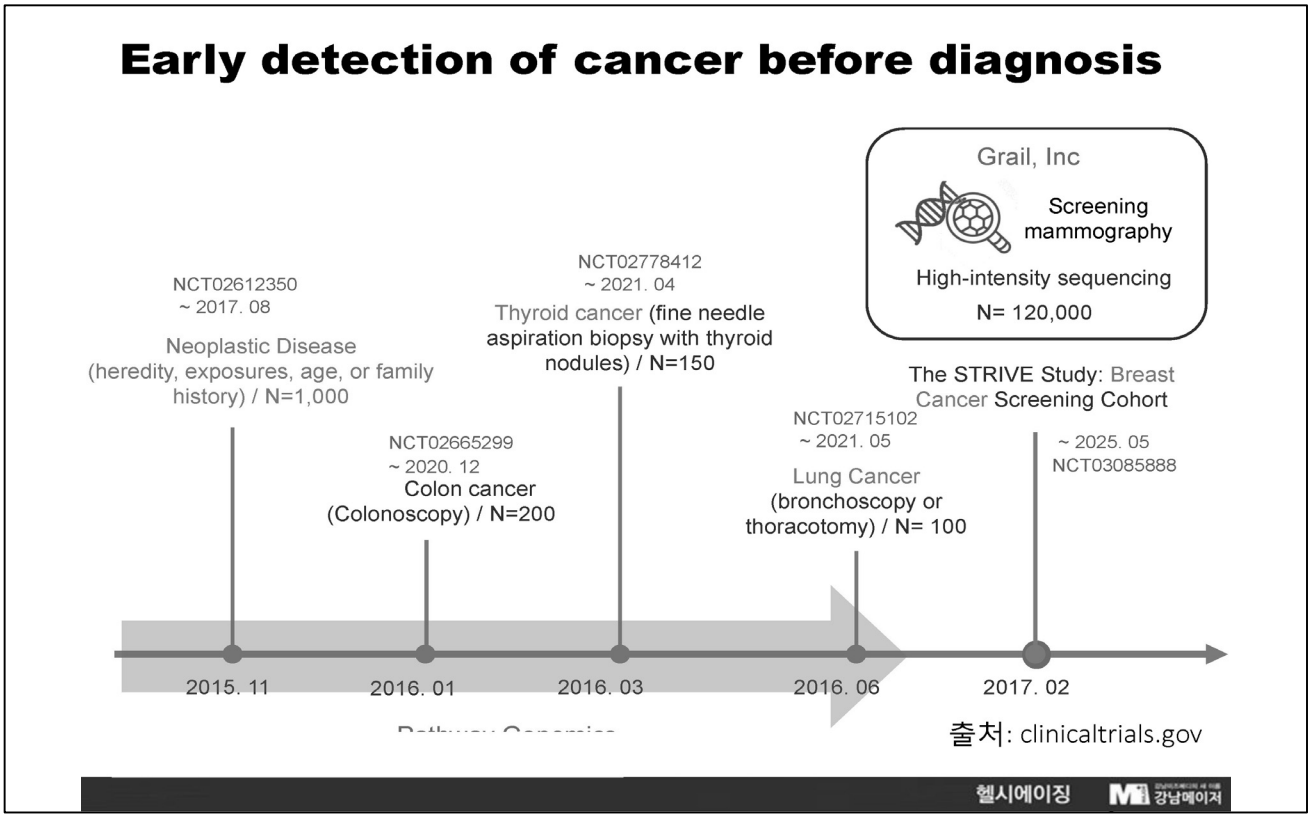
Early detection of cancer before diagnosis
Grail, Inc
Screening mammography
High-intensity sequencing
N= 120,000
NCT02612350
~ 2017. 08
Neoplastic Disease (heredity, exposures, age, or family history) / N=1,000
NCT02778412
~ 2021. 04
Thyroid cancer (fine needle aspiration biopsy with thyroid nodules) / N=150
The STRIVE Study: Breast Cancer Screening Cohort
NCT02715102
~ 2021. 05
Lung Cancer (bronchoscopy or thoracotomy) / N= 100
NCT02665299
~ 2020. 12
Colon cancer (Colonoscopy) / N=200
~ 2025. 05
NCT03085888
2015. 11
2016. 01
2016. 03
2016. 06
2017. 02
출처: clinicaltrials.gov
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전세계 동향

Magic Leap
A startup is betting more than half a billion dollars that it will dazzle you with its approach to creating 3-D imagery.

Nano-Architecture
A Caltech scientist creates tiny lattices with enormous potential.

Car-to-Car Communication
A simple wireless technology promises to make driving much safer.

Project Loon
Billions of people could get online for the first time thanks to helium balloons that Google will soon send over many places cell towers don't reach.

Liquid Biopsy
Fast DNA-sequencing machines are leading to simple blood tests for cancer.

Megascale Desalination
The world's largest and cheapest reverse-osmosis desalination plant is up and running in Israel.

Apple Pay
A clever combination of technologies makes it faster and more secure to buy things with a wave of your phone.

Brain Organoids
A new method for growing human brain cells could unlock the mysteries of dementia, mental illness, and other neurological disorders.

Supercharged Photosynthesis
Advanced genetic tools could help boost crop yields and feed billions more people.

Internet of DNA
A global network of millions of genomes could be medicine's next great advance.

GUARDANT360
Test. Take Action.

genomeweb

Business & Policy | Technology | Research | Diagnostics | Disease Areas | Applied Markets

Home » Business, Policy & Funding » Business News » Guardant Health Expands Guardant360 Panel, Reports Increased Sensitivity

Guardant Health Expands Guardant360 Panel, Reports Increased Sensitivity

Nov 03, 2016 | Molika Ashford

Premium | Save for later

NEW YORK (GenomeWeb) – Guardant Health this week said that it has made new improvements to its Guardant360 comprehensive liquid biopsy test.

Although it has not yet made public the validation data supporting these numbers, the company now lists a reportable range for its assay down to 0.04 percent mutant allele fraction for SNPs and 0.02 MAF for indels.

The firm also updated the Guardant360 panel to 73 genes from 70 in its most recent iteration. Guardant CEO Helmy Eltoukhy told GenomeWeb this week that the content improvements also include a more comprehensive representation of gatekeeper, or acquired resistance, mutations, expanding the power of the test to inform patient management at different time points in their disease progression and care.

FierceBiotech

BIOTECH | COVID-19 | RESEARCH | CRO | CELL & GENE THERAPY | MEDTECH | SPECIAL REPO

MedTech

Guardant Health unveils epigenetic research test for early-stage cancer

by Conor Hale | Jan 8, 2019 9:07am

First mover

	SNV(mutation)	Methylation
민감도	낮음	높음
암종 구분	어려움	가능
난이도	3/5	5/5

GRAIL

FierceBiotech

BIOTECH | COVID-19 | RESEARCH | CRO | CELL & GENE THERAPY | MEDTECH | SPECIAL RE

MedTech

Grail's third VC round brings funding total to $1.5B

by Conor Hale | May 22, 2018 1:42pm

FierceBiotech

BIOTECH | COVID-19 | RESEARCH | CRO | CELL & GENE THERAPY | MEDTECH

MedTech

Grail nets FDA breakthrough designation, turns eye toward DNA methylation for early cancer detection

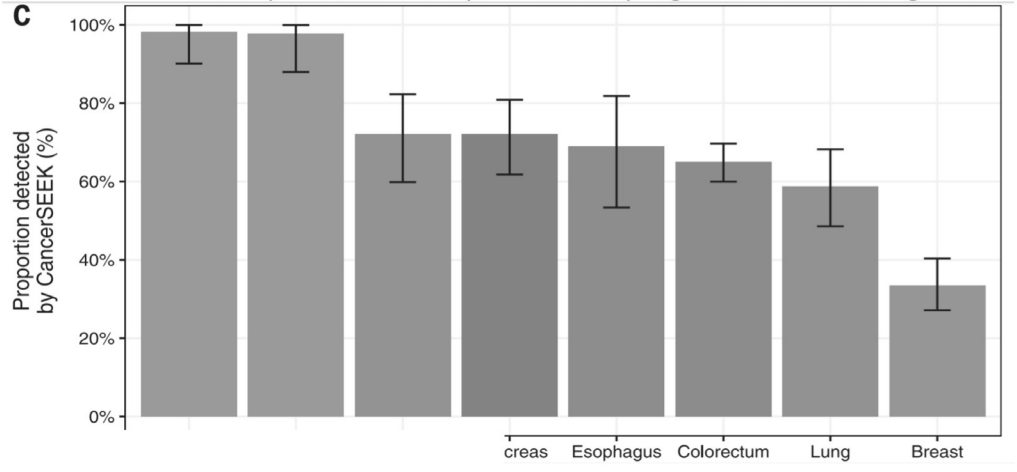
by Conor Hale | May 14, 2019 2:05pm

CANCER

Detection and localization of surgically resectable cancers with a multi-analyte blood test

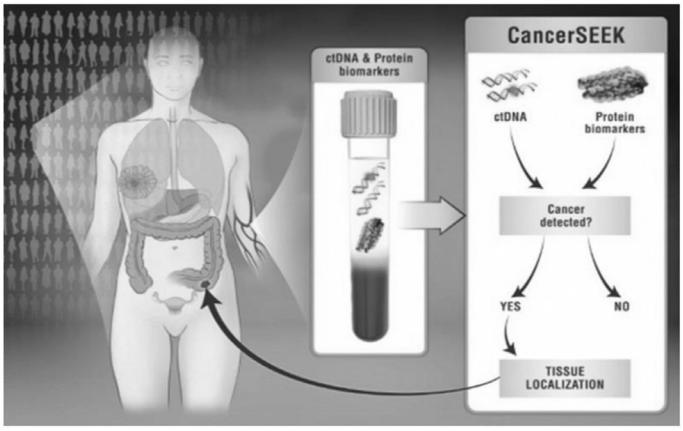
Cohen *et al.*, *Science* **359**, 926–930 (2018)

1005 patients with nonmetastatic, clinically detected cancers of the ovary, liver, stomach, pancreas, esophagus, colorectum, lung, or breast



헬시에이징 강남메이저

CancerSEEK



16개의 암 유전자를 포함해 기존의 단백질 방식의 암표지자 검사와 비교를 했다. 기존에 암 표지자 단백질 검사가 없는 난소암, 위암, 췌장암, 식도암 등의 경우에는 69~98%의 민감도를 보였고, 특이도는 99%로 매우 높았다. 암 병기(stage)별로는 3기 암의 민감도가 78%이고 2기 암은 73%인 반면, 1기암은 43%로 다소 낮았다.

Science 2018

헬시에이징 강남메이저

Limitation of Liquid Biopsy (Cancer Screening)

1. 낮은 Sensitivity (Specificity 는 높음), 특히 1기 (stage 1)에서는 민감도가 30~50%에 불과함.
2. 특정 유전자의 체세포 변이 (Somatic mutation)를 발견했음도 어느 암종 인지를 정확이 알기 어려움 (Cancer of Origin)
3. 다양한 암을 커버하기 위해서는 많은 유전자에 대한 변이를 한번에 검사해야 함 (NGS 방식) 그로 인한 비용 증가 (50~100만 원)
4. 혈장의 양이 많이 필요함 (20~50ml의 혈액)

대규모 임상 시험 및 Combination Diagnosis 를 통해 한계를 극복하고자 함

헬시에이징 강남메이저

Liquid biopsy: Early Detection for Cancer Cure

Pioneer of Cancer Genomics & Liquid biopsy

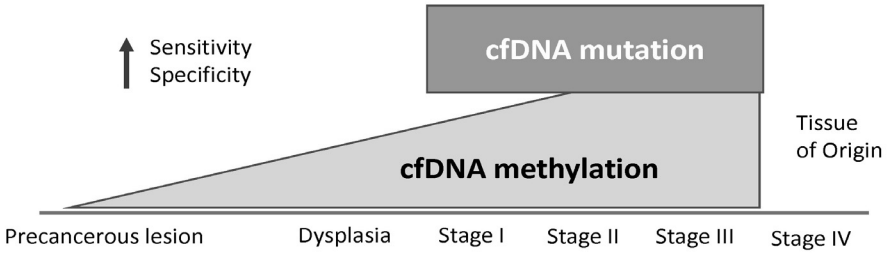
Liquid Biopsy Combination

1. Somatic mutations
2. DNA methylation changes
3. Cancer-specific mRNAs
4. Cancer-specific siRNAs
5. Cancer-specific proteins
6. Cancer-specific metabolisms

Cirina (cfDNA methylation)

액체생검 컴비네이션
(Liquid Biopsy Combination)

1. Somatic mutations
2. DNA methylation changes
3. Cancer-specific mRNAs
4. Cancer-specific siRNAs
5. Cancer-specific proteins
6. Cancer-specific metabolism



Techniques of DNA Methylation Analysis

Table 1. DNA methylation analysis methods

Pretreatment	Resolution: Global quantitative	Locus-specific PCR-based	Genome-wide	
			array-based	NGS-based
Sodium bisulfite conversion	Alu/LINE-1-PCR-pyrosequencing Alu/LINE-1-HRM	MSP-PCR MethyLight SMART-MSP MS-HRM Pyrosequencing MassARRAY	Infinum Golden Gate	RRBS WGSBS oxBS-Seq
Enzyme restriction digestion	HPLC LC-MS LUMA	HpaII-PCR MS-MLPA MS-FLAG	DMH MCAM HELP CHARM MMASS	Methyl-Seq MCA-Seq MSCC HELP-Seq
Affinity enrichment	5-mC ELISA 5-mHC ELISA	MeDIP-PCR MIRA	MeDIP-chip MIRA-chip	MeDIP-Seq MethylCap-Seq MIRA-Seq

J Nutrigenet Nutrigenomics 2013

The Methylation Profile of Circulating Cell Free DNA Can Be a Novel Marker for the Early Detection of Gastric Cancer and the Effect of Surgical Resection

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Results: The cfDNA concentration of the control group, pre-operative cancer patients, and post-operative cancer patients were 79.42±7.38, 113.07±12.81, and 77.90±5.67 ng/ml, respectively (ANOVA, $p<0.01$). 15 out of 32 cancer-associated genes of the cancer subjects demonstrated hypermethylation compared to the methylation pattern of the controls. The methylation panel of *PYCARD, APAF1, MINT3,* and *BRAC1* genes showed 97.3% sensitivity and 66.4% specificity for the presence of gastric cancer. Twenty one methylated genes turned out to be unmethylated after surgical resection ($p<0.05$).

Conclusions: The blood concentration of cfDNA and aberrant methylation pattern of tumor suppressor genes can be reliable biomarkers for the early detection of gastric cancer and to determine the efficacy of surgical resection.

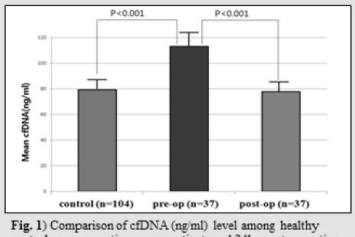

Fig. 1) Comparison of cfDNA (ng/ml) level among healthy controls, pre-operative cancer patients and 24hr post-operative cancer patients. ($p<0.01$)

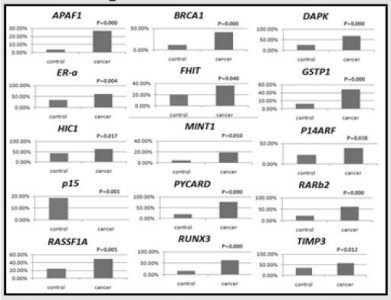

Fig. 2) Comparison of methylated genes between cfDNA levels of healthy controls and cancer patients

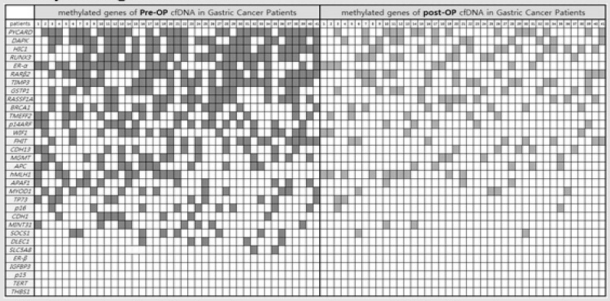

Fig. 3) Comparison of methylated genes between pre-op and post-op cfDNA levels in gastric cancer patients

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검사명	SNV	SNV-환자 맞춤형	CNV	Epigenome (Methylation)
용도	동반진단 (스크리닝)	모니터링	스크리닝 V1	스크리닝 V2
검체	혈액	암조직 + 혈액	혈액	혈액
분석 내용	**SNV, indel** - 53 Cancer related gene - TP53 whole exon	**개인 맞춤형 검사** - WES: 암조직 및 buffy coat	**CNV** + 종양표지자 5종	**Methylation** + 종양표지자, cfDNA 패턴
특징	맞춤형 항암제 선택	개인 맞춤형 프리미엄 검사, 높은 민감도	Multi-Omics 분석	조기암 분석 Tissue of Origin (종양 위치)
경쟁 서비스	Guardant360 CancerSeek (Thrive) 테라젠, 마크로젠, 지니너스	SigNatera (Natera)	녹십자지놈 개발중	Grail

SNV **CNV** **Methylation**

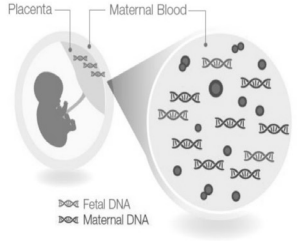
Cell free DNA 의 시대

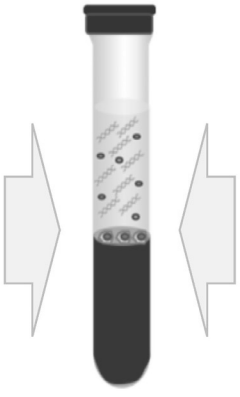
조직, 세포
gDNA

→ 1초당 약 50만개 파괴 →

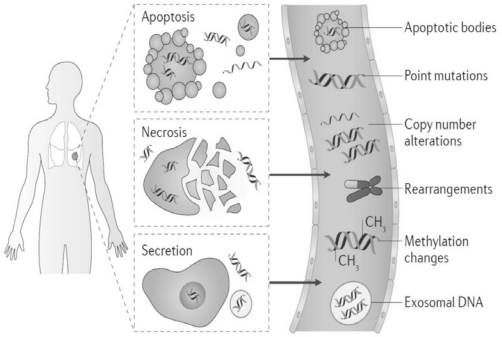
혈액
cfDNA (cell free DNA)

NIPT
비침습 산전 기형아검사





Liquid Biopsy
액체생검



헬시에이징 강남메이저