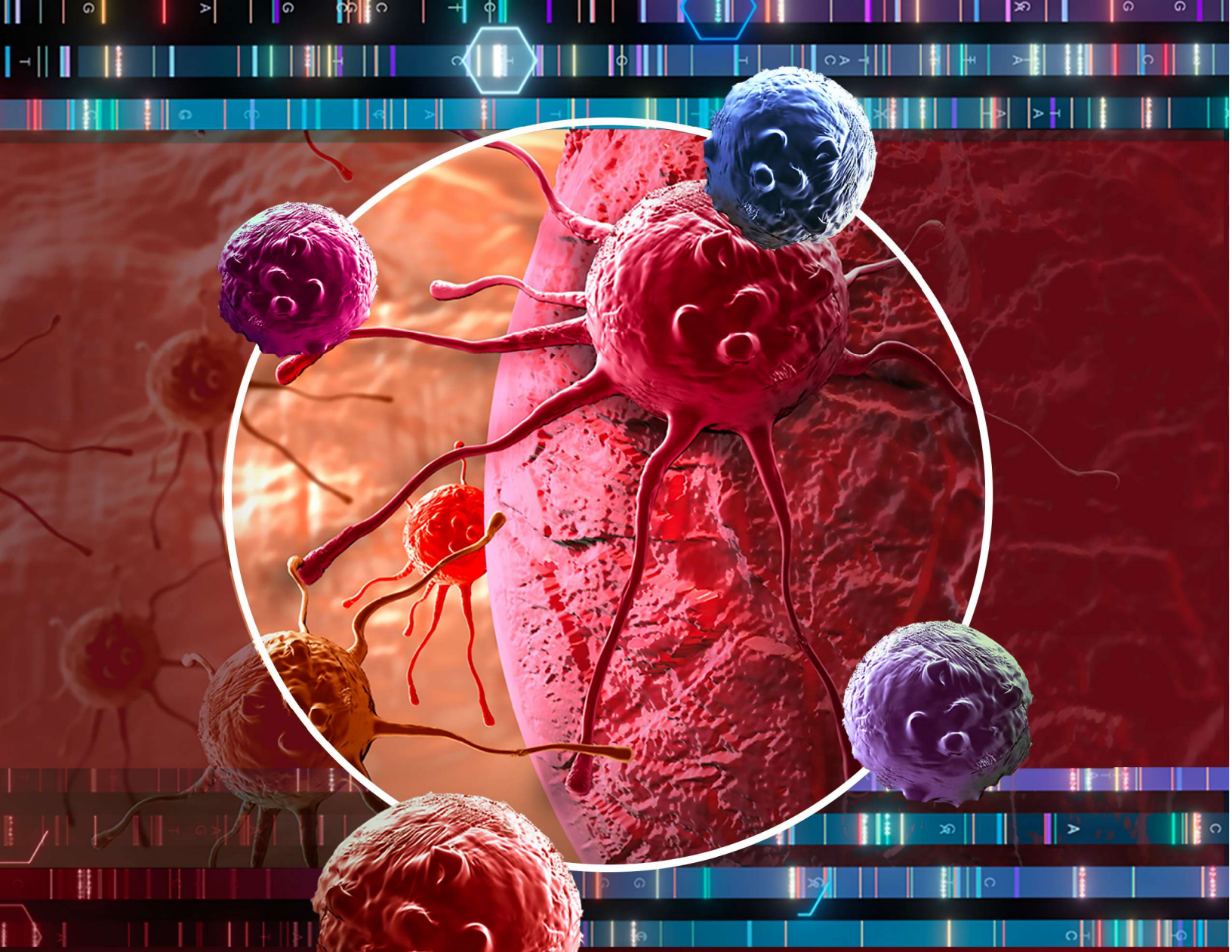

EARLIER CANCER DETECTION:

The Growing Role of Cell-Free DNA (*cf*DNA) Blood Tests in Primary Care

Wednesday, September 29, 2021
6:15 PM – 7:30 PM (ET) / 5:15 PM – 6:30 PM (CT)

FACULTY

Aparna Parikh, MD, MPH
Assistant Professor of Medicine
Harvard Medical School
Attending Oncologist
Hematology and Oncology
Massachusetts General Hospital
Boston, MA

Ryan Corcoran, MD, PhD
Associate Professor of Medicine
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Massachusetts General Hospital
Boston, MA

This event is not a part of the official AAFP FMX.

This activity is provided by Med Learning Group.
This activity is co-provided by Ultimate Medical Academy/Complete Conference Management (CCM).
This activity is supported by an educational grant from Grail, Inc.

Earlier Cancer Detection:
The Growing Role of Cell-Free DNA (cfDNA) Blood Tests in Primary Care

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PROGRAM OVERVIEW
This live virtual symposium is focused on the team involved in the care of the people who undergo screening for cancer.

TARGET AUDIENCE
This 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:

- Evaluate the science behind cfDNA testing along with its role in early multi-cancer detection
- Describe the potential benefits and limitations of using routine cfDNA screening to identify a variety of cancer types
- Plan strategies to integrate cfDNA blood tests and early multicancer detection into daily practice

ACCREDITATION STATEMENT: Med Learning Group is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. This CME activity was planned and produced in accordance with the ACCME Essentials.

CREDIT DESIGNATION STATEMENT: Med Learning Group designates this live virtual symposium for a maximum of 1.25 *AMA Category 1 credit™.* Physicians should claim only the credit commensurate with the extent of their participation in the live virtual activity.

NURSING CREDIT INFORMATION: Purpose: This program would be beneficial for nurses involved in the care of people who undergo screening for cancer. **Credits:** 1.25 ANCC Contact Hour.

CNE Accreditation Statement:
Ultimate Medical Academy/(CCM) is accredited as a provider of nursing continuing professional education by the American Nurses Credentialing Center's Commission on Accreditation. Awarded 1.25 contact hour of continuing nursing education of RNs and APNs.

AAFP CREDIT REQUEST

The AAFP has reviewed Earlier Cancer Detection: The Growing Role of Cell-Free DNA (cfDNA) Blood Tests in Primary Care and deemed it acceptable for up to 1.25 Online Only, Live AAFP Prescribed credit. Term of Approval is from 09/29/2021 to 09/29/2021. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

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Aparna Parikh, MD reports the following relationships:

Relationship	**Manufacturer**
Consultant/Advisory Board	Pfizer, Eli Lilly, Natera, and Checkmate
Research funding (Institution)	Bristol Myers Squibb, Guardant, Array, Pfizer, Macrogenics, Puretech, PMV Pharma, Plexxikon, and Takeda
Data Safety Monitor Committee	Roche
Stock Ownership	C2i

Ryan Corcoran, MD, PhD reports the following relationships:

Relationship	**Manufacturer**
Consultant/Advisory Board	AbbVie, Amgen, Array Biopharma/Pfizer, Asana Biosciences, Astex Pharmaceuticals, AstraZeneca, Avidity Biosciences, BMS, C4 Therapeutics, Chugai, Elicio, Erasca, Fog Pharma, Genentech, Guardant Health, Ipsen, Kinnate Biopharma, LOXO, Merrimack, Mirati Therapeutics, Naterna, Navire, N-of-one/Qiagen, Novartis, nRichx, Remix Therapeutics, Revolution Medicines, Roche, Roivant, Shionogi, Shire, Spectrum Pharmaceuticals, Symphogen, Tango Therapeutics, Taiho, Warp Drive Bio, and Zikani Therapeutics.
Equity	Avidity Biosciences, C4 Therapeutics, Erasca, Kinnate Biopharma, nRichDx, Remix Therapeutics, and Revolution Medicines
Research Funding	Asana, AstraZeneca, Lilly, and Novartis

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.

Douglas Cox, MSN, MHA, RN
Ultimate Medical Academy/CCM – Lead Nurse Planner

The reviewer of this activity has nothing to disclose.

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- Ana Maria Albino, Senior Program Manager of Med Learning Group, has nothing to disclose.
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METHOD OF PARTICIPATION

There are no fees for participating and receiving CME credit for this live virtual activity. To receive CME/CNE credit participants must:

1. Read the CME/CNE information and faculty disclosures;
2. Participate in the live virtual activity; and
3. Complete pre-and-post surveys and evaluation.

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AGENDA

I. **Cancer Screening: An overview**
   a. Current practice in cancer screening (CDC and/or USPSTF recommendations)
   b. Characteristics of a good screening test
   c. Gaps in current practice and adherence to screening guidelines
   d. Cancer screening in primary care

II. **Analysis of circulating cell-free nucleic acids for early cancer detection**
   a. Different forms of liquid biopsy
   b. Use of tissue of origin signature to detect cancer using methylation signature
      i. ***Whiteboard theme: cfDNA: a molecular overview of its role in cancer biology***
   c. The Circulating Cell-free Genome Atlas (CCGA) study
   d. Using Genome-Wide Association Studies (GWAS) to detect cancer early
      i. ***Whiteboard theme: Clinical trials and future directions of next-generation cfDNA sequencing assays for multi-cancer early detection***
   e. Pan Cancer using cfDNA and machine learning

III. **Integration of cfDNA blood tests into cancer screening in clinical practice**
   a. Clinical applications of cfDNA
   b. Potential placement of cfDNA blood tests in established cancer screening paradigms and evidence-based guidance
   c. potential public health impact of widespread early screening
   d. Early cancer detection: research priorities
   e. Future opportunities

IV. **Conclusions and Questions and Answers**

Earlier Cancer Detection: The Growing Role of Cell-Free DNA (cfDNA) Blood Tests in Primary Care

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1

Disclosures

- Dr. Parikh reports the following relationships:

Relationship	Manufacturer
Consultant/Advisory Board	Pfizer, Eli Lilly, Natera, and Checkmate
Research funding (Institution)	Bristol Myers Squibb, Guardant, Array, Pfizer, Macrogenics, Puretech, PMV Pharma, Plexxikon, and Takeda
Data Safety Monitor Committee	Roche
Stock Ownership	C2i

- Dr. Corcoran reports the following relationships:

Relationship	Manufacturer
Consultant/Advisory Board	AbbVie, Amgen, Array Biopharma/Pfizer, Asana Biosciences, Astex Pharmaceuticals, AstraZeneca, Avidity Biosciences, BMS, C4 Therapeutics, Chugai, Elicio, Erasca, Fog Pharma, Genentech, Guardant Health, Ipsen, Kinnate Biopharma, LOXO, Merrimack, Mirati Therapeutics, Naterna, Navire, N-of-one/Qiagen, Novartis, nRichx, Remix Therapeutics, Revolution Medicines, Roche, Roivant, Shionogi, Shire, Spectrum Pharmaceuticals, Symphogen, Tango Therapeutics, Taiho, Warp Drive Bio, and Zikani Therapeutics.
Equity	Avidity Biosciences, C4 Therapeutics, Erasca, Kinnate Biopharma, nRichDx, Remix Therapeutics, and Revolution Medicines
Research Funding	Asana, AstraZeneca, Lilly, and Novartis

- 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, Inc.

2

Learning Objectives

- Evaluate the science behind cfDNA testing along with its role in early multi-cancer detection
- Describe the potential benefits and limitations of using routine cfDNA screening to identify a variety of cancer types
- Plan strategies to integrate cfDNA blood tests and early multicancer detection into daily practice

3

What's the Problem?

4

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.

5

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.

6

Cancer Screening

7

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 C	Women aged 50 to 74 Women aged 40 to 49	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.

All recommendations available at: https://www.uspreventiveservicestaskforce.org/uspstf/.

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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.

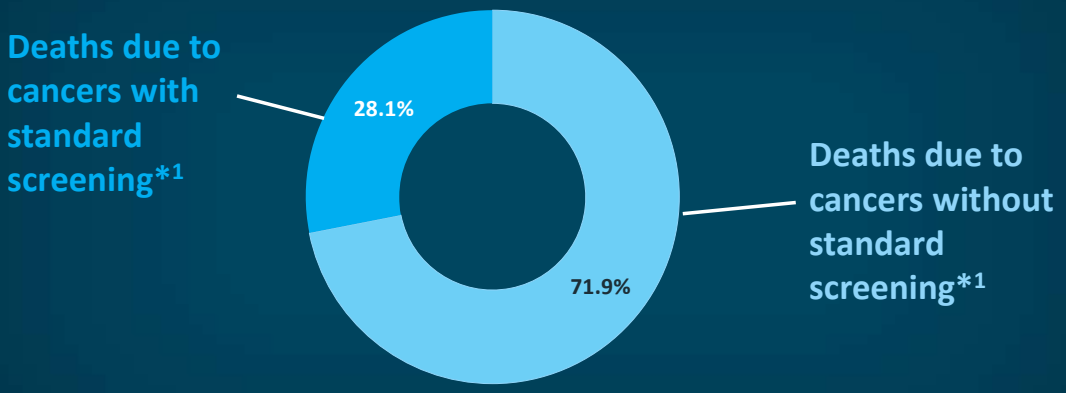
9

Characteristics of Good Screening Test

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

10

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]

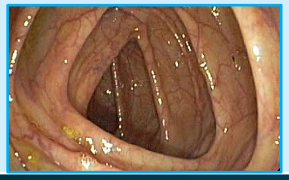
1. ACS Cancer Facts & Figures 2021. (www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf). Accessed 1/21/2021. 2. Data on file from Surveillance, Epidemiology, and End Results (SEER) 18 Regs Research Data, Nov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

11

Adherence to Screening Recommendations Can Be Suboptimal

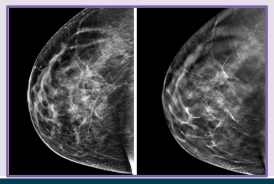
CRC (colonoscopy)

- 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



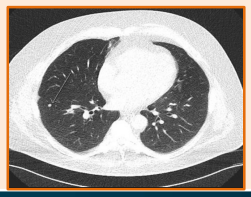
Breast (mammography)

- 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%)



Lung (low-dose CT)

- 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.

12

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

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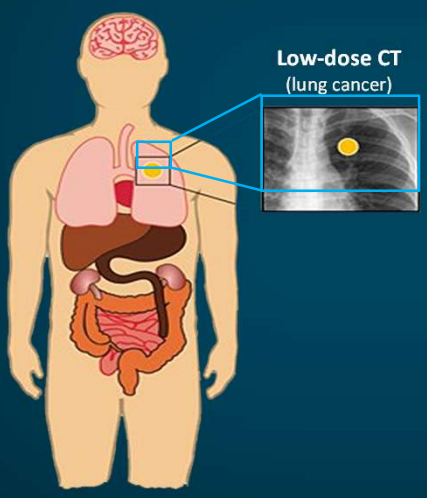
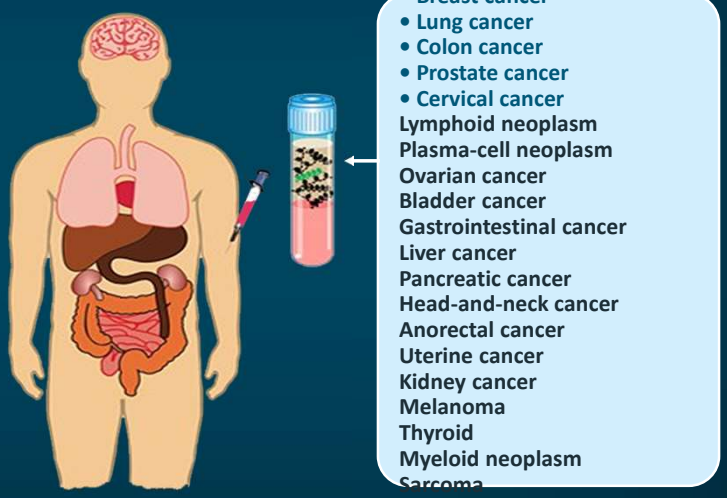
Single vs Multi-Cancer Screening

"One test-one cancer" approach



"One test-many cancers" approach



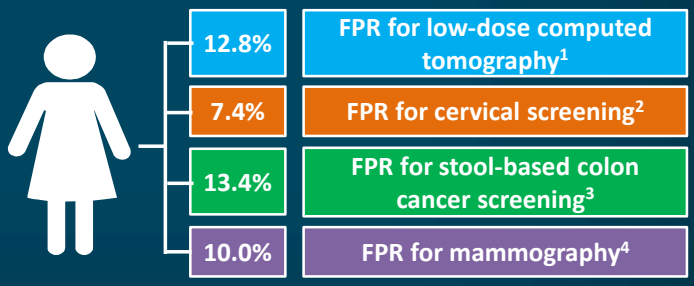
Ofman JJ, et al. *Nat Res.* 2020. (www.nature.com/articles/d42473-020-00079-y). Accessed 1/20/2021.

14

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.

15

Multi-Cancer Early Detection Using cfDNA/ctDNA

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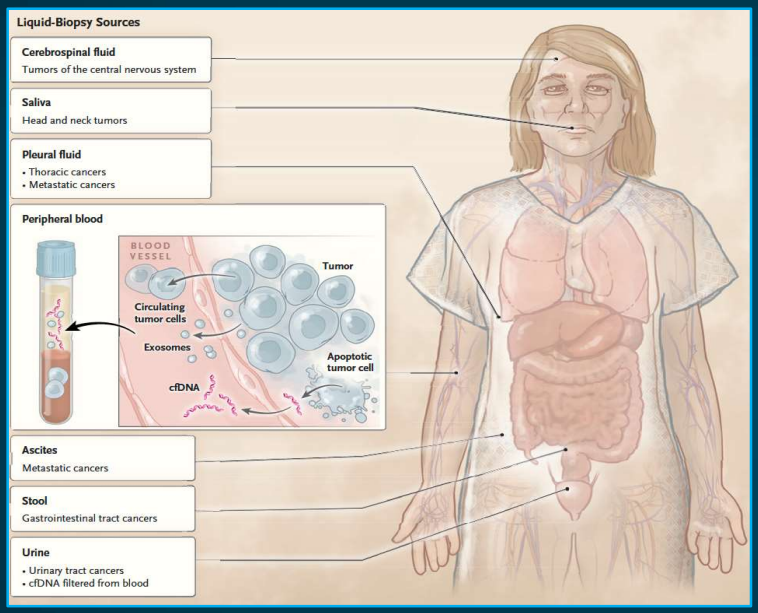
Video 1
cfDNA/ctDNA Overview

17

What Is a "Liquid Biopsy"?



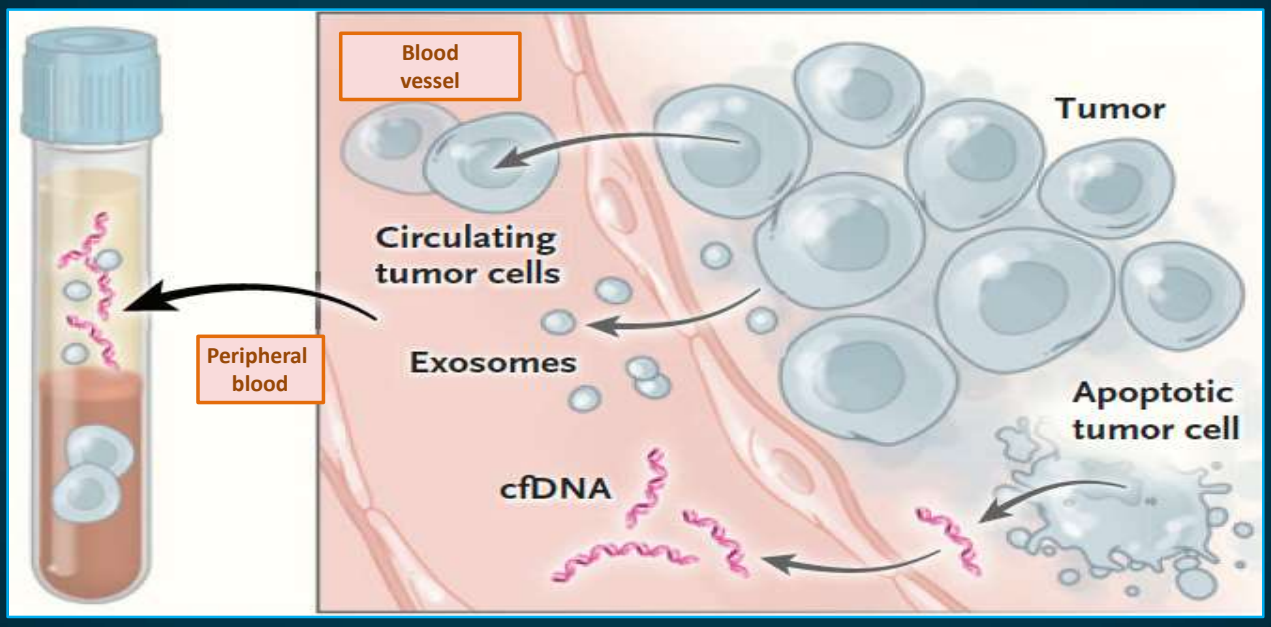
- Liquid-blood biopsy includes isolating circulating tumor cells, exosomes (membrane-bound vesicles released by tumor cells), and cell-free DNA (released by apoptotic or necrotic tumor cells)
- Other bodily fluids can also be used

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

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

18

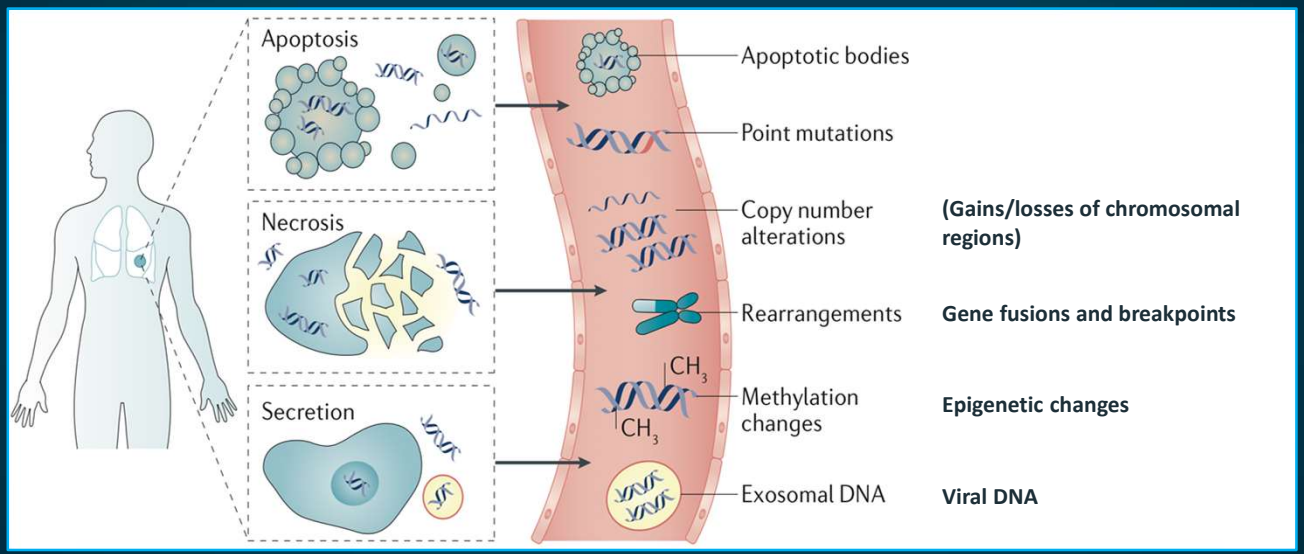
Different Forms of Liquid Biopsy



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

19

Origins and Range of Alterations in Cell-Free DNA

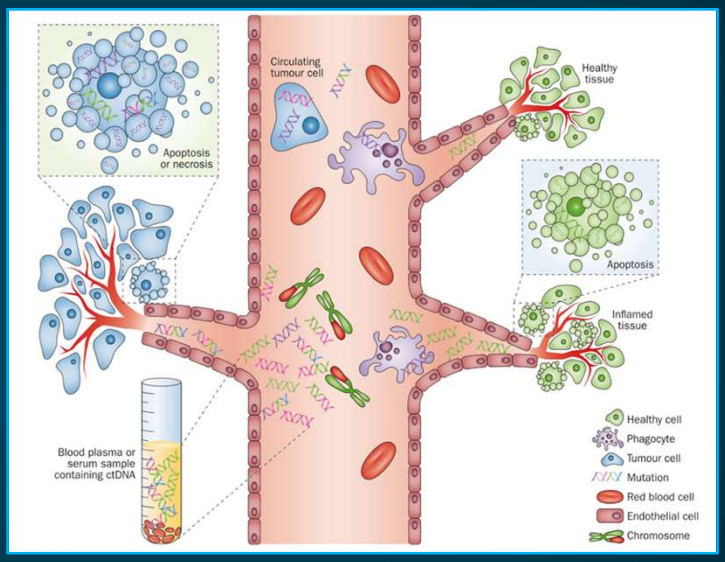


Adapted from Wan JCM, et al. *Nat Rev Cancer.* 2017;17:223-238.

20

Circulating Tumor Nucleic Acids

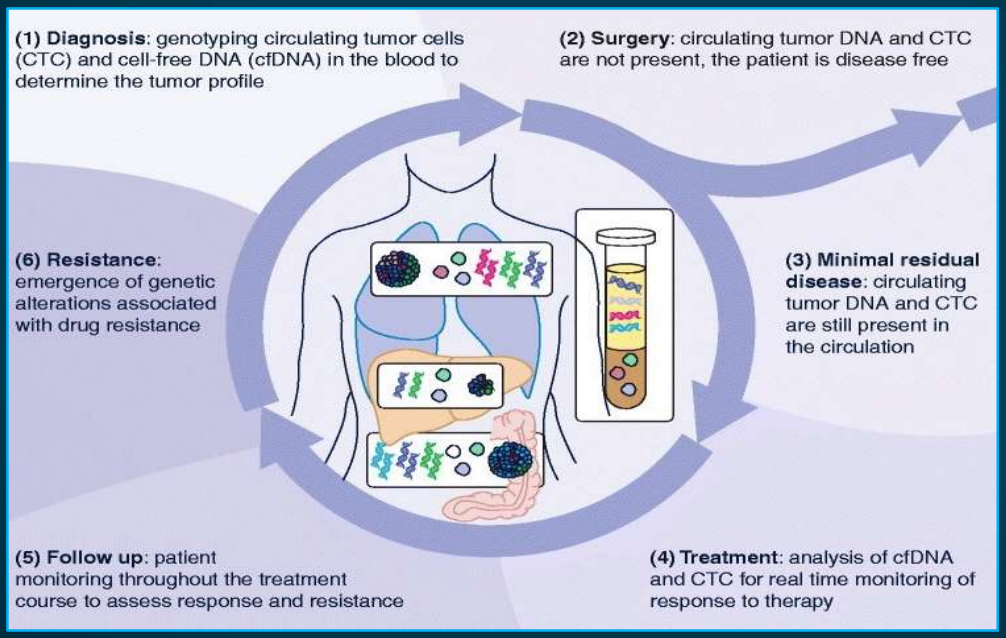
- Origins of cfDNA
  - apoptosis
  - necrosis
  - phagocytosis
  - active secretion
- cfDNA is enclosed in vesicles
  - protects from degradation
  - prevents activation of immune system
  - half-life 0.25–2.5 hours
- cfDNA cleared from blood
  - via nuclease digestion
  - renal excretion (urine)



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.

21

Clinical Applications of cfDNA

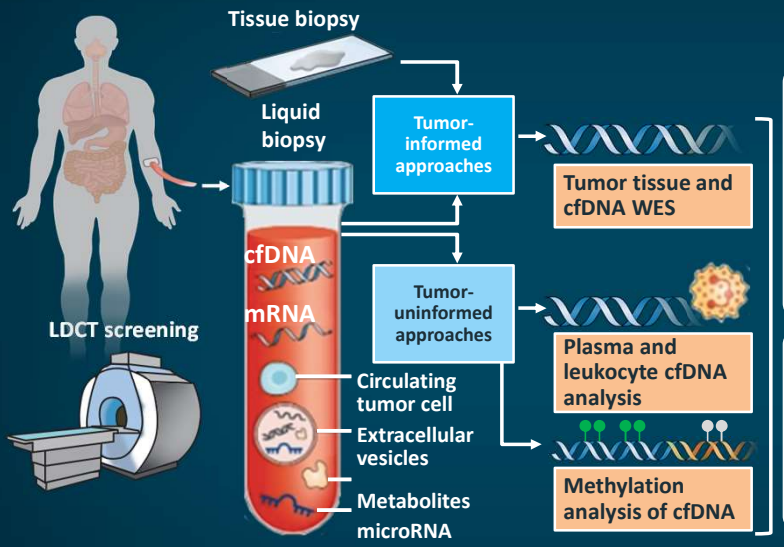


cfDNA can also be used for ***early detection***

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

22

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

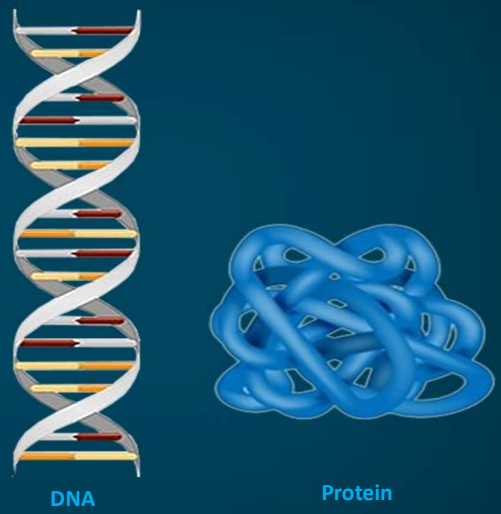
mRNA = messenger ribonucleic acid; WES = whole-exome sequencing; CHIP = clonal hematopoiesis of indeterminate potential.

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

23

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.

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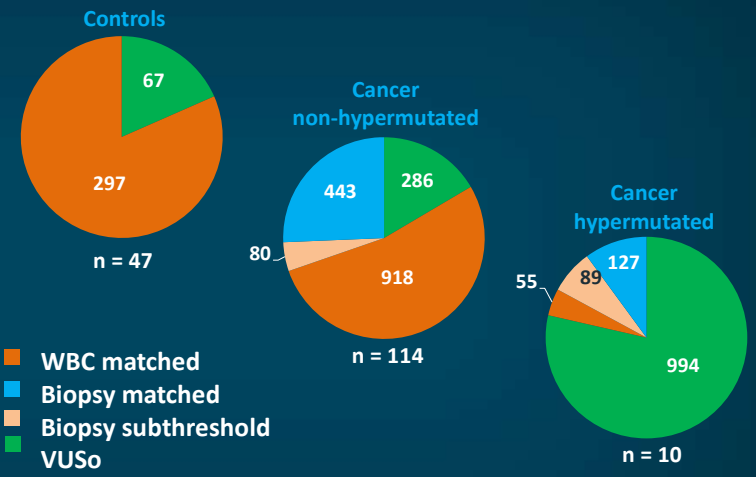
Be Cautious of CHIP

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.

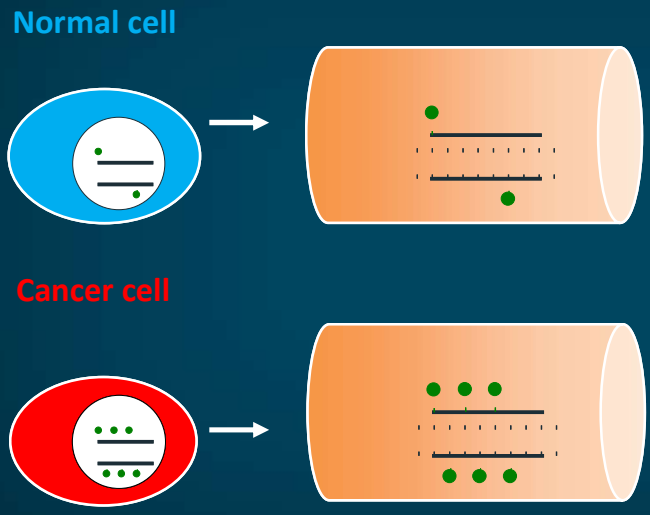
25

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

26

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.

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Video 2
cfDNA Assays

28

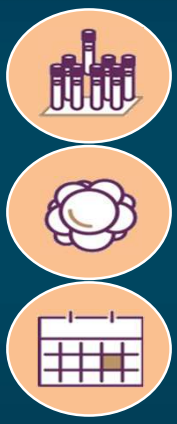
Circulating Cell-Free Genome Atlas (CCGA) Study

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)

- 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. 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.

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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
Cancer (n = 2,823)
Non-Cancer (n = 1,254)

Whole-genome methylation
Identify key informative methylation regions

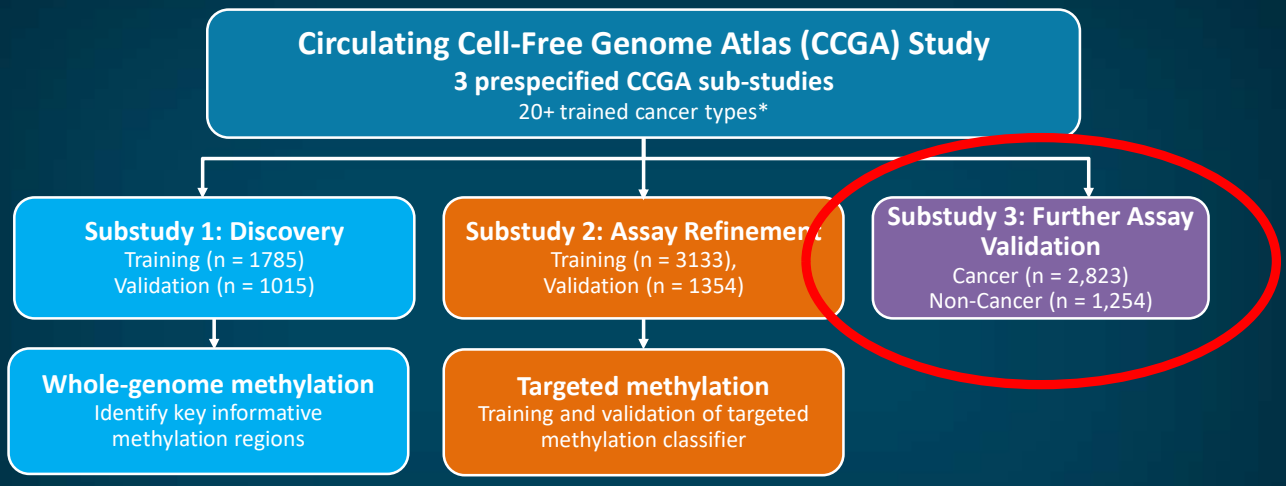
Targeted methylation
Training and validation of targeted methylation classifier

***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.

30

The CCGA: Overview of Substudies



*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.

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CCGA: Sub-study 3 Background

- Pre-specified sub-study of 4,077 participants in an independent validation set
  - –2,823 cancer
  - –1,254 non-cancer
- Specificity, sensitivity, and CSO prediction accuracy were measured
- Follow up for 5 years

Klein EA, et al. *Annals Oncol.* 2021;32:1167-77.

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MCED Performance: Overall Sensitivity and Specificity

MCED test performance: Sensitivity/Specificity for Cancer Detection

	Cancer (n=2823)	Non-cancer (n=1254)	Total (n=4077)
Test positive	1453	6	1459
Test negative	1370	1248	2618
	Sensitivity = 1453/2823 51.5% (49.6%-53.3%)	**Specificity = 1248/1254** 99.5% (99.0%-99.8%)	

The 2 x 2 contingency table summarizes overall sensitivity and specificity.

Two-sided 95% Wilson confidence intervals were calculated.

Klein EA, et al. *Annals Oncol.* 2021;32:1167-77.

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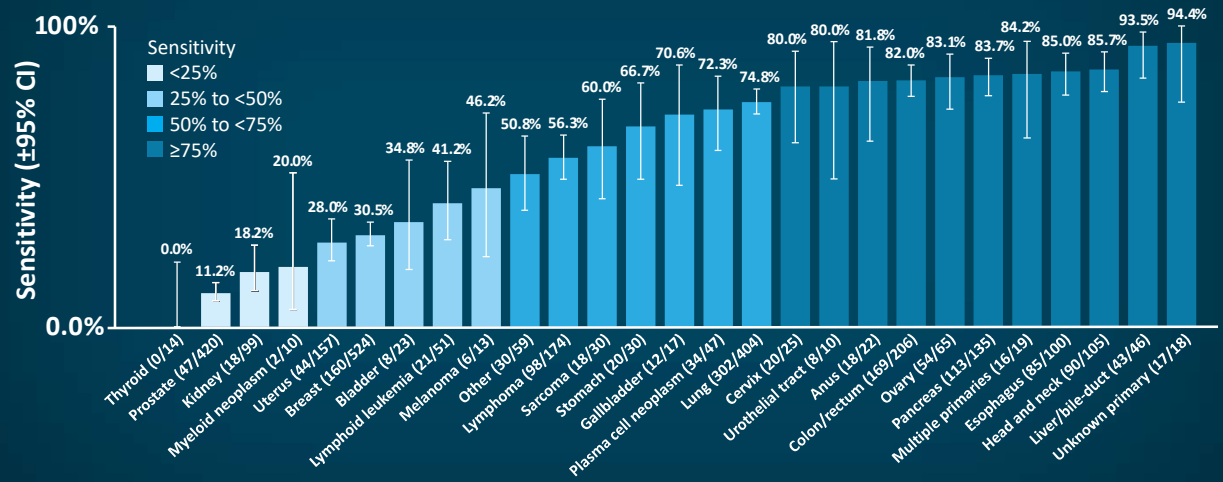
MCED Performance: Sensitivity Analysis by Cancer Class

MCED Performance for Cancer Signal Detection by Cancer Class



Sensitivity (y-axis) by cancer class based on individual cancer classes (x-axis), including other, unknown primary, and multiple primaries. Cancer classes are ordered based on increasing sensitivity; bars indicate 95% CI.

Klein EA, et al. *Annals Oncol.* 2021;32:1167-77.

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Cancer Signal Detection by Cancer Stage

Clinical stage	Total *N*	Test Positive	Sensitivity % (95% CI)[a]
All	2823	1453	51.5 (49.6% to 53.3%)
I	849	143	16.8 (14.5% to 19.5%)
II	703	284	40.4 (36.8% to 44.1%)
III	566	436	77.0 (73.4% to 80.3%)
IV	618	557	90.1 (87.5% to 92.2%)
I-II	1552	427	27.5 (25.3% to 29.8%)
I-III	2118	863	40.7 (38.7% to 42.9%)
I-IV	2736	1420	51.9 (50.0% to 53.8%)
III-IV	1184	993	83.9 (81.7% to 85.9%)
Not expected to be staged	67	23	34.3 (24.1% to 46.3%)
Missing	20	10	50.0 (29.9% to 70.1%)

CI, confidence interval.

a Two-sided 95% Wilson CIs were calculated.

Klein EA, et al. *Annals Oncol.* 2021;32:1167-77.

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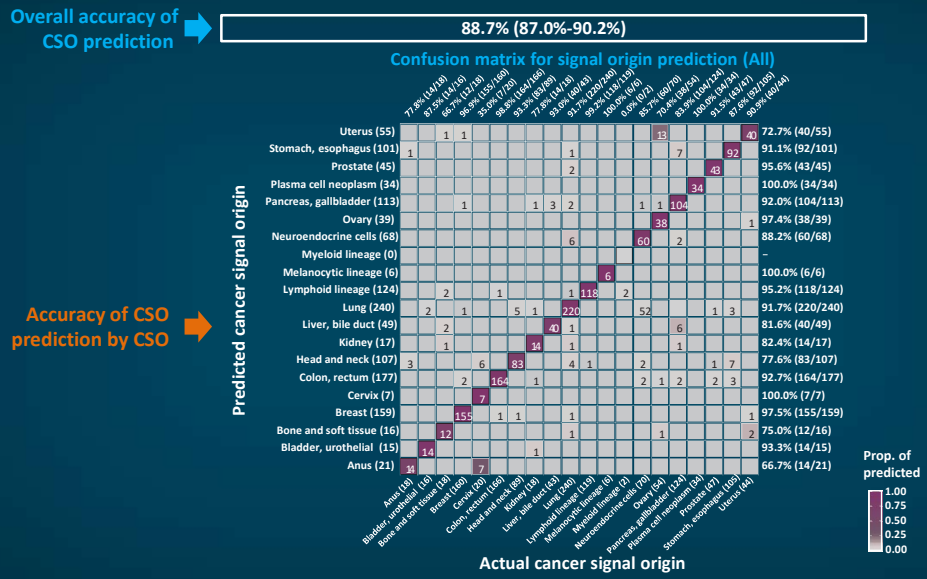
Accuracy of CSO prediction (Confusion Matrix)



Confusion matrix shows accuracy (top horizontal axis) and precision of CSO prediction by CSO (right vertical axis) among true positive participants with a known cancer signal origin. The proportion of each call is indicated by the strength of the colored signal within each individual box. Correct CSO calls are indicated on the diagonal.

CSO, cancer signal origin; Prop., proportion.

Klein EA, et al. *Annals Oncol.* 2021;32:1167-77.

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PATHFINDER: Interim Results – ASCO 2021

- PATHFINDER: prospective study with results from an early version of the MCED test (MCED-E)
  - 6662 participants from 7 centers, >50 years of age
  - 2 cohorts: with additional cancer risk factors or none
- Prespecified interim analysis, samples were retrospectively processed with the MCED-Scr; only MCED-E test results (detection and cancer signal origin prediction) were returned to the physician
- Test performance characteristics of the MCED-Scr test, including the rate of cancer signal detection, PPV, and cancer signal origin prediction were evaluated and compared to the performance for the MCED-E test

Goal: develop an MCED test with performance that make it a useful cancer screening tool in practice

Beer T, et al. ASCO 2021; June 4-8. Poster #3070.

37

Conclusions

- MCED-Scr detected cancer signals with 40% PPV and maintained a high accuracy of cancer signal origin prediction relative to the MCED-E
- MCED-Scr detected a broad range of early and advanced stage cancers
- Refinements of MCED-E test reduced the number of hematologic cancer signal origin predictions, particularly false positives, and streamlined test report to include no more than two cancer signal origins
- Updated results, specificity, and negative predictive value of MCED-Scr and MCED-E will be reported after all participants have been observed for 12 months

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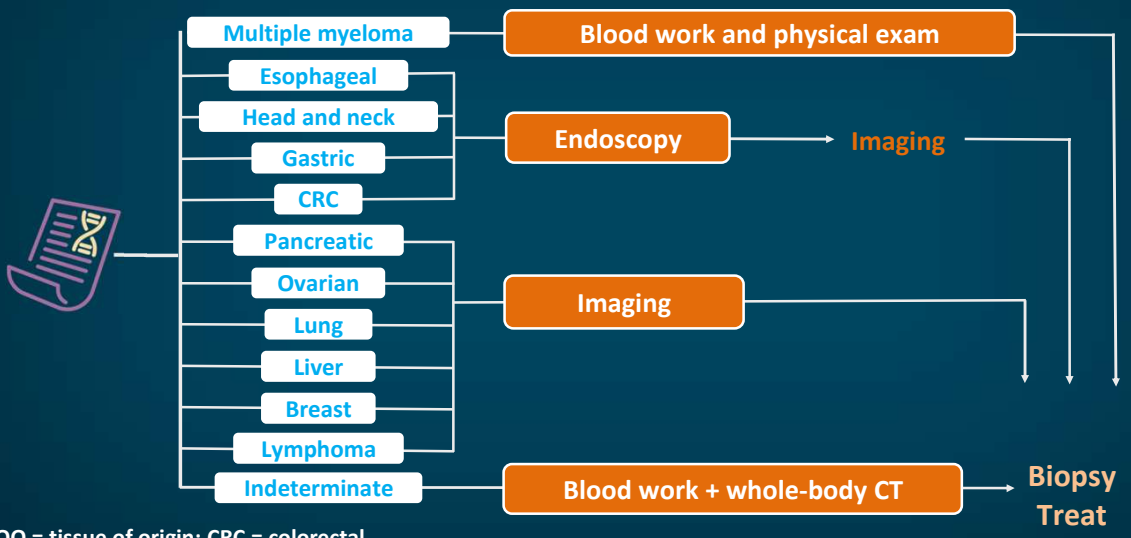
Workup of Positive Liquid Biopsy With TOO



TOO = tissue of origin; CRC = colorectal.

Graphic provided courtesy of Dr. Sana Raoof.

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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

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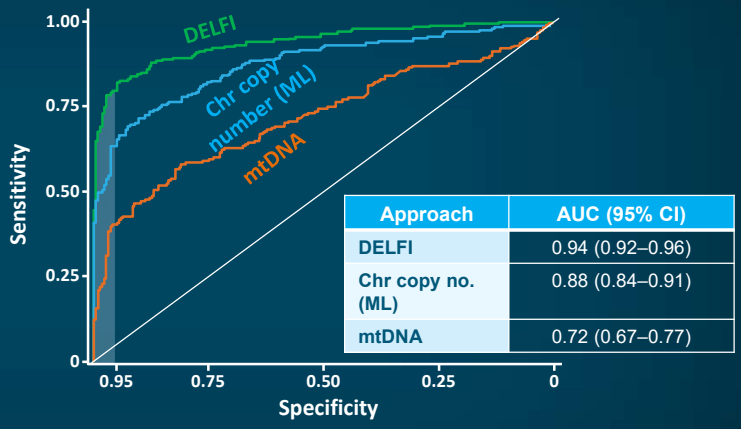
Cancer Detection Using DELFI

- 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.

41

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.

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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.

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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.

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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.

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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.

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Detection of Minimal Residual Disease

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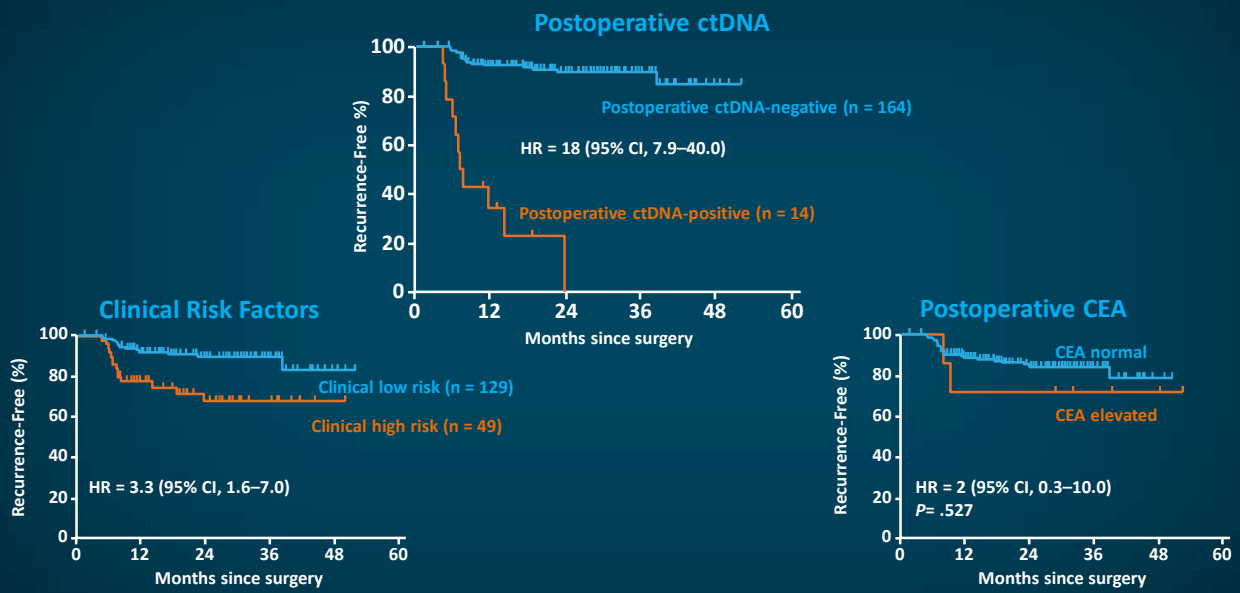
Prediction of Relapse in Stage II CRC



HR = hazard ratio; CEA = carcinoembryonic antigen.

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

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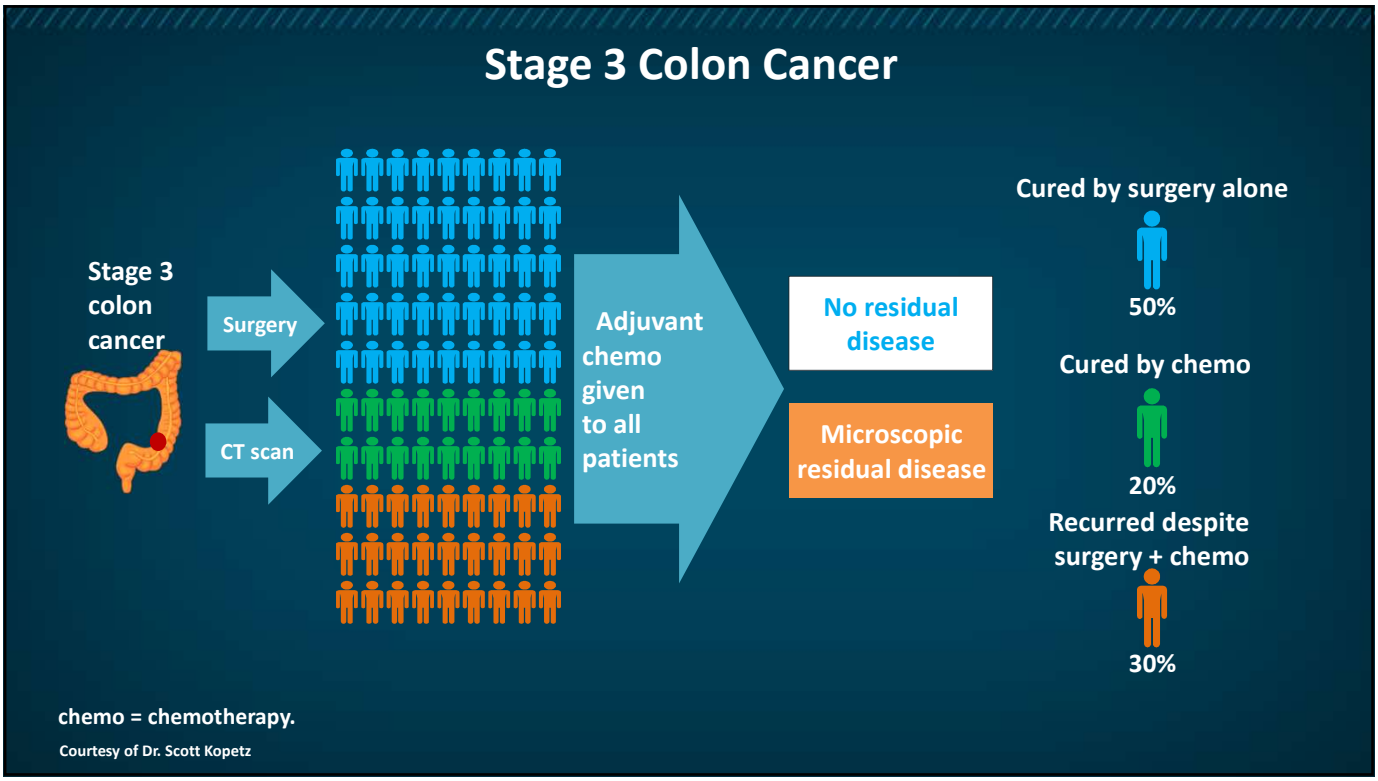
Stage 3 Colon Cancer
Stage 3 colon cancer
Surgery
CT scan
Adjuvant chemo given to all patients
No residual disease
Microscopic residual disease
Cured by surgery alone
50%
Cured by chemo
20%
Recurred despite surgery + chemo
30%
chemo = chemotherapy.
Courtesy of Dr. Scott Kopetz

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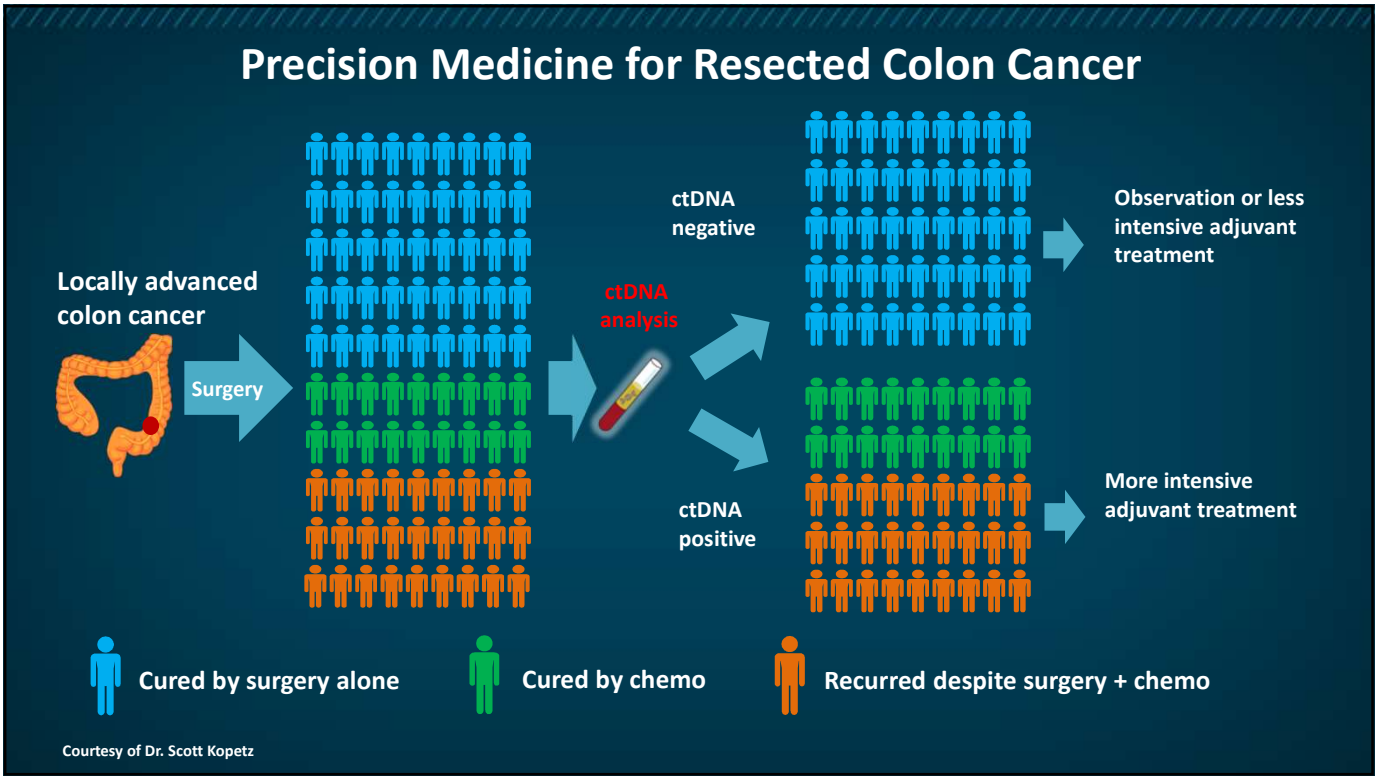
Precision Medicine for Resected Colon Cancer
Locally advanced colon cancer
Surgery
ctDNA analysis
ctDNA negative
ctDNA positive
Observation or less intensive adjuvant treatment
More intensive adjuvant treatment
Cured by surgery alone
Cured by chemo
Recurred despite surgery + chemo
Courtesy of Dr. Scott Kopetz

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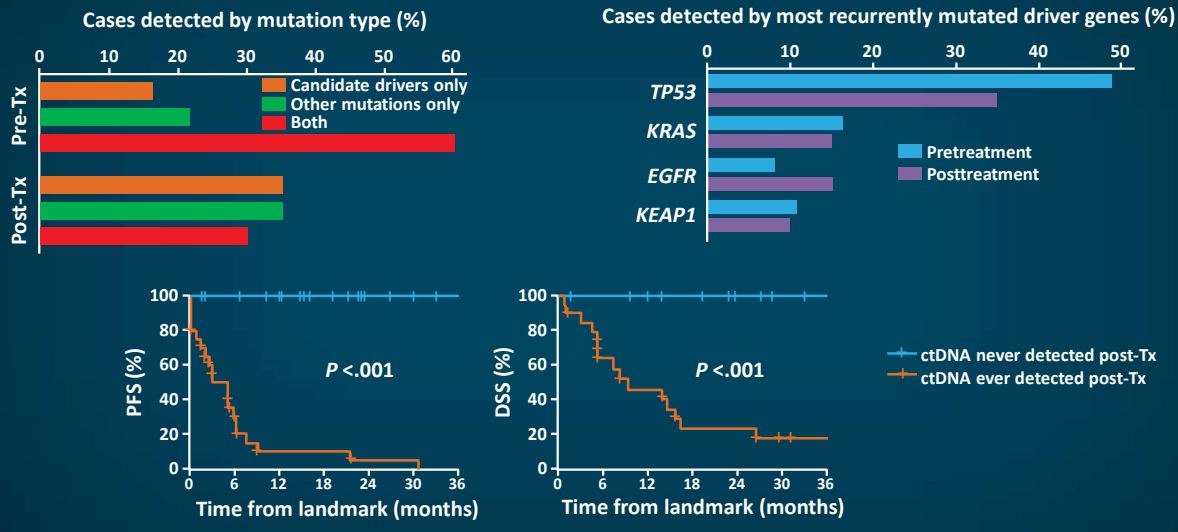
Other Applications—MRD
(continued 2)



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

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

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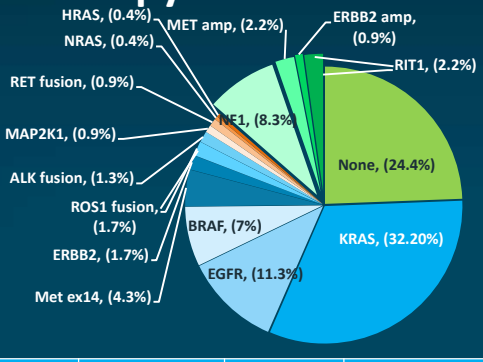
Use of Liquid Biopsy at Initial Diagnosis

52

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.

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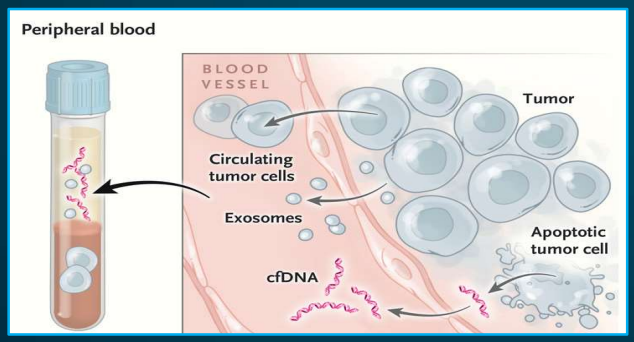
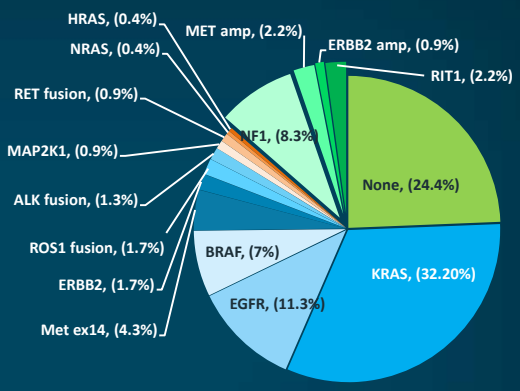
The "Power of Plasma"

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.

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Future Directions

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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. Facing racial/cultural/religious/gender/behavioral disparities • *E.g., screening is lower in black/latinx/indigenous; would an easy blood test help?*
5. Relevance of genetic testing

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

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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.

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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.

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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/

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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

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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

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Thank You!

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EARLIER CANCERDETECTION:

The Growing Role of Cell-Free DNA (*cf*DNA) Blood Tests in Primary Care

YOUTUBE 360º VIRTUAL ANIMATIONS

Use your device's QR code scanner to view the whiteboard animation content in the YOUTUBE APP!

cfDNA/ctDNA Overview
https://youtu.be/PYcflbrZUyQ

cfDNA Assays
https://youtu.be/98lC1Cp5GwU

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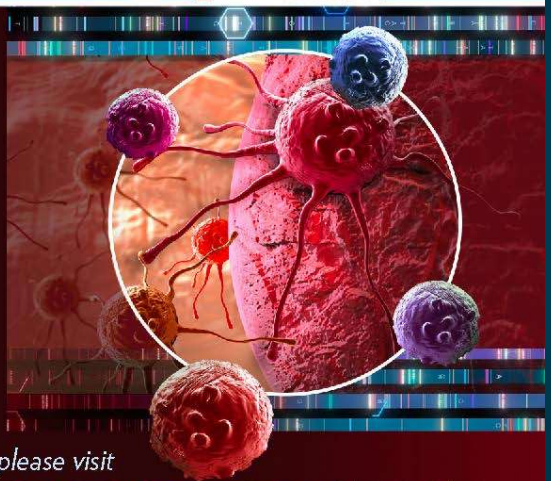
Complimentary poster for the office!

Supplement your Course Learning. It's fast and easy.

We'll ship it to you directly free of charge

EARLIER CANCER DETECTION:

The Growing Role of Cell-Free DNA (*cf*DNA) Blood Tests in Primary Care



For more information and additional resources please visit

HTTPS://CFDNA.POSTERPROGRAM.COM

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