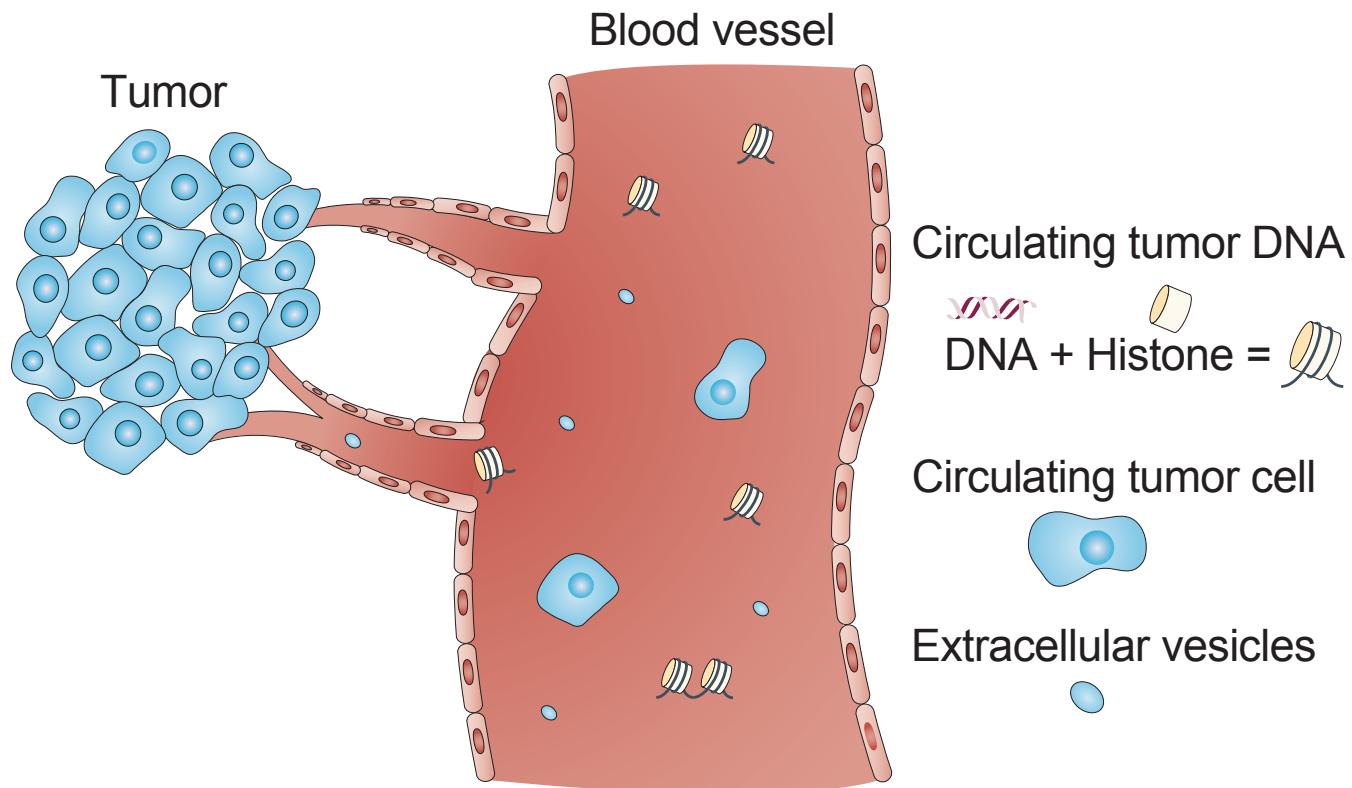


Liquid biopsies

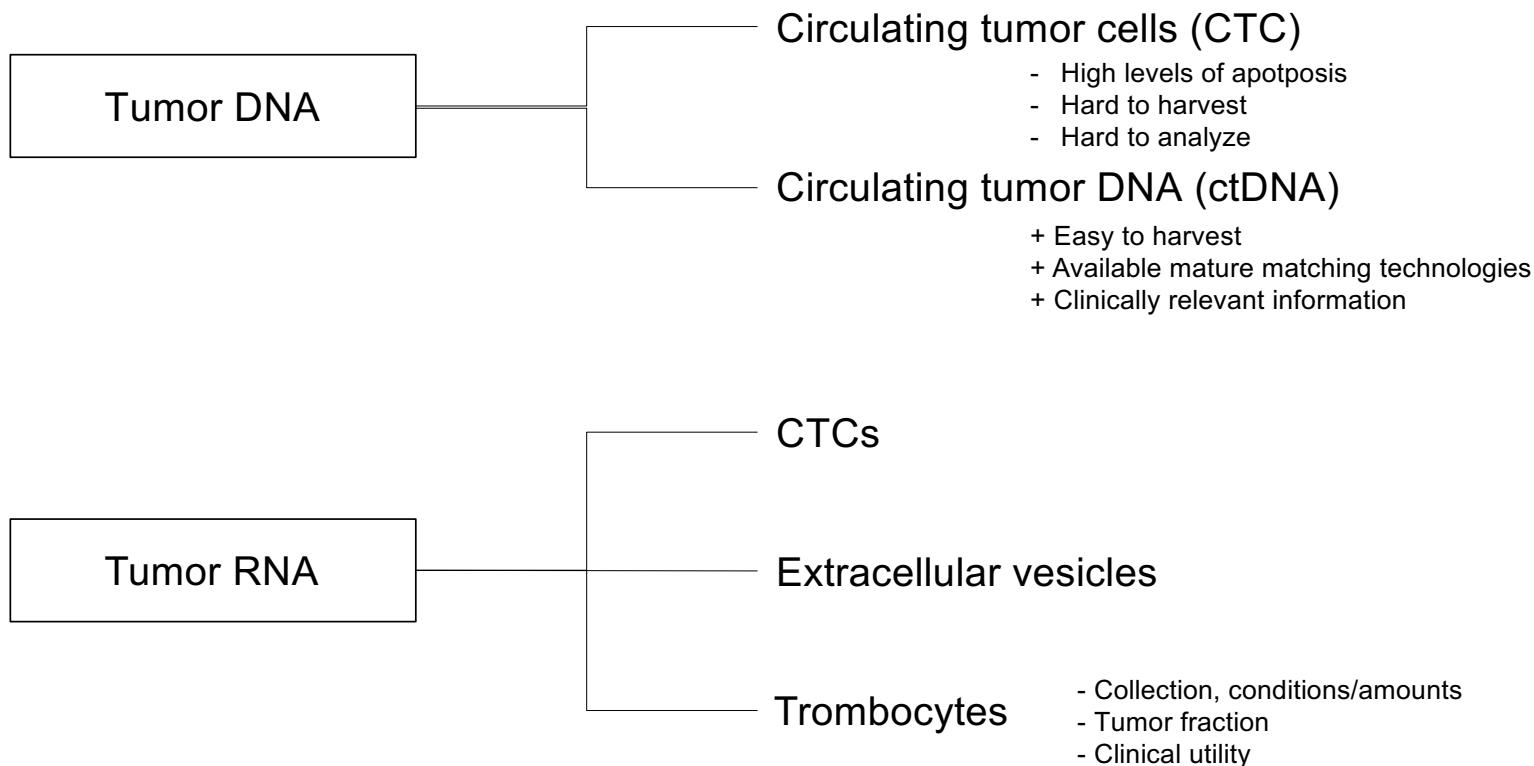


Liquid biopsies - DNA, cells or other debris that originates from the tumor tissue and is shed into the circulation



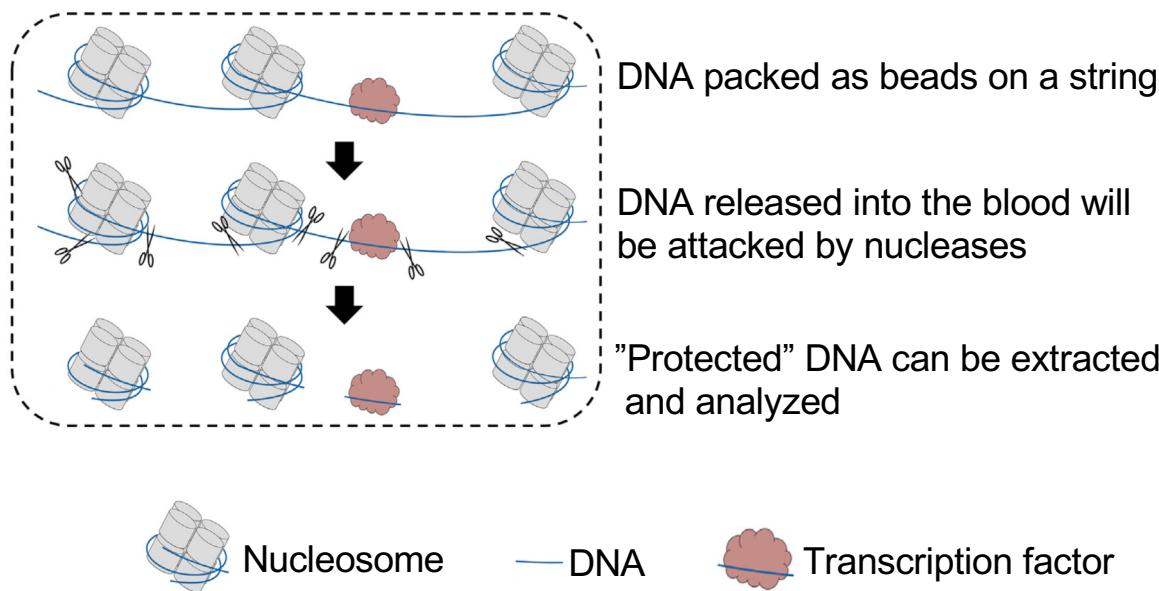
Adopted from Cell-free nucleic acids as biomarkers in cancer patients, Nat Rev Can 2011

Liquid biopsy types



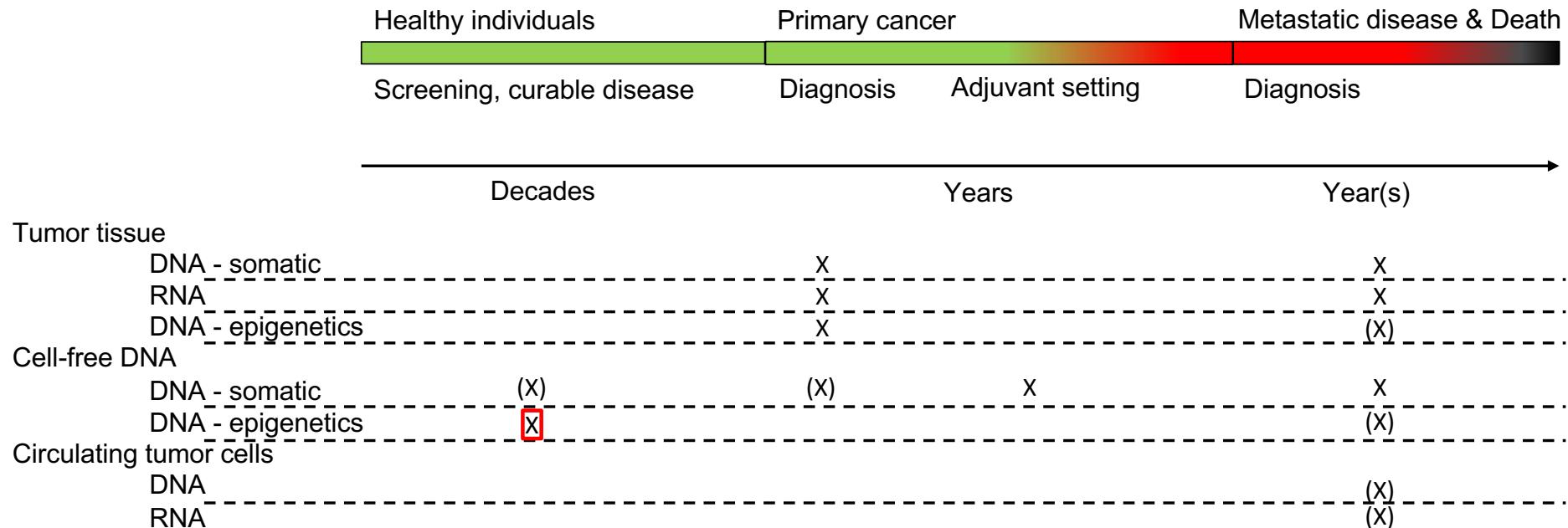
Cell-free DNA preserves tissue-specific epigenetic information

- 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)

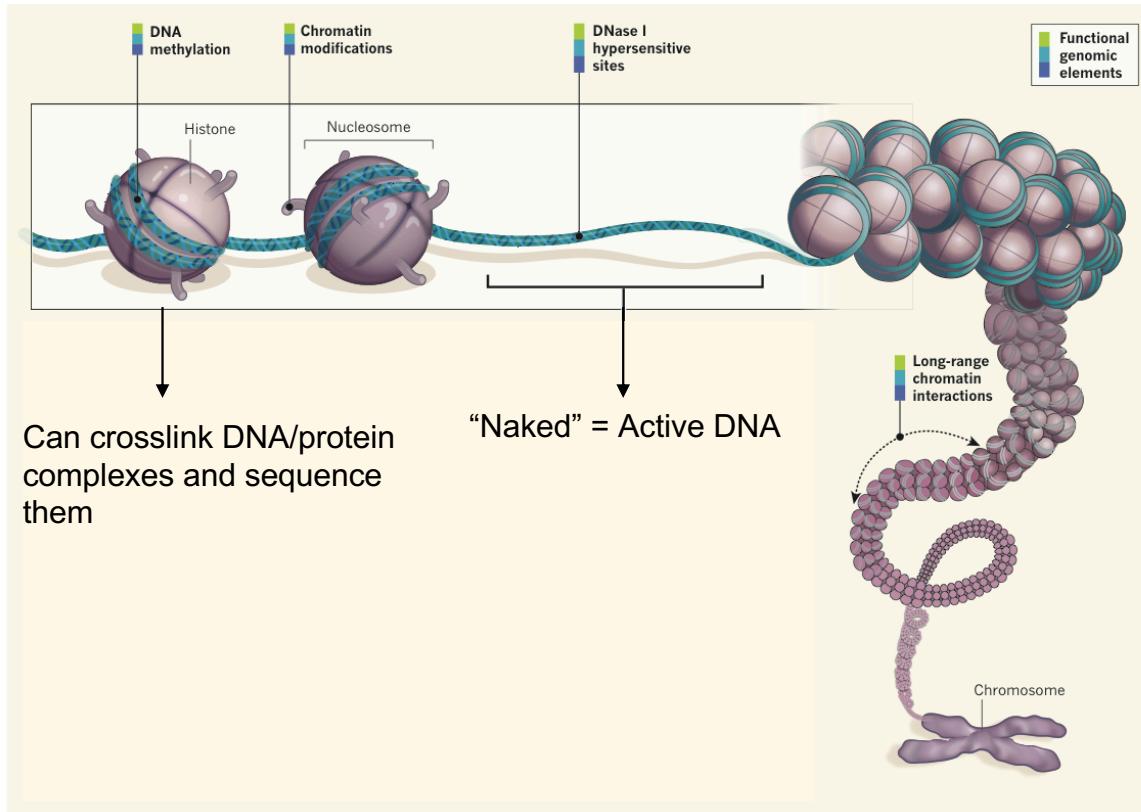


Snyder et al, Cell 2016

Tissue, analyte and context ...



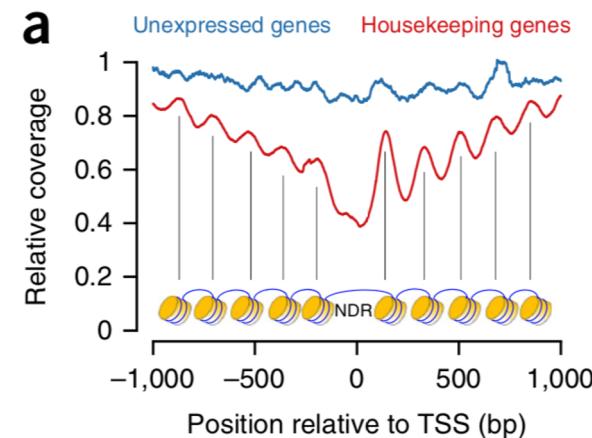
Epigenetic regulation



Epigenetic state of circulating tumor DNA

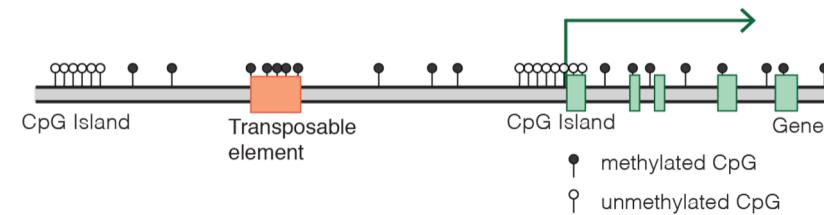
- Nucleosome occupancy can be applied to predict expressed genes

- 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

A photograph of a woman with blonde hair tied back, wearing a pink scarf, looking out over a dense city skyline. The sky is hazy, suggesting dawn or dusk.

freenome

Thrive.
Earlier Detection

Early detection/screening for localized cancer



GRAIL

Science Clinical Studies About News

Join the Team

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

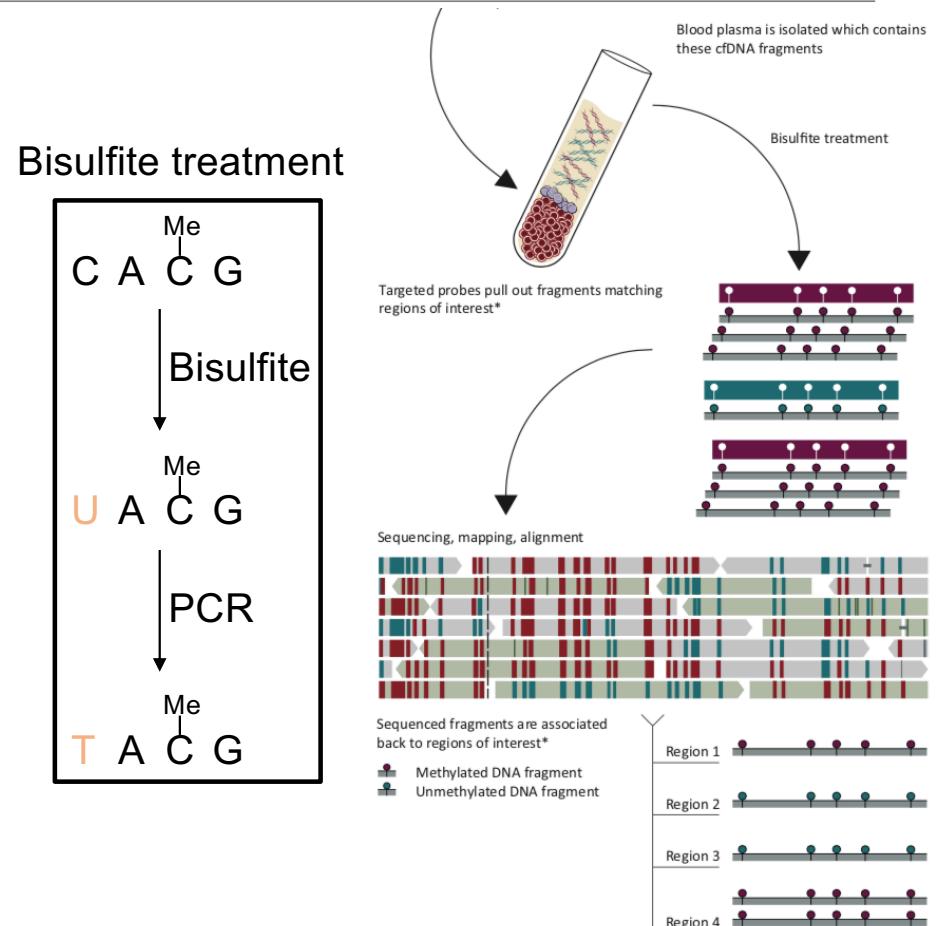
Early detection/screening for localized cancer



- 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.

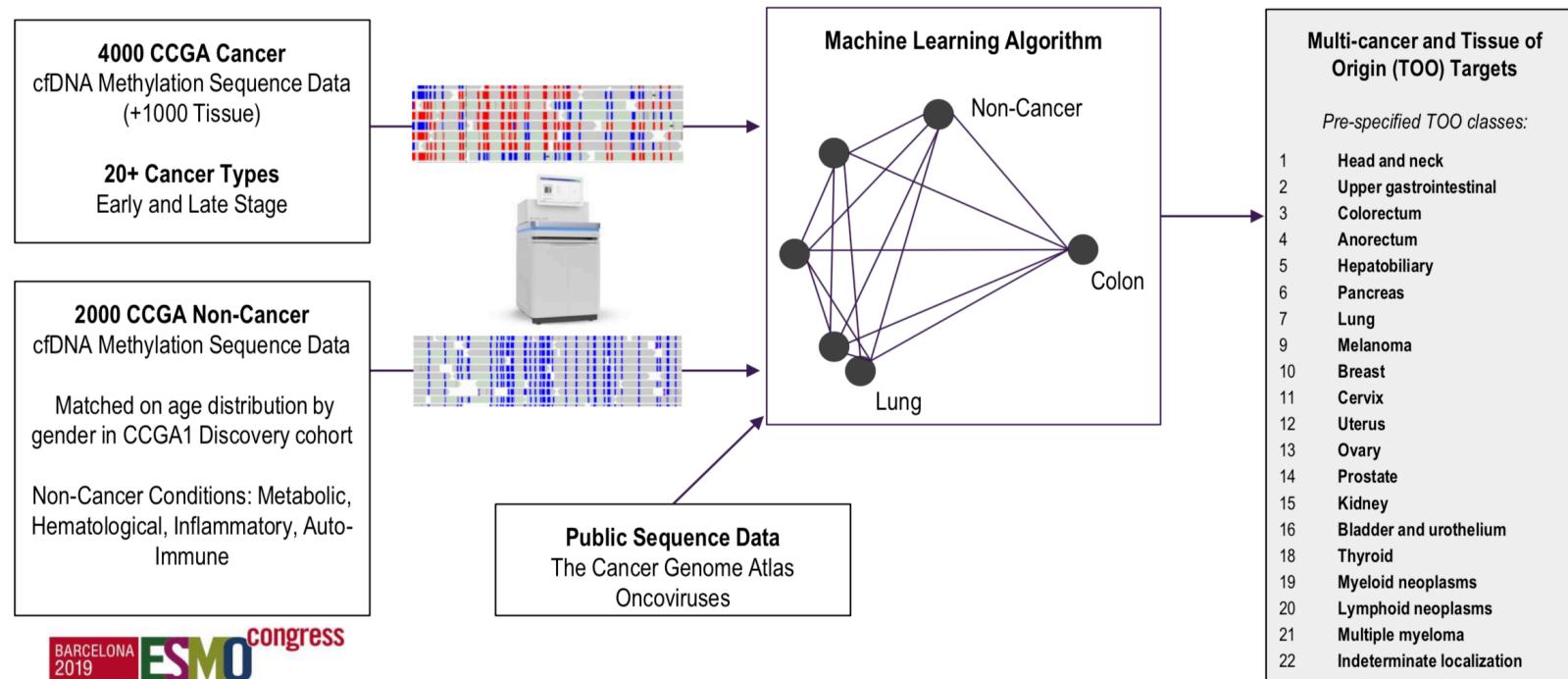


*previously defined from analysis of existing datasets from cfDNA, tissue from GRAIL trials and public databases

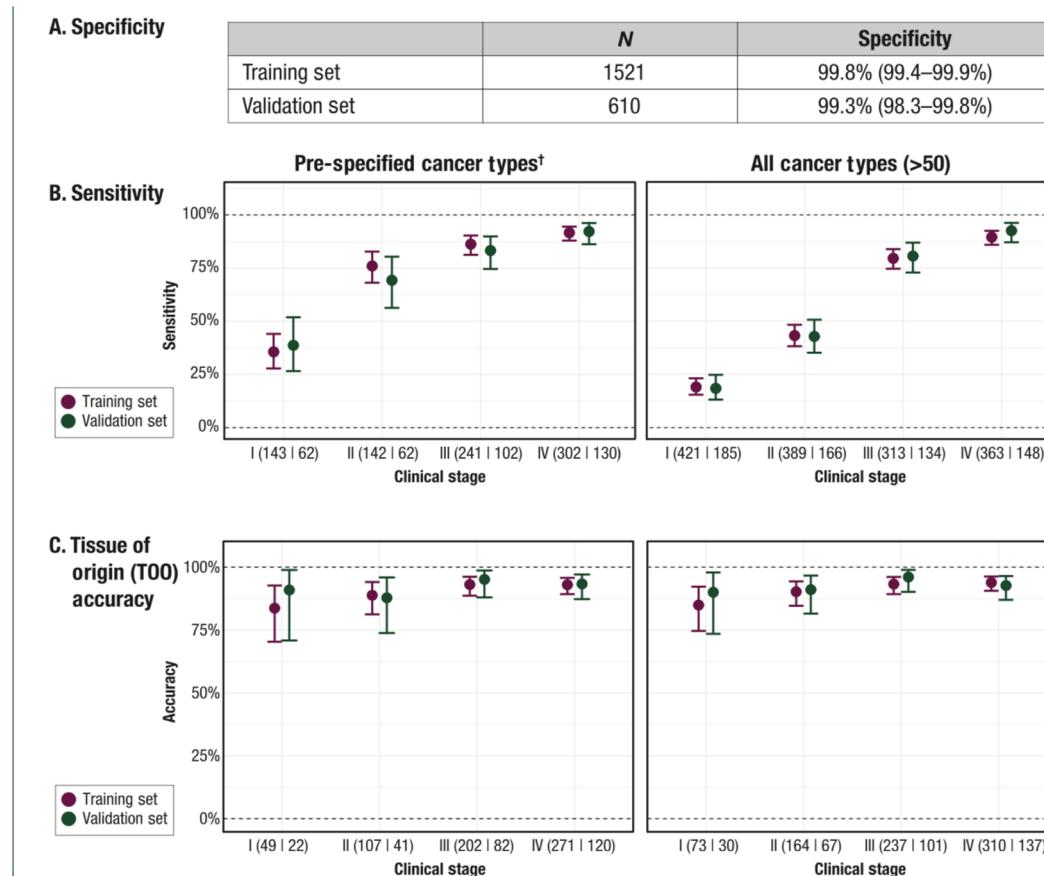
Early detection/screening for localized cancer

Target Selection using Machine Learning Algorithm

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

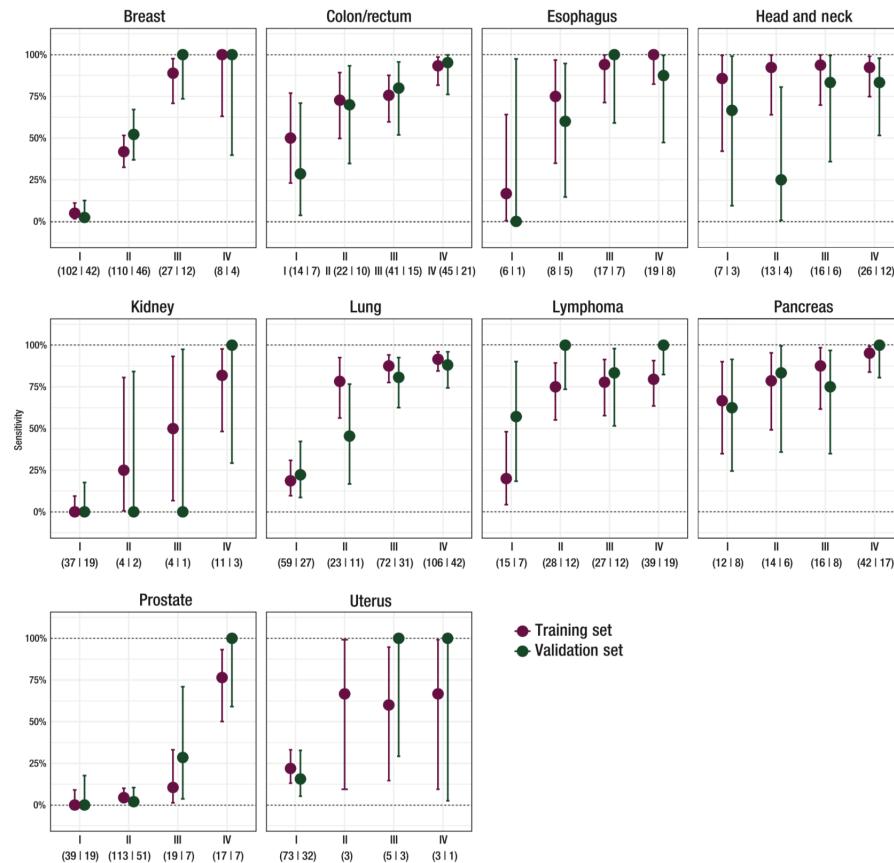


Early detection/screening for localized cancer



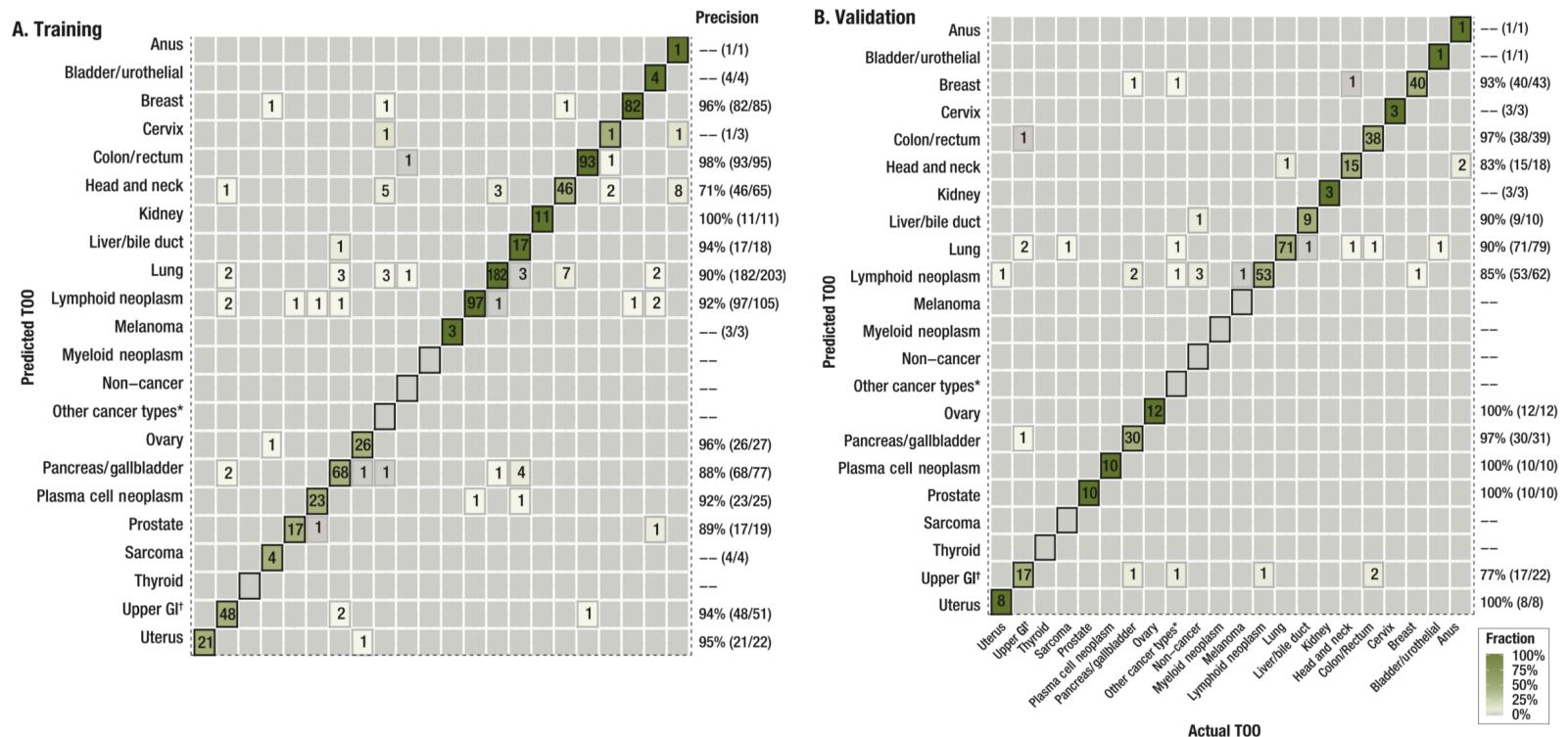
Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA, Annals of Oncology 2020.

Early detection/screening for localized cancer



Good enough?
Thoughts?
How to implement?

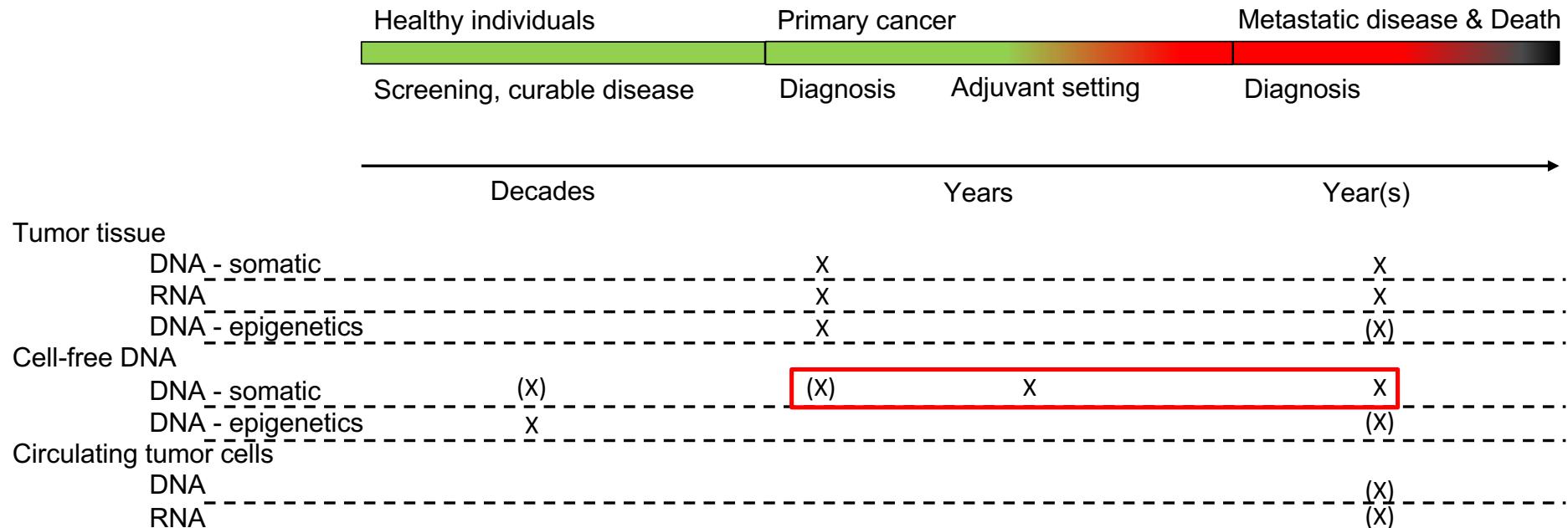
Early detection/screening for localized cancer



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?

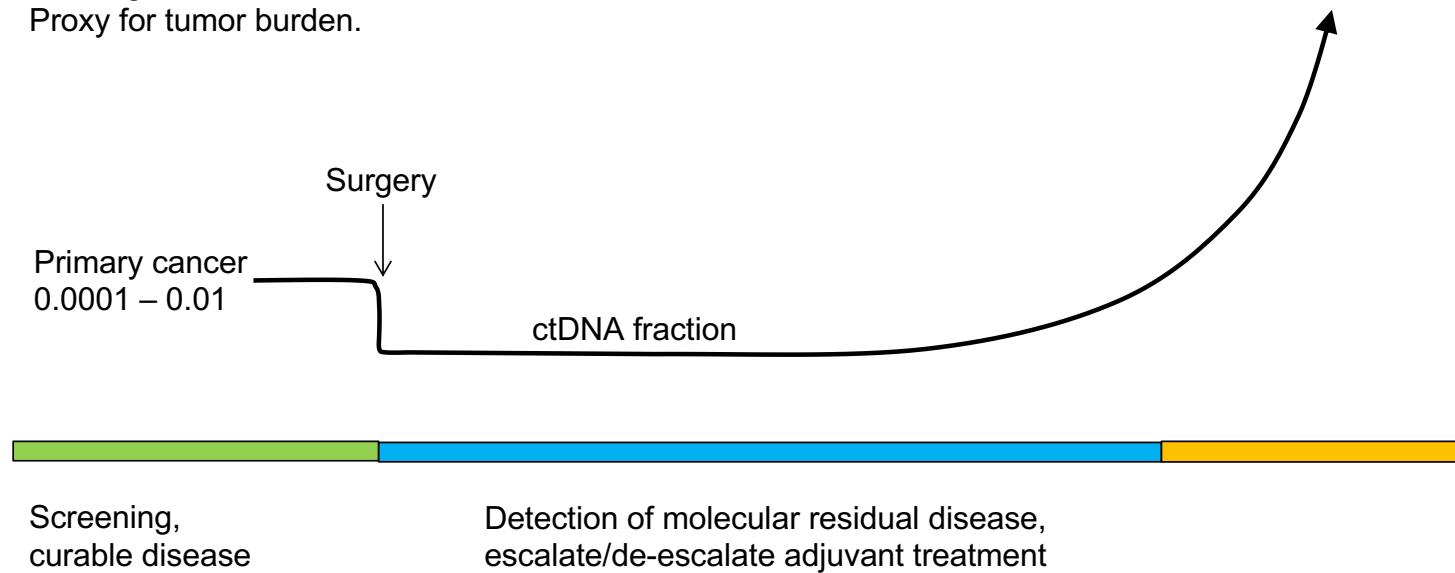
Tissue, analyte and context ...



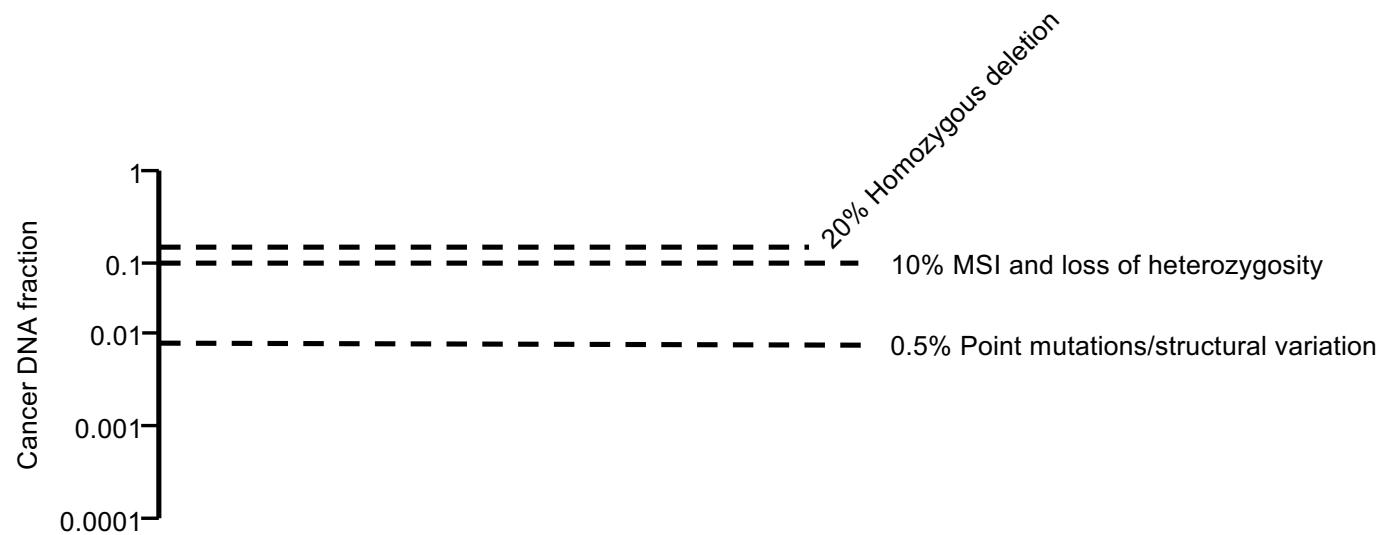
Cancer DNA fraction and consequence for genomic profiling using sequencing

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



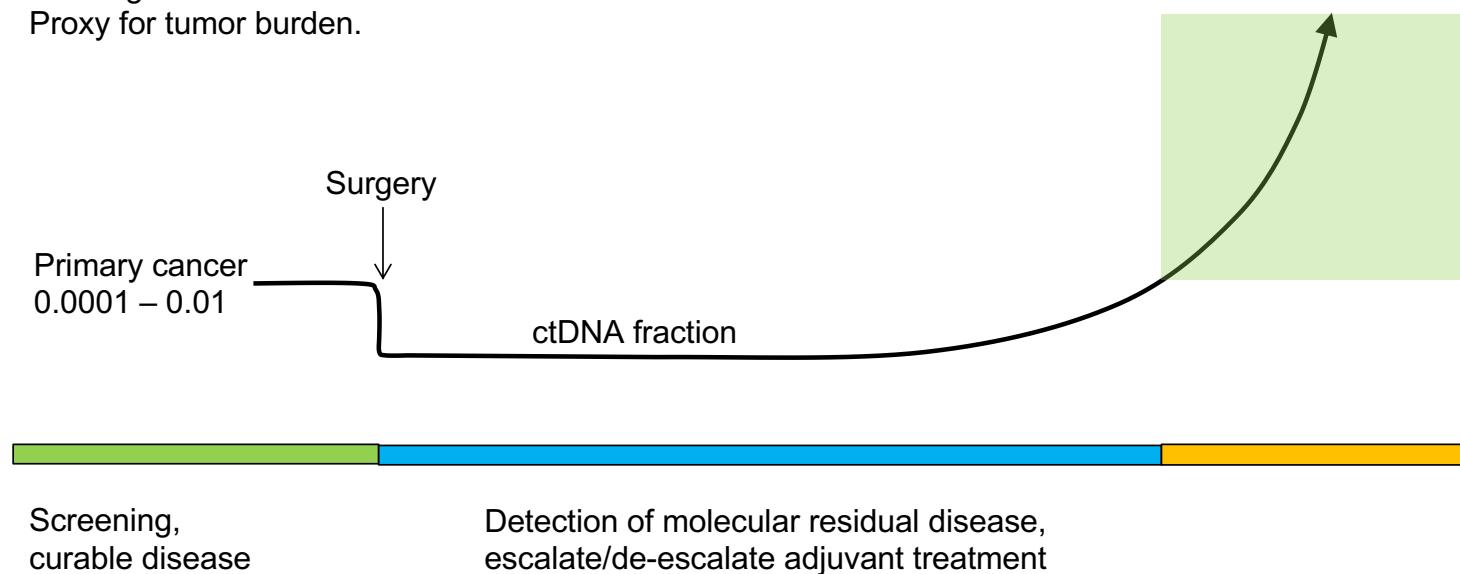
Cancer DNA fraction and consequence for genomic profiling using sequencing



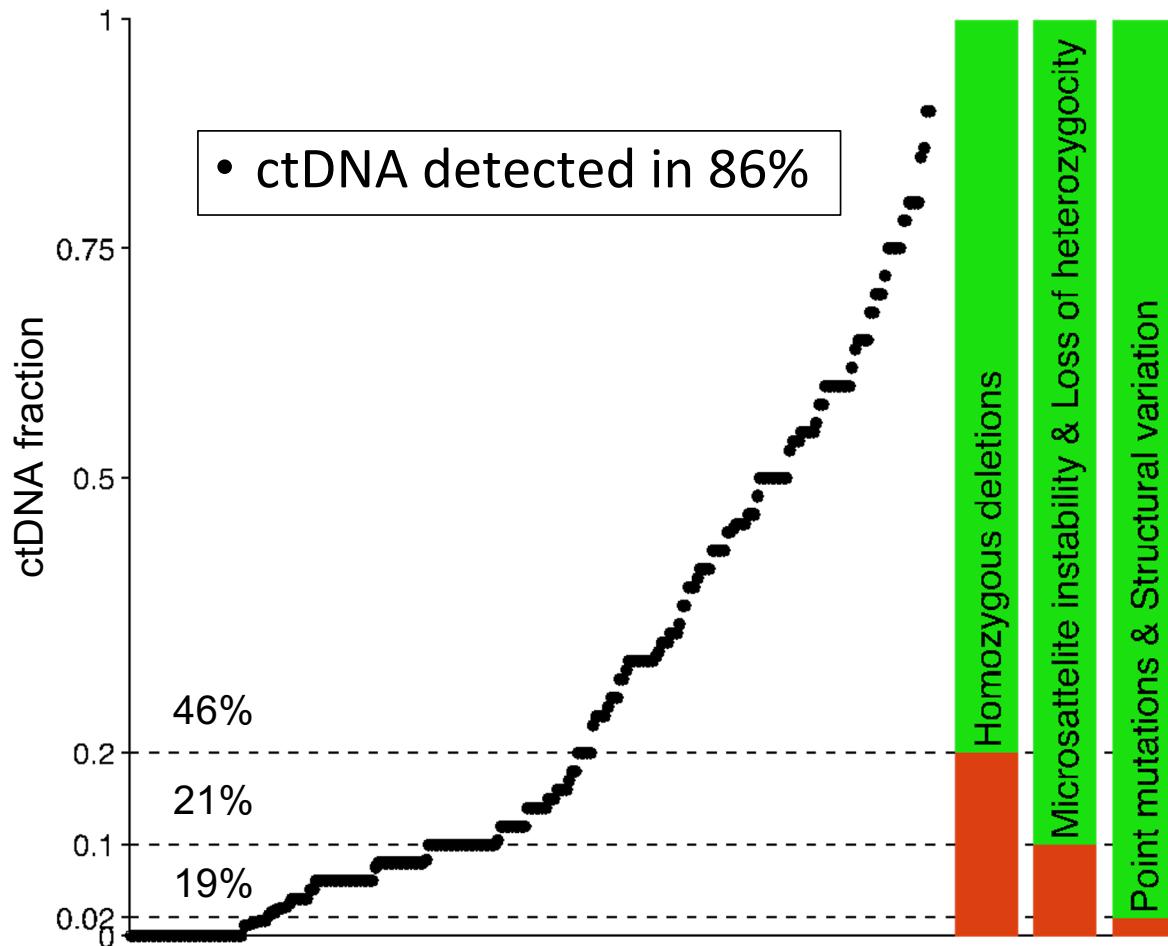
Cancer DNA fraction and consequence for genomic profiling using sequencing

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

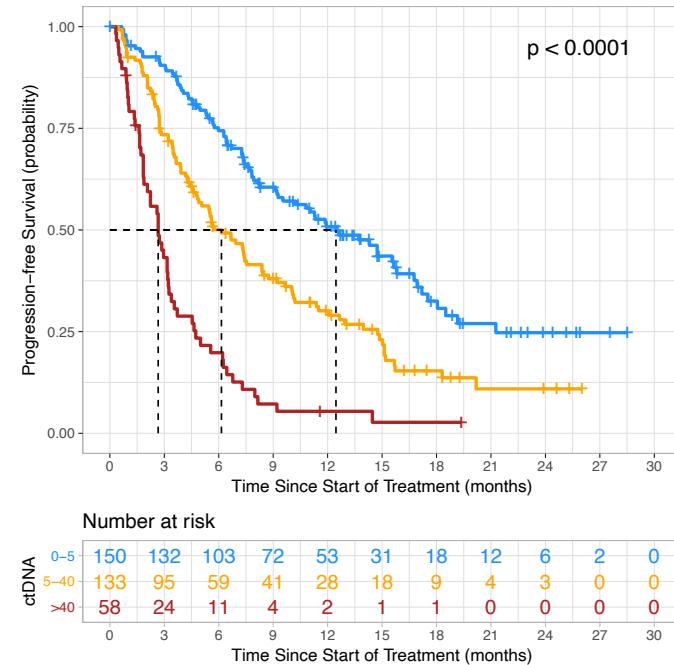


ctDNA fraction in 217 men with mCRPC



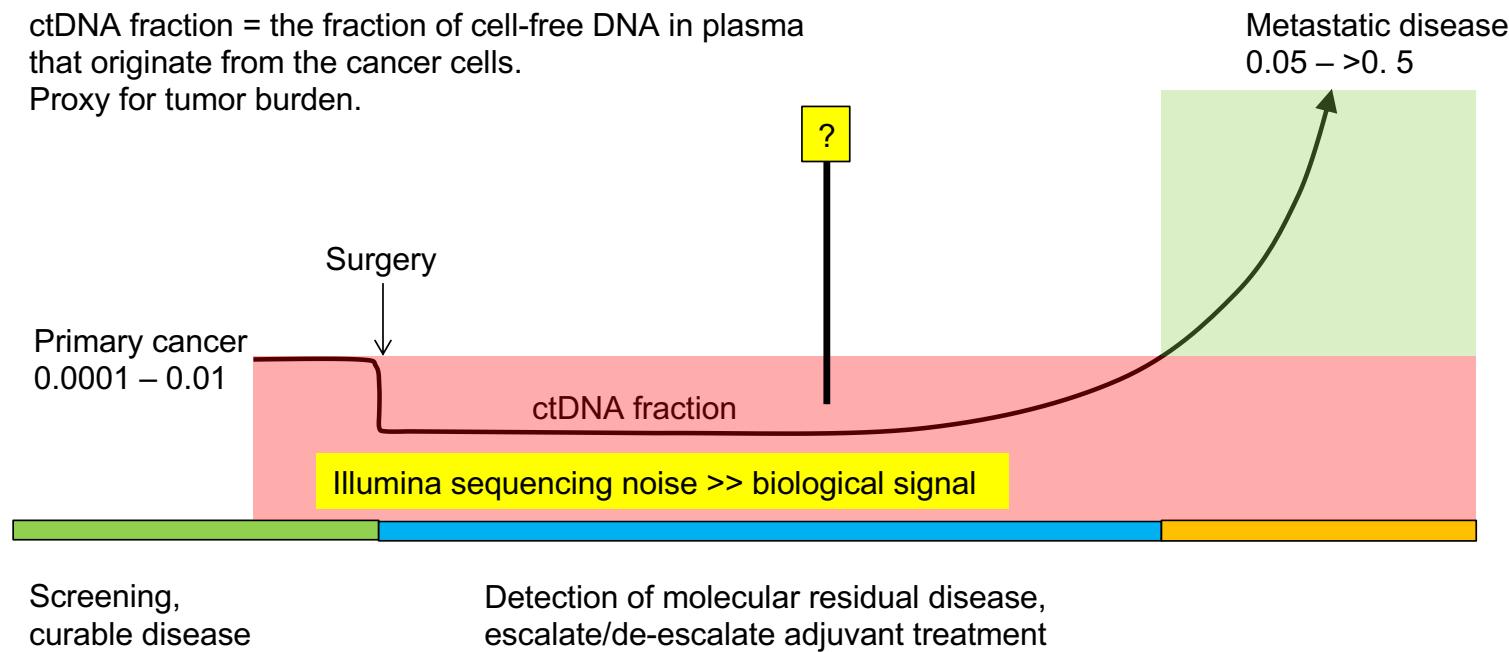
ctDNA fraction and prognosis

- Catch-22: high ctDNA fraction means poor prognosis



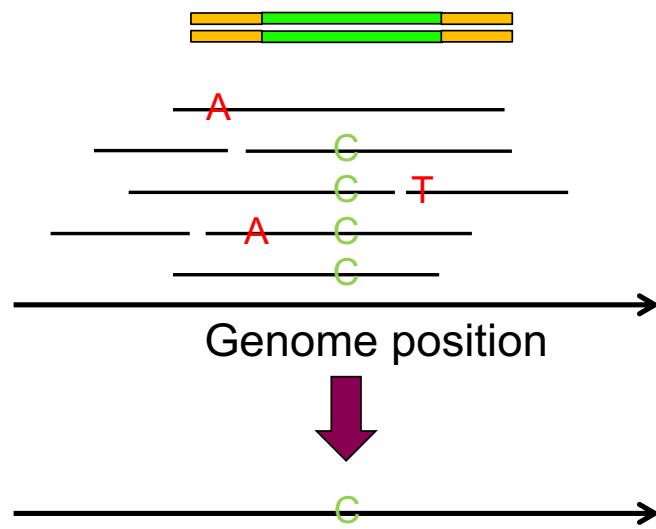
Cancer DNA fraction and consequence for genomic profiling using sequencing

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

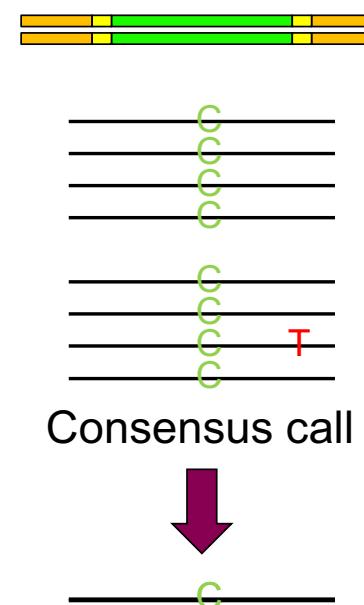


Cancer DNA fraction and consequence for genomic profiling using sequencing

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

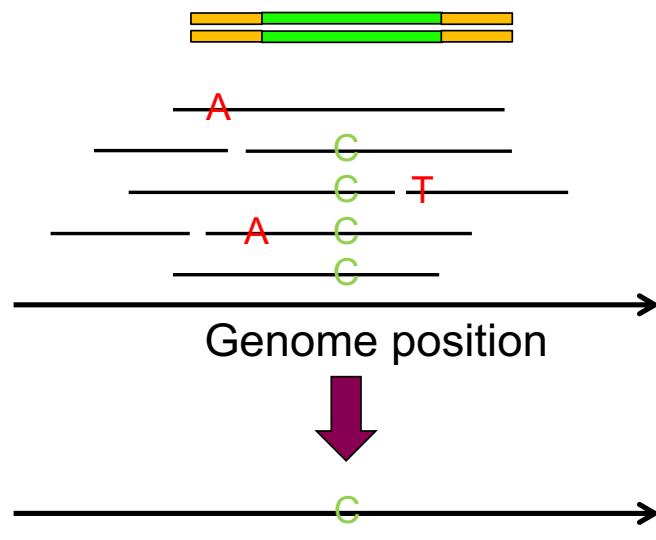


Low purity – sequence each DNA fragment ~10 times.
Error suppression, tunable sensitivity.



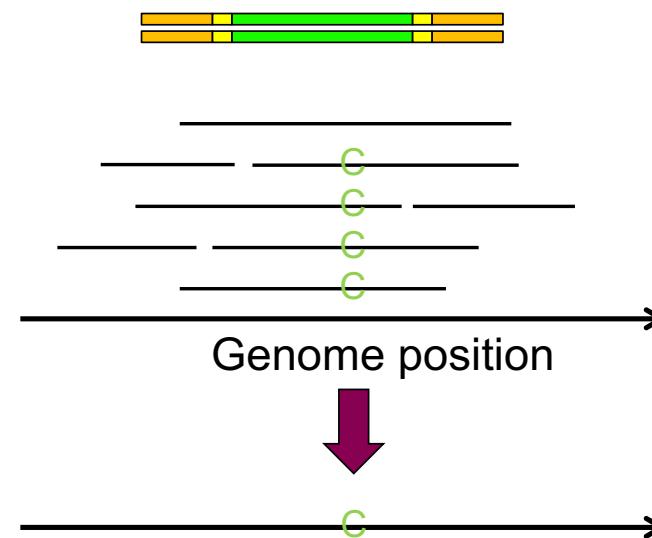
Cancer DNA fraction and consequence for genomic profiling using sequencing

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



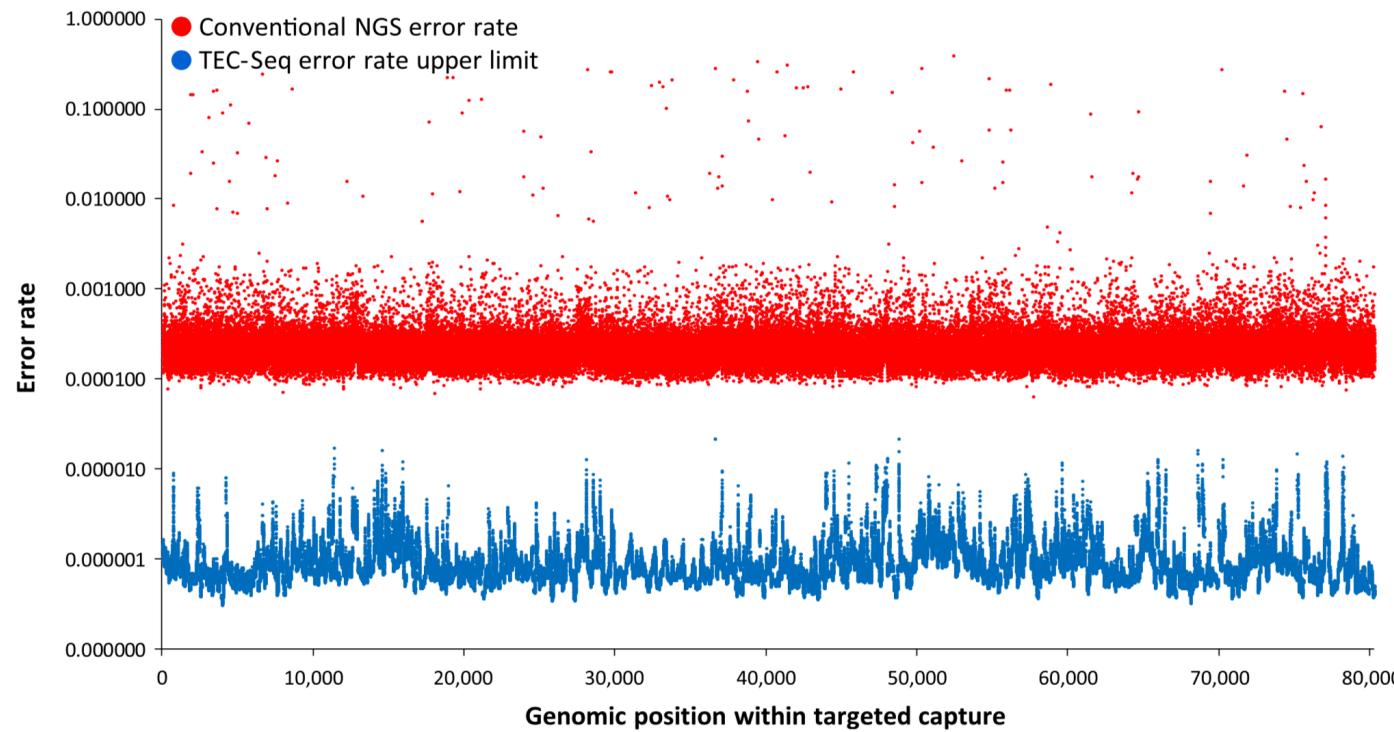
Target coverage <2000X

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

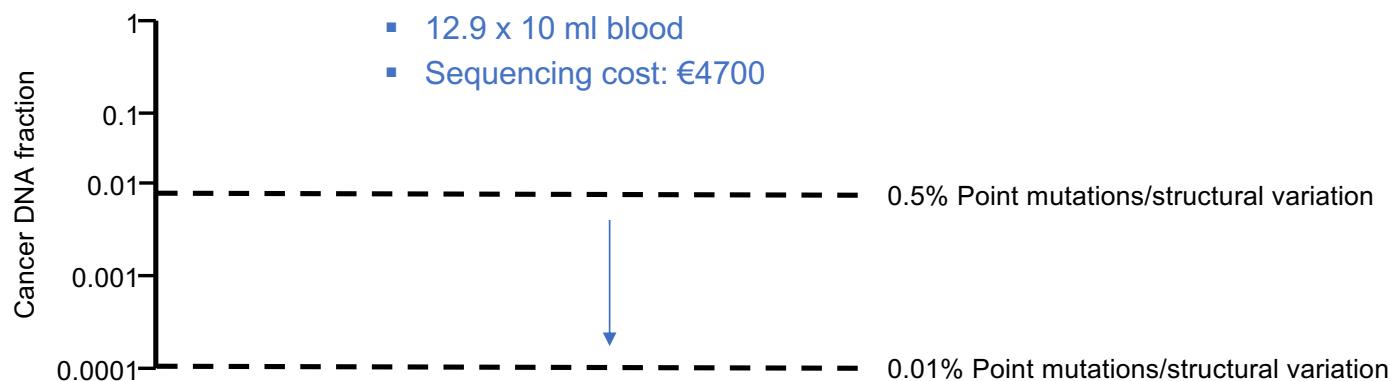
- 1.3 x 10 ml blood
- Sequencing cost: €470

→ 1/5000

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

→ 1/10000

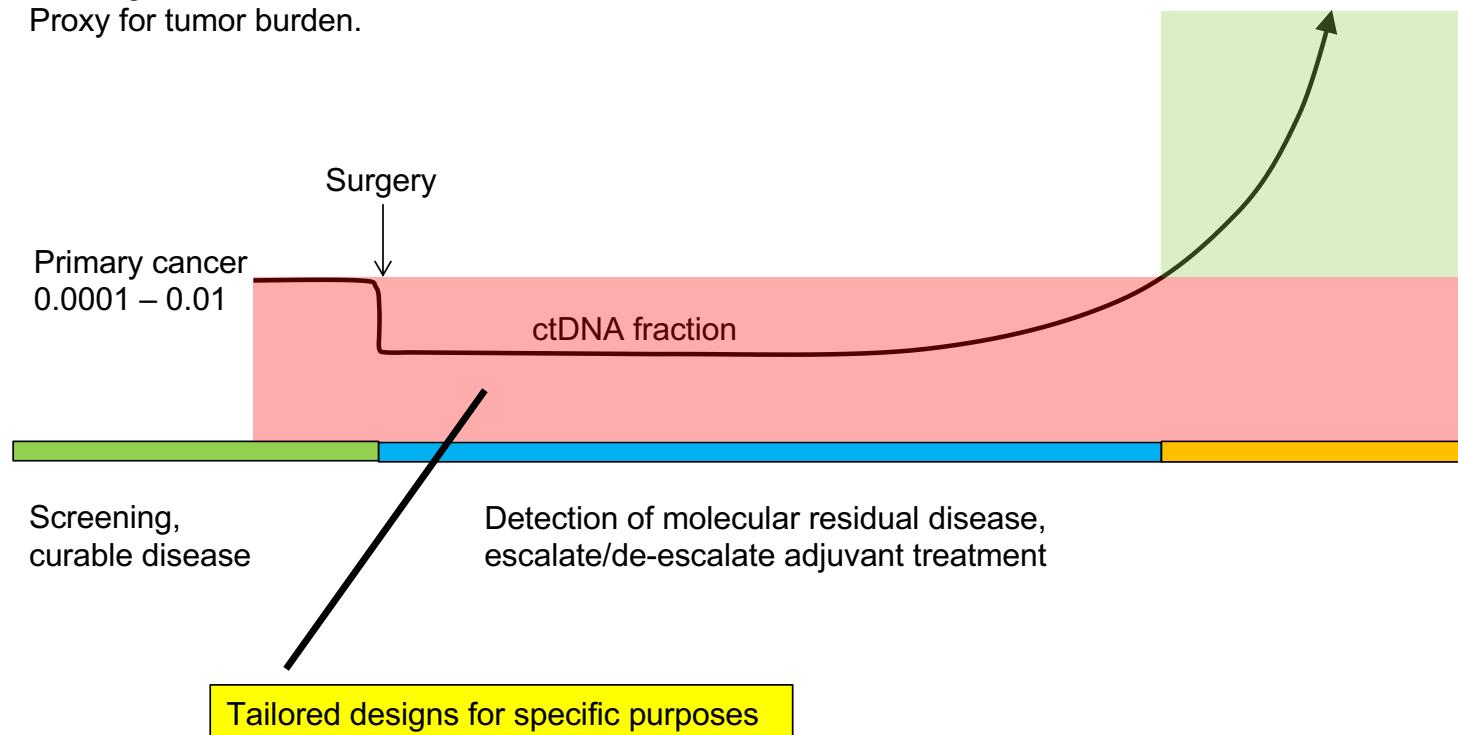
- 12.9 x 10 ml blood
- Sequencing cost: €4700



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



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”

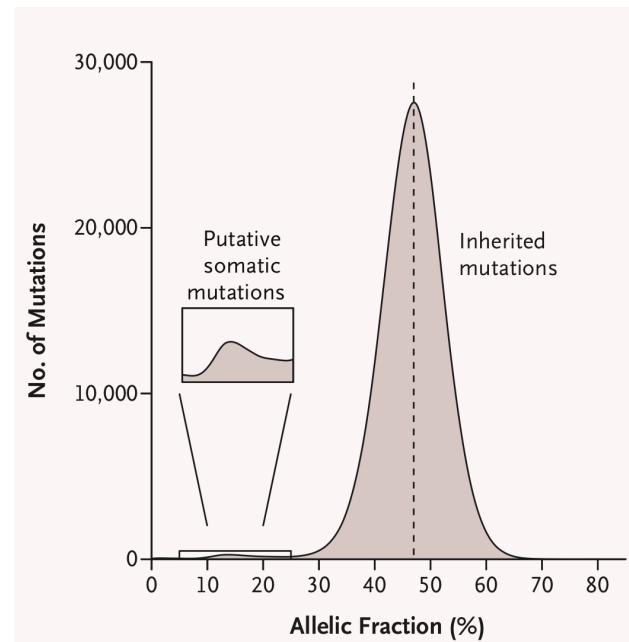
Clonal expansions in white blood cells

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



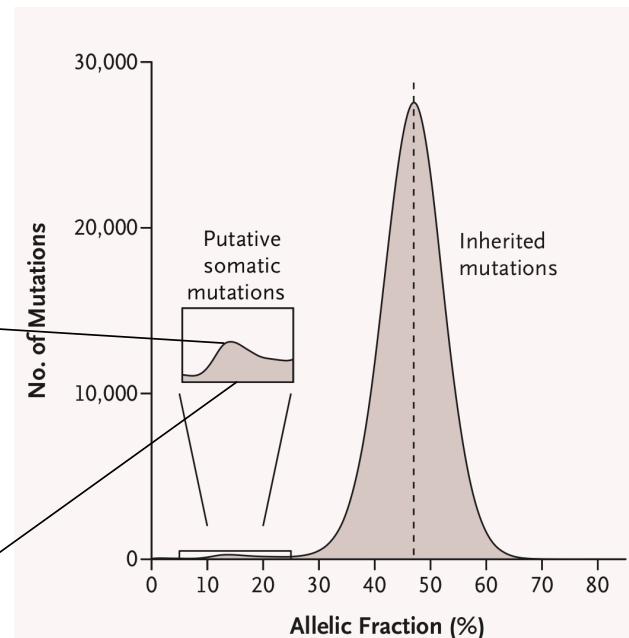
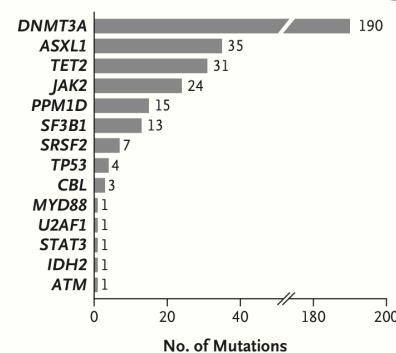
Clonal expansions in white blood cells

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

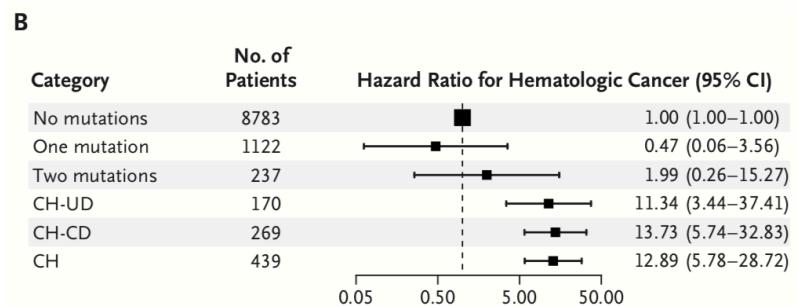
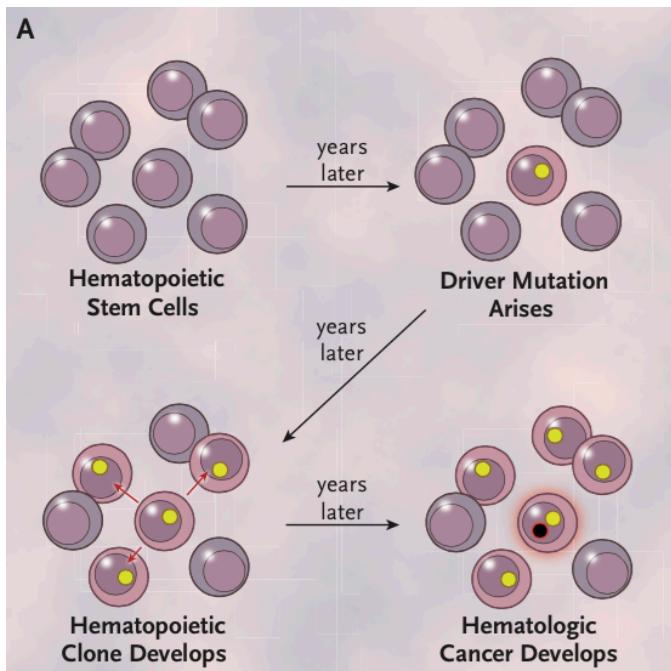


Clonal expansions in white blood cells

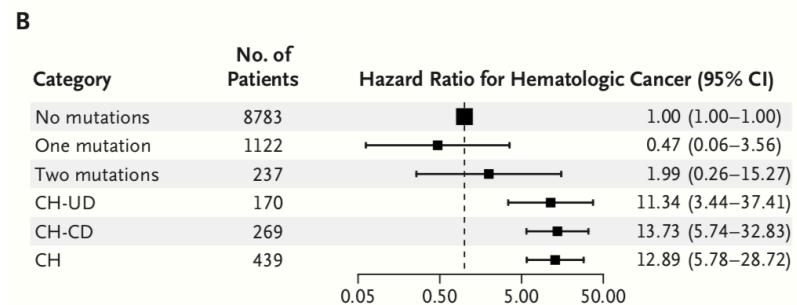
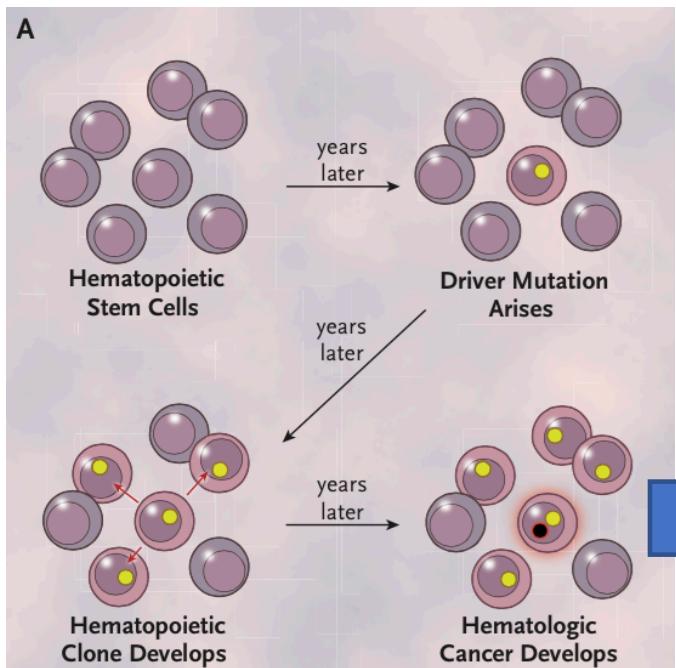
- Goal to associate germ-line variation to schizophrenia/bipolar disorder
- A technical artifact



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, Anton Berthon⁸, Ajazuddin Syed⁹, Mariko Yabe⁹, 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 Glodzik³, 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 Gillie^{24,25}, Markus Ball^{25,26}, Eric Padron²⁵, David M. Hyman^{10,27}, Jose Baselga²⁸, Larry Norton^{10,27}, Stuart Gards^{10,27}, Virginia M. Klimick^{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^{21,30}, Martin Tallman¹, Montserrat Garcia-Closas¹⁴, Nilanjana Chatterjee³¹, Luis A. Diaz Jr^{10,32,33}, Ross L. Levine³¹, Lindsay M. Morton¹⁴, Ahmet Zehir^{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



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

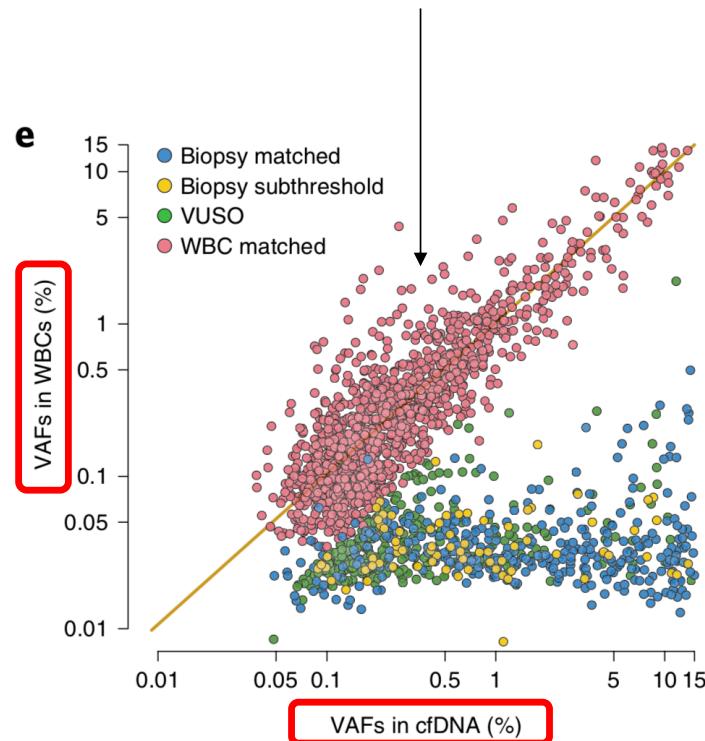
High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants

Pedram Razavi^{1,2,13*}, Bob T. Li^{1,13}, David N. Brown^{3,13}, Byoungsok Jung⁴, Earl Hubbell⁴, Ronglai Shen⁵, Wassim Abida¹, Krishna Juluru⁶, Ino De Brujin⁷, 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. Hall⁴, Angie Santiago-Zayas¹, William F. Novotny^{4,12}, James M. Isbell⁶, Valerie W. Rusch⁹, George Plitas⁸, Alexandra S. Heerdt⁸, Marc Ladanyi³, David M. Hyman¹, David R. Jones⁸, Monica Morrow^{1,8}, Gregory J. Riely¹, Howard I. Scher¹, Charles M. Rudin¹, Mark E. Robson¹, Luis A. Diaz Jr.¹, David B. Solit^{1,2,7}, Alexander M. Aravanis⁴ and Jorge S. Reis-Filho^{1,2,3*}

- 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

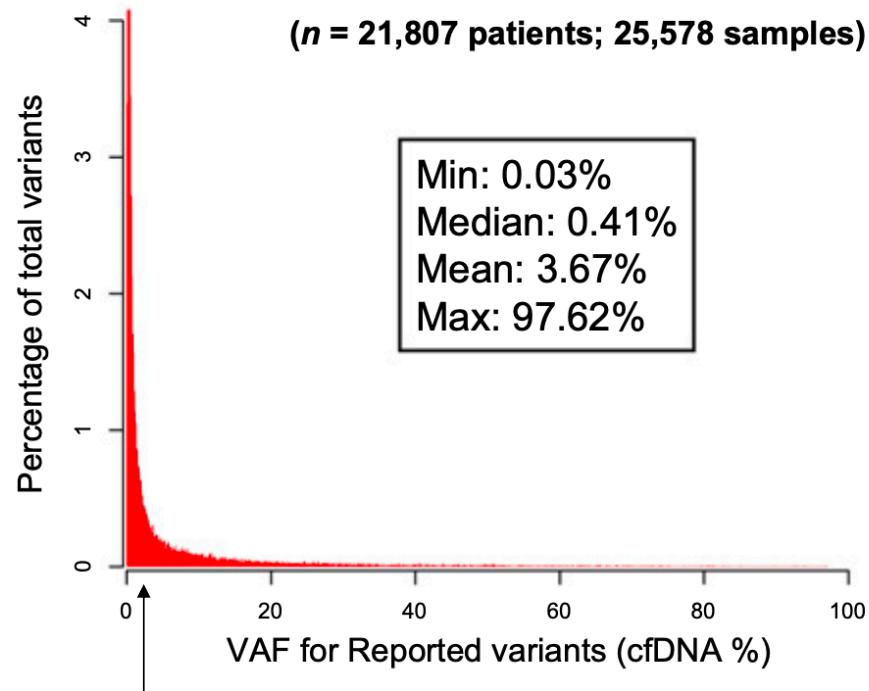
Oliver A. Zill¹, Kimberly C. Banks¹, Stephen R. Fairclough¹, Stefanie A. Mortimer¹, James V. Vowles¹, Reza Mokhtari¹, David R. Gandara², Philip C. Mack², Justin I. Odsgaard¹, Rebecca J. Nagy¹, Arthur M. Baca¹, Helmy Eltoukhy¹, Darya I. Chudova¹, Richard B. Lanman¹, and AmirAli Talasaz¹

Clinical
Cancer
Research

Check for updates

- 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

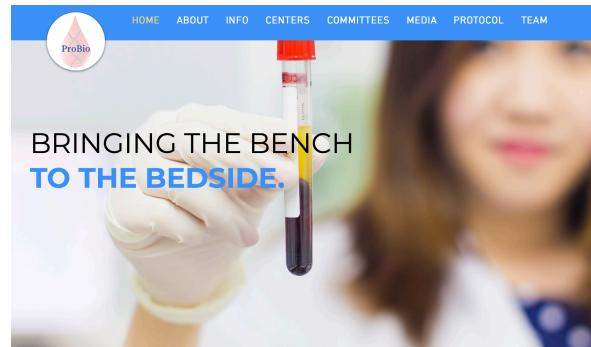
- In Sweden, broad genomic profiling is not reimbursed
- Patients go to Docrates (Finland) and bring back FMI reports
- An oncologist at St Göran reached out and asked for assistance to interpret a report from Foundation Medicine
 - Patient wanted Pembrolizumab
- Patient was included into a study at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

ProBio = a prospective clinical trial in metastatic prostate cancer

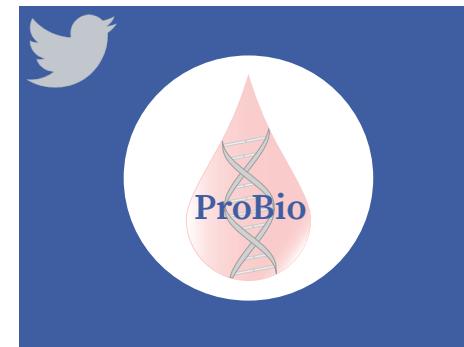
STUDY PROTOCOL Open Access

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



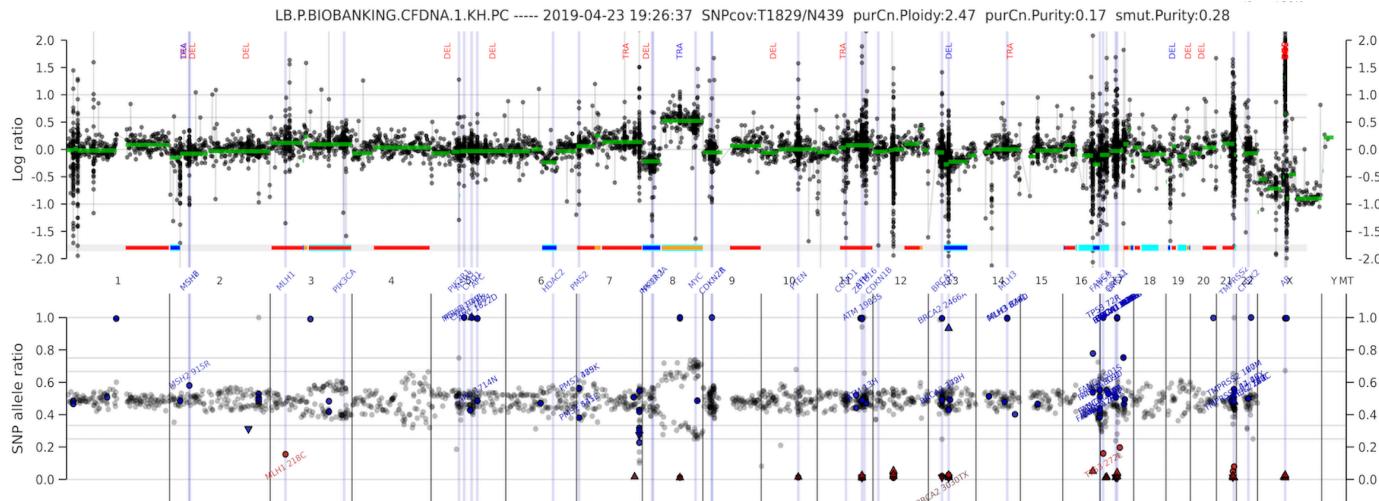
► www.probiotrial.org



► @ProBioTrial

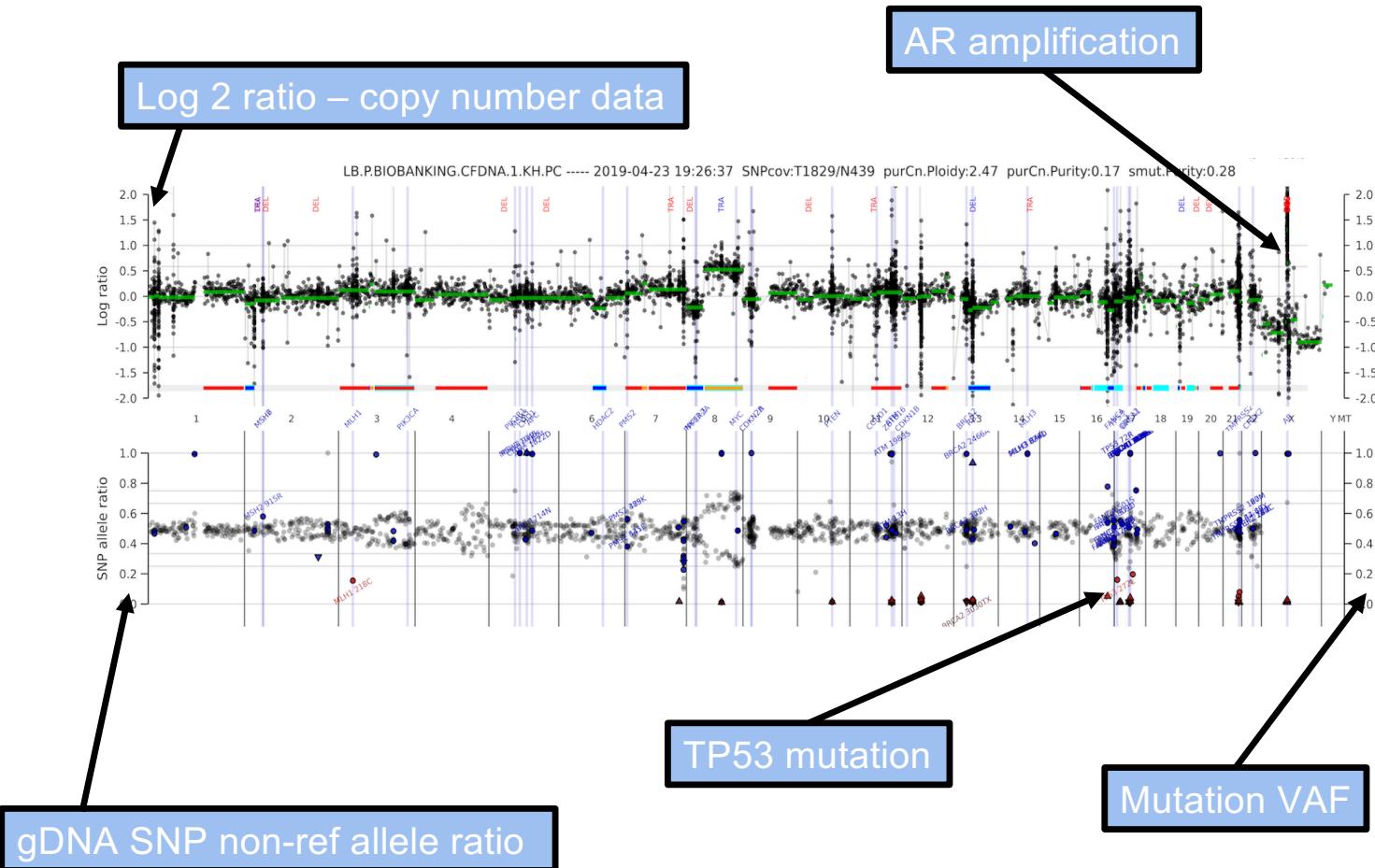
ProBio = a prospective clinical trial in metastatic prostate cancer

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 03-2020-00026951 12/12/1954

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

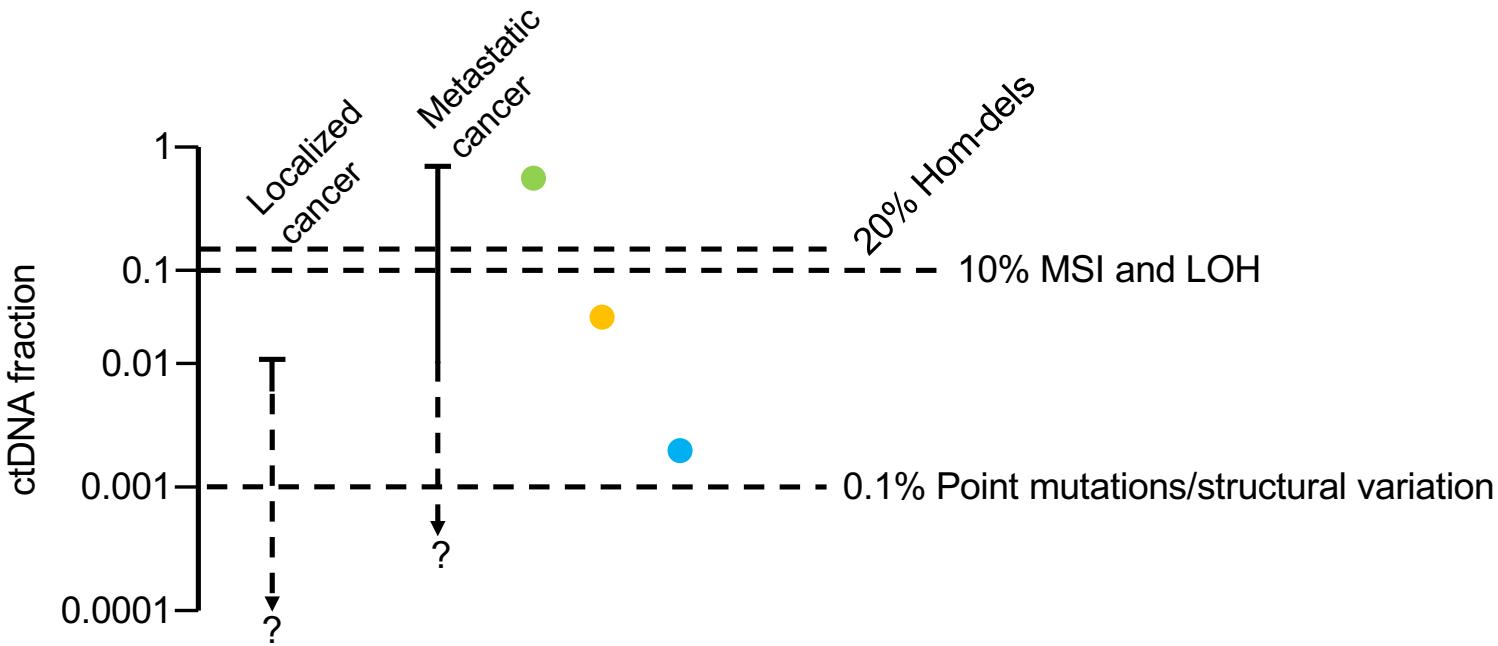
No therapies or clinical trials. See Genomic Signatures section

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.

)

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.



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 03-2020-00026951 12/12/1954

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

due to insufficient evidence of genomic

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

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



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 03-2020-00026951 12/12/1954

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

AR L702H, H875Y

CDK12 K482fs*14 →

ALK deletion exons 2-12

TP53 C275Y

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

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.



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 03-2020-00026951 12/12/1954

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%

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.





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 03-2020-00026951 12/12/1954

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

Found with ProBio assay

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.



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 03-2020-00026951 12/12/1954

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

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

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

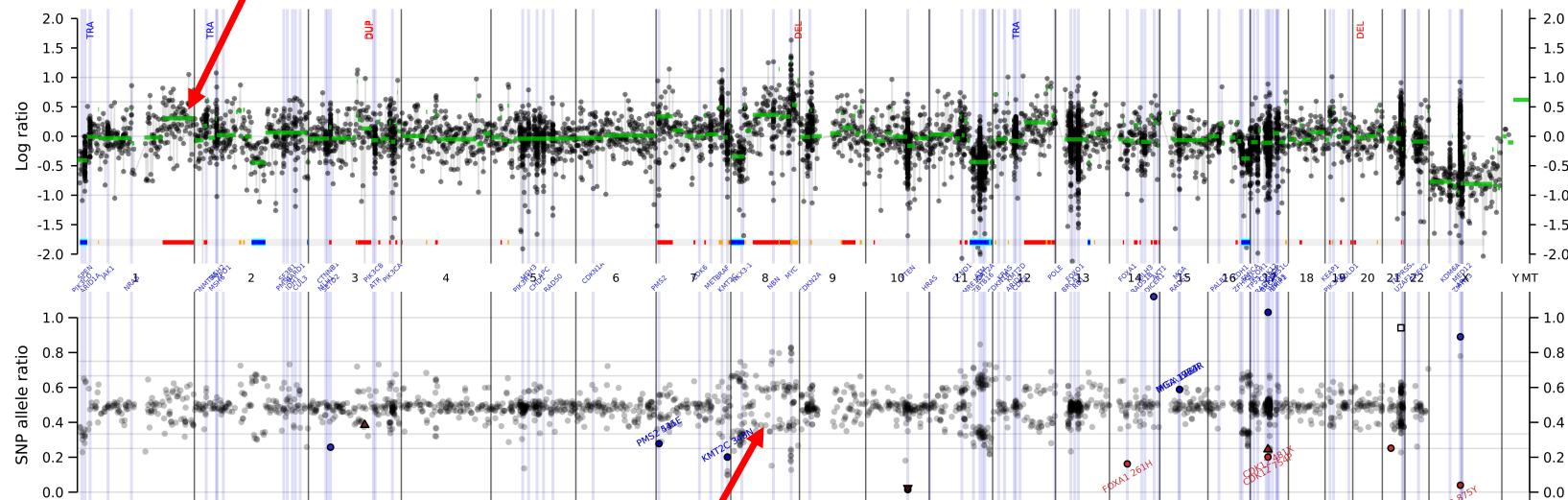
No therapies or clinical trials. See Genomic Signatures section

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.

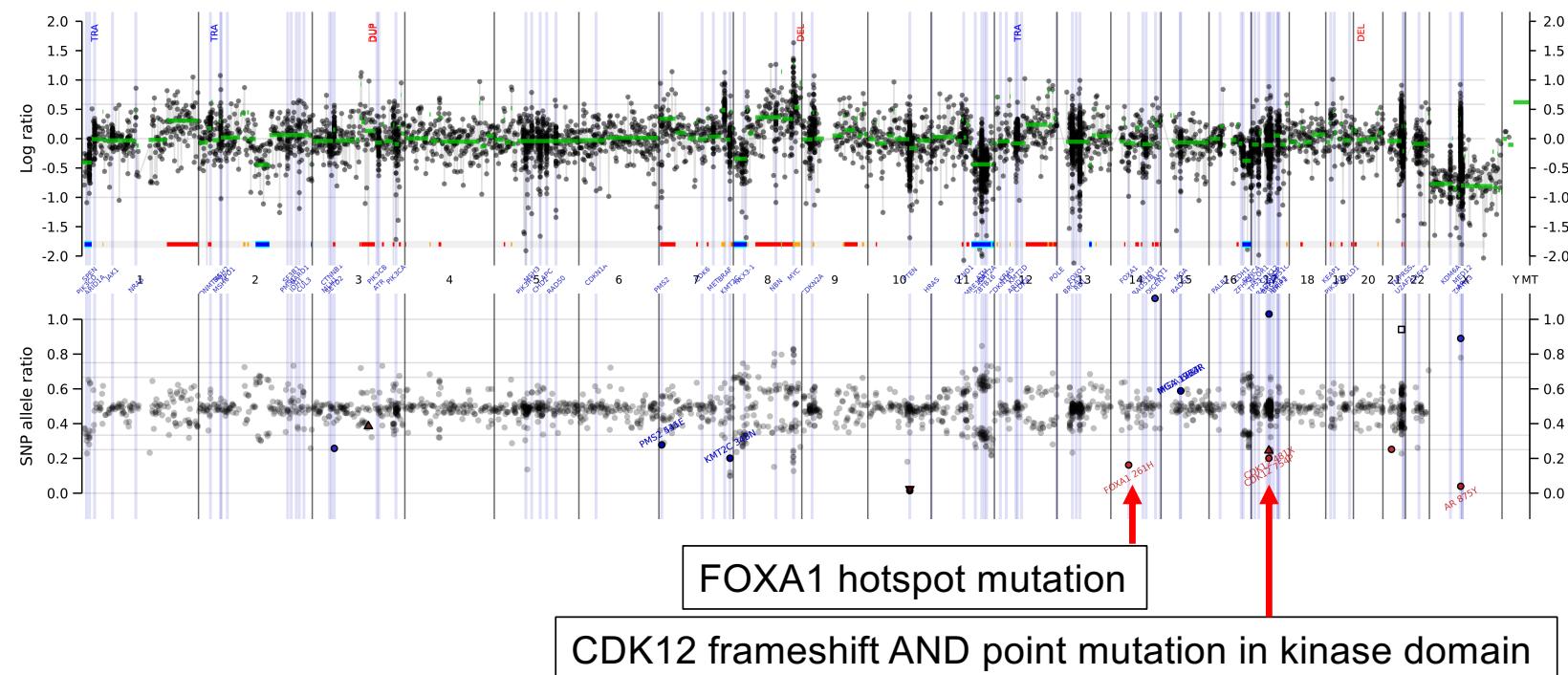
ProBio – liquid biopsy

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



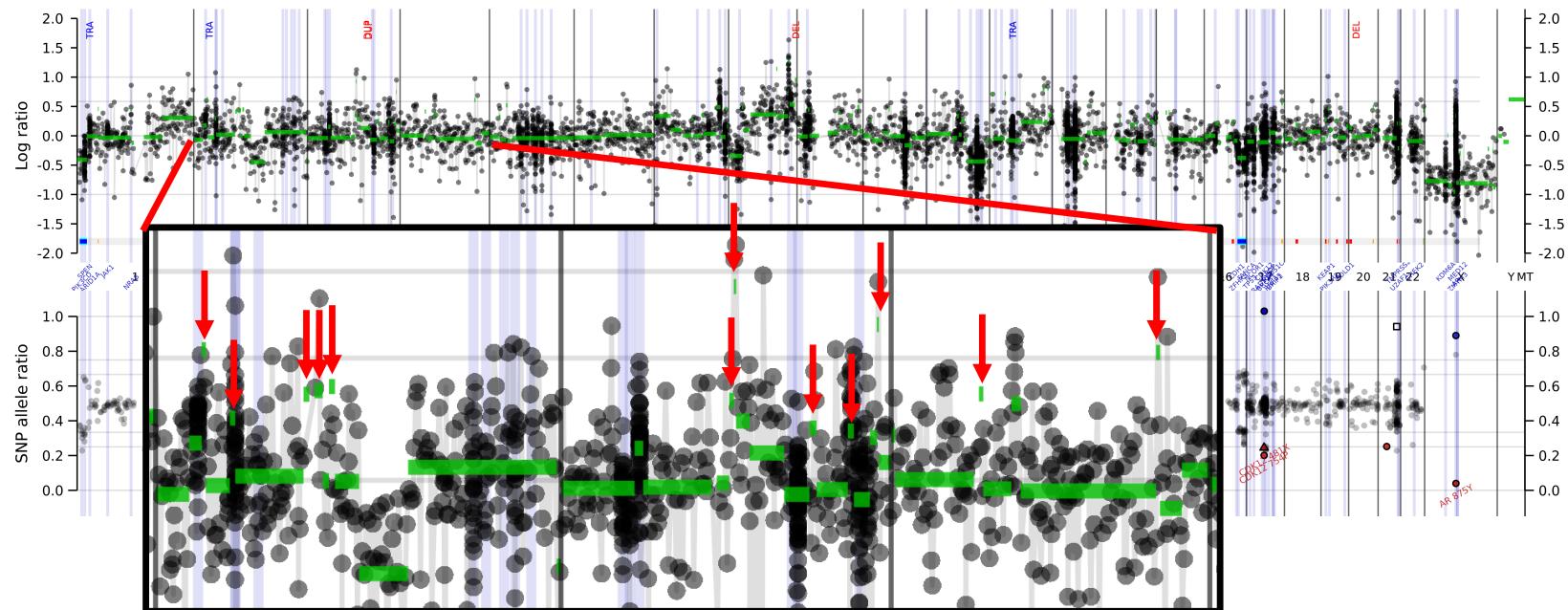
Single nucleotide polymorphism B-allele ratio supporting copy-number data

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.





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 03-2020-00026951 12/12/1954

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 →

Completely irrelevant

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.

)



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 03-2020-00026951 12/12/1954

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

False positive clonal hematopoiesis variant

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

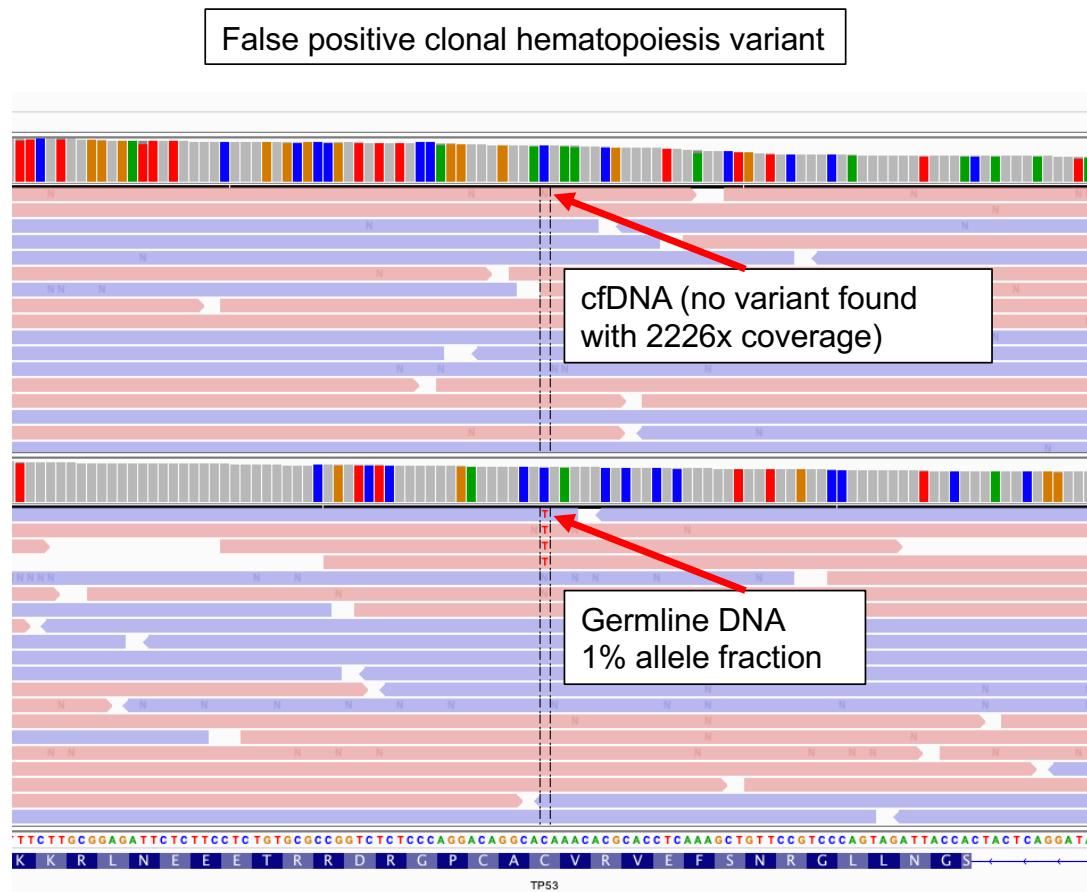
No therapies or clinical trials. See Genomic Signatures section

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.

)

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 03-2020-00026951 12/12/1954

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.

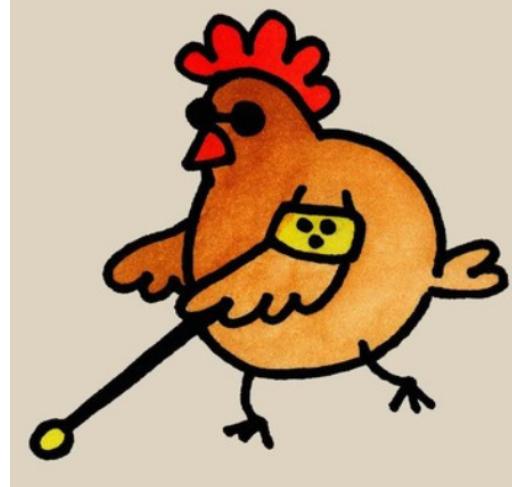
No
Unaltered
Instability

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.

FoundationOne Liquid CDx run on a mCRPC case

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

FoundationOne Liquid CDx



Questions?

