

Liquid biopsies

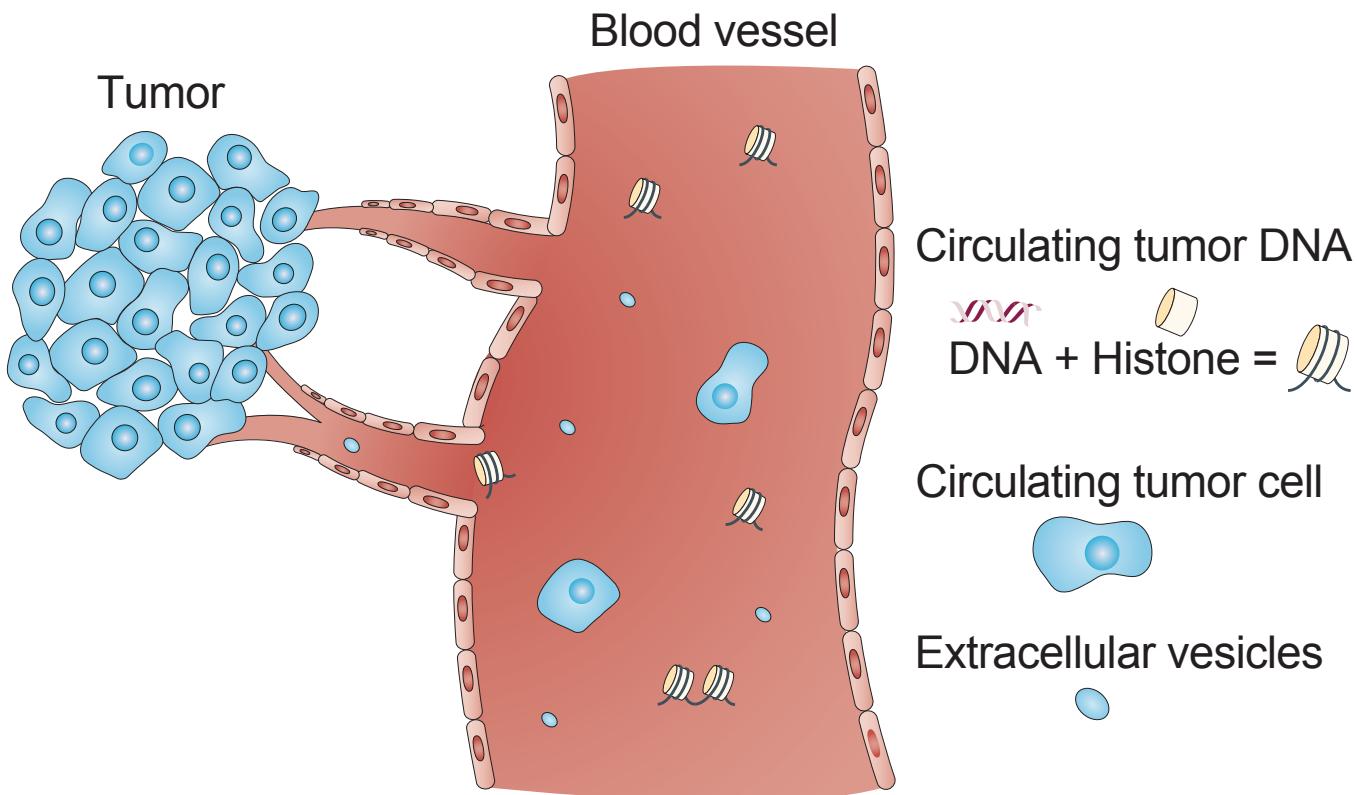


Karolinska
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Learning outcomes

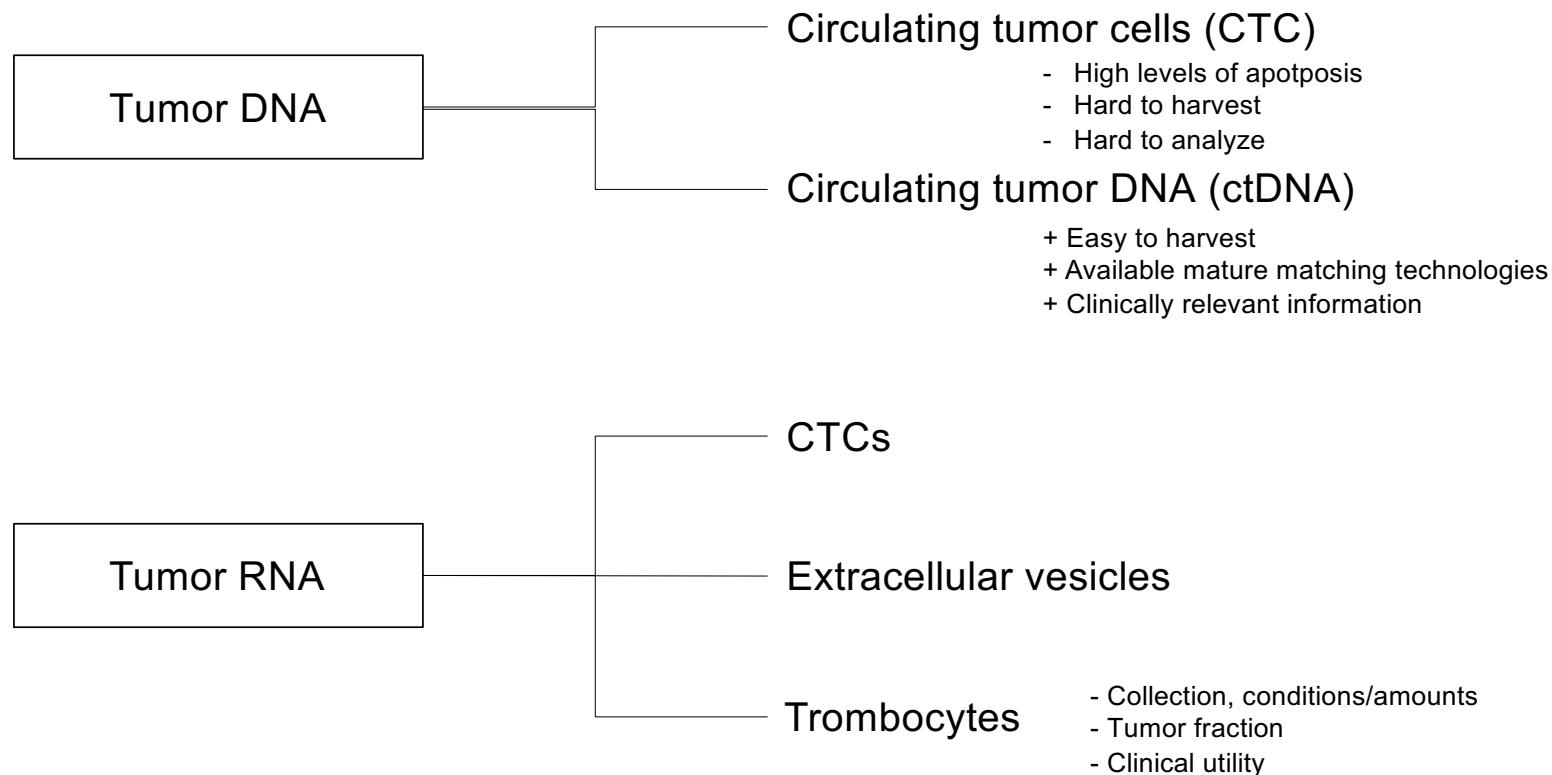
- show a basic insight into the cancer genome.
- understand how the cancer genome can be interrogated through tissues and liquid biopsies.
- understand how to apply technology to obtain relevant information from the cancer genome.

Liquid biopsies - DNA, cells or other debris that originates from the tumor tissue and is shed into the circulation



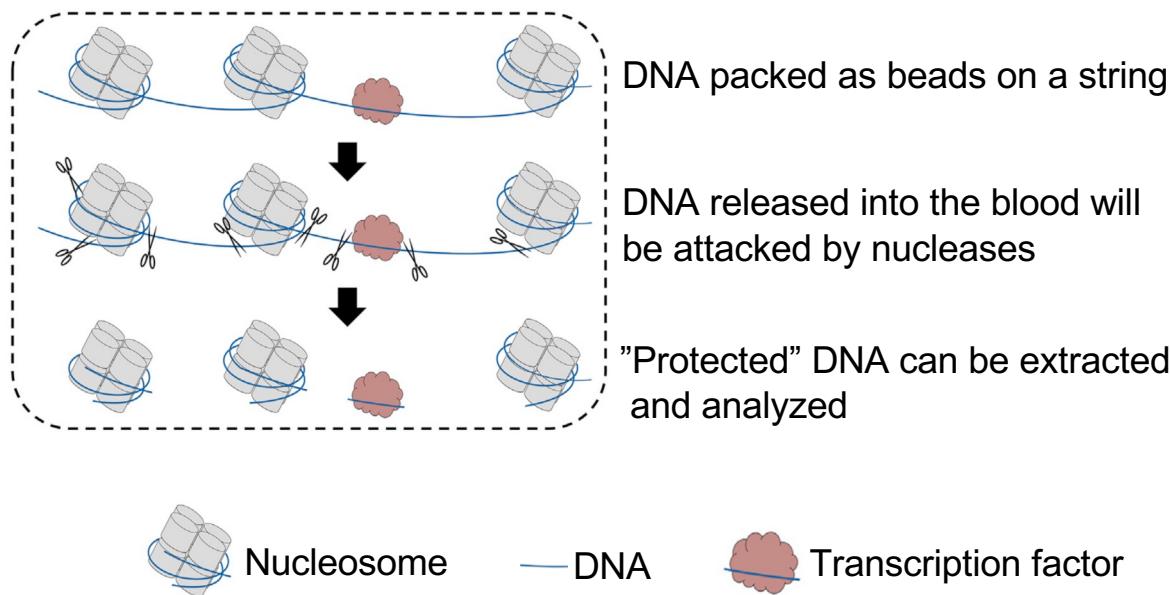
Adopted from Cell-free nucleic acids as biomarkers in cancer patients, Nat Rev Can 2011

Liquid biopsy types



Cell-free DNA preserves tissue-specific epigenetic information

- All healthy individuals harbor cell-free DNA (cfDNA) in blood/urine
- cfDNA originate (mostly) from apoptotic cells
- cfDNA fragments are short, ~167 bp (nucleosome + linker histone)



What does ctDNA represent?

Article

Deep whole-genome ctDNA chronology of treatment-resistant prostate cancer

<https://doi.org/10.1038/s41586-022-04975-9>

Received: 13 November 2021

Accepted: 14 June 2022

Published online: 20 July 2022

Cameron Herberts^{1,2}, Matti Annala^{1,2,3}, Joonatan Sipola^{2,3}, Sarah W. S. Ng⁴, Xinyi E. Chen¹,

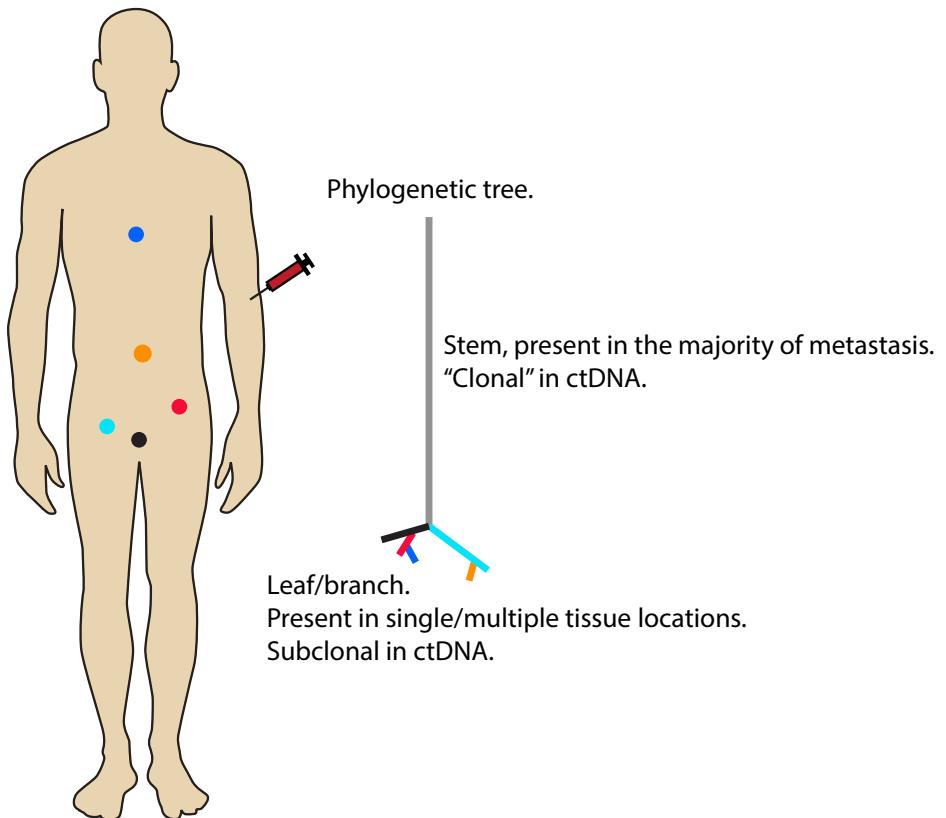
Anssi Nurminen², Olga V. Korhonen², Asli D. Munzur¹, Kevin Bojic¹, Elena Schinlau¹,

Cecily Q. Bernales¹, Elie Ritch¹, Jack V. W. Bacon¹, Nathan A. Lack^{1,3,4}, Matti Nykter⁵,

Rahul Aggarwal^{1,6}, Eric J. Small^{1,6}, Martin E. Gleave¹, SU2C/PCF West Coast Prostate Cancer

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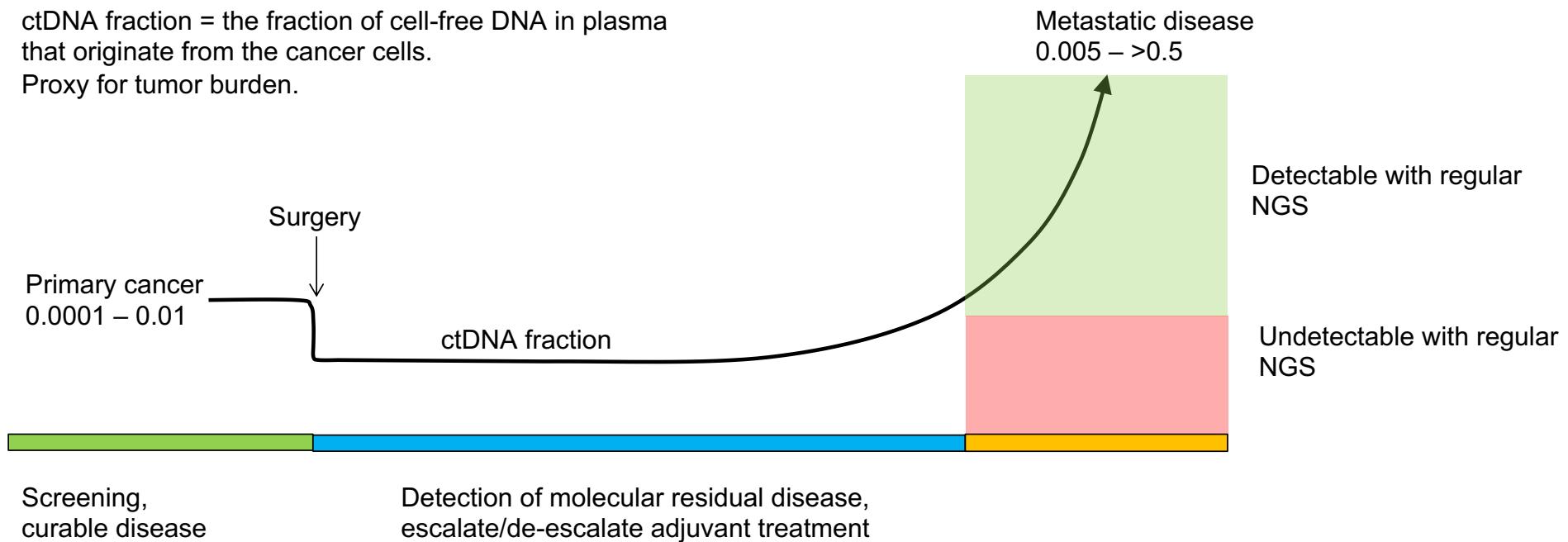
Deep whole-genome ctDNA chronology of treatment-resistant prostate cancer, Nature, 2022.

Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer, Nat Comm 2015

Cancer DNA fraction and consequence for genomic profiling using sequencing

ctDNA fraction = the fraction of cell-free DNA in plasma that originate from the cancer cells.

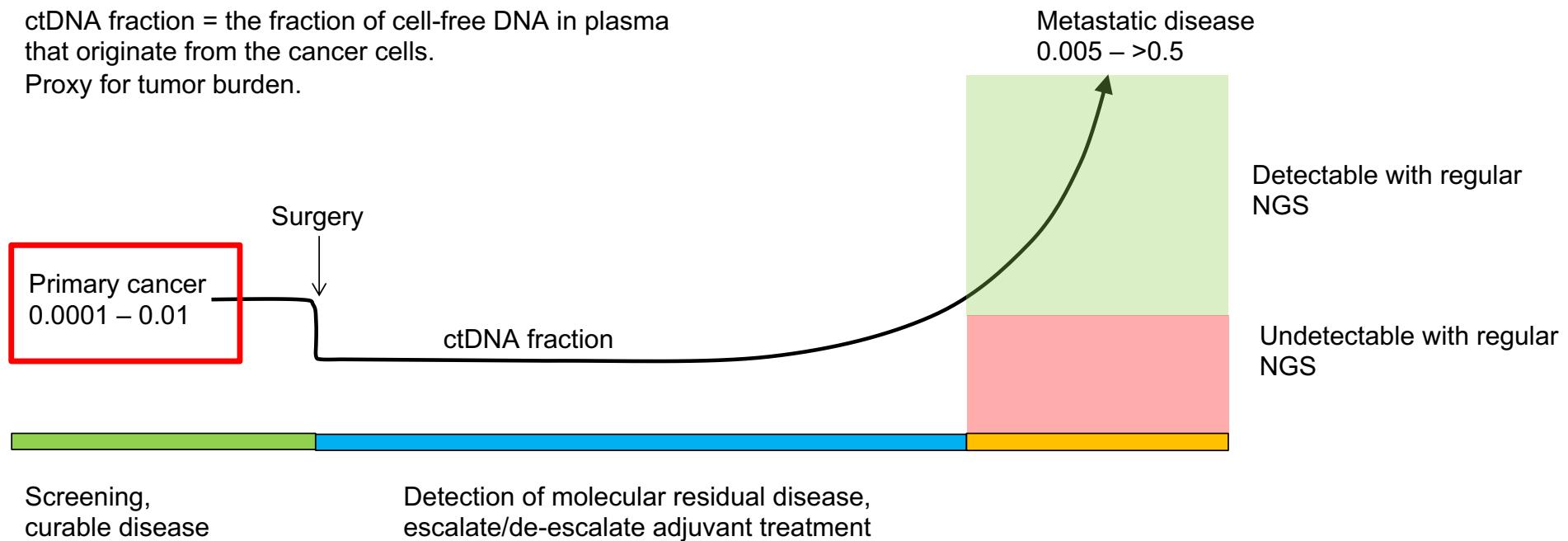
Proxy for tumor burden.



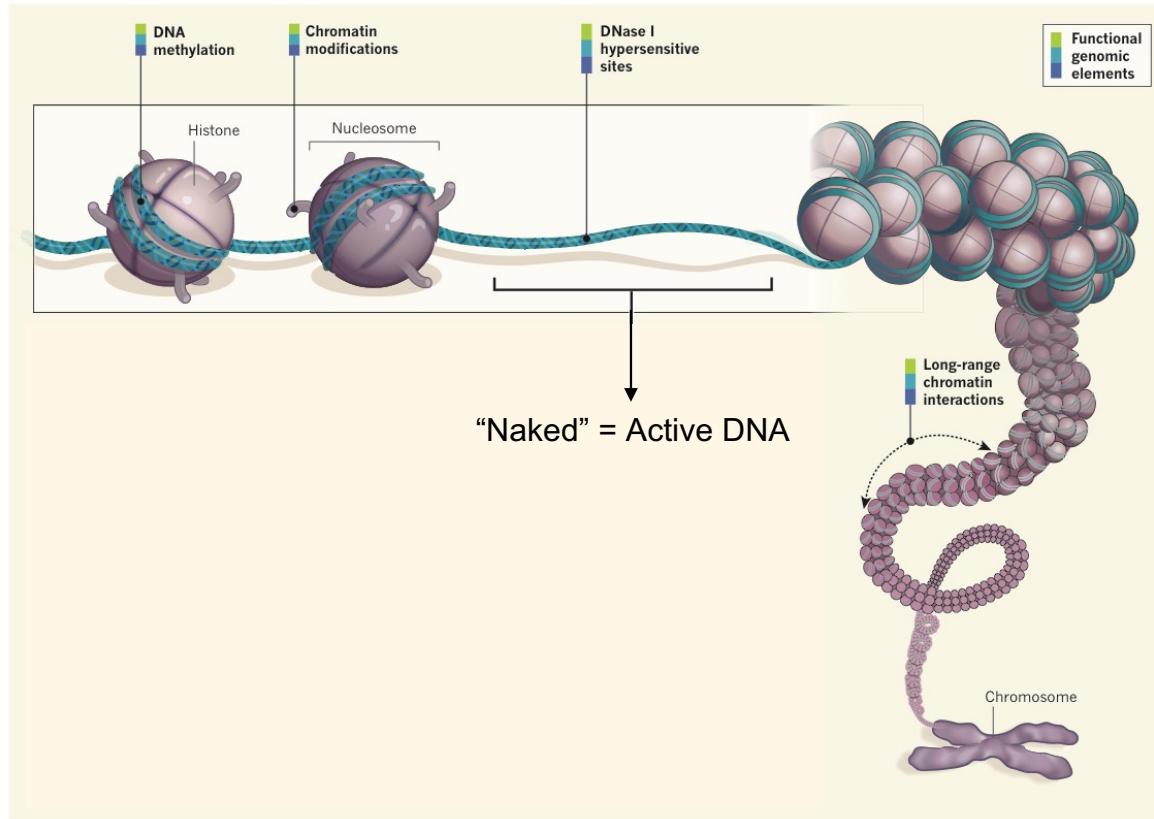
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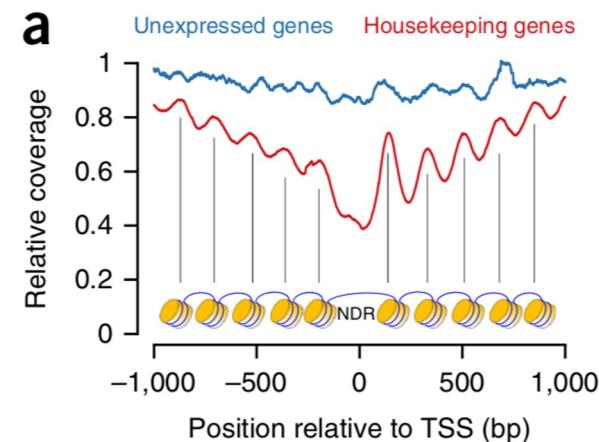
Epigenetic regulation



Epigenetic state of circulating tumor DNA

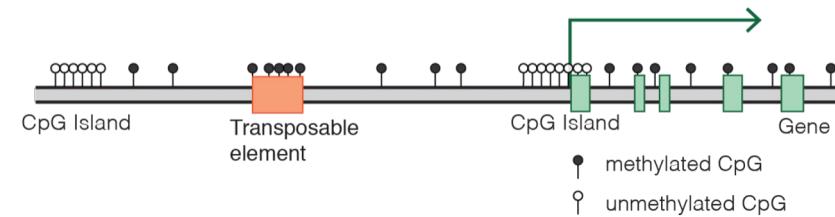
- Nucleosome occupancy can be applied to predict expressed genes

- Inferring expressed genes by whole-genome sequencing of plasma DNA, Nature genetics 2016*



- Antibody-based methylation enrichment and sequencing

- Sensitive tumour detection and classification using plasma cell-free DNA methylomes, Nature 2018*



Early detection/screening for localized cancer

- Mutation profiling
- Mutations with protein biomarkers
- Nucleosome mapping
- Methylation sequencing



GRAIL

Science Clinical Studies About News Join the Team

GRAIL's mission is to detect cancer early, when it can be cured



freenome

Thrive.
Earlier Detection

Early detection/screening for localized cancer

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GRAIL's mission is to detect cancer early, when it can be cured



- Illumina owned company
- Circulating cell-free Genome Atlas study
 - Learn how to detect non-metastatic cancers early using liquid biopsies
- Genomic, transcriptomic and methylation sequencing of cfDNA

Early detection/screening for localized cancer

- The pilot
 - Methylation profiling (sequencing), CNV and mutations (€8000/sample)
 - 15.000 individuals
 - 10,500 with cancer
 - 4,500 without cancer
 - 80 ml of blood/participant
- Follow up studies (prospective, observational, longitudinal)
 - 100.000 women having mammography
 - 50.000 men and women between 50-77 years
 - >6000 men prospectively receiving results in an interventional study



Early detection/screening for localized cancer



ORIGINAL ARTICLE

Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA

M. C. Liu^{1†}, G. R. Oxnard^{2†}, E. A. Klein³, C. Swanton^{4,5}, M. V. Seiden^{6*} & on behalf of the CCGA Consortium[†]

¹Division of Medical Oncology, Department of Oncology, Mayo Clinic, Rochester; ²Lowe Center for Thoracic Oncology, Dana Farber Cancer Institute, Boston; ³Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, USA; ⁴Cancer Evolution and Genome Instability Laboratory, The Francis Crick Institute; ⁵Cancer Evolution and Genome Instability Laboratory, University College London Cancer Institute, London, UK; ⁶US Oncology Research, US Oncology, The Woodlands, USA

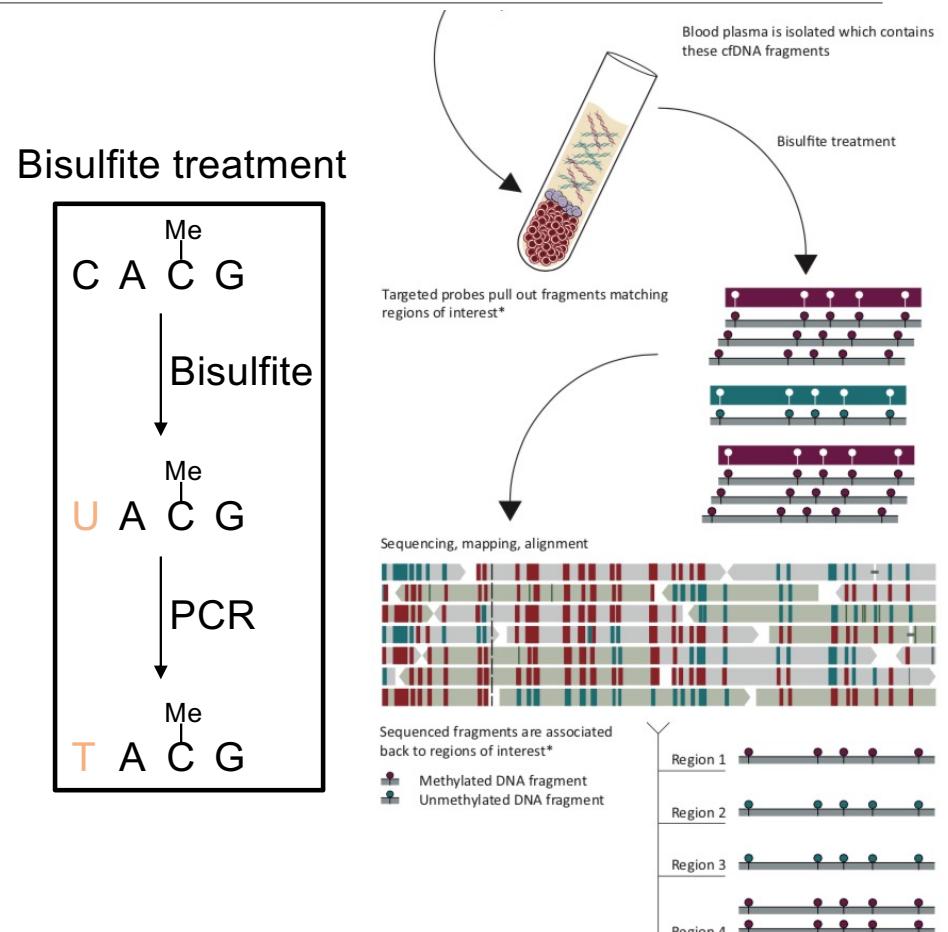
Available online XXX

Early detection/screening for localized cancer

- 6689 participants (mix of samples from all their collections..)
 - 2482 cancer (>50 cancer types)
 - 4207 non-cancer
- 80 ml of blood/participant
- Separated into training and validation set
- Targeted sequencing of >100 000 informative methylation regions.
- A classifier was developed and validated for cancer detection and tissue of origin localization.
- Classification goal: >99% specificity with >90% confidence

Early detection/screening for localized cancer

- Whole genome bisulfite sequencing
 - 3508 analyzable samples
 - 1493 cancer; 1135 non-cancer
- TCGA methylation array data
- 103 456 distinct regions (17.2 Mb) were selected.
- Plasma cfDNA (up to 75 ng) -> bisulfite conversion -> library prep using accel-NGS Methyl-Seq DNA kits
- Capture using the Twist panel (17.2 Mb)
- Cancer specific targets were selected to target 100% hyper- or hypo methylated regions = methylation specific capture
- 113e6 150 bp r-p per sample (139 X coverage) -> would cost 5700 sek/sample at Clinical genomics.

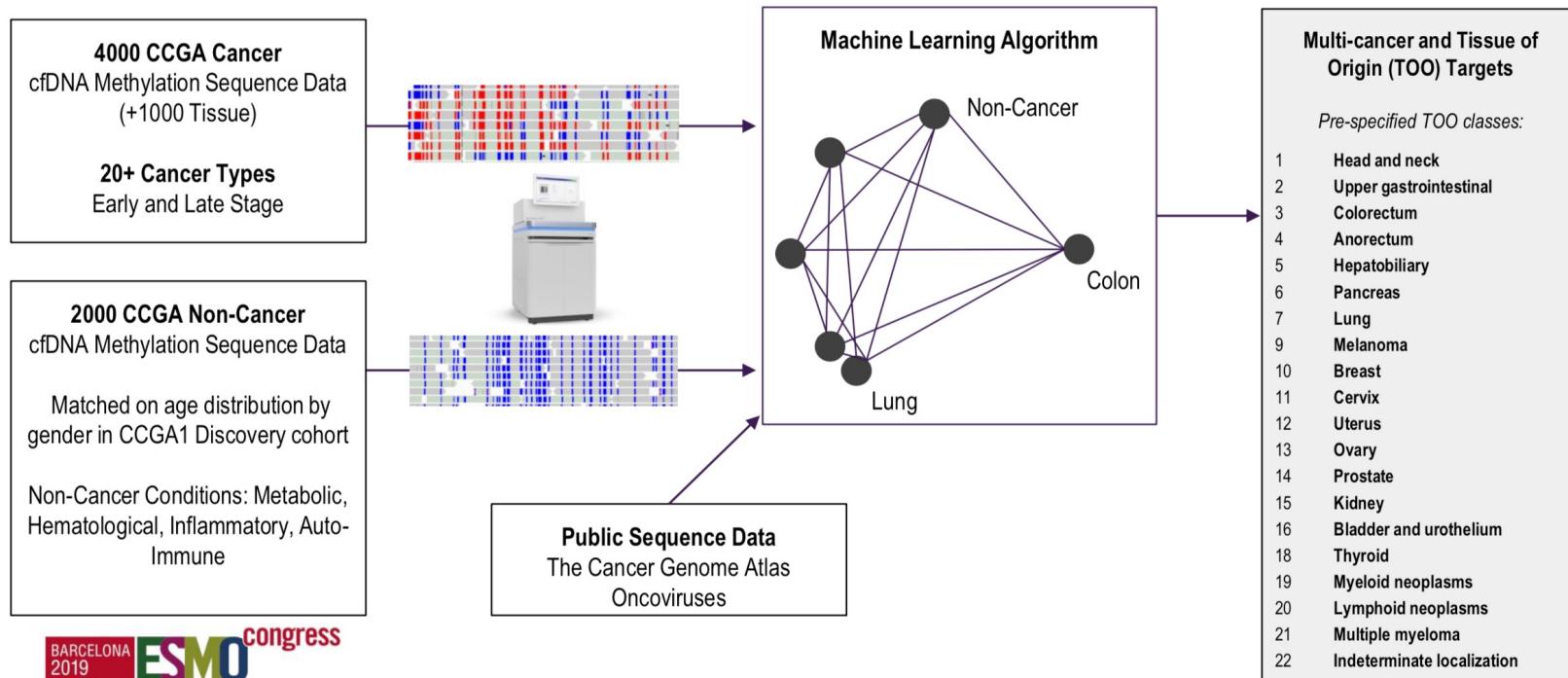


*previously defined from analysis of existing datasets from cfDNA, tissue from GRAIL trials and public databases

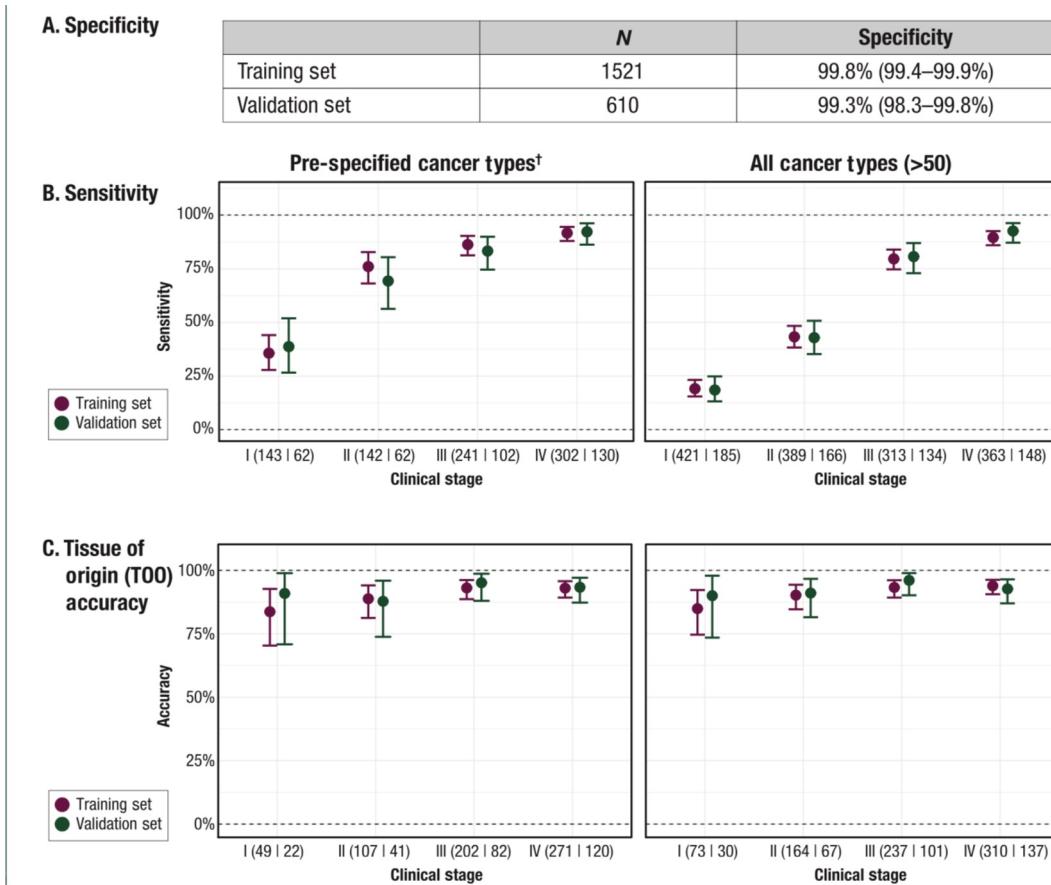
Early detection/screening for localized cancer

Target Selection using Machine Learning Algorithm

Targeted methylation panel developed through generation and analysis of an extensive database of plasma and tissue methylation patterns

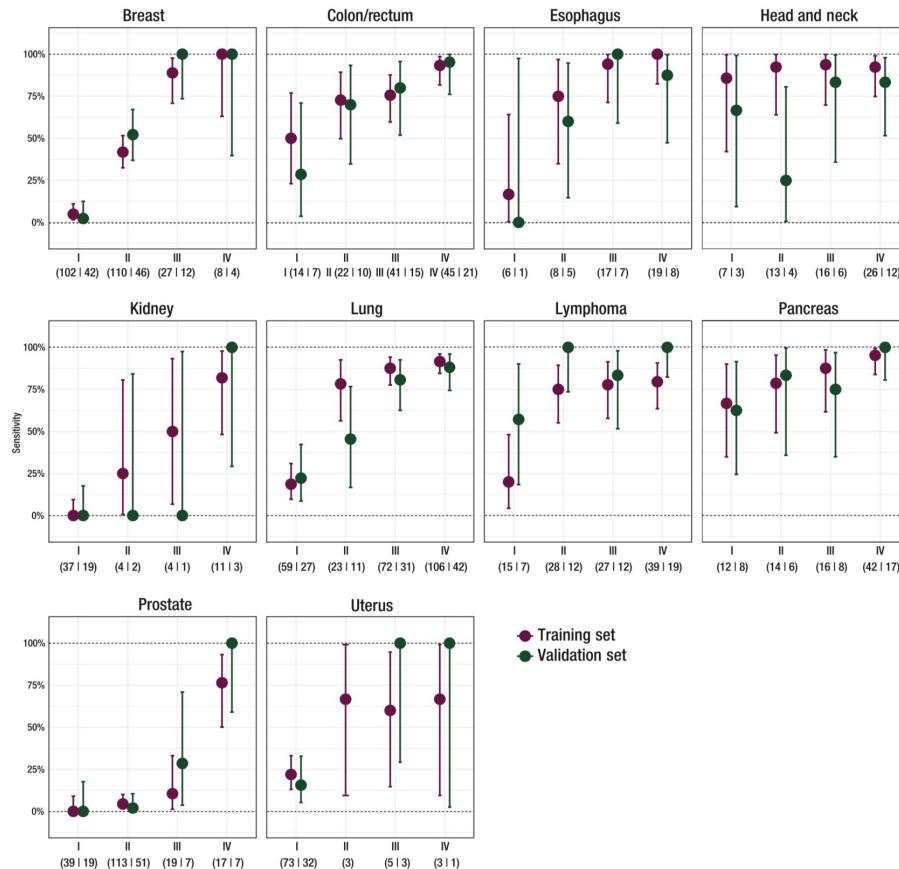


Early detection/screening for localized cancer



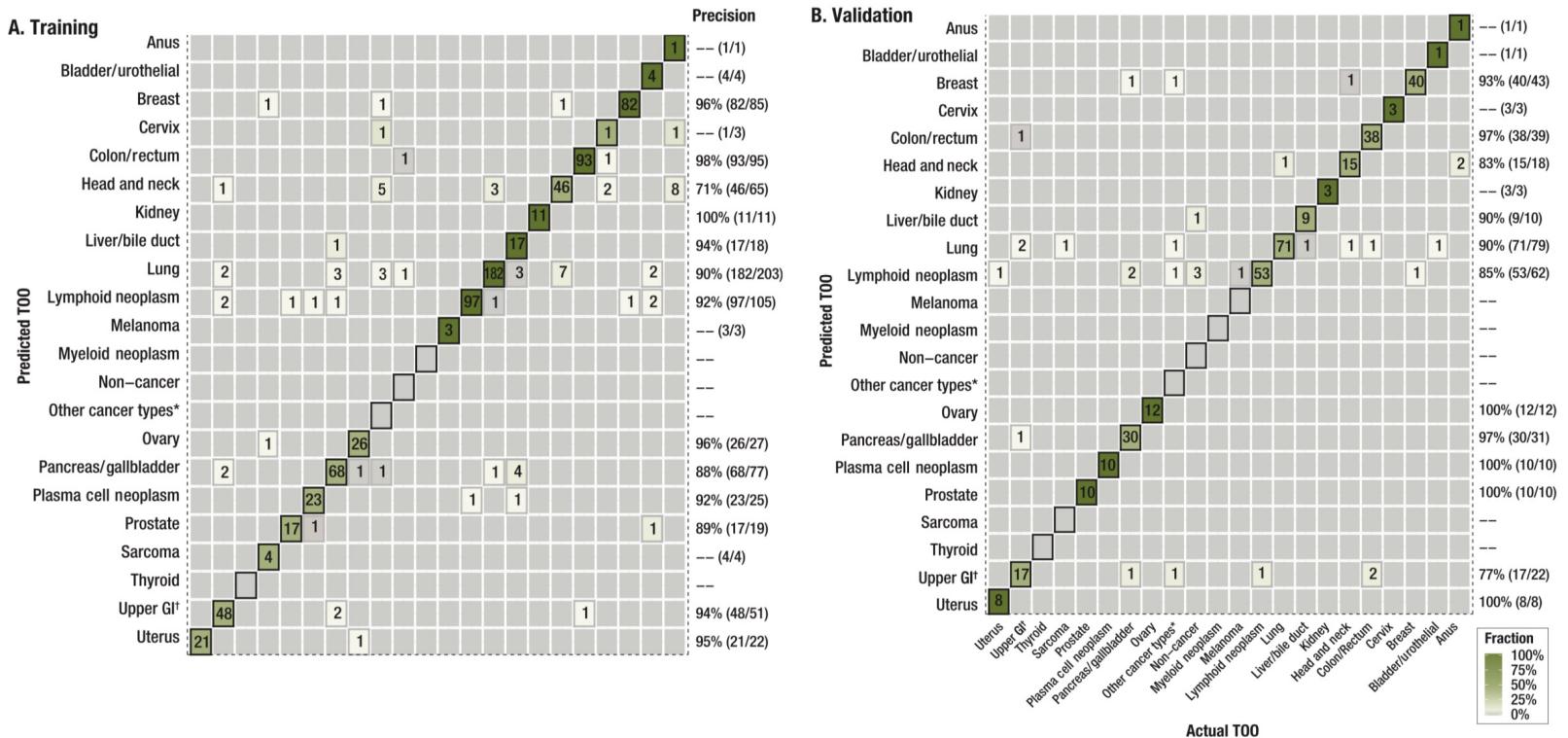
Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA, Annals of Oncology 2020.

Early detection/screening for localized cancer



Good enough?
Thoughts?
How to implement?

Early detection/screening for localized cancer



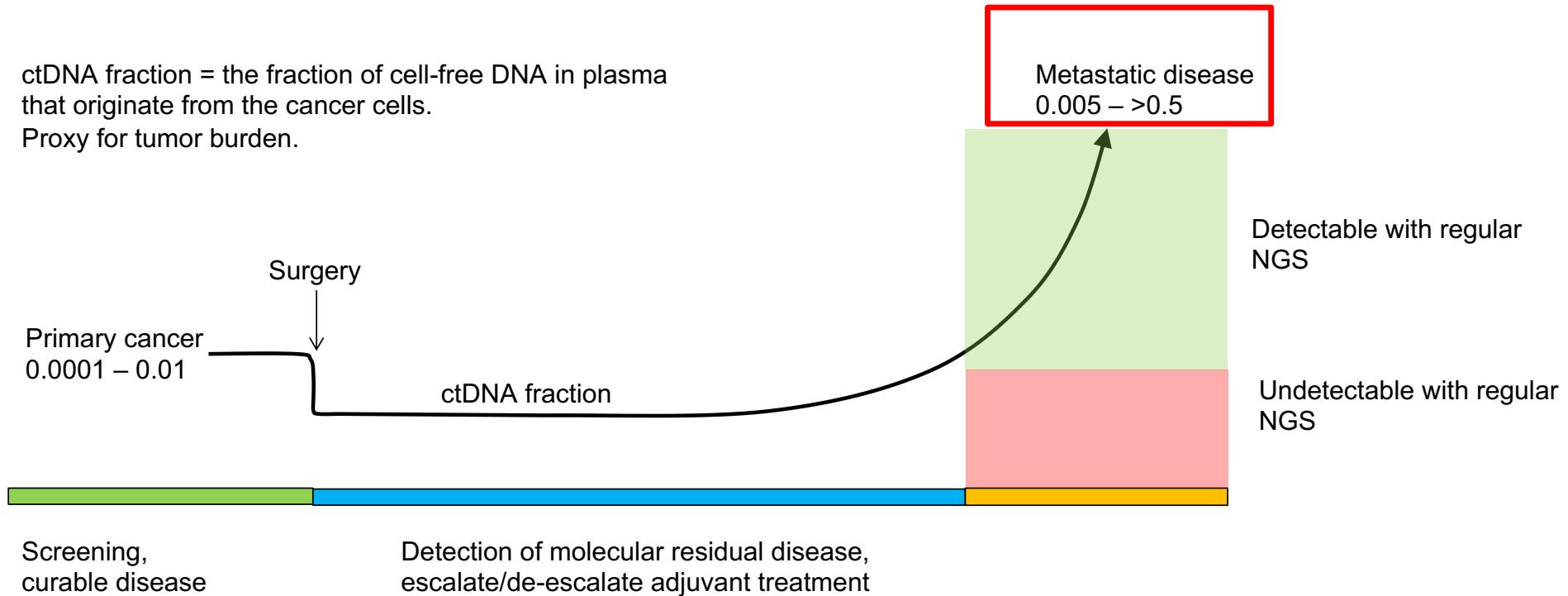
Summary

- Methylation based cfDNA profiling is currently the most promising liquid-biopsy based screening approach as it informs on tissue origin.
- May lower mortality by screening but many questionmarks remain.
 - How to avoid the prostate cancer issues with overtreatment?
 - 10-year prospective trial needed?

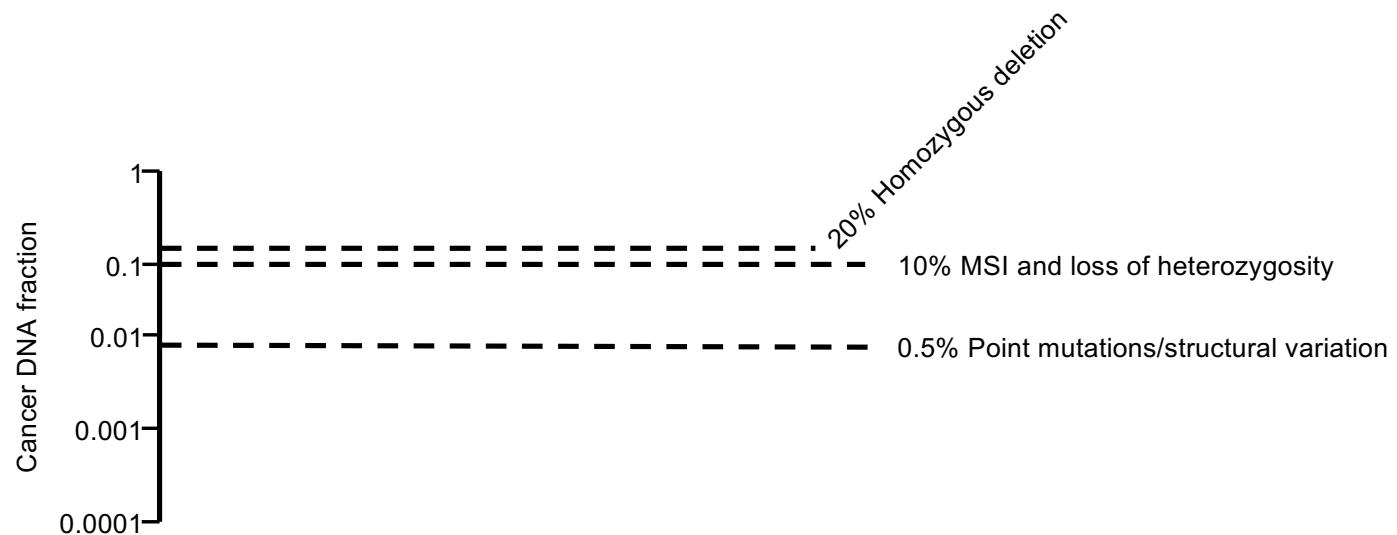
Cancer DNA fraction and consequence for genomic profiling using sequencing

ctDNA fraction = the fraction of cell-free DNA in plasma that originate from the cancer cells.

Proxy for tumor burden.

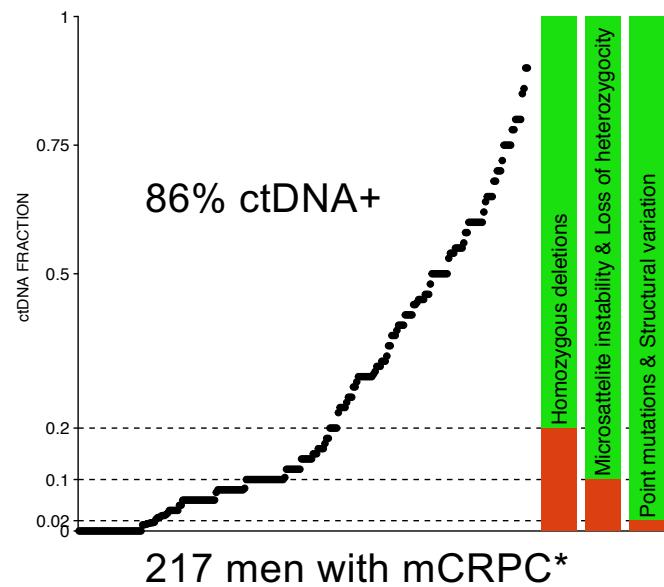


Cancer DNA fraction and consequence for genomic profiling using sequencing



Cancer DNA fraction – the key metric in cancer genomics

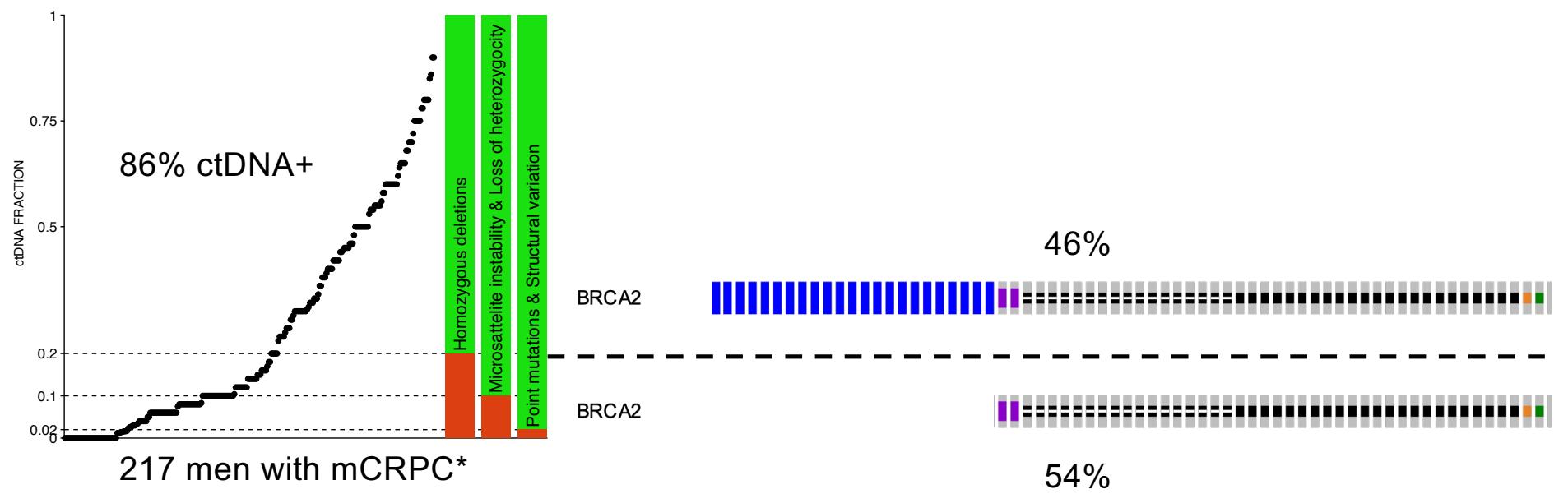
- Different types of somatic alterations require different cancer DNA fraction for detection.
- Affects tumor biopsy analysis as well ctDNA.
 - Macrodissection of biopsies can be applied to increase cancer DNA fraction.



* Mayrhofer et al, Genome Medicine 2018

Cancer DNA fraction – the key metric in cancer genomics

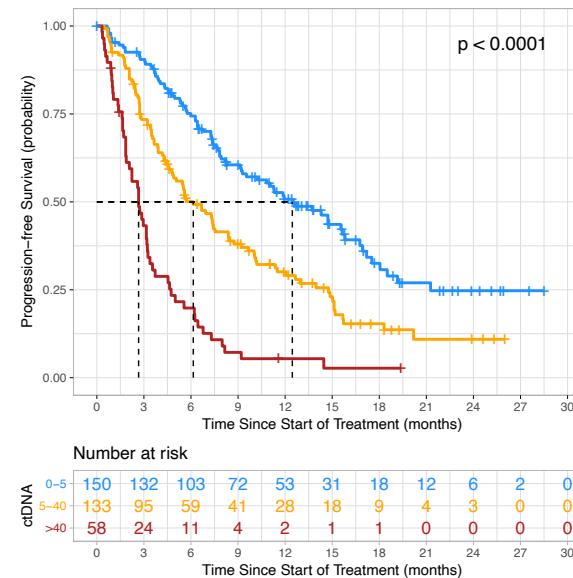
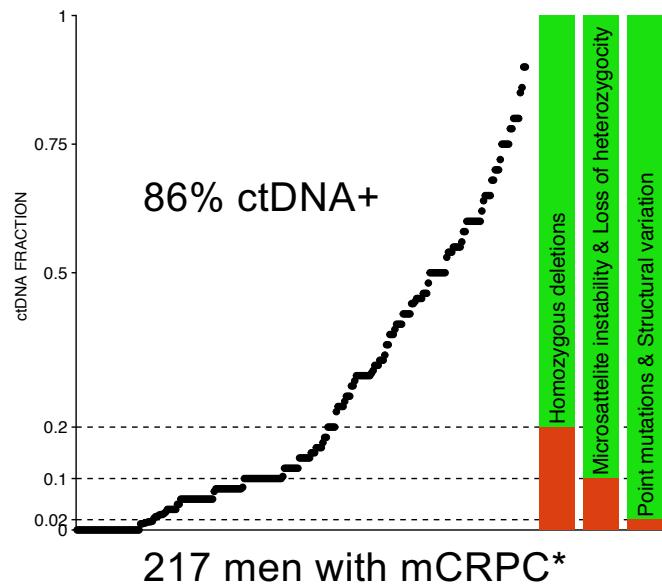
- False negatives is a challenge, must be clearly stated for clinical reporting.
- Important with honest reporting!



* Mayrhofer et al, Genome Medicine 2018

Cancer DNA fraction – the key metric in cancer genomics

- Catch 22 for ctDNA, high cancer DNA fraction = poor prognosis.
- Important to maximize sensitivity for low ctDNA-fraction cases.



* Mayrhofer et al, Genome Medicine 2018

Tissue vs. ctDNA



CLINICAL CANCER RESEARCH | CLINICAL TRIALS: TARGETED THERAPY

Detection of *BRCA1*, *BRCA2*, and *ATM* Alterations in Matched Tumor Tissue and Circulating Tumor DNA in Patients with Prostate Cancer Screened in PROfound



Kim N. Chi¹, Alan Barnicle², Caroline Sibilla³, Zhongwu Lai⁴, Claire Corcoran³, J. Carl Barrett⁴, Carrie A. Adelman⁴, Ping Qiu⁵, Ashley Easter⁶, Simon Dearden³, Geoffrey R. Oxnard⁷, Neeraj Agarwal⁸, Arun Azad⁹, Johann de Bono¹⁰, Joaquin Mateo¹¹, David Olmos¹², Antoine Thiery-Vuillemin¹³, and Elizabeth A. Harrington²

Table 2. BRCA/ATM variant subtype detection sensitivity in ctDNA and tissue.

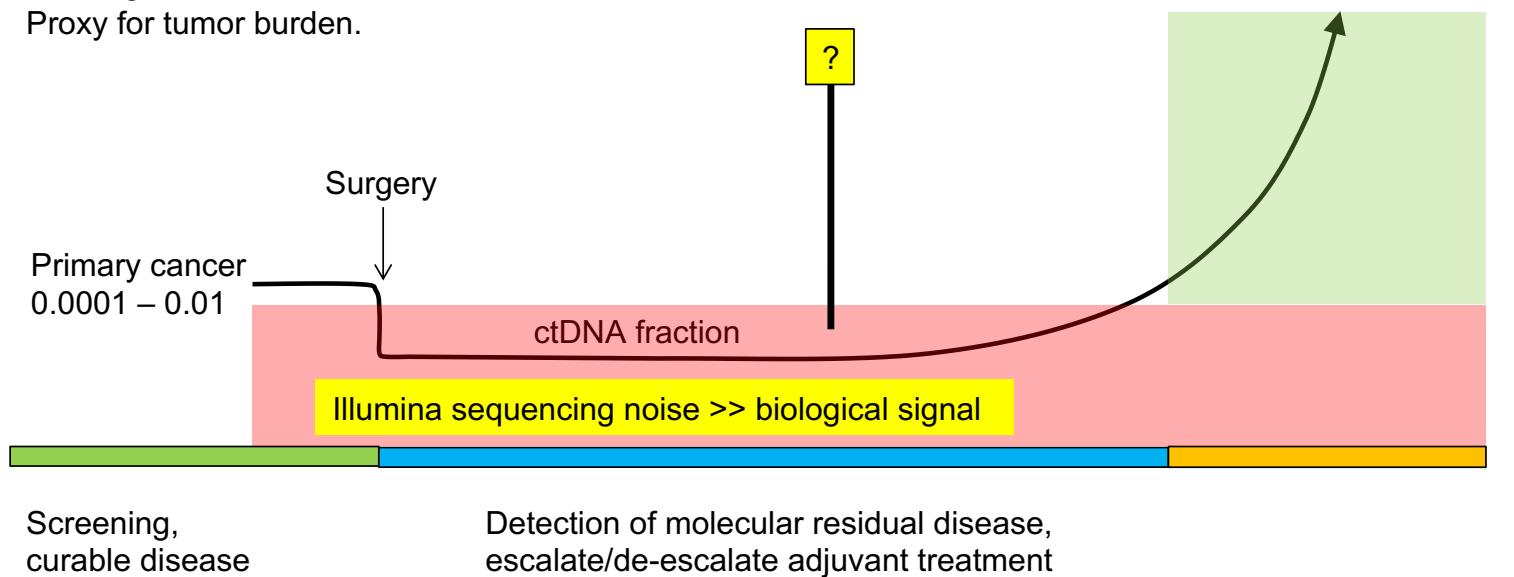
Variant types	Sensitivity of detection in tumor tissue			Sensitivity of detection in ctDNA		
	Detected in tumor tissue	Detected in tumor tissue and ctDNA	Detected in tumor tissue only	Detected in ctDNA	Detected in tumor tissue and ctDNA	Detected in ctDNA only
Frameshift/indel	96	83 (86%)	13 (14%)	110	83 (75%)	27 (25%)
Homozygous loss	30	8 (27%)	22 (73%)	7	7 (100%)	0
Large rearrangement	24	15 (63%)	9 (37%)	23	16 (70%)	7 (30%)
Nonsense	28	26 (93%)	2 (7%)	32	26 (81%)	6 (19%)
Splice	15	13 (87%)	2 (13%)	26	13 (50%)	13 (50%)
Missense	4	1 (25%)	3 (75%)	9	1 (11%)	8 (89%)
Total	197	146 (74%)	51 (26%)	207	146 (71%)	61 (29%)

491 patients who had a biomarker result for both tumor tissue and ctDNA
Note: homdel limit of detection was likely >0.30 ctDNA fraction.

Cancer DNA fraction and consequence for genomic profiling using sequencing

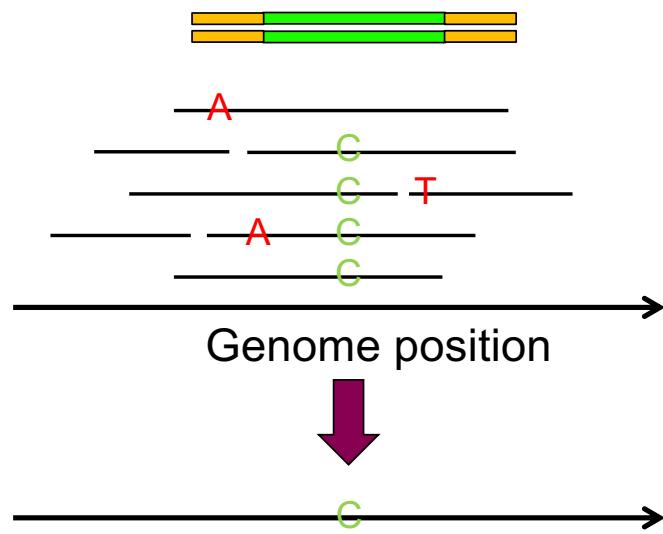
ctDNA fraction = the fraction of cell-free DNA in plasma that originate from the cancer cells.

Proxy for tumor burden.

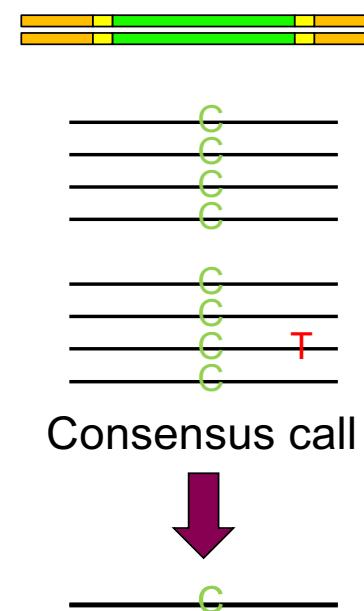


Cancer DNA fraction and consequence for genomic profiling using sequencing

High purity – sequence each DNA fragment once.
~0.5% sensitivity.

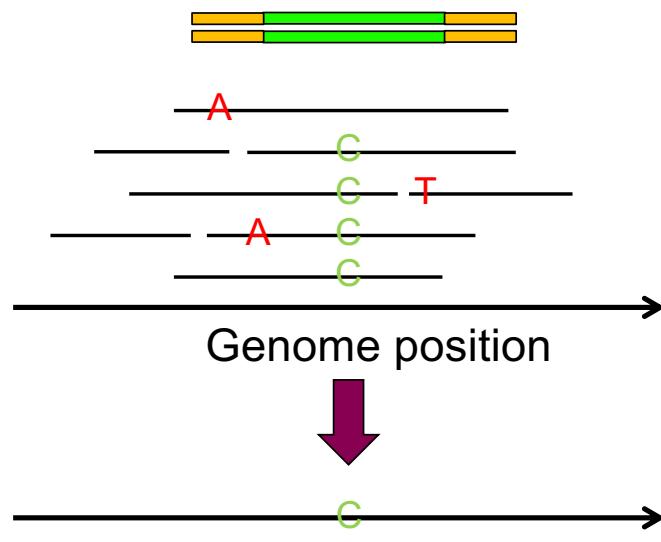


Low purity – sequence each DNA fragment ~10 times.
Error suppression, tunable sensitivity.



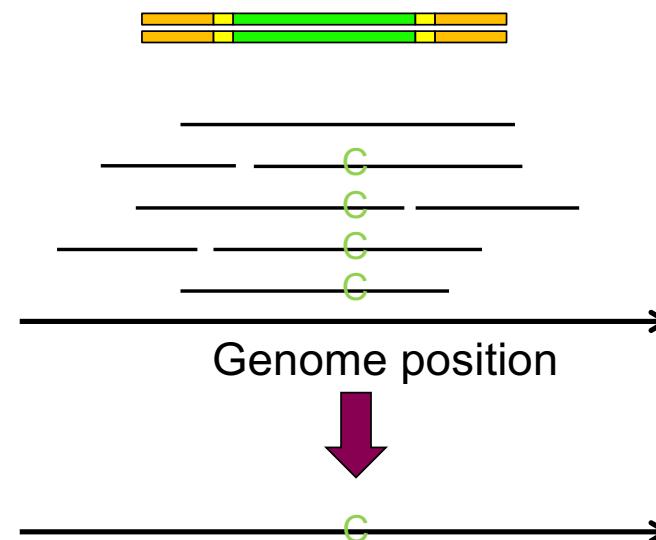
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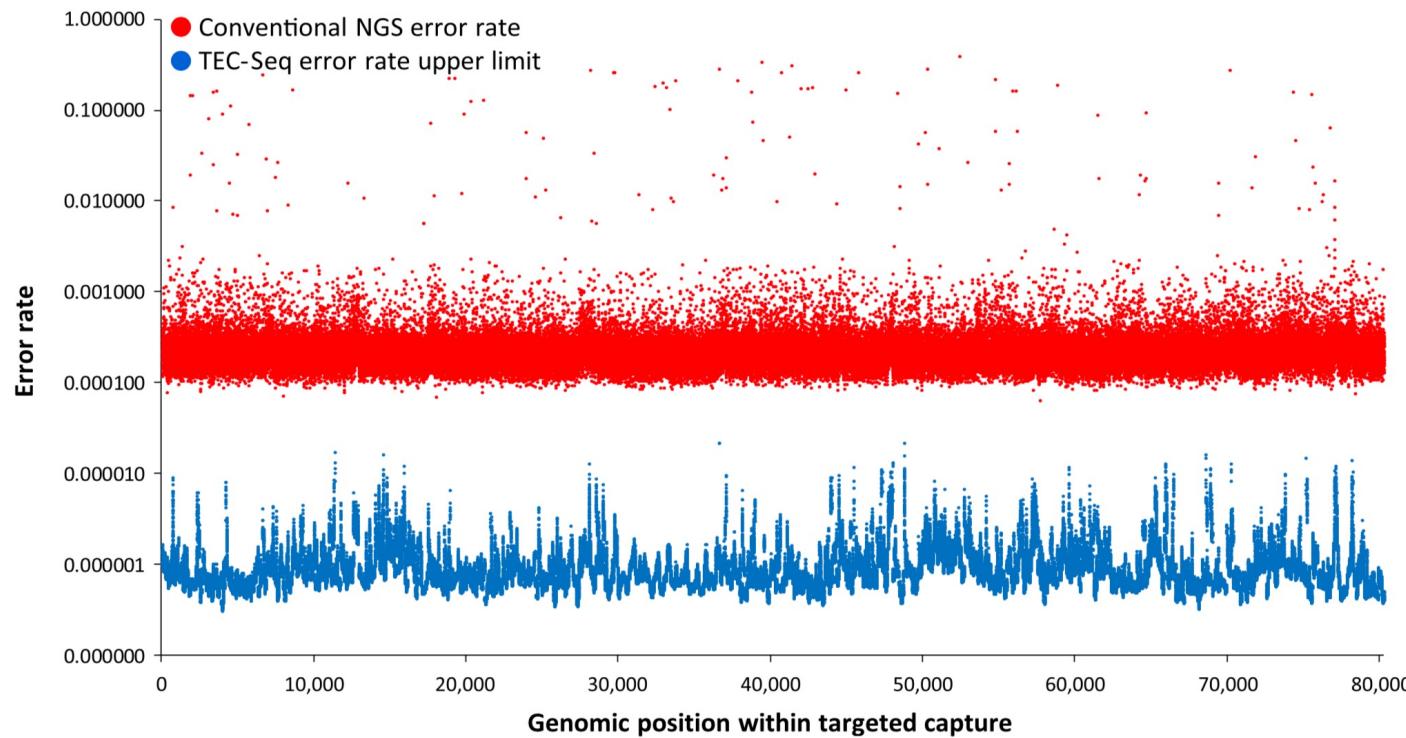
Target coverage <2000X

Low purity – sequence each DNA fragment ~10 times.
Error suppression, tunable sensitivity.



Target coverage 40.000X

Error suppression in a small panel



>99% sensitivity to detect somatic variants at 0.5% allele fraction
>99.9999% specificity with implemented thresholds = 0.05% for hotspot and 0.1% outside hotspot locations.

Direct detection of early-stage cancers using circulating tumor DNA, Sci Trans 2017

Cancer DNA fraction and consequence for genomic profiling using sequencing

- Mini-design: 28 genes chosen for Prostate Cancer (0.1 Mb design).

- Sensitivity:

→ 1/1000

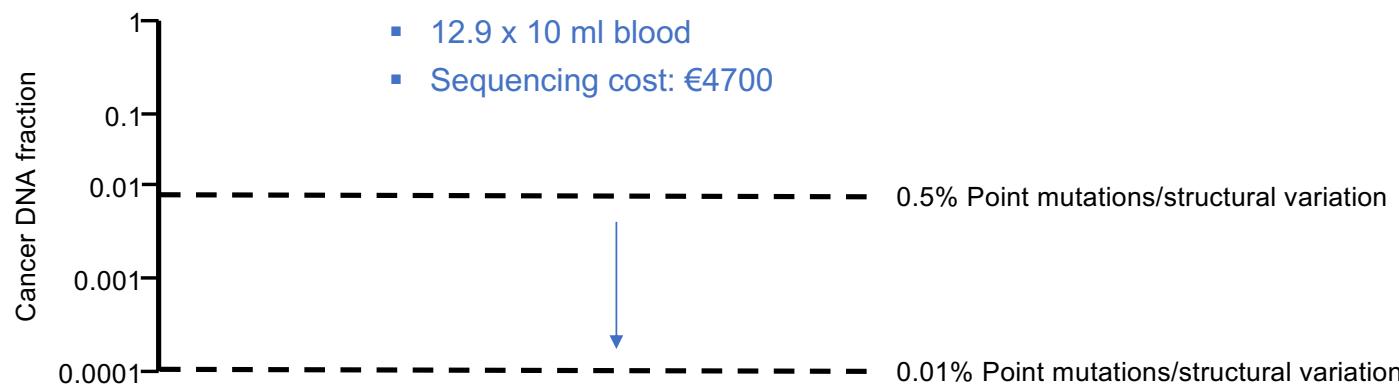
- 1.3 x 10 ml blood
- Sequencing cost: €470

→ 1/5000

- 6.4 x 10 ml blood
- Sequencing cost: €2350

→ 1/10000

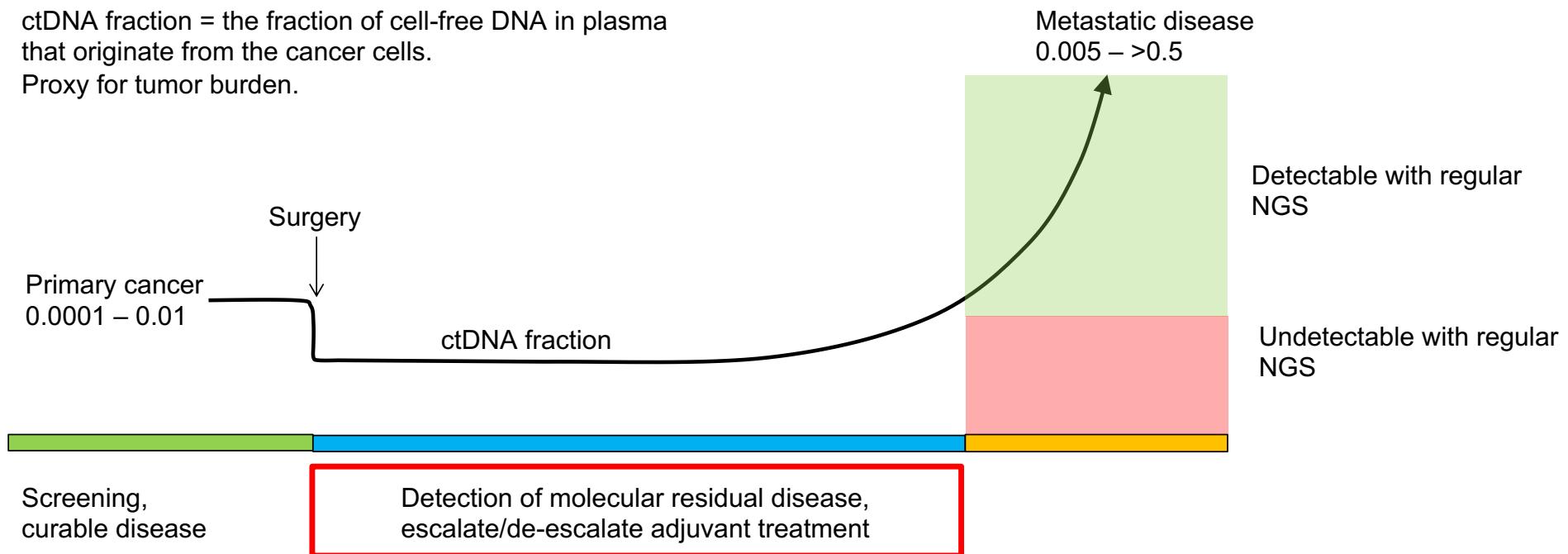
- 12.9 x 10 ml blood
- Sequencing cost: €4700



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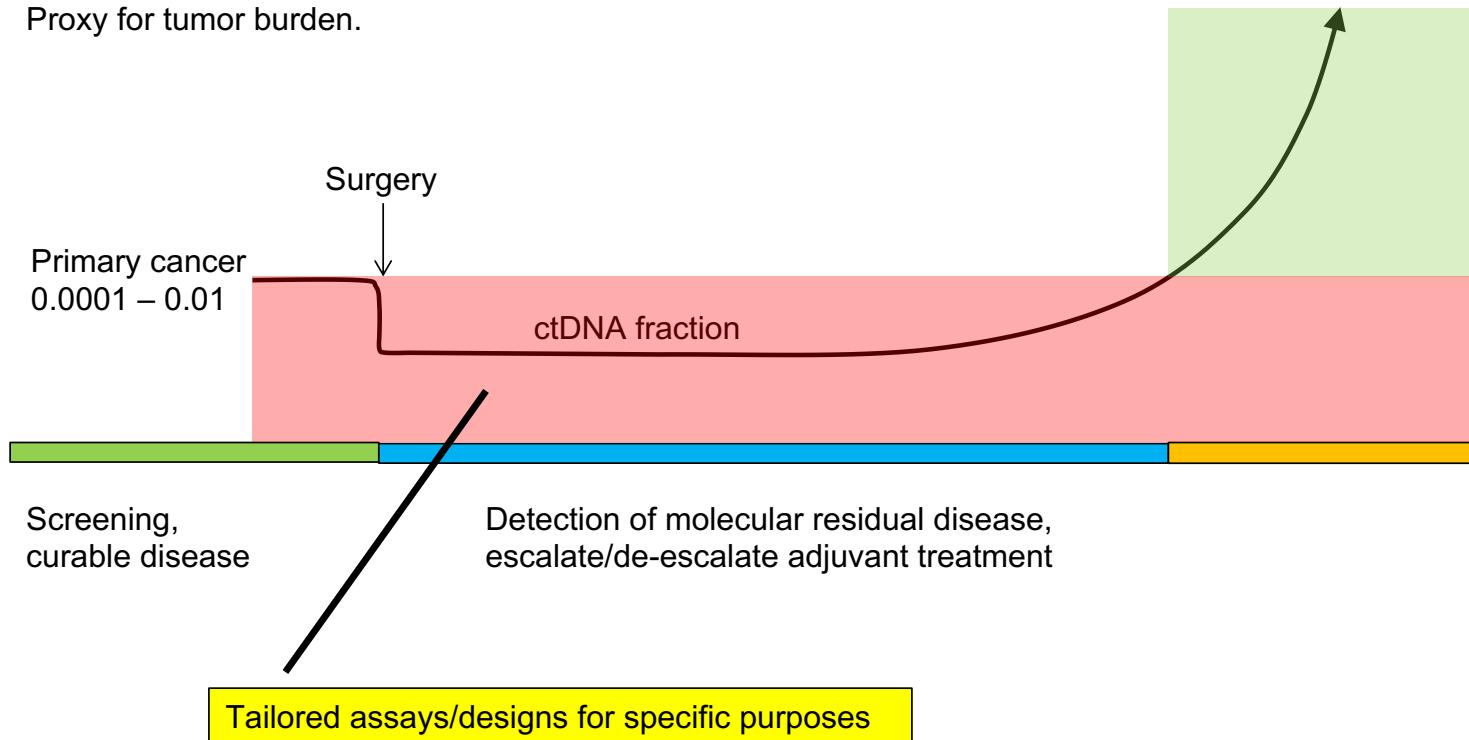
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Cancer DNA fraction and consequence for genomic profiling using sequencing

ctDNA fraction = the fraction of cell-free DNA in plasma that originate from the cancer cells.
Proxy for tumor burden.

Metastatic disease
0.05 – >0.5



MRD sequencing to reduce overtreatment



- Goal of cancer treatment: Live longer and better.
- Chemo has serious side effects
- The role of adjuvant chemotherapy in stage II colon cancer continues to be debated.
- Clinical trial DYNAMIC:
 - assess whether a ctDNA-guided approach could reduce the use of adjuvant chemotherapy without compromising recurrence risk.
- For ctDNA-guided management:
 - ctDNA+ at 4 or 7 weeks prompted chemotherapy.
 - ctDNA- no chemotherapy.
- The primary efficacy end point was recurrence-free survival at 2 years.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

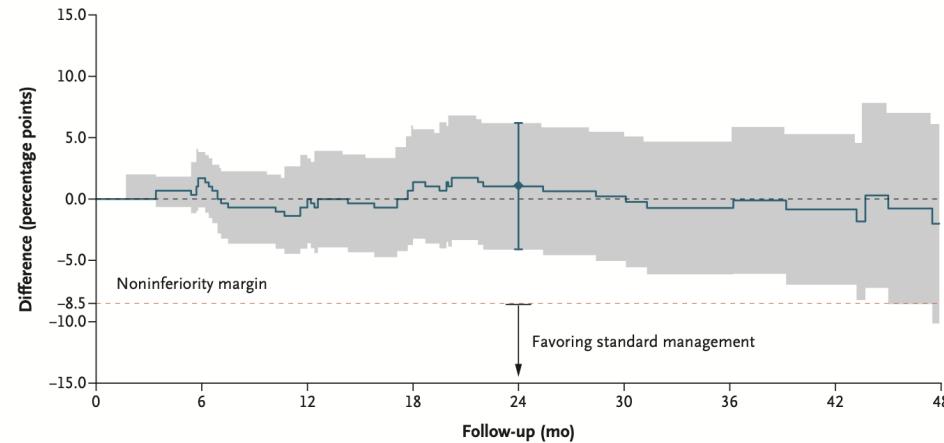
Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer

Jeanne Tie, M.D., Joshua D. Cohen, M.Phil., Kamel Lahouel, Ph.D., Serigne N. Lo, Ph.D., Yuxuan Wang, M.D., Ph.D., Suzanne Kosmider, M.B., B.S., Rachel Wong, M.B., B.S., Jeremy Shapiro, M.B., B.S., Margaret Lee, M.B., B.S., Sam Harris, M.B., B.S., Adnan Khattak, M.B., B.S., Matthew Burge, M.B., B.S., Marion Harris, M.B., B.S., James Lynam, M.B., B.S., Louise Nott, M.B., B.S., Fiona Day, Ph.D., Theresa Hayes, M.B., B.S., Sue-Anne McLachlan, M.B., B.S., Belinda Lee, M.B., B.S., Janine Ptak, M.S., Natalie Silliman, B.S., Lisa Dobbyn, B.A., Maria Popoli, M.S., Ralph Hruban, M.D., Anne Marie Lennon, M.D., Ph.D., Nicholas Papadopoulos, Ph.D., Kenneth W. Kinzler, Ph.D., Bert Vogelstein, M.D., Cristian Tomasetti, Ph.D., and Peter Gibbs, M.D., for the DYNAMIC Investigators*

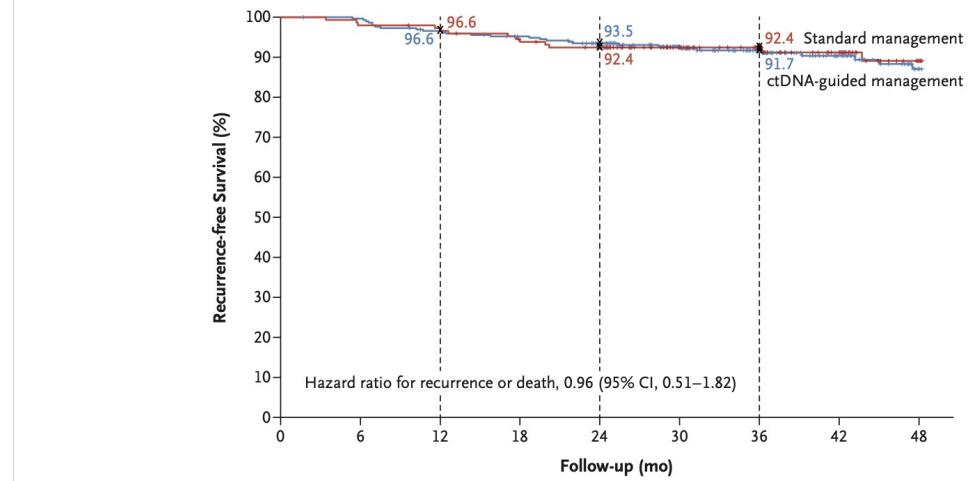
MRD sequencing to reduce overtreatment

- The trial had a positive result

A Between-Group Differences in Recurrence-free Survival



B Kaplan-Meier Estimates of Recurrence-free Survival



No. at Risk

	Standard management	ctDNA-guided management
147	144	142
294	292	281
136	136	128
273	259	207
97	97	78
57	57	109
33	33	64

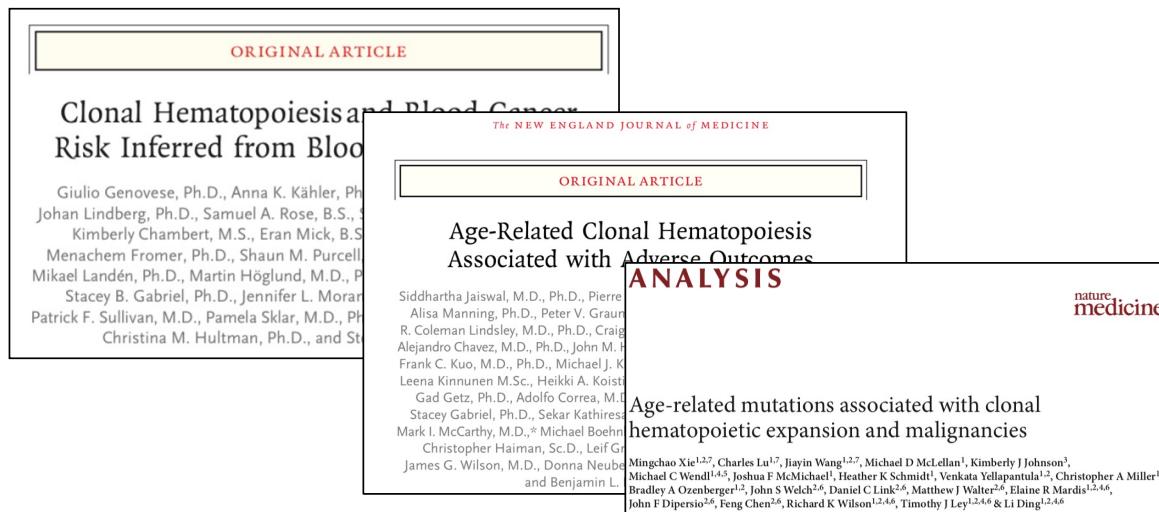
Hazard ratio for recurrence or death, 0.96 (95% CI, 0.51–1.82)

Summary

- Cancer DNA fraction determines what can be detected
- Error correction using molecular barcodes may increase accuracy of mutation calling using illumina sequencing
 - Expensive
 - Limits the scope of genomic profiling
 - Does not help for e.g. copy-number calling
 - Very useful for e.g. minimal residual disease tracking
- Commercial companies therefore goes for mutations – easiest way to motivate “value”
- Detection of ctDNA after surgery is an effective way of detecting early disease recurrence and can be used to escalate/de-escalated treatment.

Clonal expansions in white blood cells

- First described in 2014
- Clonal hematopoiesis
- Associated with risk for hematological cancers



The NEW ENGLAND JOURNAL OF MEDICINE

ORIGINAL ARTICLE

Clonal Hematopoiesis and Risk Inferred from Blood

Julio Genovese, Ph.D., Anna K. Kähler, Ph.D.,
Johan Lindberg, Ph.D., Samuel A. Rose, B.S.,
Kimberly Chamberl, M.S., Eran Mick, B.S.,
Menachem Fromer, Ph.D., Shaun M. Purcell,
Mikael Landén, Ph.D., Martin Höglund, M.D., P.
Stacey B. Gabriel, Ph.D., Jennifer L. Moran,
Patrick F. Sullivan, M.D., Pamela Sklar, M.D., Ph.D.,
Christina M. Hultman, Ph.D., and St

Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes ANALYSIS

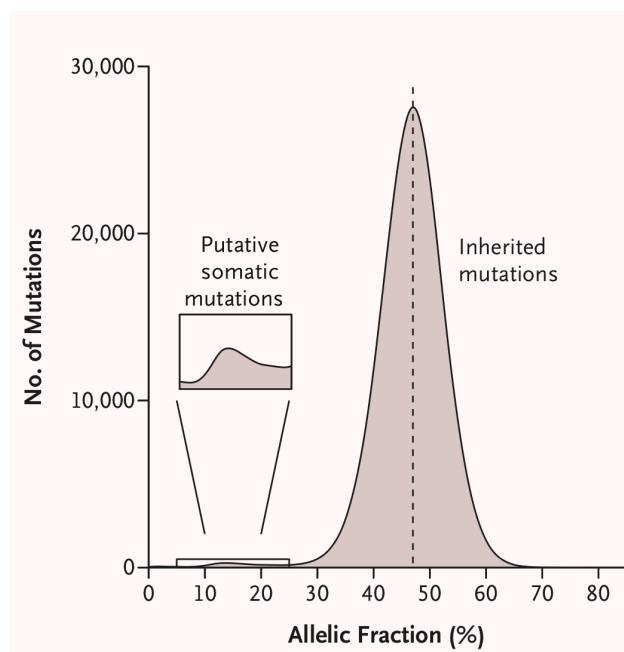
Siddhartha Jaiswal, M.D., Ph.D., Pierre
Alisa Manning, Ph.D., Peter V. Graur,
R. Coleman Lindsley, M.D., Ph.D., Craig
Alejandro Chavez, M.D., Ph.D., John M. H.
Frank C. Kuo, M.D., Ph.D., Michael J. K.
Leena Kinnunen M.Sc., Heikki A. Koist
Gad Getz, Ph.D., Adolfo Correa, M.D.,
Stacey Gabriel, Ph.D., Sekar Kathiresan,
Mark I. McCarthy, M.D., Michael Boehn,
Christopher Haiman, Sc.D., Leif Gr
James G. Wilson, M.D., Donna Neub
and Benjamin L.

nature medicine

Mingchao Xie^{1,2,7}, Charles Lu^{1,7}, Jayin Wang^{1,2,7}, Michael D McLellan¹, Kimberly J Johnson³,
Michael C Wendt^{1,4,5}, Joshua F McMichael¹, Heather K Schmidt⁴, Venkata Yellapantula^{1,2}, Christopher A Miller¹,
Bradley A Ozenberger^{1,2}, John S Welch^{2,6}, Daniel C Link^{2,6}, Matthew J Walter^{2,6}, Elaine R Mardis^{1,2,4,6},
John F DiPersio^{2,6}, Feng Chen^{2,6}, Richard K Wilson^{1,2,4,6}, Timothy J Ley^{1,2,4,6} & Li Ding^{1,2,4,6}

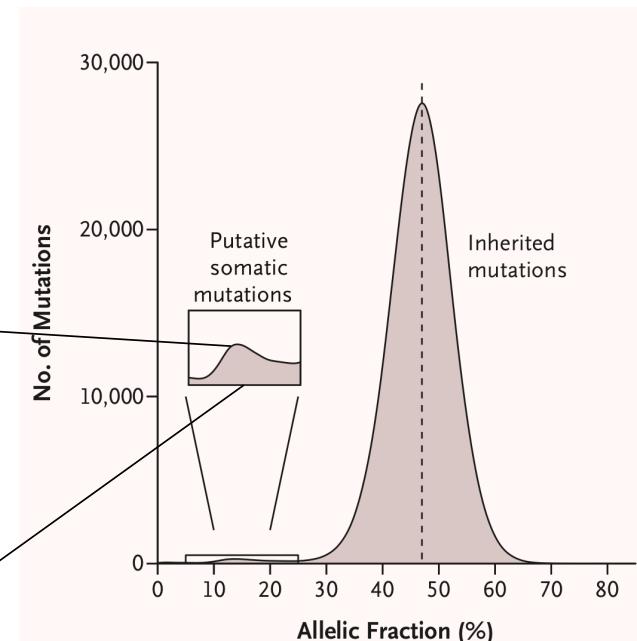
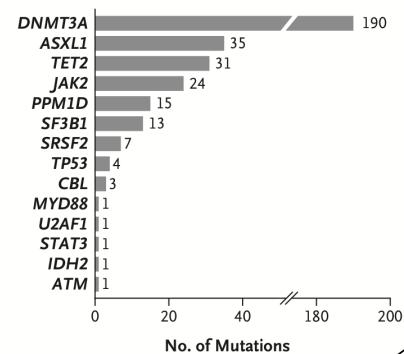
Clonal expansions in white blood cells

- Goal to associate germ-line variation to schizophrenia/bipolar disorder
- A technical artifact

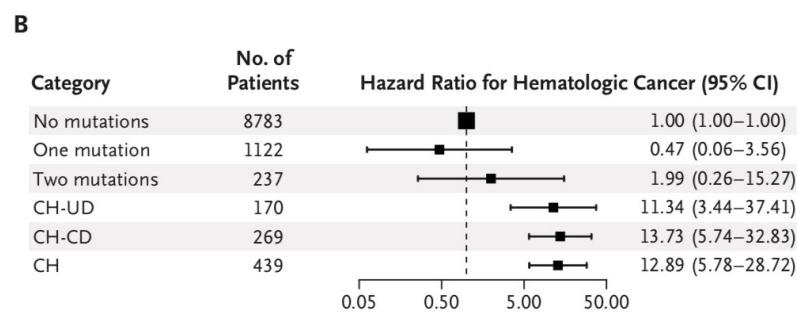
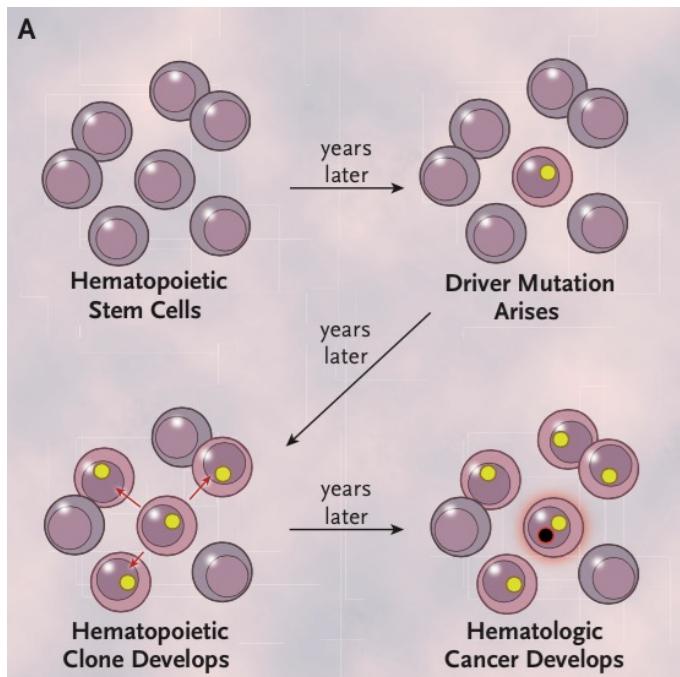


Clonal expansions in white blood cells

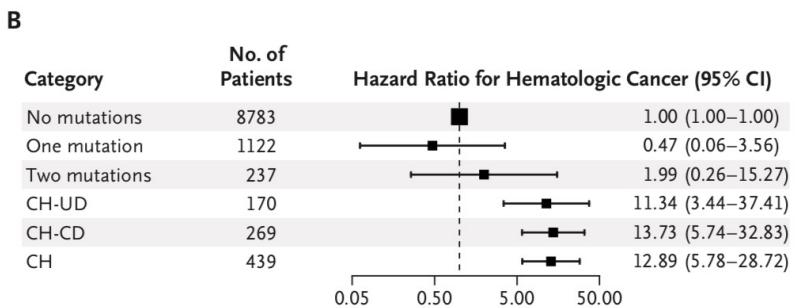
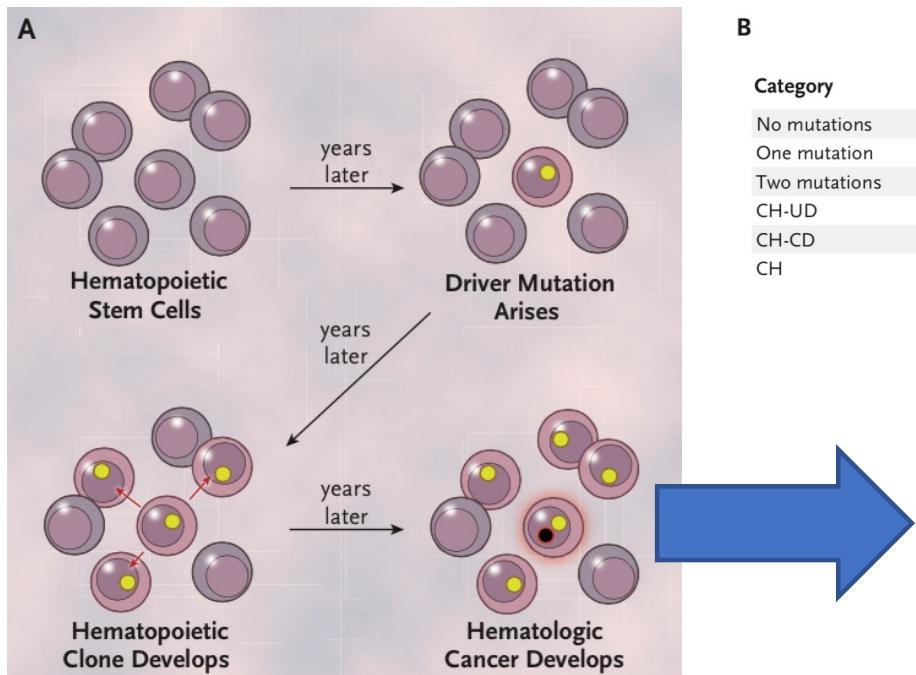
- Goal to associate germ-line variation to schizophrenia/bipolar disorder
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Clonal expansions in white blood cells



Clonal expansions in white blood cells



Ends up in plasma!
Remedy – sequence white blood cell DNA, case closed?

How frequent is CH in a relevant population?



Cancer therapy shapes the fitness landscape of clonal hematopoiesis

Kelly L. Bolton¹, Ryan N. Ptashkin^{2,34}, Teng Gao^{3,34}, Lior Braunstein⁴, Sean M. Devlin⁵, Daniel Kelly⁶, Minal Patel⁷, Antonin Berthon³, Ajiazuddin Syed², Mariko Yabe⁸, Catherine C. Coombs⁹, Nicole M. Caltabellotta⁷, Mike Walsh¹⁰, Kenneth Offit¹⁰, Zsofia Stadler¹¹, Diana Mandelker², Jessica Schulman⁷, Akshar Patel⁷, John Philip¹², Elsa Bernard¹³, Gunes Gundem³, Juan E. Arango Ossa⁷, Max Levine¹³, Juan S. Medina Martinez¹³, Noushin Farnoud⁷, Dominik Glodzik², Sonya Li¹⁰, Mark E. Robson¹⁰, Choonsik Lee¹⁴, Paul D. P. Pharoah^{15,16}, Konrad H. Stöpsack¹⁰, Barbara Spitzer¹³, Simon Mantha¹⁷, James Fagin^{10,18}, Laura Boucail¹⁹, Christopher J. Gibson²⁰, Benjamin L. Ebert²⁰, Andrew L. Young²¹, Todd Druley²², Koichi Takahashi²³, Nancy Gillis^{24,25}, Markus Ball^{25,26}, Eric Padron²⁵, David M. Hyman^{10,27}, Jose Baselga²⁸, Larry Norton^{10,27}, Stuart Gardos^{10,27}, Virginia M. Klimek^{10,27}, Howard Scher^{10,27}, Dean Bajorin^{10,27}, Eder Paraiso^{19,29}, Ryma Benayed², Maria E. Arcila², Marc Ladanyi², David B. Solit^{10,19,30}, Michael F. Berger^{2,19,30}, Martin Tallman¹, Montserrat Garcia-Closas¹³, Nilanjana Chatterjee³¹, Luis A. Diaz Jr^{10,32,33}, Ross L. Levine¹, Lindsay M. Morton¹⁴, Ahmet Zehir^{1,2,34,35} and Elli Papaemmanuil^{1,3,34,35}

- 24146 cancer patients
- 75% >50 years old
- MSK-IMPACT
 - Broad panel sequencing
 - Tumor 750x coverage
 - Normal 500x coverage
- CH in 30% of patients
- CH variant allele fraction
 - Median 5.0% (range, 2–78%)
- 50% of CH variants are drivers in cancer genes

Commercial assays are commonly not running germline analysis

- Why?
 - Cost
 - Ethical issues with germline
- Leads to false positives

Letters

RESEARCH LETTER

Patient-Paired Sample Congruence Between 2 Commercial Liquid Biopsy Tests

Torga et al, Jama Oncology 2017

COMMENT & RESPONSE

Regarding the Congruence Between 2 Circulating Tumor DNA Sequencing Assays

To the Editor Torga and Pienta¹ reported low congruence between

COMMENT & RESPONSE

Regarding the Congruence Between 2 Circulating Tumor DNA Sequencing Assays

To the Editor The recent Research Letter¹ comparing 2 cell-free

COMMENT & RESPONSE

Regarding the Congruence Between 2 Circulating Tumor DNA Sequencing Assays

To the Editor We read with interest the article by Torga and

COMMENT & RESPONSE

Regarding the Congruence Between 2 Circulating Tumor DNA Sequencing Assays

To the Editor The blood-diagnostic field has evolved in the past

COMMENT & RESPONSE

Regarding the Congruence Between 2 Circulating Tumor DNA Sequencing Assays

To the Editor The recent analysis by Torga and Pienta¹ attempted

Deeper sequencing identifies more clonal hematopoiesis



nature
medicine

ARTICLES

<https://doi.org/10.1038/s41591-019-0652-7>

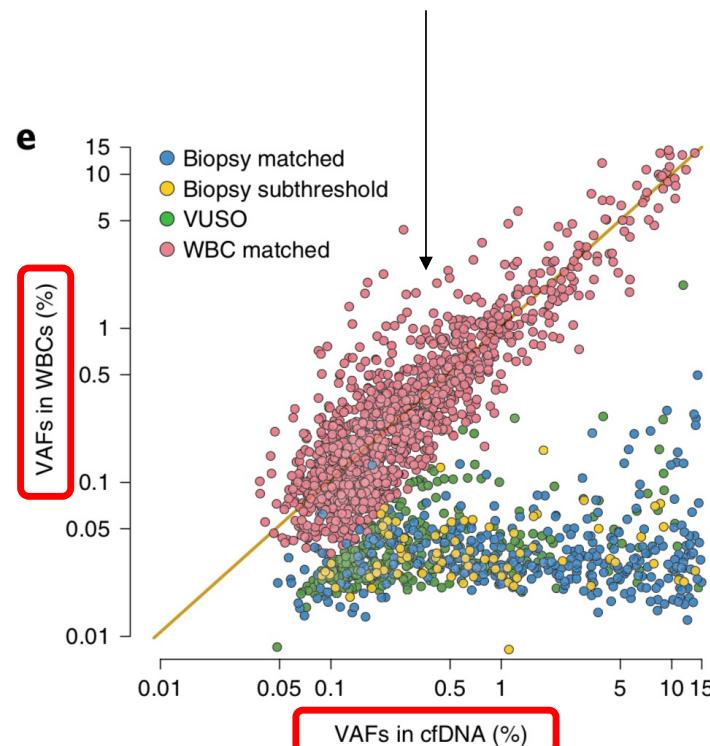
High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants

Pedram Razavi^{①,2,13*}, Bob T. Li^{②,13}, David N. Brown^{3,13}, Byoungsok Jung⁴, Earl Hubbell⁴, Ronglai Shen⁵, Wassim Abida⁶, Krishna Juluru^{③,6}, Ino De Brujin^⑦, Chenlu Hou⁴, Oliver Venn⁴, Raymond Lim³, Aseem Anand¹, Tara Maddala⁴, Sante Gnerre⁴, Ravi Vijaya Satya⁴, Qinwen Liu⁴, Ling Shen⁴, Nicholas Eattock⁴, Jeanne Yue⁴, Alexander W. Blocker^{④,9}, Mark Lee^{4,10}, Amy Sehnert^{④,11}, Hui Xu⁴, Megan P. Hall^④, Angie Santiago-Zayas¹, William F. Novotny^{4,12}, James M. Isbell⁸, Valerie W. Rusch⁸, George Plitas⁸, Alexandra S. Heerdt⁸, Marc Ladanyi¹³, David M. Hyman^①, David R. Jones⁸, Monica Morrow^⑤, Gregory J. Riely¹, Howard I. Scher¹, Charles M. Rudin^①, Mark E. Robson^①, Luis A. Diaz Jr.^①, David B. Solit^{①,2,7}, Alexander M. Aravanis⁴ and Jorge S. Reis-Filho^{②,3*}

- GRAIL and MSK collaboration
- Broad AND deep sequencing
 - 2 Mb panel
 - 60.000x = €8.000 / sample!
- Main finding
 - The vast majority of cfDNA mutations (81.6% in controls and 53.2% in patients with cancer) were due to CH

Clonal hematopoiesis - a real problem

False positive variants with Foundation Medicine OR guardant360



A commercial cfDNA-only solution and variant allele fractions

Precision Medicine and Imaging

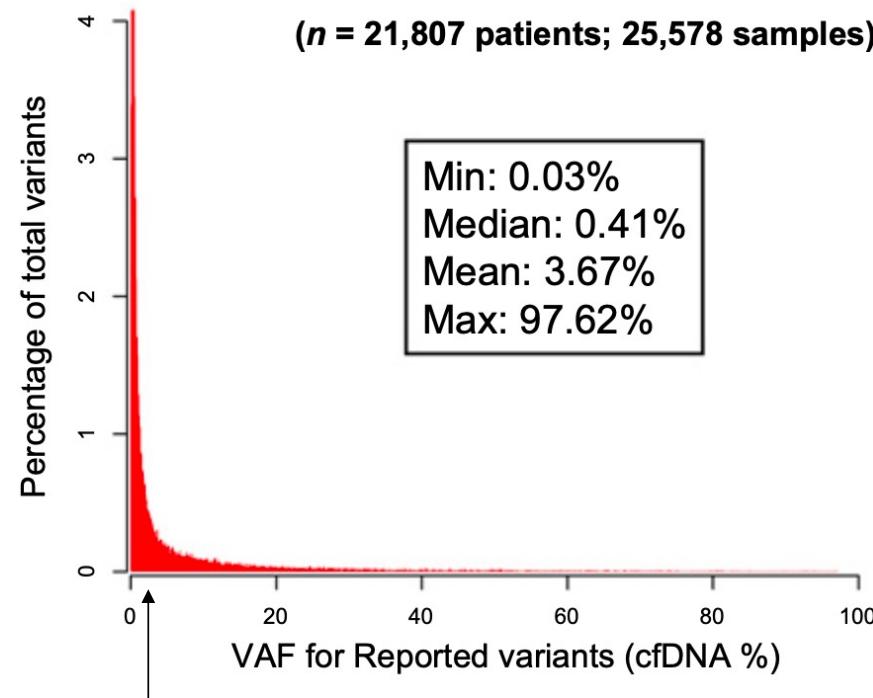
The Landscape of Actionable Genomic Alterations in Cell-Free Circulating Tumor DNA from 21,807 Advanced Cancer Patients 

Oliver A. Zill¹, Kimberly C. Banks¹, Stephen R. Fairclough¹, Stefanie A. Mortimer¹, James V. Vowles¹, Reza Mokhtari¹, David R. Gandara², Philip C. Mack², Justin I. Odegaard¹, Rebecca J. Nagy¹, Arthur M. Baca¹, Helmy Eltoukhy¹, Darya I. Chudova¹, Richard B. Lanman¹, and AmirAli Talasaz¹

Clinical
Cancer
Research

- Guardant360
- cfDNA ONLY sequencing
- 70 cancer genes
- 21,807 late-stage cancers across >50 cancer types

A commercial cfDNA-only solution and variant allele fractions



- No way of doing a good job in the lower VAF range

Clonal hematopoiesis - a real problem

Research

JAMA Oncology | Brief Report

Association of Clonal Hematopoiesis in DNA Repair Genes With Prostate Cancer Plasma Cell-free DNA Testing Interference

Kendal Jensen, MD, PhD; Eric Q. Konnick, MD; Michael T. Schweizer, MD; Alexandra O. Sokolova, MD; Petros Grivas, MD, PhD; Heather H. Cheng, MD, PhD; Nola M. Klemfuss, MHA; Mallory Beightol, BS, MB; Evan Y. Yu, MD; Peter S. Nelson, MD; Bruce Montgomery, MD; Colin C. Pritchard, MD, PhD

- 69 mCRPC cases
- ctDNA + gDNA panel sequencing
- Seven men had CHIP variants in DNA repair genes used to determine PARPi candidacy
 - ATM (n = 5), BRCA2 (n = 1), and CHEK2 (n = 1)
 - ~50% of somatic DNA-repair variants
- The BRCA2 patient was also tested with a commercial assay and was recommended PARPi.

Summary

- Clonal expansions in the white blood cells need to be taken into account when performing liquid biopsy base profiling to avoid false positive findings
 - False positive treatment recommendations will happen
 - Detected DNA-repair variant due to CH
 - False negative treatment recommendations will happen
 - ctDNA is below detection limit but CH variants are detected and interpreted as ctDNA-variants
 - The MD should then be recommended to acquire tissue
 - Suboptimal identification of gDNA variants

FoundationOne Liquid CDx run on a mCRPC case



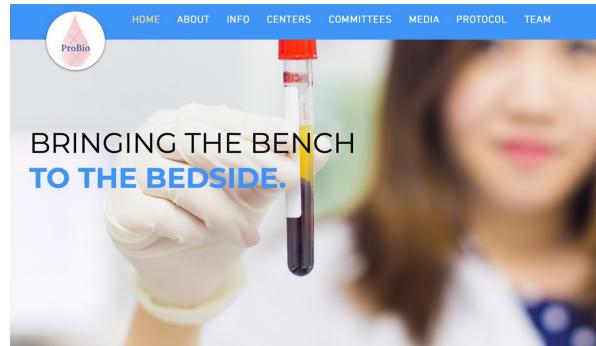
- In Sweden, broad genomic profiling is not reimbursed
- Patients go to Docrates (Finland) and bring back FMI reports
- An oncologist at St Görans reached out and asked for assistance to interpret a report from Foundation Medicine
 - Patient wanted Pembrolizumab
- Patient was included into a study at Karolinska and analysed using the ProBio assay

ProBio = a prospective clinical trial in metastatic prostate cancer

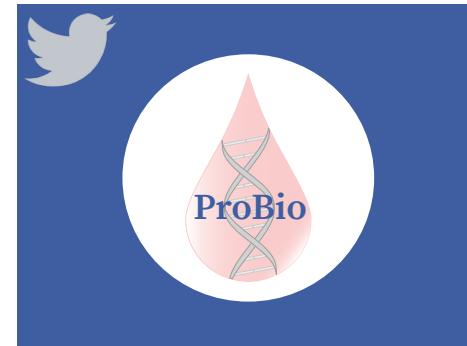
STUDY PROTOCOL Open Access

The ProBio trial: molecular biomarkers for advancing personalized treatment decision in patients with metastatic castration-resistant prostate cancer

Alessio Crippa^{1*} , Bram De Laere^{1,2}, Andrea Discacciati¹, Berit Larsson¹, Jason T. Connor^{3,4}, Erin E. Gabriel¹, Camilla Thellenberg⁵, Elin Jänes⁶, Gunilla Enblad⁷, Anders Ullen⁸, Marie Hjälm-Eriksson⁹, Jan Oldenburg¹⁰, Piet Ost¹¹, Johan Lindberg¹, Martin Eklund¹ and Henrik Grönberg¹



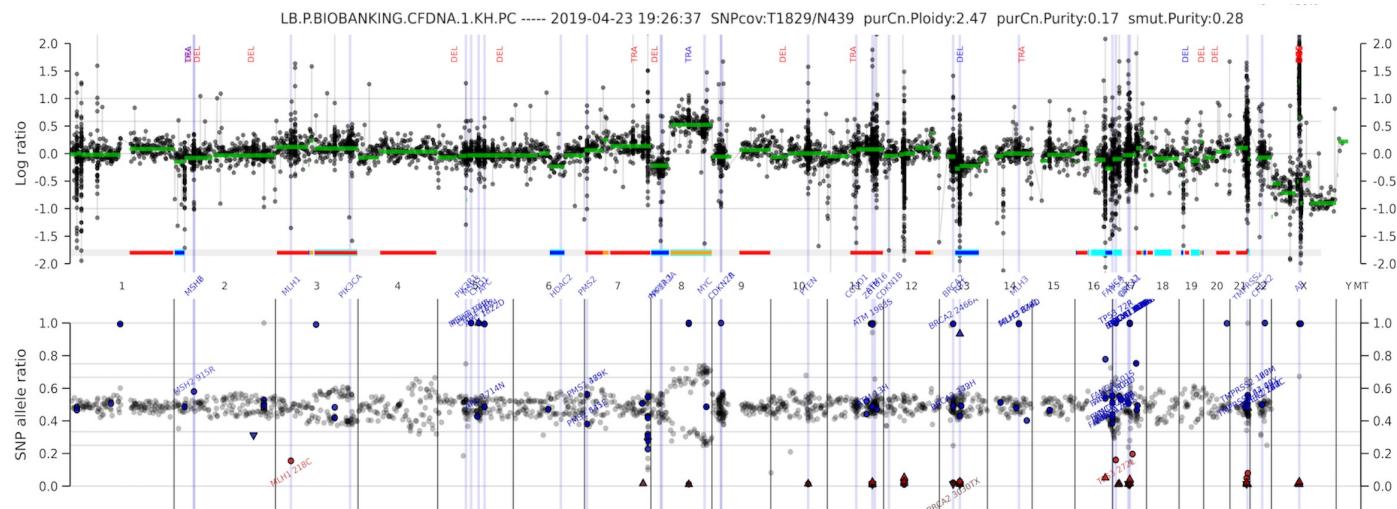
► www.probiotrial.org



► @ProBioTrial

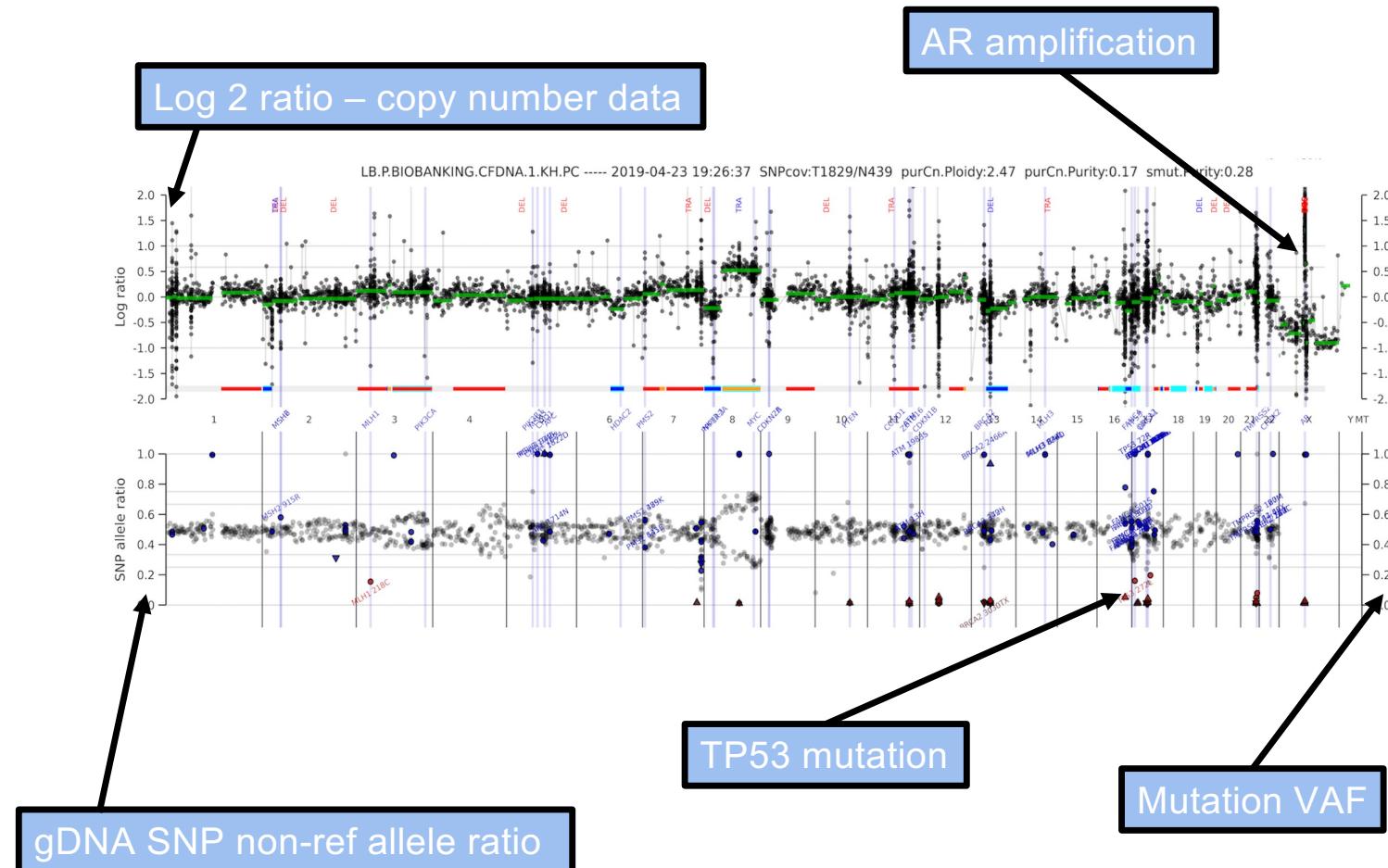
ProBio = a prospective clinical trial in metastatic prostate cancer

ProBio panel = Custom ctDNA panel for metastatic prostate cancer (1.48 Mb)



- **Mutations** in 78 genes
 - **Structural rearrangements** in 10 genes
 - SNP backbone for genome-wide **copy number alternations**
 - 63 microsatellites to infer **MSI**
 - Tumor Mutational Burden (**TMB**) estimation
 - Estimation of circulating tumor burden by **ctDNA fraction**

ProBio – liquid biopsy





FOUNDATIONONE® LIQUID CDx

PATIENT
03-2020-00026951, FI

TUMOR TYPE
Prostate cancer (NOS)
COUNTRY CODE
FI

REPORT DATE
16 Sep 2020
ORDERED TEST #
ORD-0892843-01

ABOUT THE TEST FoundationOne®Liquid CDx is a next generation sequencing (NGS) assay that identifies clinically relevant genomic alterations in circulating cell-free DNA.

PATIENT

DISEASE Prostate cancer (NOS)

NAME 03-2020-00026951, FI

DATE OF BIRTH [REDACTED]

SEX Male

MEDICAL RECORD # Not given

PHYSICIAN

ORDERING PHYSICIAN [REDACTED]

MEDICAL FACILITY Docrates Syopasairaala

ADDITIONAL RECIPIENT None

MEDICAL FACILITY ID [REDACTED]

PATHOLOGIST Provided, Not

SPECIMEN

SPECIMEN ID 03-2020-0002695112/12/1954

SPECIMEN TYPE Blood

DATE OF COLLECTION 28 August 2020

SPECIMEN RECEIVED 02 September 2020

Sensitivity for the detection of alterations and genomic signatures is reduced due to sample quality.

Genomic Signatures

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Gene Alterations

For a complete list of the genes assayed, please refer to the Appendix.

AR L702H, H875Y

CDK12 K482fs*14

ALK deletion exons 2-12

TP53 C275Y

14 Therapies Approved in the EU

20 Clinical Trials

3 Therapies with Lack of Response

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

THERAPY AND CLINICAL TRIAL IMPLICATIONS

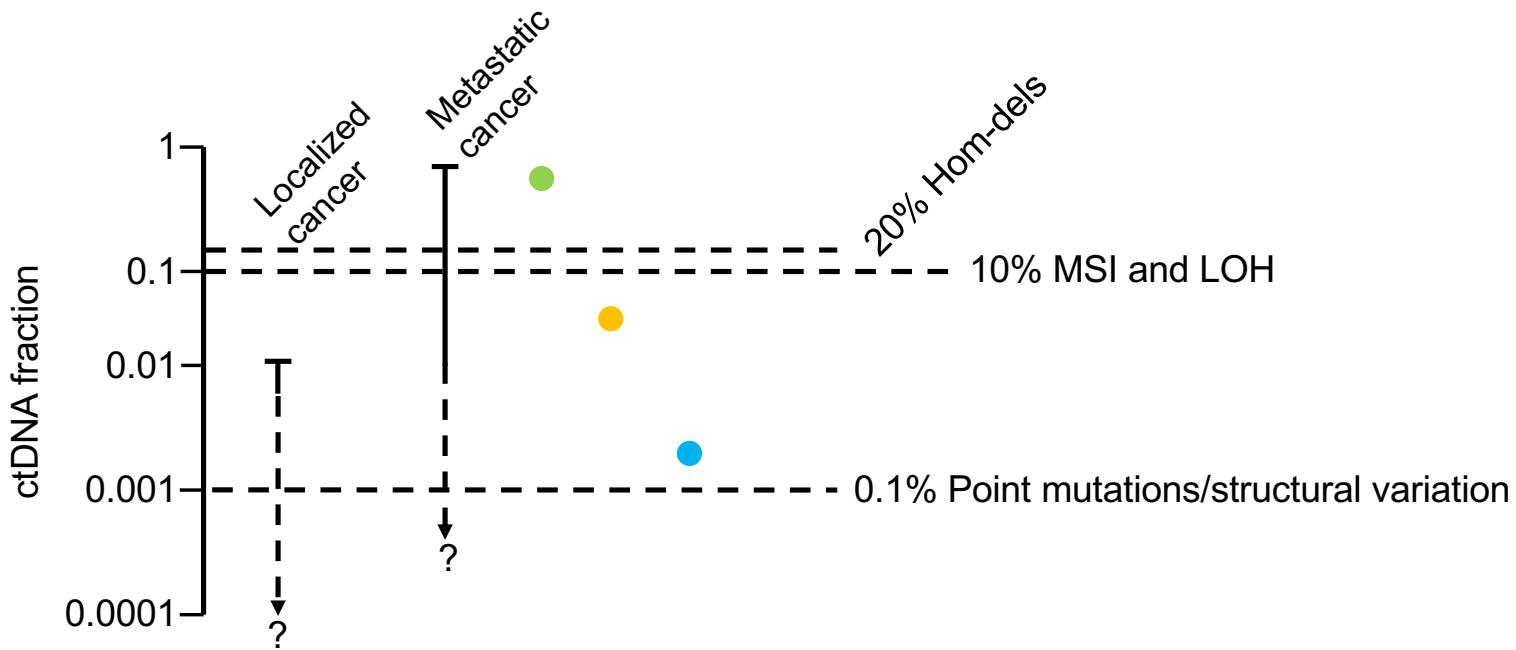
No therapies or clinical trials. See Genomic Signatures section

Unable to determine Microsatellite status due to insufficient evidence of genomic instability.

Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.

i

Figure for the ESMO Precision Medicine Working Group guidelines paper



- Example case 1: The ctDNA fraction is high enough to detect all types of somatic alterations. If a variant is present but not detected, then it is a false negative.
- Example case 2: The ctDNA fraction is too low to detect homozygous deletions, MSI or LOH. Therefore the test is non-informative for these types of somatic alterations. The MD should be recommended to do tissue biopsy for testing of e.g. homdel of BRCA2
- Example case 3: The ctDNA fraction is high enough to detect mutations, however, only 20 ng of cfDNA was used as input which caps sensitivity at approximately 1/500. Therefore the test is non-informative for mutations.



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Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Genomic Signatures section

due to insufficient evidence of genomic

percentage of circulating-tumor DNA (ctDNA) based on observed aneuploid instability.

Important, ctDNA fraction high enough to be able to find ALL types of somatic variation



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CDK12 K482fs*14 →

ALK deletion exons 2-12

TP53 C275Y

Pembro recommendation.
However 1 hit in CDK12 not
enough to recommend Pembro

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3 Therapies with Lack of Response

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Genomic Signatures section

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*CDK12 K482fs*14*

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TP53 C275Y

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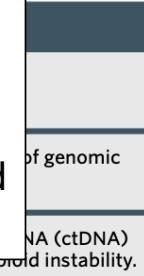
GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 →
Muts/Mb

Microsatellite status - Cannot Be
Determined

Tumor Fraction - 22%

Weird to present this TMB, very close to FDA-approved limit to allow PemBro however NO rationale whatsoever for this based on the data presented.



present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.



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ALK deletion exons 2-12

TP53 C275Y

Found with ProBio assay

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3 Therapies with Lack of Response

GENOMIC SIGNATURES

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THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Genomic Signatures section

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Tumor Fraction - 22%

Gene Alterations

For a complete list of the genes assayed

AR L702H, H875Y

CDK12 K482fs*14 →

ALK deletion exons 2-12

TP53 C275Y

Found with ProBio assay
AND
CDK12 second hit
AND
CDK12 tandem duplication phenotype

14 Therapies Approved in the EU

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GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

THERAPY AND CLINICAL TRIAL IMPLICATIONS

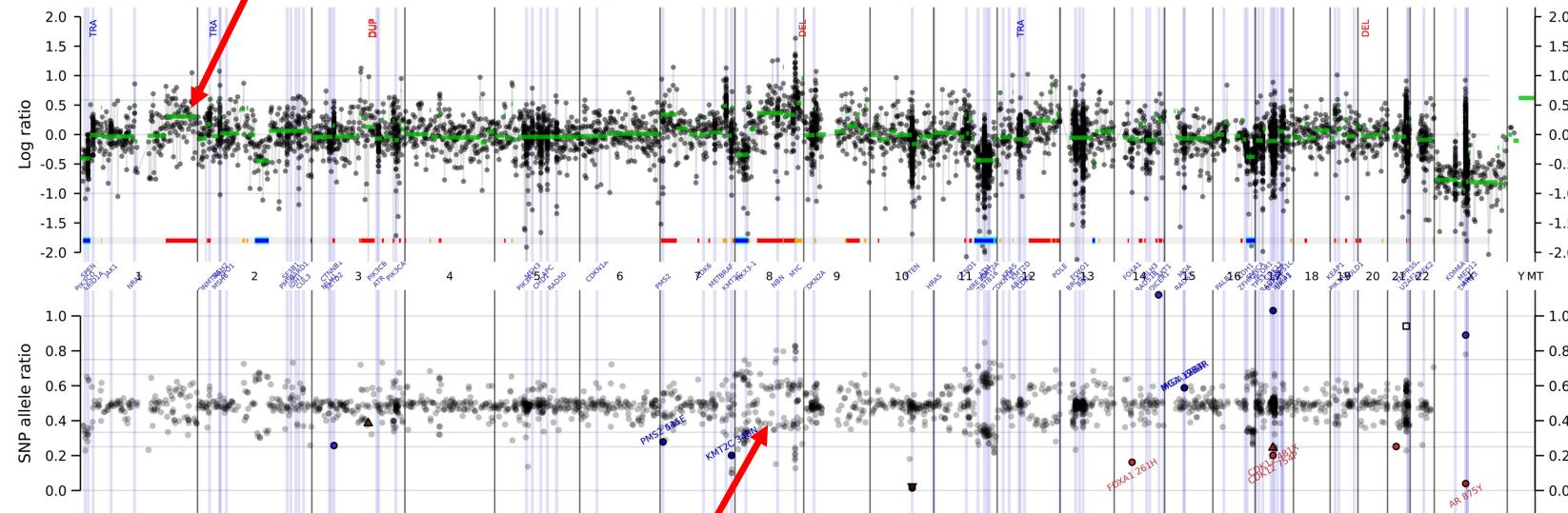
No therapies or clinical trials. See Genomic Signatures section

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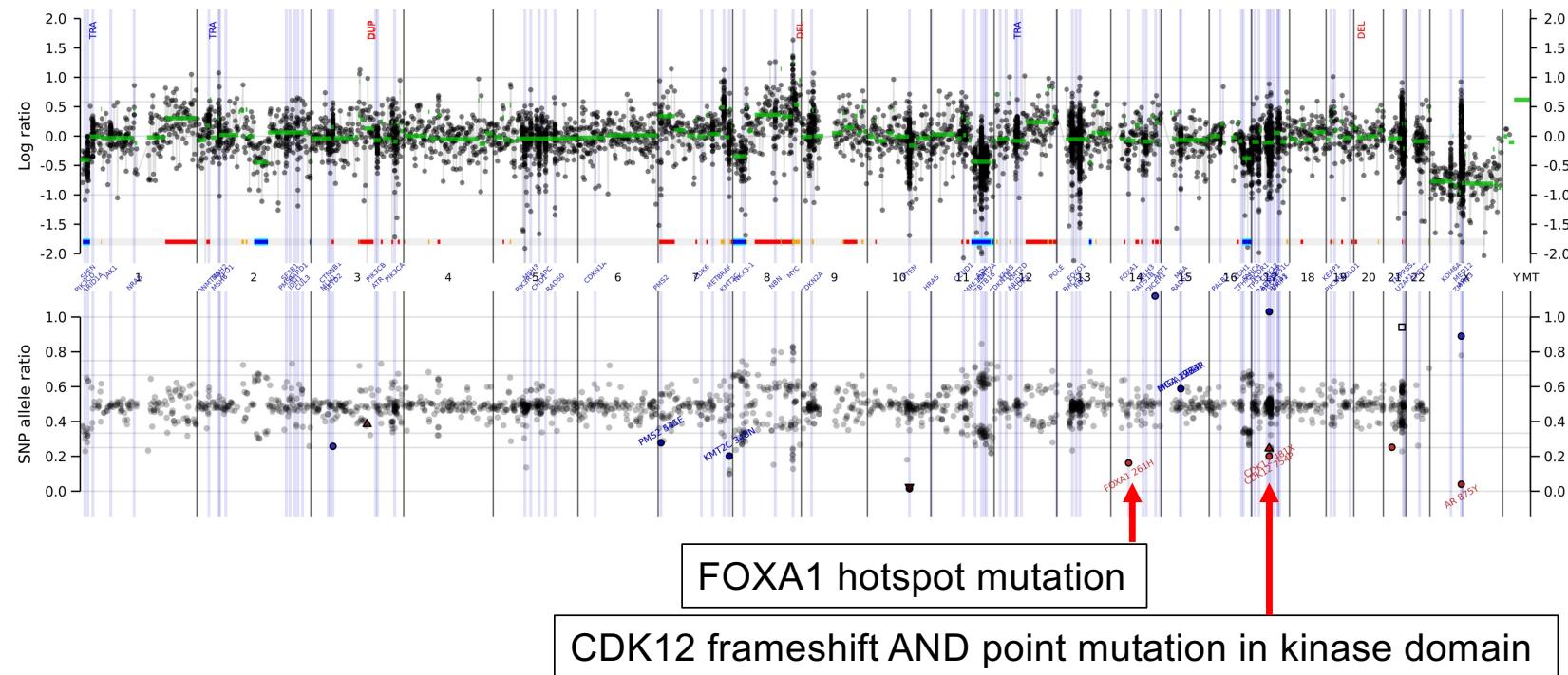
ProBio – liquid biopsy

Genome wide copy number alterations, provided in autoseq curation interface, static and interactive version



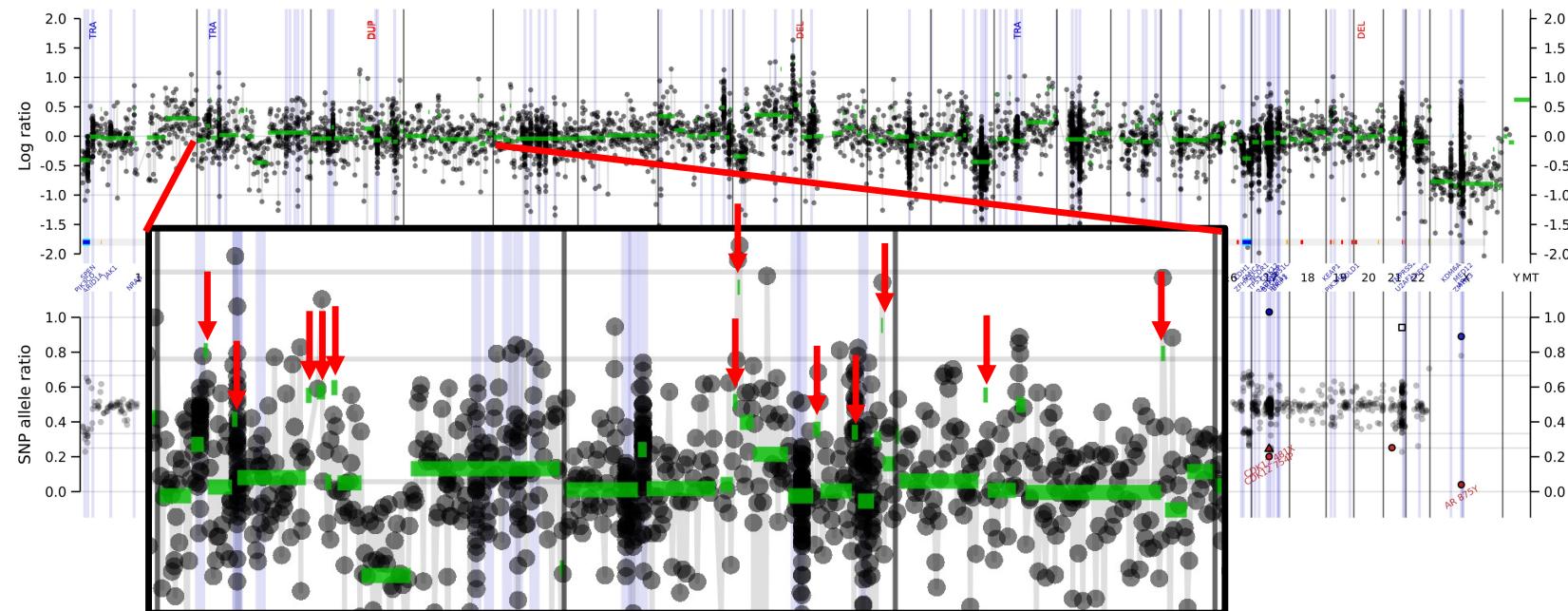
Single nucleotide polymorphism B-allele ratio supporting copy-number data

ProBio – liquid biopsy



ProBio – liquid biopsy

Tandem duplication phenotype = small amplifications genome wide
 Leads to increased amount of fusion proteins = novel antigens on the cell surface.





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MEDICAL FACILITY Docrates Syopasairaala

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ALK deletion exons 2-12 →

TP53 C275Y

Completely irrelevant

GENOMIC SIGNATURES

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THERAPY AND CLINICAL TRIAL IMPLICATIONS

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MEDICAL RECORD # Not given

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MEDICAL FACILITY ID [REDACTED]

PATHOLOGIST Provided, Not

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TP53 C275Y

False positive clonal hematopoiesis variant

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20 Clinical Trials

3 Therapies with Lack of Response

GENOMIC SIGNATURES

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THERAPY AND CLINICAL TRIAL IMPLICATIONS

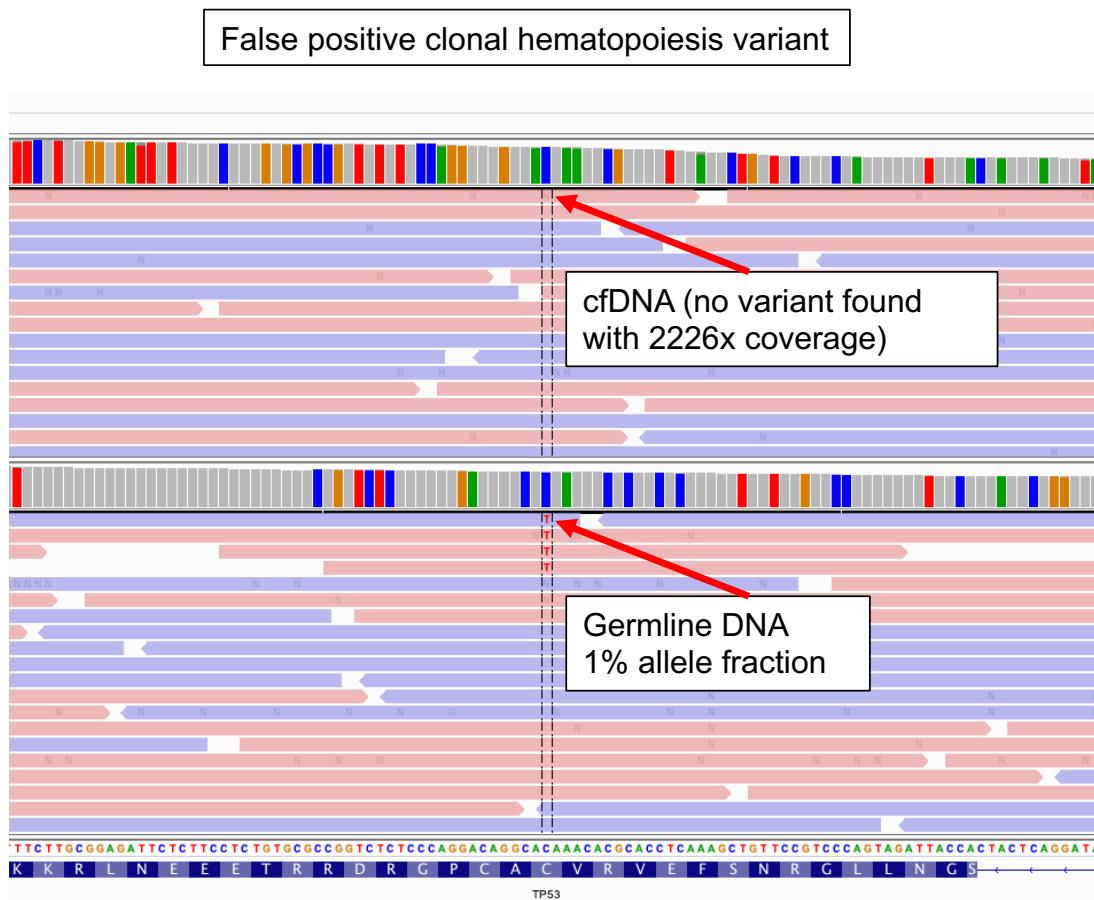
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FoundationOne Liquid CDx run on a mCRPC case





FOUNDATIONONE® LIQUID CDx

PATIENT
03-2020-00026951, FI

TUMOR TYPE
Prostate cancer (NOS)
COUNTRY CODE
FI

REPORT DATE
16 Sep 2020
ORDERED TEST #
ORD-0892843-01

ABOUT THE TEST FoundationOne®Liquid CDx is a next generation sequencing (NGS) assay that identifies clinically relevant genomic alterations in circulating cell-free DNA.

PATIENT

DISEASE Prostate cancer (NOS)

NAME 03-2020-00026951, FI

DATE OF BIRTH [REDACTED]

SEX Male

MEDICAL RECORD # Not given

PHYSICIAN

ORDERING PHYSICIAN [REDACTED]

MEDICAL FACILITY Docrates Syopasairaala

ADDITIONAL RECIPIENT None

MEDICAL FACILITY ID [REDACTED]

PATHOLOGIST Provided, Not

SPECIMEN

SPECIMEN ID 03-2020-0002695112/12/1954

SPECIMEN TYPE Blood

DATE OF COLLECTION 28 August 2020

SPECIMEN RECEIVED 02 September 2020

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Sensitivity for the detection of alterations and genomic signatures is reduced due to sample quality.

Genomic Signatures

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Gene Alterations

For a complete list of the genes assayed, please refer to the Appendix.

AR L702H, H875Y

CDK12 K482fs*14

ALK deletion exons 2-12

TP53 C275Y

14 Therapies Approved in the EU

20 Clinical Trials

3 Therapies with Lack of Response

Based on the tumor = ctDNA fraction and the TP53 allele frequency (0.24%) the C275Y variant should NOT be presented to a physician as a relevant result on the front page.

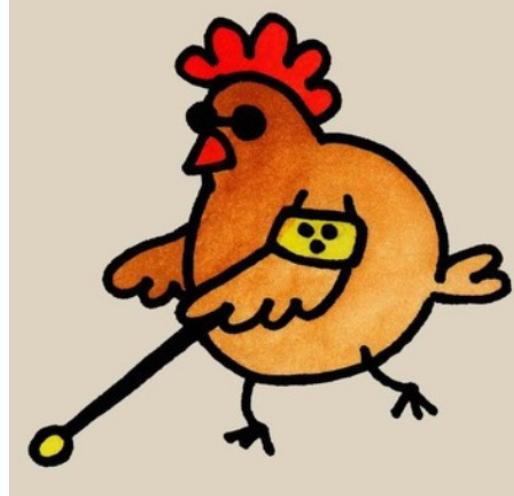
No
Unaltered
instability.

Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.

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- The Pembro recommendation was correct in the end BUT based on the wrong arguments

FoundationOne Liquid CDx



Questions?

