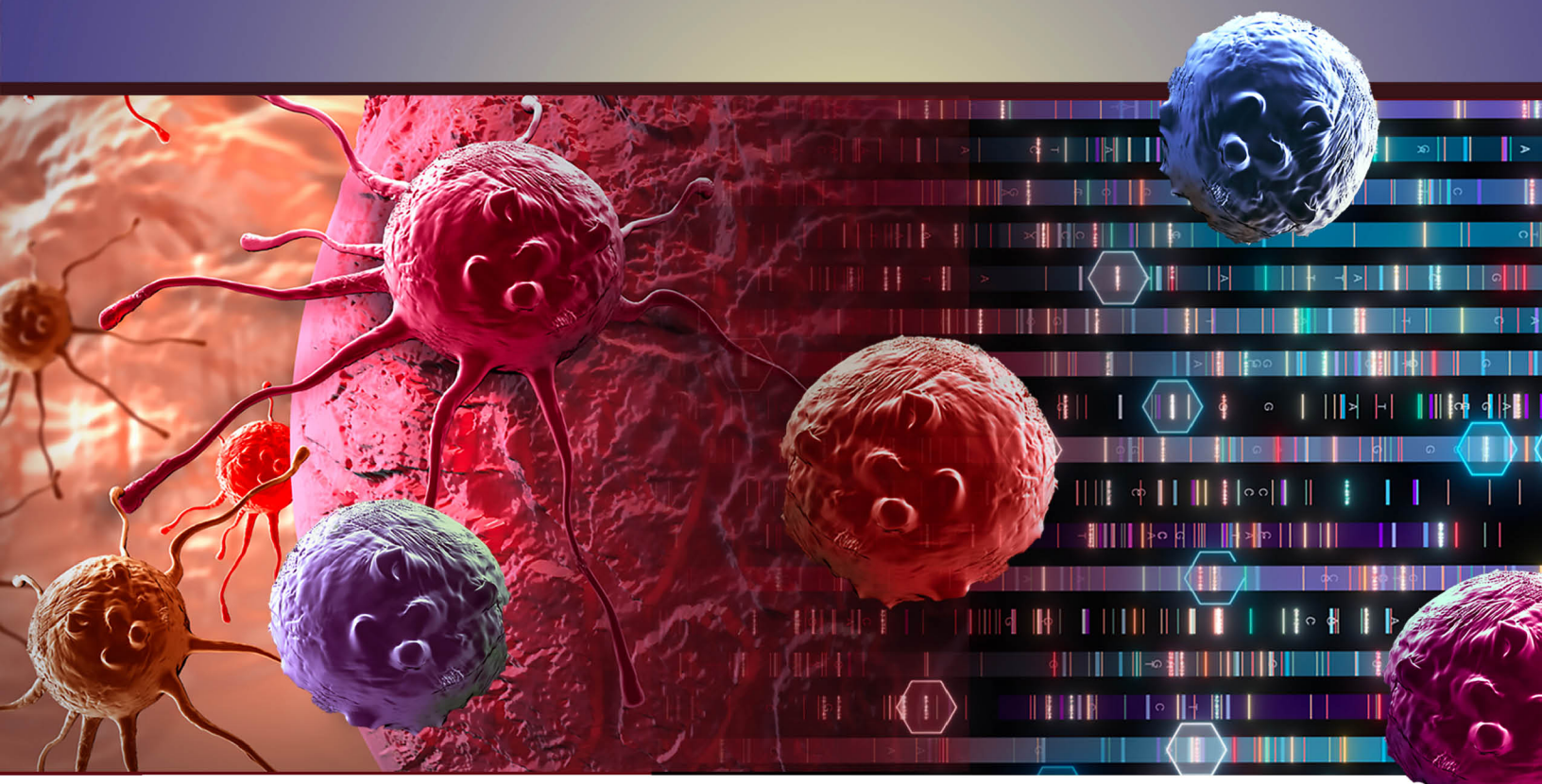
The Push to Detect Cancer Earlier:

Cell-Fee DNA (cfDNA) Blood Tests in Primary Care



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The activity is supported by an educational grant from Grail, Inc.

The Push to Detect Cancer Earlier: Cell-Free DNA (cfDNA) Blood Tests in Primary Care

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PROGRAM OVERVIEW

This live activity will cover early detection and intervention in cancer.

TARGET AUDIENCE

This live activity is intended for primary care physicians, internists, family practice physicians, and related healthcare professionals involved in the care of people who undergo screening for cancer.

LEARNING OBJECTIVES

After completing the CME activity, learners should be better able to:

- Define cfDNA and ctDNA along with their potential roles in early multi-cancer detection.
- Evaluate emerging data on the clinical validity and utility of cfDNA blood tests in early detection of cancer
- Plan strategies to integrate cfDNA blood tests and early multi-cancer detection into daily practice

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NURSING CREDIT INFORMATION

Purpose: This program would be beneficial for nurses involved in caring for patients with cancer.

Credits: 1.0 ANCC Contact Hour

CNE Accreditation Statement: Ultimate Medical Academy/CCM is accredited as a provider of continuing nursing education by the American Nurses Credentialing Center's Commission on Accreditation. Awarded 1.0 contact hour of continuing nursing education of RNs and APNs.

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	Research funding (Institution)	Bristol-Myers Squibb, Guardant, Array, Pfizer, Macrogenics, and Takeda
Eric Klein, MD	Consultant	Grail, Inc
Deepti Behl, MD	Consultant/Advisory Board and Speaker	Guardant
Kristen Ciombor, MD, MSCI	Consultant	Merck
	Research Funding (Institution)	Bristol-Myers Squibb, Array, Incyte, Daiichi Sankyo, Nucana, Merck, Calithera/Pfizer

CME content review

The content of this activity was independently peer reviewed.

The reviewer of this activity has nothing to disclose.

CNE Content Review

The content of this activity was peer reviewed by a nurse reviewer.

The reviewer of this activity has nothing to disclose.

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2. Participate in the live activity.
3. Submit the pre- and post-test and evaluation form to Med Learning Group.

You will receive your certificate as a downloadable file.

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AGENDA

I. Cancer Screening: An Overview

a. Current practice in cancer screening (CDC and USPSTF recommendations)
b. Gaps in current practice
c. Survival based on cancer stage at diagnosis

II. Analysis of Circulating Cell-Free Nucleic Acids for Early Cancer Detection

a. Characteristics of good screening tests
b. Adherence to screening recommendations
c. The “liquid biopsy”
d. cfNAs, cfDNA, ctDNA
e. Next-generations assays in development for cfDNA analysis for multi-cancer early detection
f. The concept of “minimal residual disease”

III. Integration of cfDNA Blood Tests into Cancer Screening in Clinical Practice

a. Emerging data from observational and interventional clinical studies on the validity and utility of cfDNA blood tests in early cancer detection
b. Potential placement of cfDNA blood tests in established cancer screening paradigms and evidence-based guidance
c. Communication of cancer risk information to patients
d. Navigating the complexities and challenges associated with integrating multi-cancer early detection in clinical practice
e. Monitoring outcomes

V. Conclusions

VI. Questions and answers

VII. Adjournment

The Push to Detect Cancer Earlier: Cell-Free DNA (cfDNA) Blood Tests in Primary Care

Disclosures

- During the course of this activity, faculty will be discussing investigational cancer-detection methods that do not have FDA approval.
- Acknowledgement: special thank you to Dr. Charu Aggarwal and Dr. Sana Raoff for some slide content.

This activity is supported by an educational grant from GRAIL

Learning Objectives

- Define cfDNA and ctDNA along with their potential roles in early multi-cancer detection
- Evaluate emerging data on the clinical validity and utility of cfDNA blood tests in early detection of cancer
- Plan strategies to integrate cfDNA blood tests and early multi-cancer detection into daily practice

What's the Problem?

Where Are We Now?

2021 ACS Facts and Figures

- Cancer is the leading cause of death among Americans under 80[1]
- 1.9 million Americans are diagnosed with cancer annually[2]
- 608,570 Americans die of cancer annually[2]
- 5-year cancer-specific survival across 20 cancer types: **81% at local stages, 22% at advanced stages**[3]

1. Siegel RL, et al. *CA Cancer J Clin*. 2020;70:7-30. 2. Siegel RL, et al. *CA Cancer J Clin*. 2021;71:7-33. American Cancer Society (ACS). Cancer Facts & Figures 2021 (www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf). Accessed 1/21/2021.

Cancers Detected Earlier Do Better

Five-year relative survival rates (%) by stage at diagnosis, US, 2010–2016

	All Stages %	Local %	Regional %	Distant %
Breast (female)	90	99	86	28
Colon & rectum	65	90	72	14
Colon	63	91	72	14
Rectum	67	89	72	16
Esophagus	20	47	25	5
Kidney	75	93	70	13
Larynx	61	78	45	34
Liver	20	34	12	3
Lung and bronchus	21	59	32	6
Melanoma of skin	93	99	66	27

	All Stages %	Local %	Regional %	Distant %
Oral cavity & pharynx	66	85	67	40
Ovary	49	93	75	30
Pancreas	10	39	13	3
Prostate	98	>99	>99	30
Stomach	32	70	32	6
Testis	95	99	96	73
Thyroid	98	>99	98	55
Urinary bladder	77	69	37	6
Uterine cervix	66	92	58	17
Uterine corpus	81	95	69	17

ACS. *Cancer Facts & Figures 2021* (www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf). Accessed 1/20/2021.

Cancer Screening

USPSTF Recommendations for Cancer Screening

Cancer	Grade	Population	Modality/ Recommendation	Pathway and Outcome
Cervical[1]	A	Women aged 21 to 65	Regular screening (3–5 years) using cervical cytology and/or HPV tests	HPV testing: USPSTF → CMS National Coverage Determination (NCD)
Colorectal[2]	A B	Adults aged 50 to 75 Adults aged 45-49*	Regular annual screening, multiple effective methods available	Legislation → CMS NCD Also has USPSTF "A" rating
Breast[3]	B	Women aged 50 to 74	Biennial screening mammography	Mandate for coverage with no cost sharing (Balanced Budget Act of 1997, Sec 4101)
Lung[4]	B	Adults aged 55–80, with history of smoking	Annual low-dose computed tomography (LDCT) screening	USPSTF → CMS NCD
Prostate[5]	C	Men aged 55 to 69	Periodic PSA screening on **case-by-case basis**	Not applicable

***Draft recommendation – in progress.**
HPV = human papillomavirus; CMS = Centers for Medicare & Medicaid Services; PSA = prostate-specific antigen.

1. USPSTF. *JAMA.* 2018;320:674-686. 2. USPSTF. Available at: https://uspreventiveservicestaskforce.org/uspstf/draft-recommendation/colorectal-cancer-screening3. 3. USPSTF. *Ann Intern Med.* 2016;164:279-296. 4. USPSTF. *Ann Intern Med.* 2014;160:330-338. 5. USPSTF. *JAMA.* 2018;319:1901-1913.

Non-Standard Cancer Screens

- High-risk screening
  - Pancreatic cancer in patients with genetic syndromes, family history
  - Lung cancer in patients with a history of heavy smoking
  - Esophageal cancer in patients with Barrett's esophagus
  - Liver cancer in patients with underlying liver diseases
- Most deaths in these cancer types occur in patients who were not enrolled in special surveillance, i.e., they do not meet screening criteria or know they are at high risk

Ahlquist DA. *NPJ Precis Oncol.* 2018;2:23.

Characteristics of Good Screening Test

- Inexpensive
- Easy to administer
- Minimally invasive
- Reliable (consistent)
- Valid (accurately identifies positives)
- High sensitivity and extremely specific

Problems With Current Screening Tools

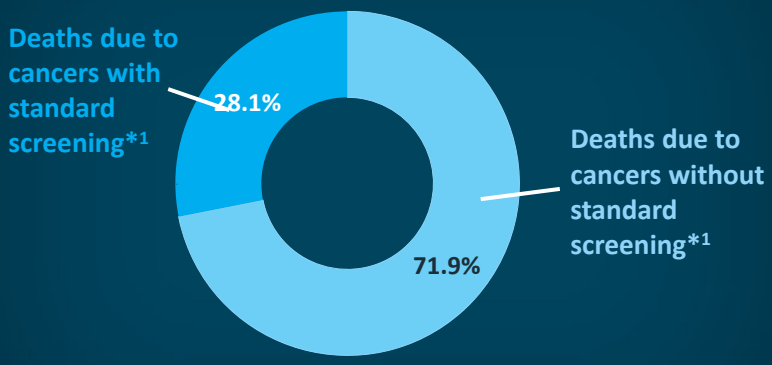
- Some screening modalities can be invasive
- Adherence can be suboptimal
- Lack of screening for certain lethal cancers beyond high-risk populations can lead to increases in metastatic disease

Importance of Cancer Screening

Screening is associated with earlier stage at diagnosis and improved outcomes

Kim J, et al. *Cancer Res.* 2011;71(24 suppl): abstract P5-14-02. Plumb AA, et al. *Eur Radiol*. 2016;26:4313-4322.

Cancers Without Screening Tests Account for 72% of All Cancer Deaths in US

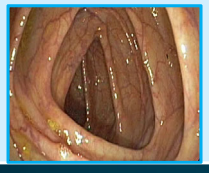


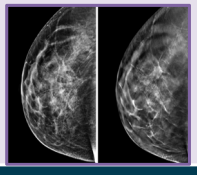
*USPSTF-recommended standard screening includes breast, cervical, colorectal, prostate, and 27% of lung cancer, based on estimated proportion of lung cancers that occur in screen-eligible individuals older than 40 years.[2]

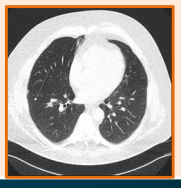
1. ACS Cancer Facts & Figures 2021. (www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf). Accessed 1/21/2021. 2. Data on file from Surveillance, Epidemiology, and End Results (SEER) 18 Regs Research Data, Nov 2017 Submission. Includes persons aged 50–79.

Adherence to Screening Recommendations Can Be Suboptimal

CRC (colonoscopy)	Breast (mammography)	Lung (low-dose CT)
• Of 151,638 subjects in an insured cohort, only 64% were adherent with current CRC screening recommendations • Avg age at screening with any test was 3 years past recommendation	• Of 159,123 women, 76–81% were adherent to USPSTF guidelines • Increases with age, with highest screened in women ages 65–69 • Adherence to mammography remains poor in women with low access to health insurance (<50%)	• VA cohort; of 1120 eligible for repeat annual LDCT, 880 underwent follow-up scan • 77.6% adherence rate from annual screening in those with a normal baseline scan







CRC = colorectal cancer; Avg = average; CT = computed tomography (scan); VA = Veterans Administration.

Cyhaniuk A, Coombes ME. *Am J Manag Care*. 2016;22:105-111. Narayan A, et al. *Breast Cancer Res Treat*. 2017;164:719-725. CHEST 2018 (www.ascopost.com/News/59355). Accessed 1/21/2021.

Why Are Patients Not Getting Screened?

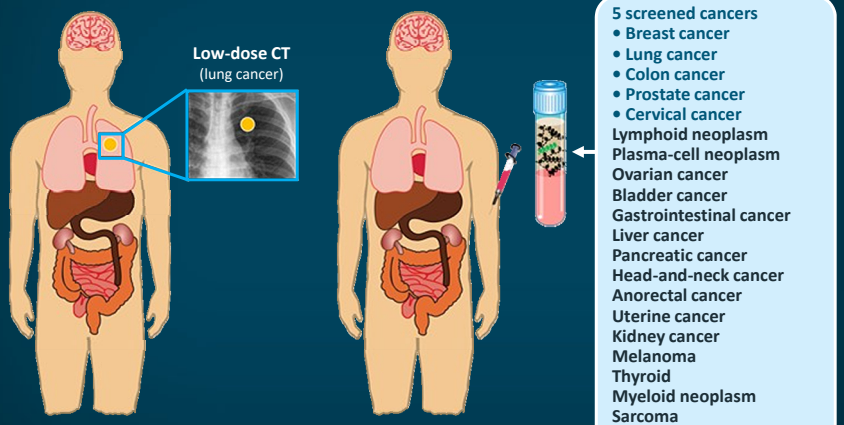
Even among cancers that are screened for, many people are not being screened due to…

- Inconvenience, missing work
- Discomfort
- Lack of awareness
- Fear of radiation exposure
- Lack of nearby radiology facility
- Oversight by medical team
- Disparities in screening for certain populations

Single vs Multi-Cancer Screening

"One test-one cancer" approach

"One test-many cancers" approach

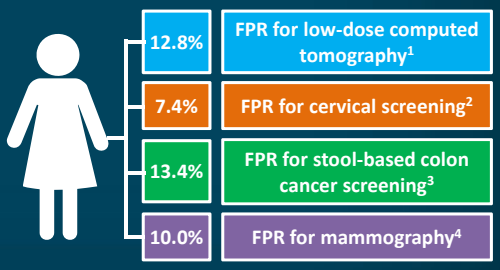


Ofman JJ, et al. *Nat Res.* 2020. (www.nature.com/articles/d42473-020-00079-y). Accessed 1/20/2021.

Cumulative False-Positive Rate From Single-Cancer Screening

- Each false positive requires follow-up tests or interventions
- Cumulative risks are not well understood at population level because current paradigms only evaluate one cancer at a time

A 60-year-old female with a history of smoking screened for 4 cancers would have a 43.6% false positive rate (FPR)[1–4]

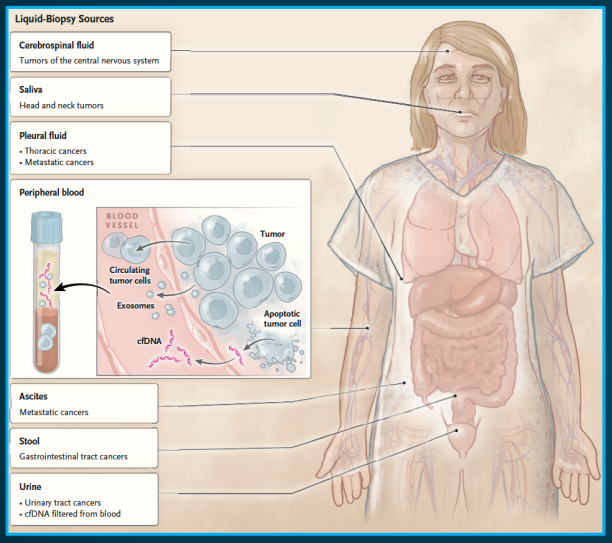


1. Pinsky PF, et al. *Ann Intern Med*. 2015;162:485-491. 2. Melnikow J, et al. *JAMA*. 2018;320:687-705. 3. US Food and Drug Administration (FDA) premarket approval (PMA) P130017 (www.accessdata.fda.gov/cdrh_docs/pdf13/P130017b.pdf). Accessed 1/21/2021. 4. Lehman CD, et al. *Radiology*. 2017;283:49-58.

Multi-Cancer Early Detection Using cfDNA/ctDNA

Video 1
cfDNA/ctDNA Overview

What Is a "Liquid Biopsy"?

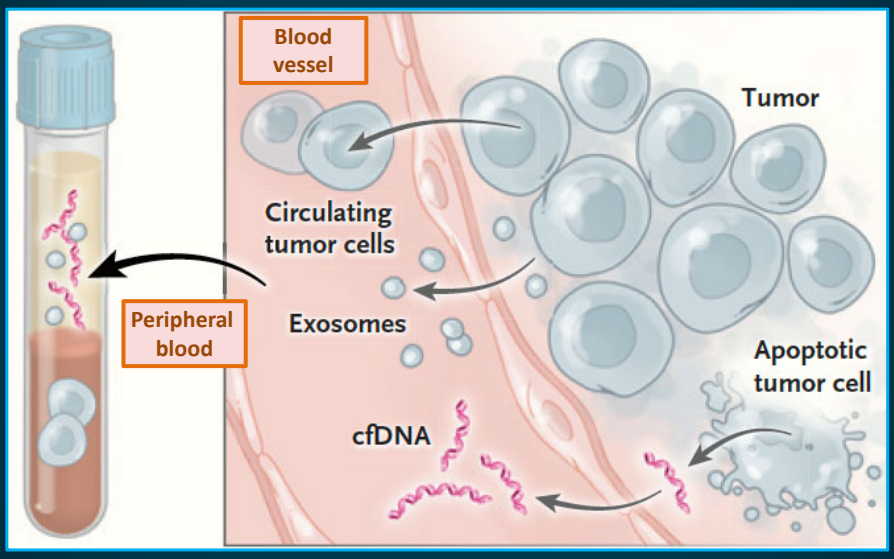


- Liquid-blood biopsy includes isolating circulating tumor cells, exosomes (membrane-bound vesicles released by tumor cells), and cell-free DNA (released by apoptotic or necrotic tumor cells)
- Other bodily fluids can also be used

DNA = deoxyribonucleic acid; cfDNA = cell-free DNA.

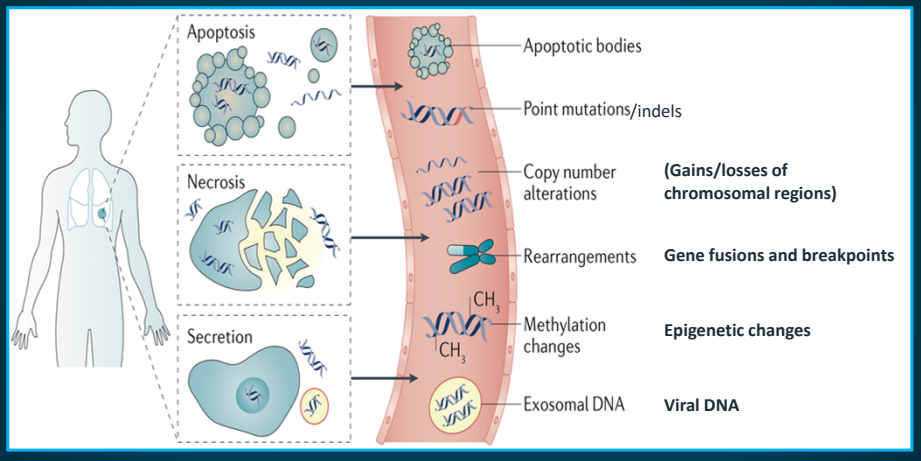
Corcoran RB, Chabner BA. *N Engl J Med.* 2018;379:1754-1765.

Different Forms of Liquid Biopsy



Adapted from Corcoran RB, Chabner BA. *N Engl J Med.* 2018;379:1754-1765.

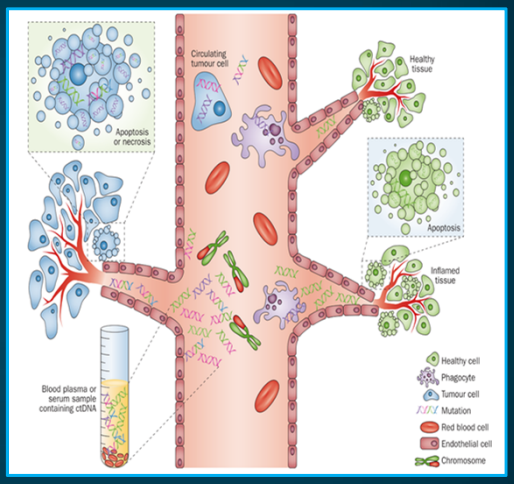
Origins and Range of Alterations in Cell-Free DNA



Adapted from Wan JCM, et al. *Nat Rev Cancer.* 2017;17:223-238.

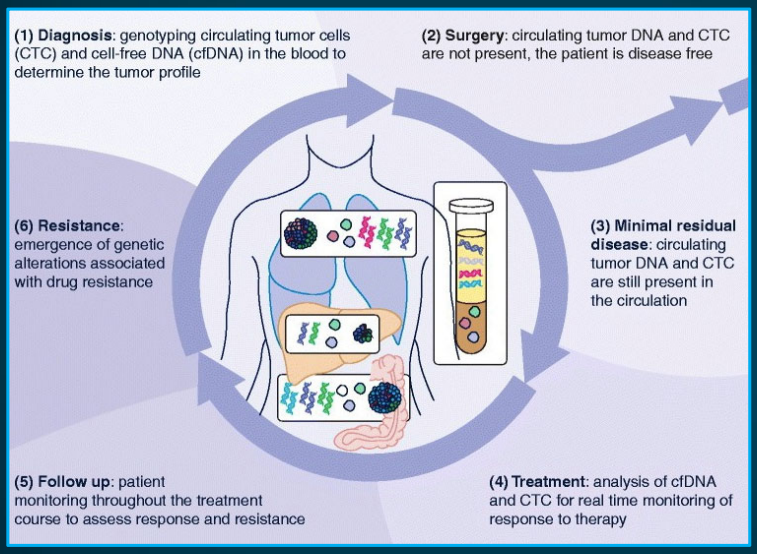
Circulating Tumor Nucleic Acids

- Origins of cfDNA
  - apoptosis
  - necrosis
  - phagocytosis
  - active secretion
- cfDNA is enclosed in vesicles
  - protects from degradation
  - prevents activation of immune system
  - half-life 0.25–2.5 hours
- cfDNA cleared from blood
  - via nuclease digestion
  - renal excretion (urine)



Crowley E, et al. *Nat Rev Clin Oncol.* 2013;10:472-484. Wan JCM, et al. *Nat Rev Cancer.* 2017;17:223-238. Santos Pessoa L, et al. *Crit Rev Oncol Hematol.* 2020;155:103109.

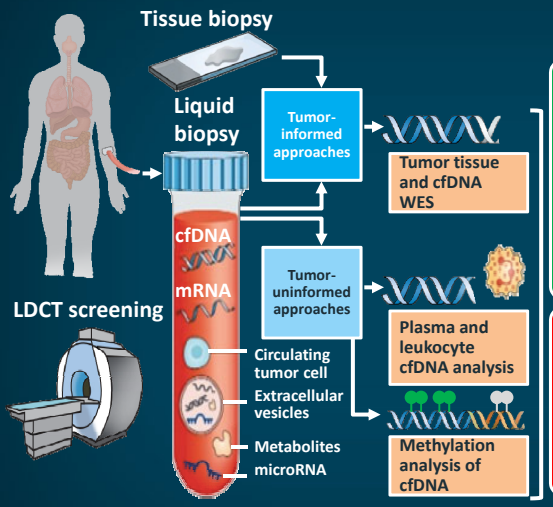
Clinical Applications of cfDNA



cfDNA can also be used for ***early detection***

Siravegna G, Bardelli A. *Genome Biol.* 2014;15:449.

Challenges



Strengths
- High sensitivity
- Minimally invasive
- Integration with other screening programs

Weaknesses
- Clonal hematopoiesis /false positives
- Costs
- Reproducibility

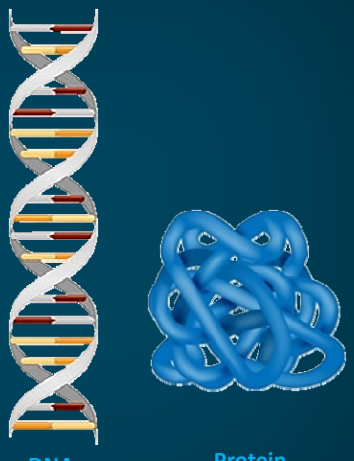
Challenges
- Low burden of disease
- Low mutation burden
- Mutations found in normal tissues
- Problem of CHIP
- High sensitivity can cause increased cost

mRNA = messenger ribonucleic acid; WES = whole-exome sequencing; CHIP = clonal hematopoiesis of indeterminate potential.

Oxnard GR, et al. *J Global Oncol.* 2019;5(suppl): abstract 44.

Science of Different Liquid Biopsy Tools

- All our cells have same DNA (except some immune cells)
  - DNA-based tests are invoking PET-CT as a reflex in clinical trials
- Using targeted NGS, 10–50% of people in their 40s and 25–75% of people aged ≥70 years have clonal hematopoiesis

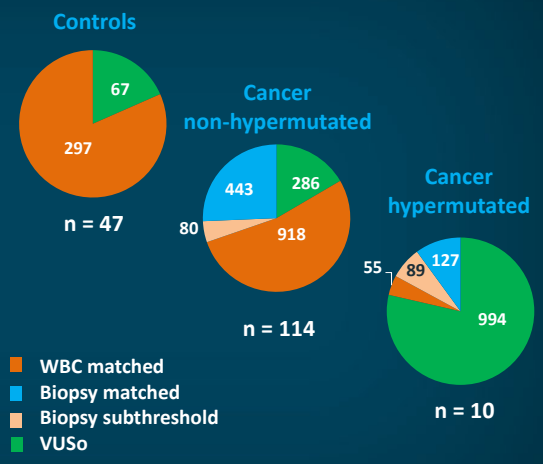


PET = positron emission tomography (scan); CT = computerized tomography (scan); NGS = next-generation sequencing.

Dor Y, Cedar H. *Lancet.* 2018;392:777-786. Camera S, et al. *Cancers[Basel].* 2020;12:C2752. Chan HT, et al. *Cancers[Basel].* 2020;12:2277.

Be Cautious of CHIP

Mutations with WBC-Matched Variants				
	Control	mBC	NSCLC	CRPC
DNMT3A	48	37	66	50
TET2	30	21	34	25
PPM1D	3	23	14	34
TP53	7	8	8	11
ASXL1	4	5	5	11
ATM	2	8	5	7
NF1	4	4	9	5
KMT2C	8	3	7	4
FAT1	2	7	4	6
PTPRT	3	3	6	4
KMT2D	4	4	3	5
CHEK2	1	1	4	9
CBL	2	5	5	2
SH2B3	3	4	4	1
ZFHX3	4	2	2	3
RAD21	4	0	1	5
GRIN2A	1	3	3	2
GNAS	3	1	2	3
TET1	1	0	5	2
ARID2	2	1	4	1
MGA	2	2	4	0
SF3B1	4	0	2	2
EP300	4	1	3	0
SETD2	4	0	2	2
PTCH1	4	1	2	1
AR	1	2	4	1
IRS1	2	3	0	2
ROS1	1	5	0	1



mBC = metastatic breast cancer; NSCLC = non-small-cell lung cancer; CRPC = castration-resistant prostate cancer; WBC = white blood cell; VUSo = variants of unknown source.

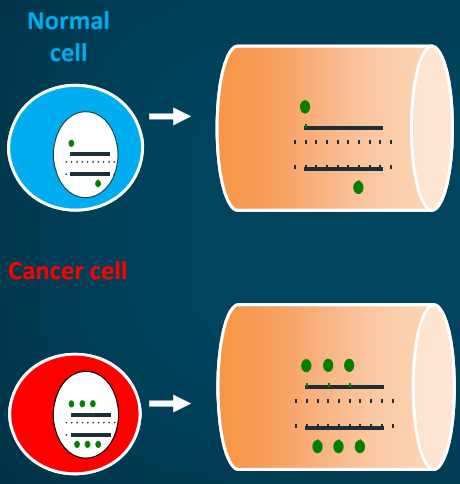
Razavi P, et al. *Nature Med*. 2019;25:1928-1937.

Use of Tissue-of-Origin Signature to Detect Cancer Using Methylation Signature

Simultaneous multi-cancer detection and tissue of origin localization using targeted bisulfite sequencing of plasma cell-free DNA

Oxnard GR, et al. *J Global Oncol*. 2019;5(suppl): abstract 44

Biology of Methylation
Integration of Genomic and Epigenomic Data



- Tumor-specific methylation patterns as potential biomarker
- Each cell type has own methylation pattern (fingerprint)
- Early event "tissue of origin"

Hao X, et al. *Proc Natl Acad Sci USA*. 2017;114:7414-7419. Mitra S, et al. *Mol Oncol*. 2020;14:933-950. Hoadley KA, et al. *Cell*. 2018;173:291-304.e6. Moss J, et al. *Nat Commun*. 2018;9:5068.

The CCGA: Overview of Substudies

Circulating Cell-Free Genome Atlas (CCGA) Study
3 prespecified CCGA sub-studies
20+ trained cancer types*

Substudy 1: Discovery
Training (n = 1785)
Validation (n = 1015)

Substudy 2: Assay Refinement
Training (n = 3133),
Validation (n = 1354)

Substudy 3: Further Assay Validation
(~5000 participants enrolled)

Whole-genome methylation
Identify key informative methylation regions

Targeted methylation
Training and validation of targeted methylation classifier

*Anus, bladder, breast, cervix, colon/rectum, esophagus, gallbladder, head and neck, kidney, liver/bile duct, lung, lymphoid leukemia, lymphoma, melanoma, myeloma, ovary, pancreas, prostate, sarcoma, stomach, thyroid, urothelial tract, uterus, and other (including brain, mesothelioma, orbit, penis, pleura, skin cancer [not basal cell carcinoma, squamous cell carcinoma, or melanoma], small intestine, testis, thymus, urethra, vagina, and vulva)

Wolpin BM, et al. ASCO 2020: abstract/poster 283 (https://grail.com/wp-content/uploads/ASCO-GI-2020-GI-Cancer-TOO-Wolpin-POS-Final-1.pdf). Accessed 1/23/2021.

Circulating Cell-Free Genome Atlas (CCGA) Study

Supporting Development of a Multi-Cancer Test

Prospective, observational, longitudinal, case-control study for the discovery, training, and validation of a multi-cancer test

15,254 participants
with and without cancer
—
142 sites

Fully enrolled

Blood samples
(from all participants)

Tissue samples
(cancer only)

Follow-up for 5 years
(vital status and cancer status)

Liu MC, et al. *Ann Oncol*. 2020;31;745-759. Oxnard GR, et al. *J Global Oncol.* 2019;5(suppl): abstract 44. Wolpin BM, et al. American Society of Clinical Oncology (ASCO) gastrointestinal cancer symposium. 2020: abstract/poster 283 (https://grail.com/wp-content/uploads/ASCO-GI-2020-GI-Cancer-TOO-Wolpin-POS-Final-1.pdf). Accessed 1/23/2021.

Multi-Cancer Detection Using Methylation Signatures: Study Design

- 6689 participants (2482 cancer [>50 cancer types], 4207 non-cancer) training and validation sets
- Bisulfite sequencing of plasma cfDNA: panel of >100,000 methylation regions
- Classifier developed for cancer detection and TOO localization

Liu MC, et al. *Ann Oncol*. 2020;31;745-759.

Multi-Cancer Detection Using Methylation Signatures: Specificity and Sensitivity

- In validation, specificity 99.3% (95% CI, 98.3–99.8%; 0.7% false-positive rate)
- Stage I–III sensitivity
  - 67.3% (95% CI, 60.7–73.3%) for 12 prespecified cancer types* that account for ~63% of US cancer deaths each year
  - 43.9% (95% CI, 39.4–48.5%) in all cancer types
- Sensitivity increased with increasing stage
  - Prespecified types: 39% (95% CI, 27–52%) in stage I; 69% (95% CI, 56–80%) in stage II; 83% (95% CI, 75–90%) in stage III; 92% (95% CI, 86–96%) in stage IV
  - All cancers: 18% (95% CI, 13–25%) in stage I; 43% (95% CI, 35–51%) in stage II; 81% (95% CI, 73–87%) in stage III; and 93% (95% CI, 87–96%) in stage IV.
  - TOO was predicted in 96% of samples with cancer-like signal; TOO localization was accurate in 93% of them.

***Anus, bladder, colon/rectum, esophagus, head and neck, liver/bile duct, lung, lymphoma, ovary, pancreas, plasma-cell neoplasm, and stomach**

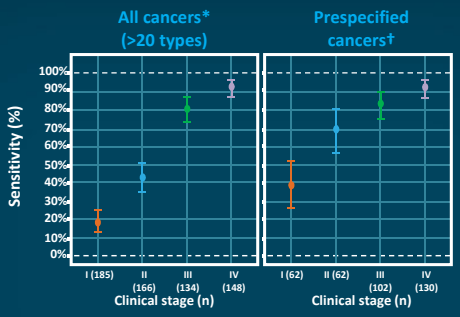
Liu MC, et al. *Ann Oncol*. 2020;31;745-759.

Multi-Cancer Detection Using Methylation Signatures: Conclusions

- cfDNA sequencing leveraging informative methylation patterns detected more than 50 cancer types across stages
- Good specificity but sensitivity not great, especially at lower stages
- Further evaluation is justified in prospective population-level studies

Liu MC, et al. *Ann Oncol*. 2020;31;745-759.

More Than 20 Cancer Types Detected at Early and Late Stages

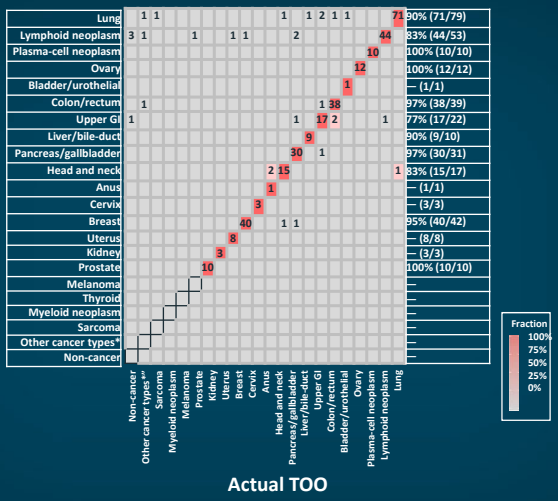


- At 99.3% specificity, sensitivity overall = 55% (53–58%); sensitivity in prespecified† cancers = 78% (75–81%)
- Cancers were detected at both early and late stages
- 96% of samples with predicted TOO; 93% of those calls were accurate

***Excludes unstaged cancers; †Includes anal, bladder, colorectal, esophageal, head and neck, liver/bile-duct, lung, lymphoma, ovary, pancreatic, plasma-cell neoplasm, and stomach cancer.**
TOO = tissue of origin.

Liu MC, et al. *Ann Oncol*. 2020;31:745-759.

20 Cancer Types: TOO Localization



***Other cancers include skin cancer (not including BSC, SCC, or melanoma), testis, seminoma, vagina, and vulva.**
GI = gastrointestinal; BSC = basal cell carcinoma; SCC = squamous cell carcinoma.

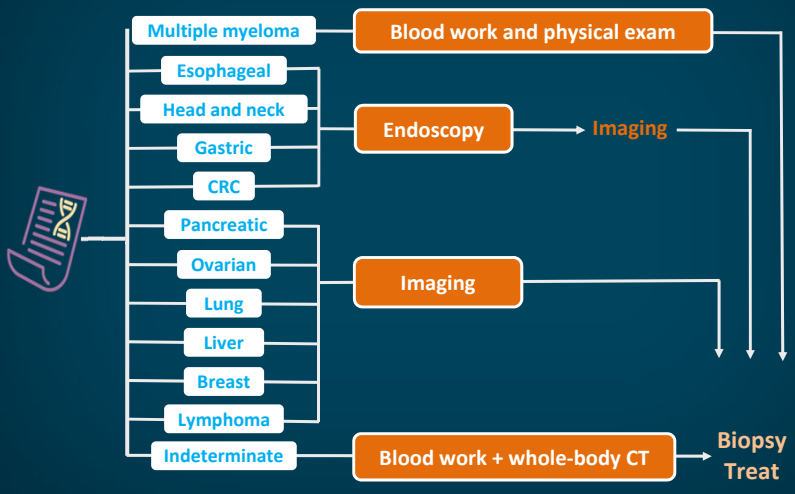
Oxnard GR, et al. *J Clin Oncol.* 2019;5(suppl): abstract 44.

Conclusions

- Multiple deadly cancer types that currently have no screening paradigm were detected across stages and simultaneously accurately localized to a TOO, using methylation signatures in plasma cfDNA
- This was achieved with trained thresholds that resulted in single, fixed, low false-positive rate (<1%) in independent validation set
- Importantly, results in independent validation set were indistinguishable from training set, demonstrating the robustness of machine-learning classifier training, with no evidence of overtraining
- This validation demonstrates feasibility of a single blood-based test that can simultaneously detect multiple cancers and supports further clinical development

Liu MC, et al. *Ann Oncol*. 2020;31;745-759. Oxnard GR, et al. *J Global Oncol*. 2019;5(suppl): abstract 44.

Workup of Positive Liquid Biopsy With TOO



TOO = tissue of origin; CRC = colorectal.

Graphic provided courtesy of Dr. Sana Raoof.

DETECT-A: Blood Test + PET-CT for CA Screening

The DETECT-A Blood Test	
Background	• Test looks at 1,933 bases on 19 genes commonly mutated in cancer, and 9 cancer-associated proteins
Patient Pop.	• 10,000 women aged 65-75 with no cancer hx, screened for asymptomatic cancers
Results	• 134 has positive result on screening; 26 found to have cancers • 10 different cancers identified (7 with no standard diagnostic test) • Conventional screening after test (e.g., mammography, colonoscopy) found 24 more cancer types • Test alone: 98.9% specificity; 19.4% PPV • Test + PET: 99.6% specificity; 28.3% PPV

Implications

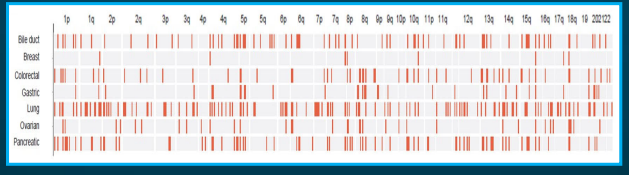
- Combining SoC with blood test augmented screening for breast, CRC, and lung sensitivity from 47% to 71%
- Sensitivity for other 7 cancer types with no screening = 31%

Lennon AM, et al. *Science*. 2020;369:eabb9601.

SoC = standard of care. PPV = positive predictive value

DELFI: Genome-Wide cfDNA Fragmentation Profiling for Early Cancer Detection

- DNA evaluation of fragments for early interception (DELFI) assay
  - Using low-pass WGS, study ratio of large fragments (151–220 bp) to small fragments (100–150 bp)
  - Ratio is quite stable in healthy controls, but variable in 236 cancer patients studied
- Regions of abnormal fragmentation vary by cancer type
  - In the figure below, orange = region where >10% of cancer samples have fragmentation profile >3SD from median of healthy controls

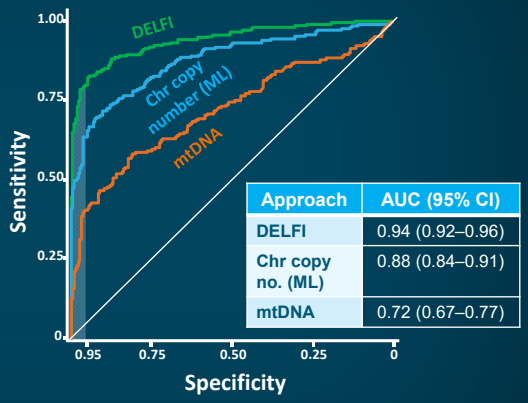


WGS = whole-genome sequencing; bp = base pair; SD = standard deviation.

Cristiano S, et al. *Nature*. 2019;570:385-389. Leal A, et al. *J Clin Oncol*. 2019;37(15 suppl): abstract 3018.

Cancer Detection Using DELFI

- DELFI comparison of 208 cancer patients with 215 healthy controls
- Cancer patients were previously untreated and 88% had stage I–III disease
- Using a machine-learning classifier, overall AUC of 0.94
- At 95% specificity, had similar sensitivity in stage I–III (79%) as stage IV (82%)



Approach	AUC (95% CI)
DELFI	0.94 (0.92–0.96)
Chr copy no. (ML)	0.88 (0.84–0.91)
mtDNA	0.72 (0.67–0.77)

AUC = area under the curve; Chr = chromosomal; ML = machine learning; mtDNA = mitochondrial DNA.

Cristiano S, et al. *Nature*. 2019;570:385-389. Leal A, et al. *J Clin Oncol*. 2019;37(15 suppl): abstract 3018.

Video 2
ctDNA Assays

Future Opportunities in Clinical Trials Design

How do we measure utility of cancer early detection?

- From a clinical-trials standpoint, late-stage treatments are more efficient because OS/mortality endpoints can be measured quickly
  - Time to reimbursement for R&D costs is shorter
  - Use of ctDNA as a biomarker of efficacy?
- In comparison, RCTs on screening healthy populations take decades to measure survival/mortality
  - PLCO studied colonoscopy for 20 years before concluding it helps
  - PSA testing was studied for 16 years before the wrong conclusion was drawn about it

OS = overall survival; R&D = research and development; ctDNA = circulating tumor DNA; RCT = randomized controlled trial; PLCO = Prostate, Lung, Colorectal, and Ovarian (cancer trial); PSA = prostate-specific antigen.

Frakt A. (www.nytimes.com/2015/12/29/upshot/why-preventing-cancer-is-not-the-priority-in-drug-development.html). Accessed 1/23/2021.

Future Opportunities in Clinical Trials Design (continued)

How do we measure utility of liquid biopsy?

- Find surrogate endpoints for OS
- Creative solutions to demonstrate value without need for 20-year studies
- Proof-of-concept studies in high-risk populations
- Regulatory feedback and reimbursement

Frakt A. (www.nytimes.com/2015/12/29/upshot/why-preventing-cancer-is-not-the-priority-in-drug-development.html). Accessed 1/23/2021.

Future Opportunities in Radiology

How do you follow up a positive test?

- If you know the tissue of origin, look there
- What if you don't see anything at TOO site? What if you find no TOO?
- Look at most common sites (lungs, breast, prostate, colon)
- CT chest/abdomen/pelvis (C/A/P)?
  - Field of view of a CT C/A/P catches about 90% of cancers by incidence and 94.5% of cancers that kill patients
- PET-CT? MRI?
- Clinical reasoning? Repeat the liquid biopsy in 3–6 months?

MRI = magnetic resonance imaging.

Future Opportunities in Radiology (continued)

Important questions following a positive test

- What are arguments for and against PET-CT as a reflex test?
- Radiation risk?
- At which PPV would you even consider a PET-CT?
- What is realistic in community centers?

PPV = positive predictive value.

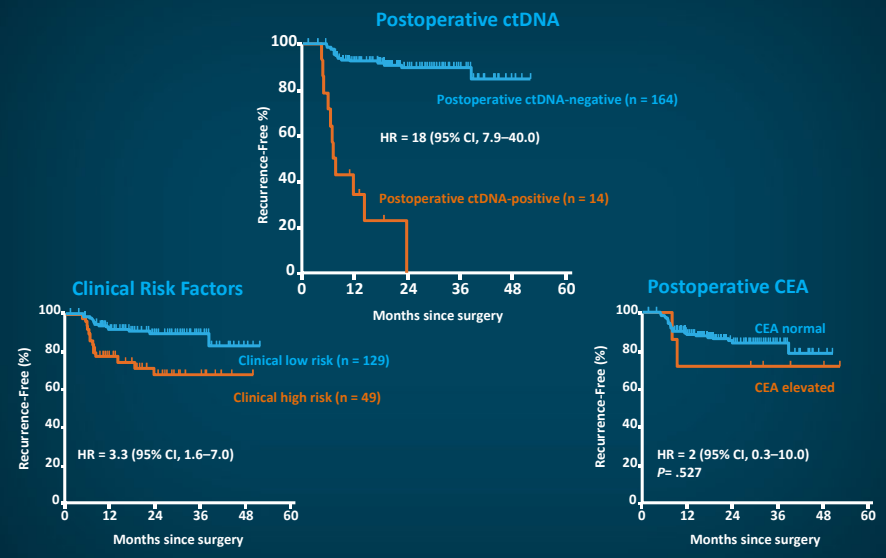
Future Opportunities in Health Policy/Economics

- How do we incentivize early detection over treatment for metastatic cancer?
- Doctors are paid 6% above drug costs in modern era, and this influences the choice to prescribe more expensive drugs and for drug-development research to dominate
- There is no reimbursement for detecting cancer early or preventing it, though cancer care represents a large burden on healthcare costs
- How can we 1) quantify and 2) incentivize early cancer detection over late-stage treatment?

Ubel P. KevinMD blog. 2012 (www.kevinmd.com/blog/2012/07/oncologists-incentive-prescribe-expensive-treatments.html). Accessed 1/23/2021.

Detection of Minimal Residual Disease

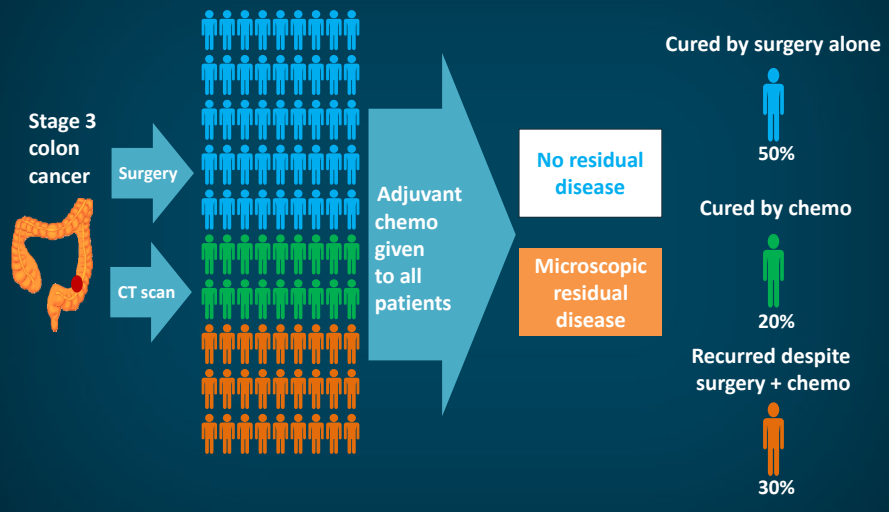
Prediction of Relapse in Stage II CRC



HR = hazard ratio; CEA = carcinoembryonic antigen.

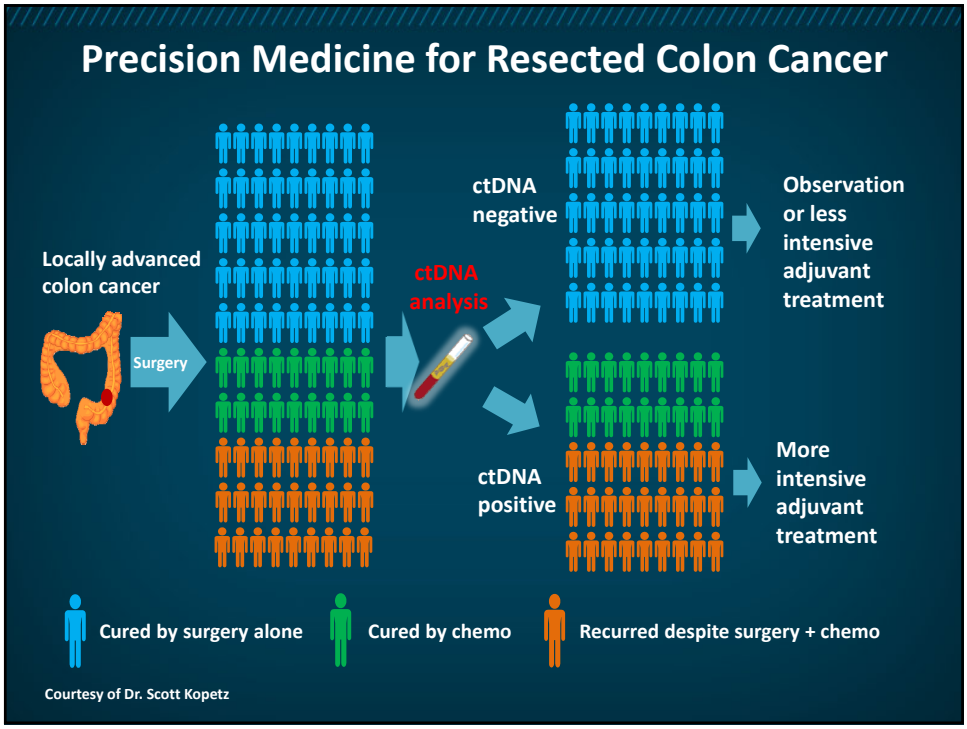
Tie J, et al. *Sci Transl Med*. 2016;8:346ra92.

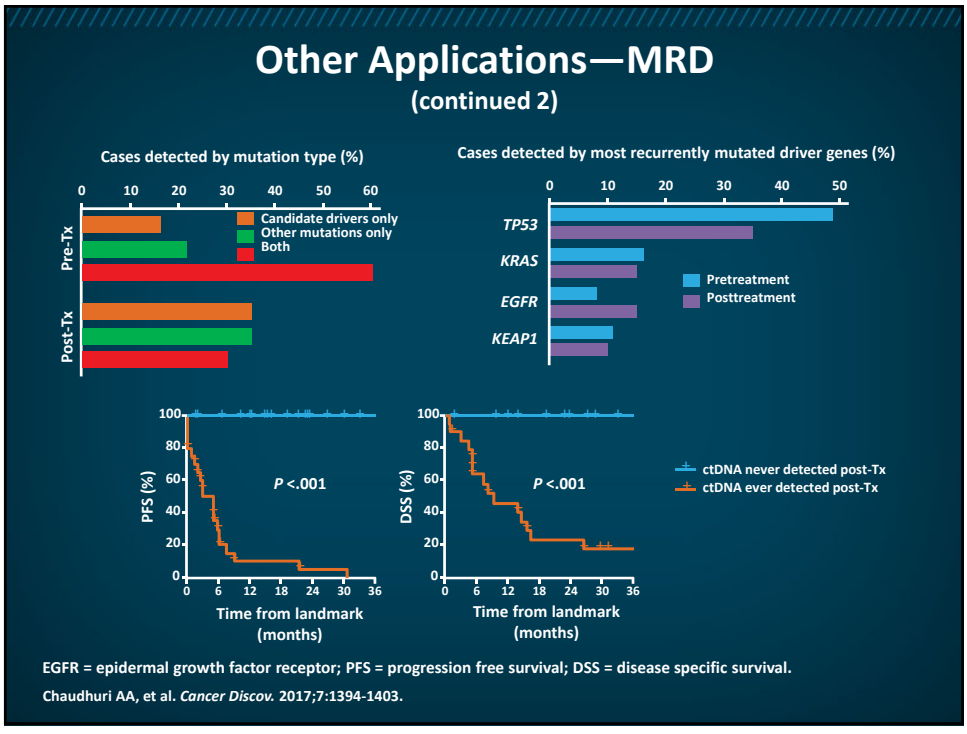
Stage 3 Colon Cancer



chemo = chemotherapy.

Courtesy of Dr. Scott Kopetz





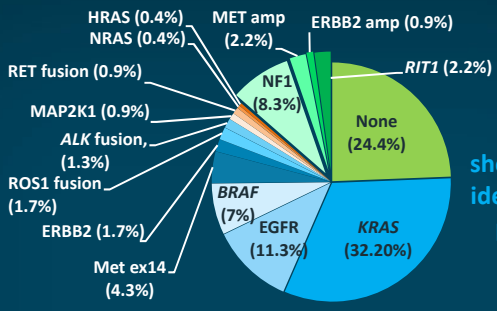
EGFR = epidermal growth factor receptor; PFS = progression free survival; DSS = disease specific survival.

Chaudhuri AA, et al. *Cancer Discov.* 2017;7:1394-1403.

Use of Liquid Biopsy at Initial Diagnosis

Targeted Therapy—How Do We Apply It In Clinic?

Lung cancer is COMPLEX



How should/do we identify these patients?

Tremendous progress has been made in personalized therapy

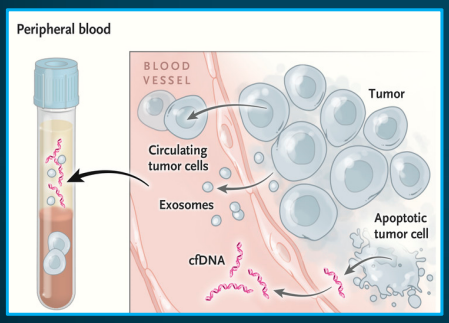
EGFR	ALK	ROS1	BRAF	MET	RET	TRK
Erlotinib	Crizotinib	Crizotinib	Dabrafenib	Crizotinib	Vandetanib	Larotrectinib
Gefitinib	Ceritinib	Entrectinib	Vemurafenib	Tepotinib	Cabozantinib	Entrectinib
Afatinib	Brigatinib		Trametinib		Selpercatinib	
Osimertinib	Alectinib				Pralsetinib	
Dacomitinib	Lorlatinib					
	Entrectinib					

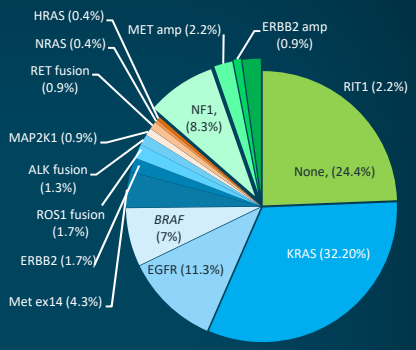
ALK = anaplastic lymphoma kinase; ROS1 = c-ros oncogene receptor tyrosine kinase; BRAF = B-raf proto-oncogene, serine/threonine kinase; MET = MET receptor tyrosine kinase; RET = rearranged-during-transfection proto-oncogene tyrosine-protein kinase receptor; TRK = tropomyosin receptor kinase.

Vargas AJ, Harris CC. *Nat Rev Cancer*. 2016;16:525-37. Prescribing information (PI) for agents listed in the table.

The "Power of Plasma"

Tumor NGS





Harness the information from plasma to deliver personalized therapy

Vargas AJ, Harris CC. *Nat Rev Cancer*. 2016;16:525-537. Corcoran RB, Chabner BA. *N Engl J Med.* 2018;379:1754-1765.

Future Directions

Early CA Detection: Research Priorities

Need/Question
1. A simple, non-invasive, painless, cost-effective, convenient test 2. How to include in routine care 3. Would increasing access for PCPs improve # of cancers detected early? 4. Addressing cultural, religious, gender, behavioral issues and disparities 5. Relevance of genetic testing

Badrick E, et al. *Lancet Public Health*. 2019;4(11):E551.

Early CA Detection: Research Priorities

Need/Question
6. Use of cancer-relevant diagnostic tools (e.g., reminders in EMR) 7. Use for cancers not currently screened (ovarian, pancreatic, etc.)? 8. Use of data from already-diagnosed patients to look for warning signs that might have been missed? 9. Coordination of information b/w healthcare sectors 10. Predictions of tumor development, reduction of unnecessary tests and overdiagnosis

Badrick E, et al. *Lancet Public Health*. 2019;4(11):E551.

Future Directions

- Several ongoing studies[1-4]
  - E.g. PREEMPT-CRC: 91% sensitivity; 94% specificity for CRC[4]
- CancerSEEK[5]
- Cost analyses for population health level efforts
- Demonstration of prospective survival benefit
- Implications in COVID-19 era (screening rates declined)[6]

1. STRIVE study: https://clinicaltrials.gov/ct2/show/NCT03085888.
2. SUMMIT study: https://www.clinicaltrials.gov/ct2/show/NCT03934866.
3. PATHFINDER study: https://clinicaltrials.gov/ct2/show/NCT04241796.
4. PREEMPT-CRC: https://clinicaltrials.gov/ct2/show/NCT04369053.
5. Cohen J, et al. *Science*. 2018;359(6378):926-30.
6. Bakouny Z, et al. *JAMA Oncology*. Jan 2021. doi:10.1001/jamaoncol.2020.7600.

BloodPAC

- ***Who:*** Consortium managed by the Center for Computational Science Research, Inc., an Illinois based non-profit
- ***Goal:*** accelerate the development, validation, and clinical use of liquid biopsy assays
- ***Mission:*** collaboration between stakeholders in industry, academia, and regulatory agencies to share information
- ***Collaborators:*** FDA, American Cancer Society, cancer treatment centers, drug/device manufacturers, biotech, many others

Develop a framework to bring liquid biopsy into routine clinical practice

BloodPAC. Available at: https://www.bloodpac.org/

Early Detection: Enormous Public Health Impact

- Today: <20% of cancers are detected by screening[1]
- In 5 years: predicted 75% detected by screening

Modeled Public Health Effects of Multi-cancer Early Detection[2]

Early testing could *intercept 485 cancers/year/100,000 persons*

⇩

This would *reduce late-stage (III+IV) incidence by 78%* in those intercepted

⇩

This could *reduce 5-year cancer mortality by 39%* in those intercepted

⇩

This would be *absolute reduction of 104 deaths/100,000*

⇩

This is 26% of all cancer deaths!

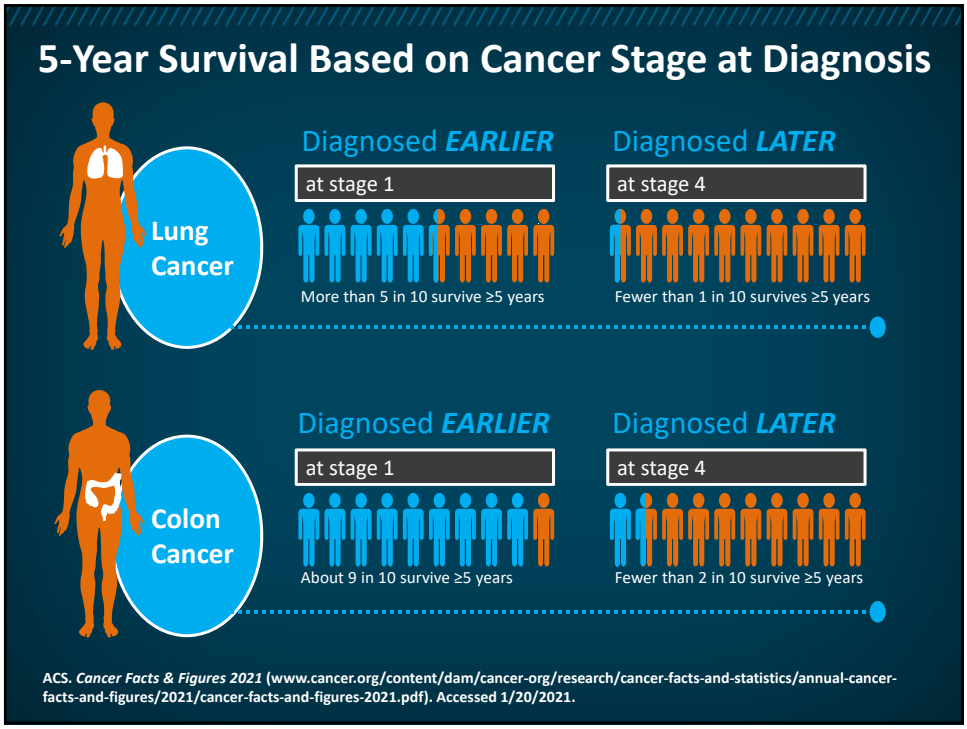
1. Vogelstein B. https://www.usatoday.com/story/news/health/2020/09/03/cancer-fda-approves-liquid-biopsy-tests-can-improve-treatment/5644829002/. 2. Hubell E, et al. *Cancer Epidemiol Biomarkers Prev*. Dec 2020. DOI: 10.1158/1055-9965.EPI-20-1134

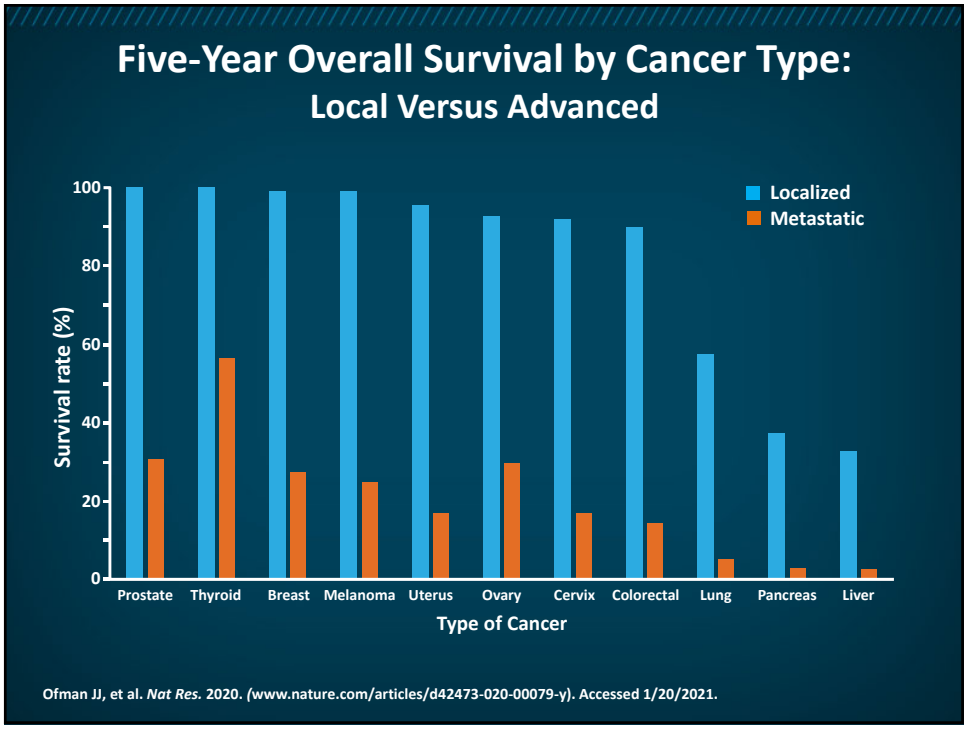
Conclusions

- Early detection is key in cancer since outcomes and quality of life vary greatly, depending on the stage of disease at the time of diagnosis
- Evidence-based modalities for cancer screening remain limited, with low adherence
- Growing information on the use of cfDNA and ctDNA for multi-cancer screening has emerged in the last decade
- These tests can detect and interpret extremely faint signals to isolate the type and origin of cancer, with the potential for routine application in primary care

Thank You!

Backup Slides


5-Year Survival Based on Cancer Stage at Diagnosis
Lung Cancer
Diagnosed EARLIER
at stage 1
More than 5 in 10 survive ≥5 years
Diagnosed LATER
at stage 4
Fewer than 1 in 10 survives ≥5 years
Colon Cancer
Diagnosed EARLIER
at stage 1
About 9 in 10 survive ≥5 years
Diagnosed LATER
at stage 4
Fewer than 2 in 10 survive ≥5 years
ACS. Cancer Facts & Figures 2021 (www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf). Accessed 1/20/2021.


Five-Year Overall Survival by Cancer Type:
Local Versus Advanced
Localized
Metastatic
Survival rate (%)
100
80
60
40
20
0
Prostate
Thyroid
Breast
Melanoma
Uterus
Ovary
Cervix
Colorectal
Lung
Pancreas
Liver
Type of Cancer
Ofman JJ, et al. Nat Res. 2020. (www.nature.com/articles/d42473-020-00079-y). Accessed 1/20/2021.

Clinical Trials

How close are liquid biopsies to clinic?

Examples of studies from GRAIL, Inc. include:

Fully enrolled

CCGA[1]	STRIVE[2]	PATHFINDER[3]	SUMMIT[4]
NCT02889978	NCT03085888	NCT04241796	NCT03934866
15,254 participants ♂♀	99,481 participants ♀	~6200 participants ♂♀	~25,000 participants ♂♀
Demonstrate feasibility of detecting cancer and predicting tissue of origin with minimal false positives	Confirm performance of cell-free nucleic acids for early detection in a population with no known active cancer diagnosis	Evaluate implementation of test in clinical practice	Additional performance in a population with no known active cancer diagnosis and clinical utility in a high-risk population

CCGA = Circulating Cell-Free Genome Atlas (study).

1. NCT02889978 (CCGA) (https://clinicaltrials.gov/ct2/show/NCT02889978?term=NCT02889978&draw=2&rank=1). 2. NCT03085888 (STRIVE) (https://clinicaltrials.gov/ct2/show/NCT03085888?term=NCT03085888&draw=2&rank=1). 3. NCT04241796 (PATHFINDER) (https://clinicaltrials.gov/ct2/show/NCT04241796?term=NCT04241796&draw=2&rank=1). 4. NCT03934866 (SUMMIT) (https://clinicaltrials.gov/ct2/show/NCT03934866?term=NCT03934866&draw=2&rank=1). All URLs accessed 1/23/2021.

CCGA Substudy 2: 447 GI Cancers

Subset of 2185 cancer participants across >20 cancer types in CCGA Substudy 2

Liver / Bile Duct

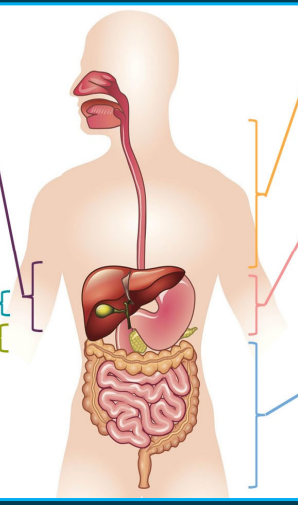
Stage	I-III	IV	All
Training	19	10	29
Validation	7	4	11
All	26	14	40

Gallbladder

Stage	I-III	IV	All
Training	7	4	11
Validation	3	0	3
All	10	4	14

Pancreas

Stage	I-III	IV	All
Training	42	42	84
Validation	22	17	39
All	64	59	123



Esophagus

Stage	I-III	IV	All
Training	31	19	50
Validation	13	8	21
All	44	27	71

Stomach

Stage	I-III	IV	All
Training	9	8	17
Validation	5	3	8
All	14	11	25

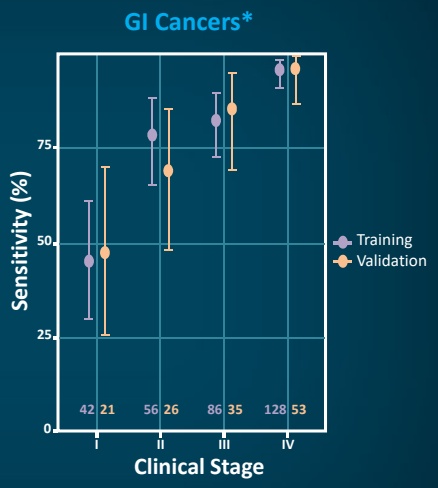
Colon / Rectum

Stage	I-III	IV	All
Training	76	45	121
Validation	32	21	53
All	108	66	174

Wolpin BM, et al. ASCO 2020: abstract/poster 283 (https://grail.com/wp-content/uploads/ASCO-GI-2020-GI-Cancer-TOO-Wolpin-POS-Final-1.pdf). Accessed 1/23/2021.

Sensitivity for GI Cancer Detection at >99% Specificity

- Classifier achieved specificity of **99.8%** in cross-validated training set and **99.3%** in independent validation set
- False positives were **3/1521 (0.2%)** in training and **4/610 (0.7%)** in validation
- Training and validation sets had similar sensitivity
- Stage I–III sensitivity was **73%** (95% CI, 66–79%; training) and **71%** (95% CI, 60–80%, validation)
- Stage I–IV sensitivity was **82%** (95% CI, 78–86%, training) and **81%** (95% CI, 73–87%, validation)



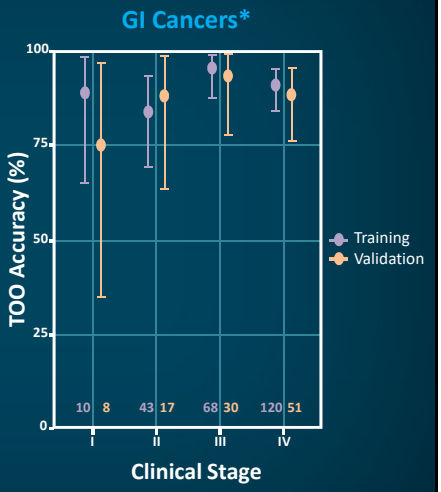
*Cancers of esophagus, stomach, liver/bile duct, pancreas, gallbladder, and colon/rectum.

CI = confidence interval.

Wolpin BM, et al. ASCO 2020: abstract/poster 283 (https://grail.com/wp-content/uploads/ASCO-GI-2020-GI-Cancer-TOO-Wolpin-POS-Final-1.pdf). Accessed 1/23/2021.

Accuracy of TOO Localization for GI Cancers

- 20+ trained cancer TOO classes enable localization to single tissue site
- **97%** of detected GI cancers were assigned a TOO in both training and validation sets
- Training and validation sets had consistent TOO accuracy across all stages
- Overall, the **predicted TOO accuracy** for patients with GI cancers was **91%** (95% CI, 86–94%) in the training and **89%** (95% CI, 81–94%) in the validation set



*Cancers of esophagus, stomach, liver/bile duct, pancreas, gallbladder, and colon/rectum.

Wolpin BM, et al. ASCO 2020: abstract/poster 283 (https://grail.com/wp-content/uploads/ASCO-GI-2020-GI-Cancer-TOO-Wolpin-POS-Final-1.pdf). Accessed 1/23/2021.

Conclusions

- Methylation analysis of plasma cfDNA simultaneously detected multiple GI cancers at high sensitivity with prespecified high specificity
  - >99% specificity maintained in independent validation set
- 97% of detected GI cancers were assigned a TOO in training and validation sets
- Highly accurate TOO localization achieved in GI cancers
- This test may be a practical method for detecting and localizing GI and other cancers, thereby directing downstream diagnostic evaluation

Wolpin BM, et al. ASCO 2020: abstract/poster 283 (https://grail.com/wp-content/uploads/ASCO-GI-2020-GI-Cancer-TOO-Wolpin-POS-Final-1.pdf). Accessed 1/23/2021.

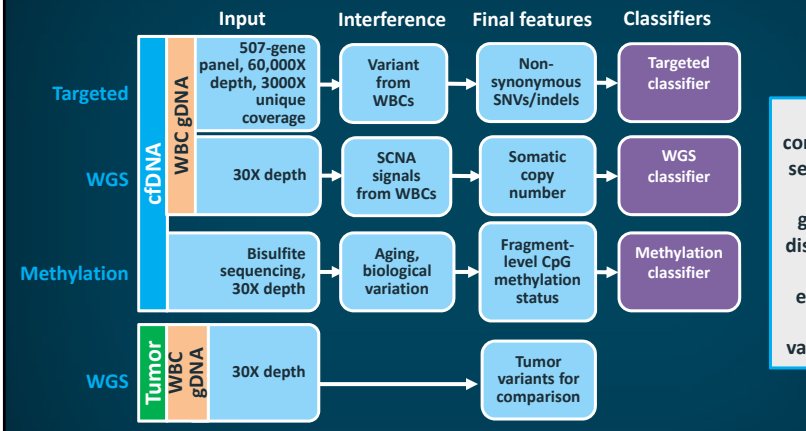
Using GWAS to Detect Early Stage NSCLC— Subset Data from the CCGA Study

Genome-wide sequencing for early stage lung cancer detection from plasma cell-free DNA: the Circulating Cancer Genome Atlas Study

Oxnard GR, et al. *J Clin Oncol.* 2018;36(18 suppl): abstract LBA8501

cfDNA GWAS for Detection of Lung Cancer

Prototype sequencing assays used to comprehensively characterize cancer-specific cfDNA signals



Using comprehensive sequencing of cfDNA generated 3 distinct signals that were evaluated in test and validation sets

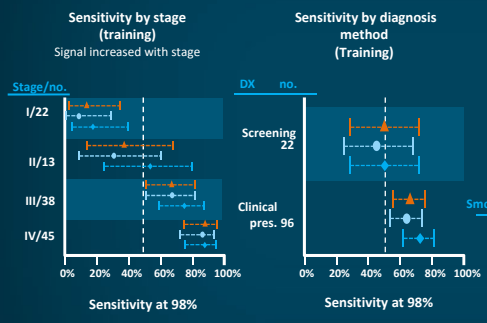
All major somatic and epigenetic cfDNA features were characterized

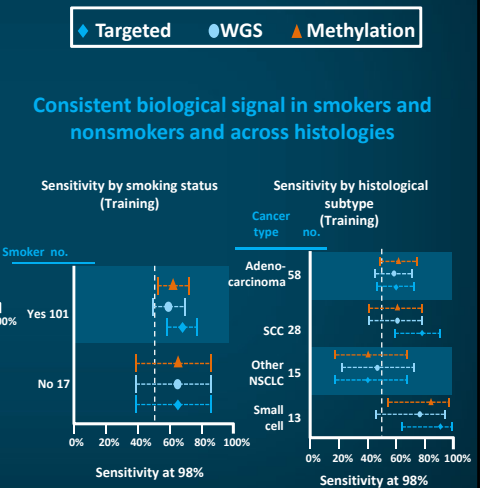
GWAS = genome-wide association study; SCNA = somatic copy number alteration; SNV = single nucleotide variant; CpG = dinucleotide in DNA with cytosine preceding guanine.

Oxnard GR, et al. *J Clin Oncol.* 2018;36(18 suppl): abstract LBA8501.

Assays Performed Within Lung Cancer Stages and by Diagnosis Method

Assays performed consistently within lung cancer stages and by diagnosis method





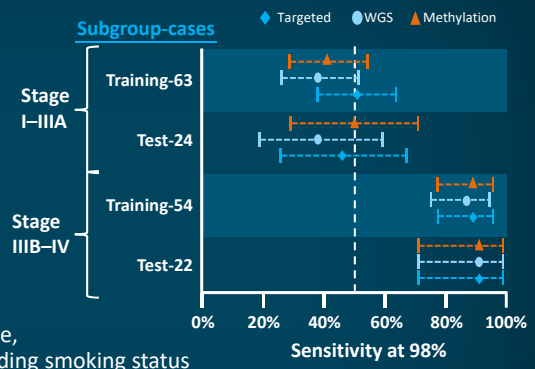
Dx = diagnosis.

Oxnard GR, et al. *J Clin Oncol.* 2018;36(18 suppl): abstract LBA8501.

Consistent Biological Signal Across Lung Cancer Stages in Training and Test Sets

Methylation score*
(Sensitivity [95% CI])

- Early stage lung cancer (stage I–IIIA)
  - Training: 41% (29–54%)
  - Test: 50% (29–71%)
- Advanced lung cancer (stage IIIB–IV)
  - Training: 89% (77–96%)
  - Test: 91% (71–99%)



- Assays are consistent across stage, histology, and populations, including smoking status
- Results replicate in an independent test set
- WBC-derived mutations are source of false positives and must be accounted for
- These data support the concept of using cfDNA as a screening modality
- Further study ongoing in CCGA and in the STRIVE clinical trial

***Results were comparable across assays.**

Oxnard GR, et al. *J Clin Oncol.* 2018;36(18 suppl): abstract LBA8501.

Pan Cancer Using cfDNA and Machine Learning

Genome-wide cell-free DNA fragmentation profiling for early cancer detection

Leal A, et al. *J Clin Oncol.* 2019;37(15 suppl): abstract 3018.

Circulating Cell-Free Genome Atlas (CCGA) Study

Prospective, longitudinal, case-control study for development of a multi-cancer test

15,254 participants
with and without cancer
—
142 sites

Fully enrolled

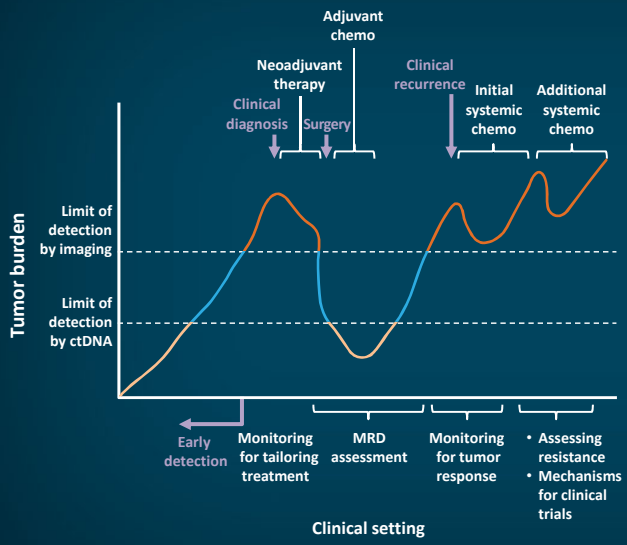
Blood samples
(from all participants)

Tissue samples
(cancer only)

Follow-up for 5 years
(vital status and cancer status)

Wolpin BM, et al. American Society of Clinical Oncology (ASCO) gastrointestinal cancer symposium. 2020: abstract/poster 283 (https://grail.com/wp-content/uploads/ASCO-GI-2020-GI-Cancer-TOO-Wolpin-POS-Final-1.pdf). Accessed 1/23/2021.

Minimal Residual Disease (MRD): Key Points

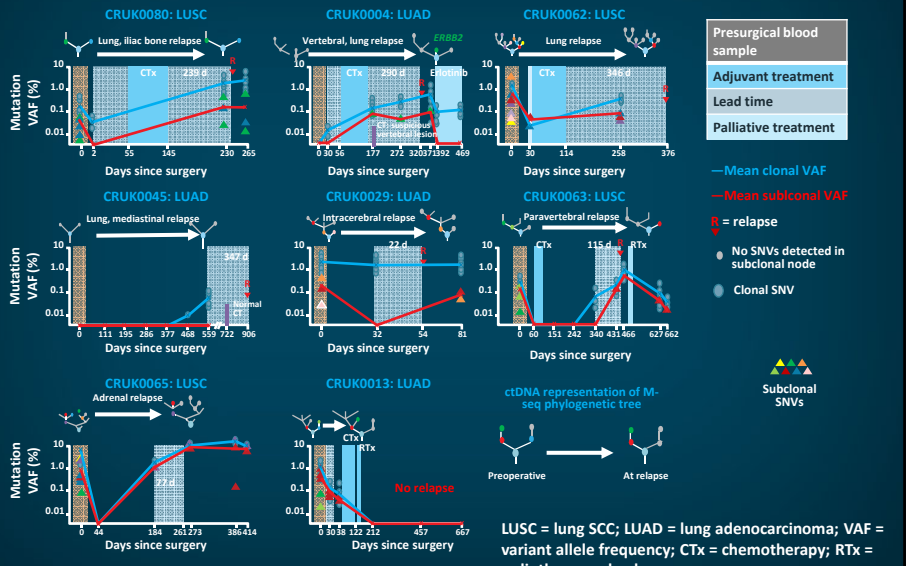


- MRD applications high positive predictive value (low false positive) for recurrent disease in patients with ctDNA detected in "adjuvant" setting
- Defines molecular persistence of disease
- Stage I–III patients with ctDNA+ after definitive interventions should be considered as stage IV MRD

Dasari A, et al. *Nat Rev Clin Oncology*. 2020;17:757-770.

Other Applications—MRD

Prediction of relapse after surgery for early-stage NSCLC by plasma ctDNA



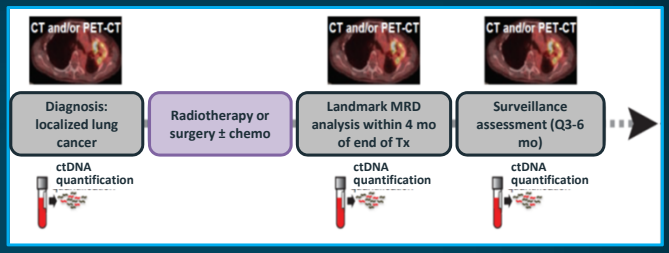
LUSC = lung SCC; LUAD = lung adenocarcinoma; VAF = variant allele frequency; CTx = chemotherapy; RTx = radiotherapy; d = days.

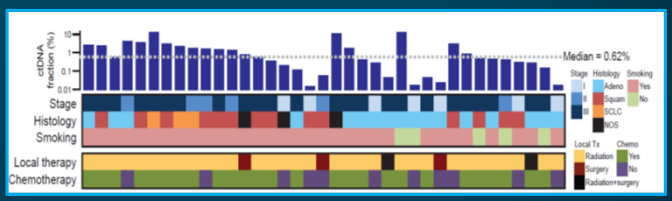
Abbosh C, et al. *Nature*. 2017;545:446-451.

Other Applications–MRD

(continued 1)

- Prospective plasma collection for ctDNA MRD and surveillance analysis
- 40 patients with localized NSCLC
  - Stage I = 18%; Stage II = 18%; Stage III = 64%
- Approach
  - CAPP-seq to identify mutations in tumor biopsy or pretreatment plasma
  - Track mutations in posttreatment plasma





CAPP-Seq = cancer personalized profiling by deep sequencing; Tx = treatment; Q = every; adeno = adenocarcinoma; Squam = squamous-cell carcinoma; NOS = not otherwise specified.

Chaudhuri AA, et al. *Cancer Discov.* 2017;7:1394-1403.

The Push to Detect Cancer Earlier:
Cell-Free DNA (cfDNA) Blood Tests in Primary Care

Siegel RL, Miller KD, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin.* 2021;71:7-33.	https://acsjournals.onlinelibrary.wiley.com/doi/10.3322/caac.21654
Smith RA, Andrews KS, Brooks D, et al. Cancer screening in the United States, 2019: A review of current American Cancer Society guidelines and current issues in cancer screening. *CA Cancer J Clin.* 2019;69(3):184-210.	https://pubmed.ncbi.nlm.nih.gov/30875085/
McPhail S, Johnson S, Greenberg D, et al. Stage at diagnosis and early mortality from cancer in England. *Br J Cancer.* 2015;112 Suppl 1:S108-115.	https://pubmed.ncbi.nlm.nih.gov/25734389/
Cho H, Mariotto AB, Schwartz LM, et al. When do changes in cancer survival mean progress? The insight from population incidence and mortality. *J Natl Cancer Inst Monogr.* 2014;2014(49):187-197.	https://pubmed.ncbi.nlm.nih.gov/25417232/
Centers for Disease Control and Prevention. Screening Tests. 2020.	https://www.cdc.gov/cancer/dcpc/prevention/screening.htm
Aravanis AM, Lee M, Klausner RD. Next-generation sequencing of circulating tumor DNA for early cancer detection. *Cell.* 2017;168(4):571-574.	https://pubmed.ncbi.nlm.nih.gov/28187279/
Salvi S, Gurioli G, De Giorgi U, et al. Cell-free DNA as a diagnostic marker for cancer: current insights. *Onco Targets Ther.* 2016;9:6549-6559.	https://doi.org/10.2147/OTT.S100901
Ulrich BC, Paweletz CP. Cell-free DNA in oncology: gearing up for clinic. *Ann Lab Med.* 2018;38(1):1-8.	https://pubmed.ncbi.nlm.nih.gov/29071812/
US Preventive Services Task Force. Lung Cancer: Screening.	https://www.uspreventiveservicestaskforce.org/Page/Document/UpdateSummaryFinal/lung-cancer-screening
US Preventive Services Task Force. Colorectal Cancer: Screening.	https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/colorectal-cancer-screening
World Health Organization. Guide to Cancer Early Diagnosis. 2017.	https://www.who.int/cancer/publications/cancer_early_diagnosis/en/
The National Academy of Sciences. Fulfilling the Potential of Cancer Prevention and Early Detection. 2003.	https://www.nap.edu/catalog/10263/fulfilling-the-potential-of-cancer-prevention-and-early-detection
Ahlquist DA. Universal cancer screening: revolutionary, rational, and realizable. *NPJ Precis Oncol.* 2018;2:23.	https://pubmed.ncbi.nlm.nih.gov/30393772/
ASCO. New Technologies Offer the	https://www.asco.org/about-asco/press-

Possibility of Identifying Cancer from a Single Blood Draw. 2019.	center/news-releases/new-technologies-offer-possibility-identifying-cancer-single
Ranucci R. Cell-Free DNA: Applications in Different Diseases. *Methods Mol Biol*. 2019;1909:3-23.	https://pubmed.ncbi.nlm.nih.gov/30580419/
Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med.* 2014;371(26):2477-2487.	https://pubmed.ncbi.nlm.nih.gov/25426838/
Razavi P, Li BT, Brown DN, et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. *Nat Med.* 2019;25(12):1928-1937.	https://pubmed.ncbi.nlm.nih.gov/31768066/
Stroun M, Maurice P, Vasioukhin V, et al. The origin and mechanism of circulating DNA. *Ann N Y Acad Sci.* 2000;906:161-168.	https://pubmed.ncbi.nlm.nih.gov/10818614/
Christensen KD, Vassy JL, Jamal L, et al. Are physicians prepared for whole genome sequencing? A qualitative analysis. *Clin Genet.* 2016;89(2):228-234.	https://pubmed.ncbi.nlm.nih.gov/26080898/
Arora NS, Davis JK, Kirby C, et al. Communication challenges for nongeneticist physicians relaying clinical genomic results. *Per Med.* 2016;14(5):423-431.	https://pubmed.ncbi.nlm.nih.gov/29181085/
Haspel RL, Saffitz JE. Genomic oncology education: An urgent need, a new approach. *Cancer J.* 2014;20(1):91-95.	https://pubmed.ncbi.nlm.nih.gov/24445771/
Lawler M, Alsina D, Adams RA, et al. Critical research gaps and recommendations to inform research prioritization for more effective prevention and improved outcomes in colorectal cancer. *Gut.* 2018;67(1):179-193.	https://pubmed.ncbi.nlm.nih.gov/29233930/
ClinicalTrials.gov. The Circulating Cell-free Genome Atlas Study (CCGA).	https://clinicaltrials.gov/ct2/show/NCT02889978
Aravanis AM, Oxnard GR, Maddala T, et al. Development of plasma cell-free DNA (cfDNA) assays for early cancer detection: first insights from the Circulating Cell-Free Genome Atlas Study (CCGA). *AACR*. 2018; 78(13):Supplement LB-343.. Accessed October 12, 2020.	https://pubmed.ncbi.nlm.nih.gov/28187279/
Oxnard GR, Klein EA, Seiden M, et al. Simultaneous multi-cancer detection and tissue of origin (TOO) localization using	https://ascopubs.org/doi/abs/10.1200/JGO.2019.5.suppl.44

targeted bisulfite sequencing of plasma cell-free DNA (cfDNA). *J Glob Oncol*. 2019; 5 (suppl):44-44.	
Oxnard GR, Klein EA, Seiden MV, et al. Simultaneous multi-cancer detection and tissue of origin (TOO) localization using targeted bisulfite sequencing of plasma cell-free DNA (cfDNA). *Ann Oncol*. 2019; 30 (suppl_5): v851-v934.	https://oncologypro.esmo.org/meeting-resources/esmo-2019-congress/Simultaneous-multi-cancer-detection-and-tissue-of-origin-TOO-localization-using-targeted-bisulfite-sequencing-of-plasma-cell-free-DNA-cfDNA
Melton C, Freese P, Bagaria S, et al. Optimized early cancer detection from whole genome sequencing of cell-free DNA. Presented at the 2019 Annual Meeting of The American Society of Human Genetics, Houston, TX.	https://www.ashg.org/wp-content/uploads/2019/10/ASHG-2019-poster-abstracts.pdf
Wolpin BM, Richards DA, Cohn AL, et al. Performance of a blood-based test for the detection of multiple cancer types. *J Clin Oncol*. 2020; 38(4_suppl):283-283.	https://ascopubs.org/doi/abs/10.1200/JCO.2020.38.4_suppl.283
ClinicalTrials.gov. The STRIVE Study: Development of a Blood Test for Early Detection of Multiple Cancer Types.	https://clinicaltrials.gov/ct2/history/NCT03085888
ClinicalTrials.gov. The SUMMIT Study: A Cancer Screening Study (SUMMIT).	https://www.clinicaltrials.gov/ct2/show/NCT03934866
ClinicalTrials.gov. Evaluation of the ctDNA LUNAR Test in an Average Patient Screening Episode (ECLIPSE).	https://clinicaltrials.gov/ct2/show/NCT04136002
ClinicalTrials.gov. Detecting Cancers Earlier Through Elective Plasma-based CancerSEEK Testing (ASCEND).	https://clinicaltrials.gov/ct2/show/NCT04213326
ClinicalTrials.gov. Assessment of the Implementation of an Investigational Multi-Cancer Early Detection Test Into Clinical Practice.	https://clinicaltrials.gov/ct2/show/NCT04241796