ProBio

From tissue to liquid biopsies - insights and technical challenges

Karolinska Institutet

AstraZeneca

Janssen

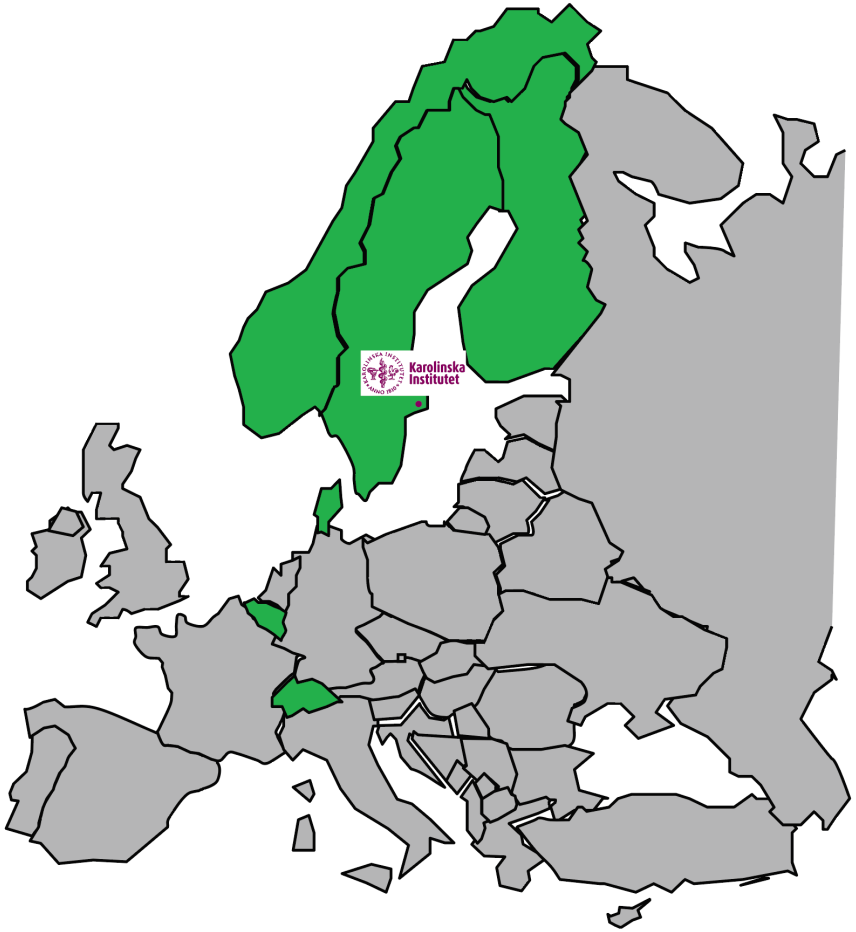
krebsliga beider basel
beraten – unterstützen – informieren

Cancerfonden

Vetenskapsrådet

Kom op tegen Kanker

Cancer genomics team at KI



ALASCCA
Adjuvant ASA vid kolorektalcancer

ProBio

iPCM

PSFF program
(Pre-Screening För Fas-I)

ctDNA for patients referred to the Phase-I Unit

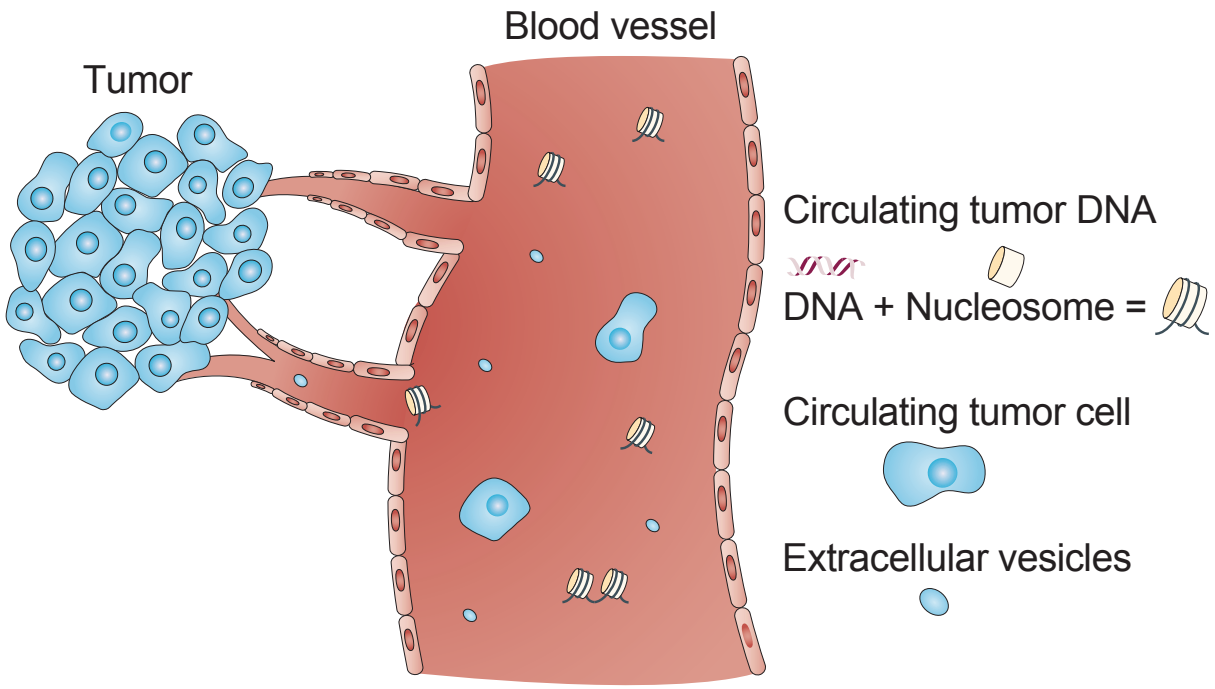
~50 hospitals in 6 European countries

Karolinska Institutet
>5000 cases analysed

Todays topic: how genomic profiling is setup for the ProBio trial

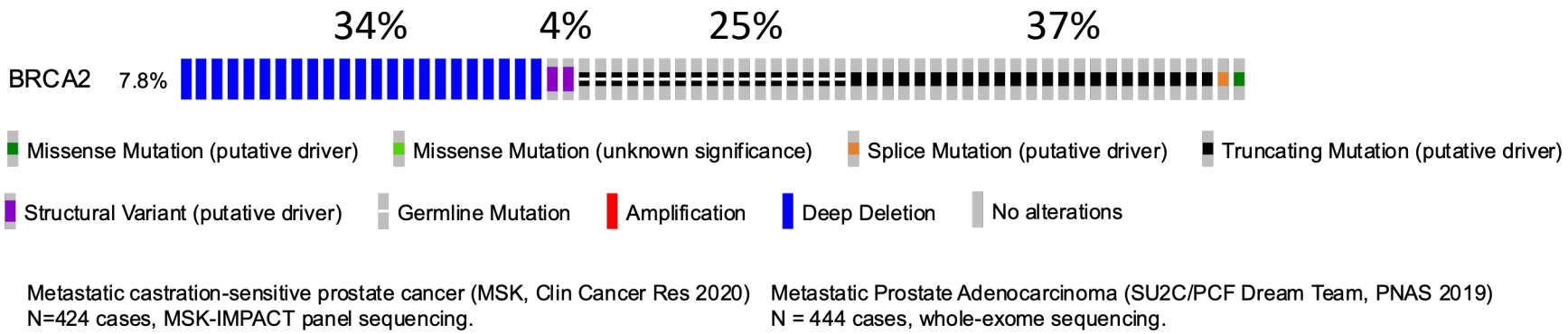
Biomarker testing – insights and technical challenges

- Tissue options:
  - Diagnostic biopsy.
  - Metastatic biopsy.
  - Circulating tumor DNA.



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

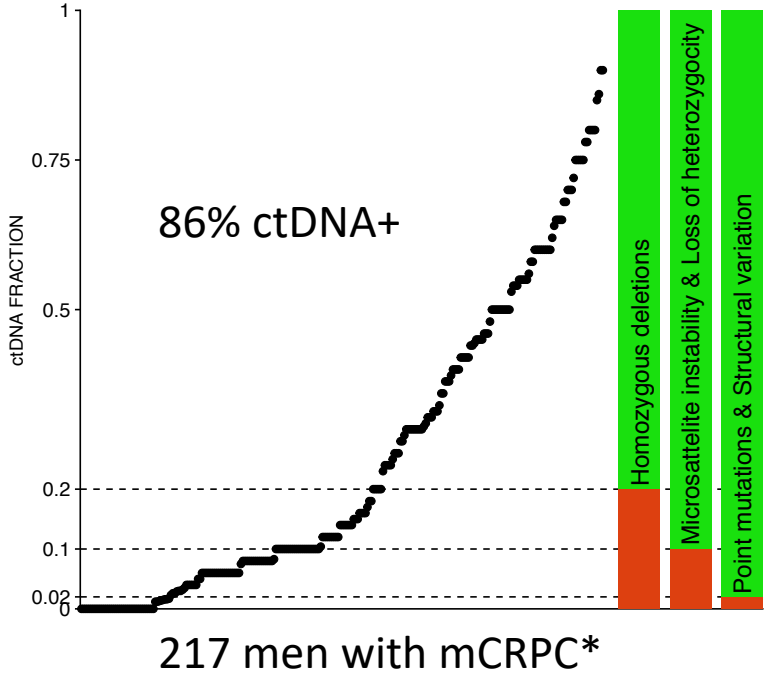
BRCA2 – expected alterations in an ”optimal” setting.



- Comment:
  - Important to identify BRCA2 homdels as these have the best responses (Biomarkers Associating with PARP Inhibitor Benefit in Prostate Cancer in the TOPARP-B Trial).

Cancer DNA fraction – the key metric in cancer genomics

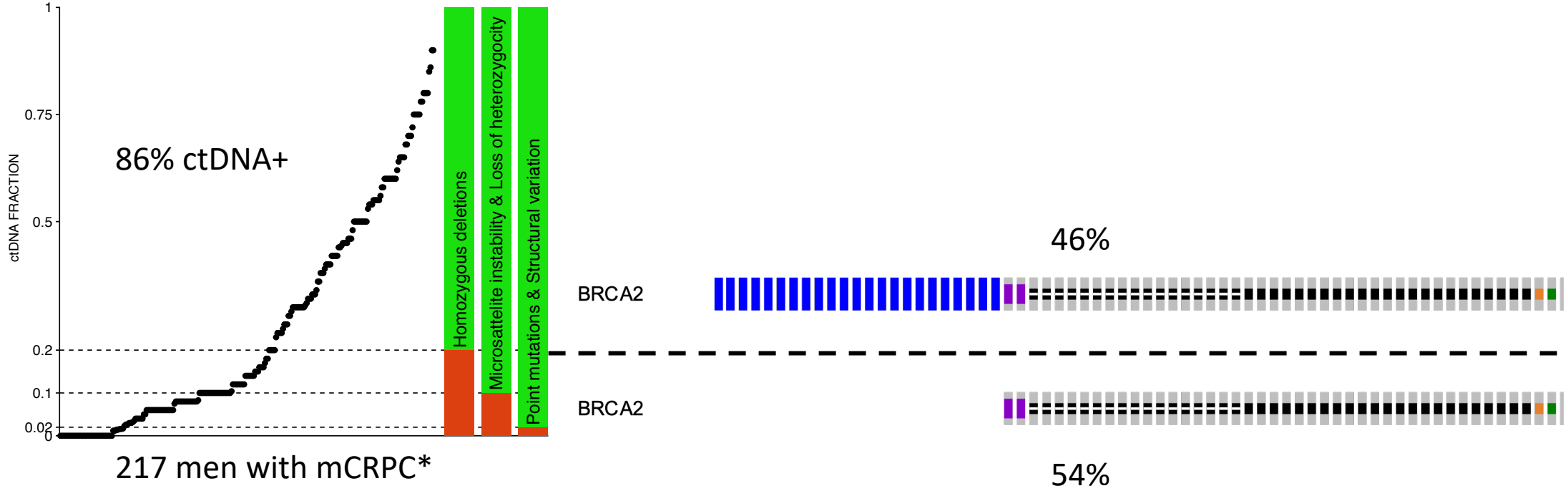
- Different types of somatic alterations require different cancer DNA fraction for detection.
- Affects tumor biopsy analysis as well ctDNA.
  - Macrodissection of biopsies can be applied to increase cancer DNA fraction.



* Mayrhofer et al, Genome Medicine 2018

Cancer DNA fraction – the key metric in cancer genomics

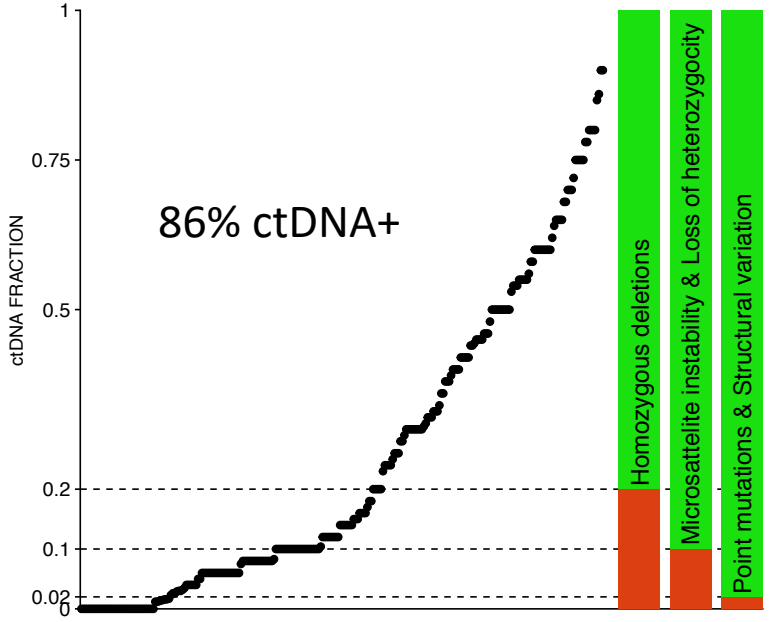
- False negatives is a challenge, must be clearly stated for clinical reporting.
- Important with honest reporting!



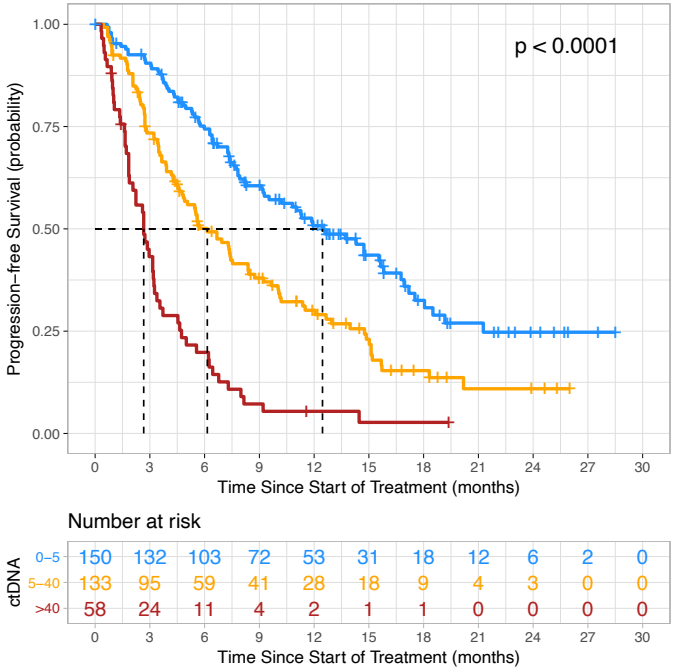
* Mayrhofer et al, Genome Medicine 2018

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.

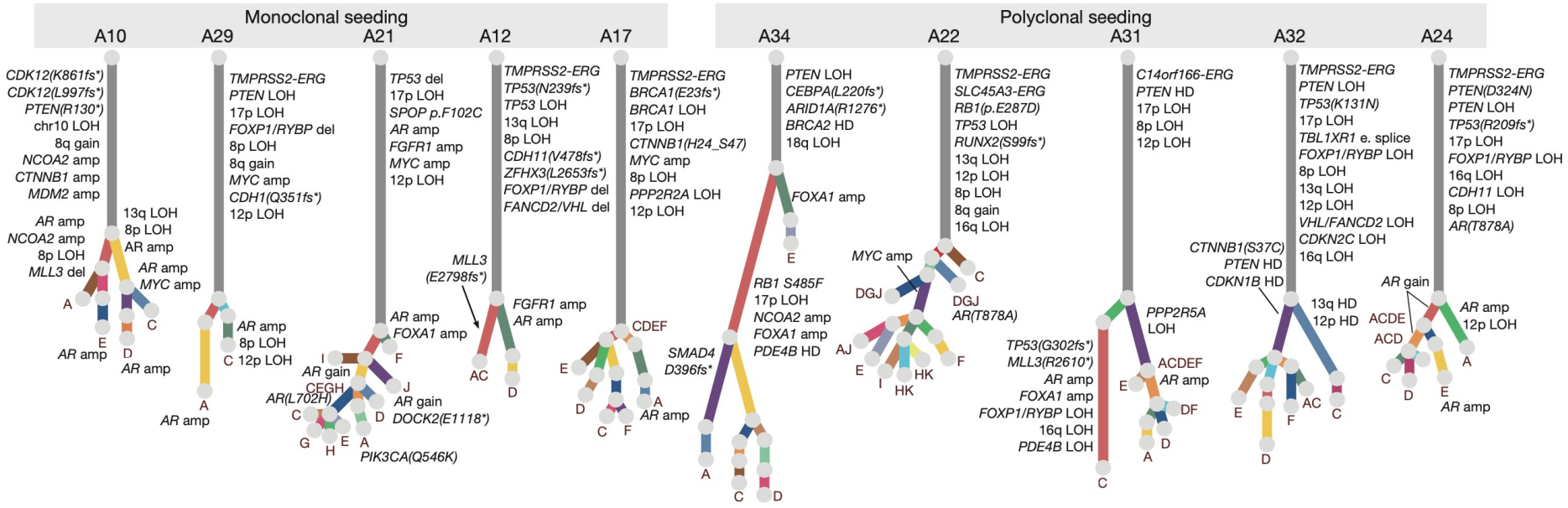


217 men with mCRPC*



* Mayrhofer et al, Genome Medicine 2018

What does ctDNA represent?



- mCRPC rapid autopsy cohort.
- WGS on multiple metastasis / case.
- Heterogeneity occurred mostly in AR, due to the evolutionary pressure of treatment.
- Supported by data from the Hartwig foundation.

The evolutionary history of lethal metastatic prostate cancer, Nature, 2015.
Limited evolution of the actionable metastatic cancer genome under therapeutic pressure, Nature Medicine, 2021.

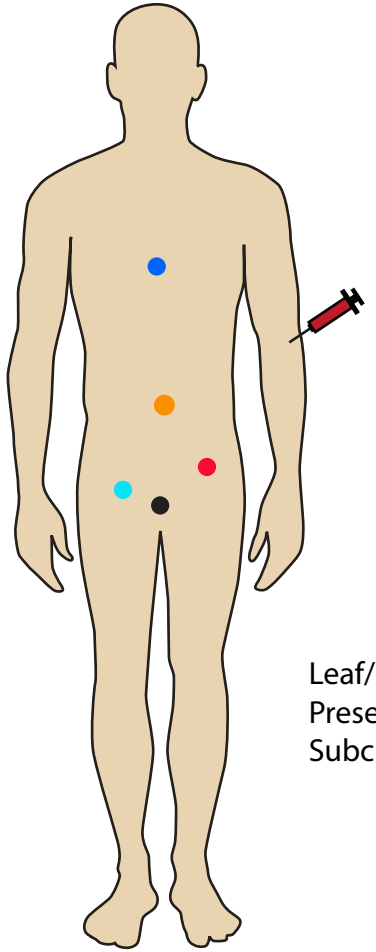
What does ctDNA represent?

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[1,12], Matti Annala[1,2,12], Joonatan Sipola[2,12], Sarah W. S. Ng[1], Xinyi E. Chen[1], Anssi Nurminen[2], Olga V. Korhonen[2], Aslı D. Munzur[1], Kevin Beja[1], Elena Schönlau[1], Cecily Q. Bernales[1], Elie Ritch[1], Jack V. W. Bacon[1], Nathan A. Lack[1,3,4], Matti Nykter[2], Rahul Aggarwal[5,6], Eric J. Small[5,6], Martin E. Gleave[1], SU2C/PCF West Coast Prostate Cancer Dream Team*, David A. Quigley[5,7,8], Felix Y. Feng[5,6,7,9,13], Kim N. Chi[1,10,13] & Alexander W. Wyatt[1,11,13]



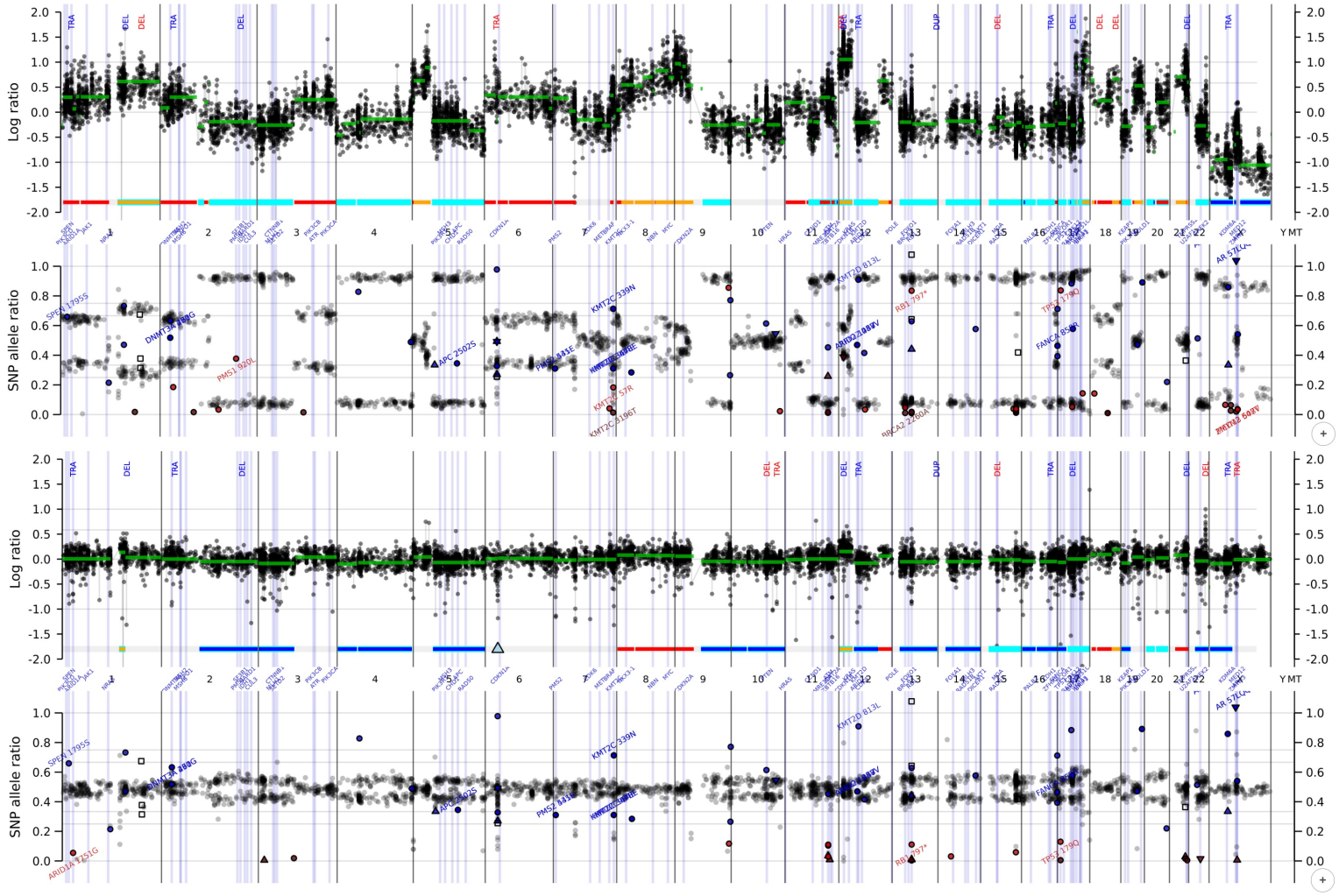
Phylogenetic tree.

Stem, present in the majority of metastasis.
"Clonal" in ctDNA.

Leaf/branch.
Present in single/multiple tissue locations.
Subclonal in ctDNA.

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

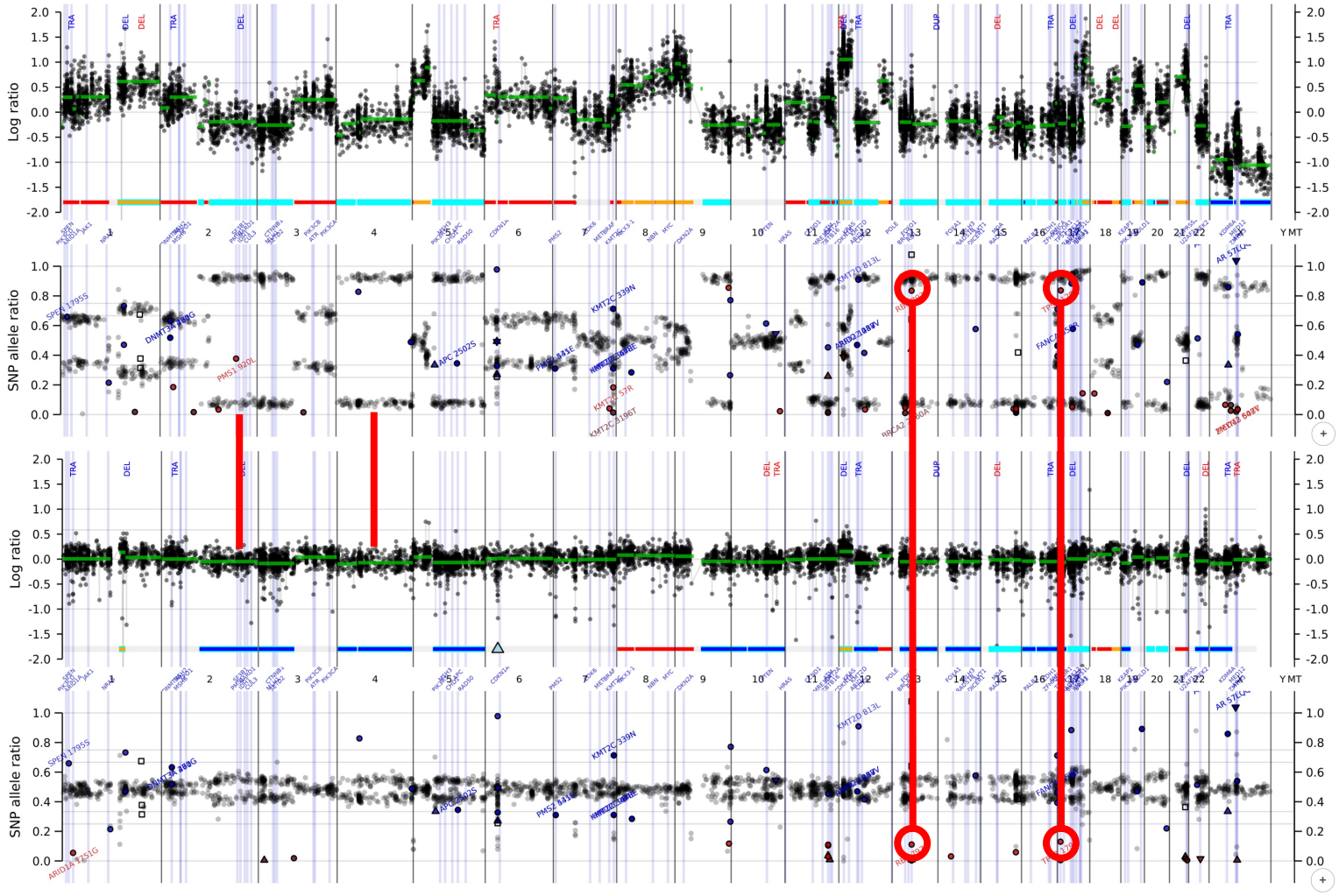
What does ctDNA represent?



Metastatic tissue biopsy
High cancer DNA fraction

ctDNA
Low cancer DNA fraction

What does ctDNA represent?



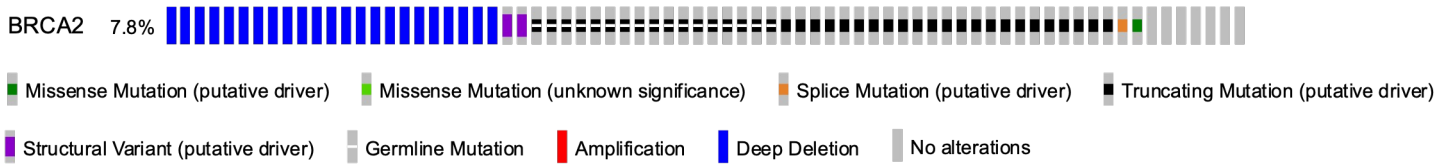
Metastatic tissue biopsy
High cancer DNA fraction

ctDNA
Low cancer DNA fraction

What assay to use?

- Make sure it covers the biomarkers of interest.

A test should cover all relevant alteration types



Metastatic castration-sensitive prostate cancer (MSK, Clin Cancer Res 2020)
N=424 cases, MSK-IMPACT panel sequencing.

Metastatic Prostate Adenocarcinoma (SU2C/PCF Dream Team, PNAS 2019)
N = 444 cases, whole-exome sequencing.

Some of the large liquid biopsy companies

- Simultaneous profiling of ctDNA & gDNA is preferred.

Clonal expansions in white blood cells (CHIP)

- Known since 2014

ORIGINAL ARTICLE

Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Kähler, Ph.D., Robert E. Handsaker, B.S., Johan Lindberg, Ph.D., Samuel A. Rose, B.S., Samuel F. Bakhoum, M.D., Ph.D., Kimberly Chambert, M.S., Eran Mick, B.S., Benjamin M. Neale, Ph.D., Menachem Fromer, Ph.D., Shaun M. Purcell, Ph.D., Oscar Svantesson, M.S., Mikael Landén, Ph.D., Martin Höglund, M.D., Ph.D., Sören Lehmann, M.D., Ph.D., Stacey B. Gabriel, Ph.D., Jennifer L. Moran, Ph.D., Eric S. Lander, Ph.D., Patrick F. Sullivan, M.D., Pamela Sklar, M.D., Ph.D., Henrik Grönberg, M.D., Ph.D., Christina M. Hultman, Ph.D., and Steven A. McCarroll, Ph.D.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes

Siddhartha Jaiswal, M.D., Ph.D., Pierre Fontanillas, Ph.D., Jason Flannick, Ph.D., Alisa Manning, Ph.D., Peter V. Grauman, B.A., Brenton G. Mar, M.D., Ph.D., R. Coleman Lindsley, M.D., Ph.D., Craig H. Mermel, M.D., Ph.D., Noel Burtt, B.S., Alejandro Chavez, M.D., Ph.D., John M. Higgins, M.D., Vladislav Moltchanov, Ph.D., Frank C. Kuo, M.D., Ph.D., Michael J. Kluk, M.D., Ph.D., Brian Henderson, M.D., Leena Kinnunen M.Sc., Heikki A. Koistinen, M.D., Ph.D., Claes Ladenvall, Ph.D., Gad Getz, Ph.D., Adolfo Correa, M.D., Ph.D., Benjamin F. Banahan, Ph.D., Stacey Gabriel, Ph.D., Sekar Kathiresan, M.D., Heather M. Stringham, Ph.D., Mark I. McCarthy, M.D.,* Michael Boehnke, Ph.D.,* Jaakko Tuomilehto, M.D., Ph.D., Christopher Haiman, Sc.D., Leif Groop, M.D., Ph.D., Gil Atzmon, Ph.D., James G. Wilson, M.D., Donna Neuberg, Sc.D., David Altshuler, M.D., Ph.D.,* and Benjamin L. Ebert, M.D., Ph.D.†

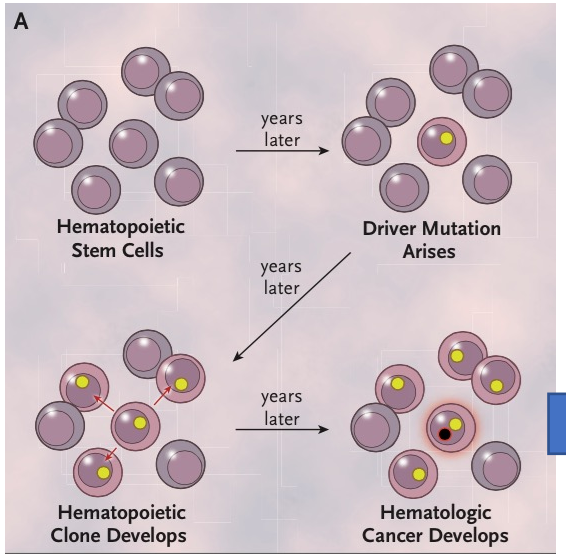
ANALYSIS

nature medicine

Age-related mutations associated with clonal hematopoietic expansion and malignancies

Mingchao Xie[1,2,7], Charles Lu[1,7], Jiayin Wang[1,2,7], Michael D McLellan[1], Kimberly J Johnson[3], Michael C Wendl[1,4,5], Joshua F McMichael[1], Heather K Schmidt[1], Venkata Yellapantula[1,2], Christopher A Miller[1], 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]

- Associated with risk for hematological cancers



Apoptotic DNA ends up in plasma!

Deeper sequencing identifies more CHIP

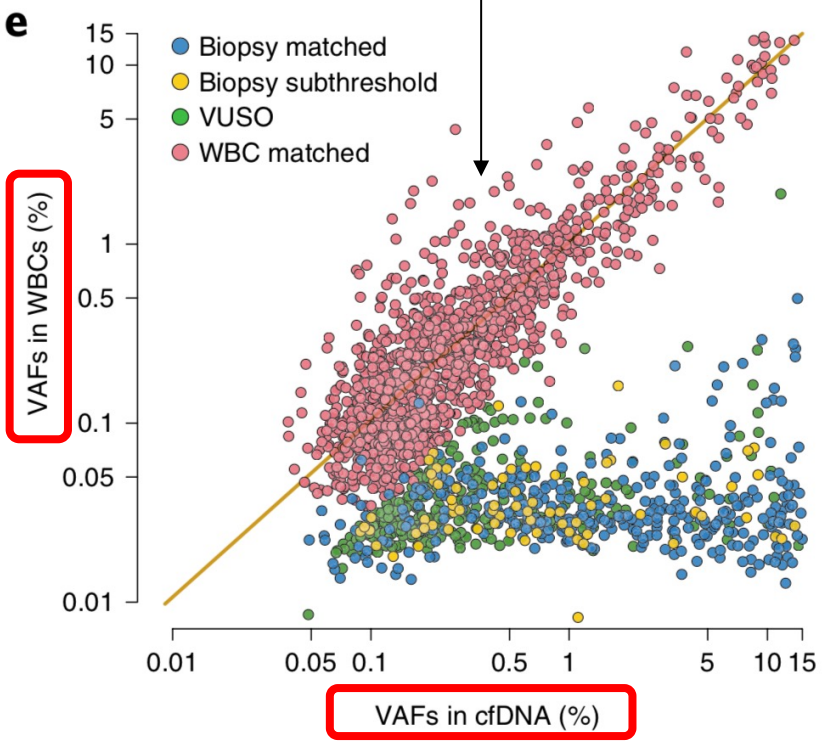
nature medicine
ARTICLES
https://doi.org/10.1038/s41591-019-0652-7

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

Pedram Razavi[1,2,13]*, Bob T. Li[1,13], David N. Brown[3,13], Byoungsok Jung[4], Earl Hubbell[4], Ronglai Shen[5], Wassim Abida[1], Krishna Juluru[6], Ino De Bruijn[7], Chenlu Hou[4], Oliver Venn[4], Raymond Lim[3], Aseem Anand[1], Tara Maddala[4], Sante Gnerre[4], Ravi Vijaya Satya[4], Qinwen Liu[4], Ling Shen[4], Nicholas Eattock[4], Jeanne Yue[4], Alexander W. Blocker[4,9], Mark Lee[4,10], Amy Sehnert[4,11], Hui Xu[4], Megan P. Hall[4], Angie Santiago-Zayas[1], William F. Novotny[4,12], James M. Isbell[8], Valerie W. Rusch[8], George Plitas[8], Alexandra S. Heerdt[8], Marc Ladanyi[3], David M. Hyman[1], David R. Jones[8], Monica Morrow[8], Gregory J. Riely[1], Howard I. Scher[1], Charles M. Rudin[1], Mark E. Robson[1], Luis A. Diaz Jr.[1], David B. Solit[1,2,7], Alexander M. Aravanis[4] and Jorge S. Reis-Filho[2,3]*

- Broad AND deep sequencing
  - 2 Mb panel
  - 60.000X coverage
- Lung, breast and prostate cancer.
- Matched tumor tissue and ctDNA.
- The majority of cfDNA mutations were due to CHIP

Potentially false positive variants with cfDNA-only testing



CHIP – 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

- mCRPC ctDNA + gDNA panel sequencing
- Large fraction of DNA-repair variants originated from CHIP in the white blood cells.
- One case carried a BRCA2 CHIP-variant.
- The patient was tested with a commercial assay and recommended PARPi.

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

SPECIAL ARTICLE

ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group

J. Pascual[1], G. Attard[2], F.-C. Bidard[3,4], G. Curigliano[5,6], L. De Mattos-Arruda[7,8], M. Diehn[9], A. Italiano[10,11,12], J. Lindberg[13], J. D. Merker[14], C. Montagut[15], N. Normanno[16], K. Pantel[17], G. Pentheroudakis[18], S. Popat[19,20], J. S. Reis-Filho[21], J. Tie[22,23], J. Seoane[24,25], N. Tarazona[26,27], T. Yoshino[28] & N. C. Turner[19,20*]

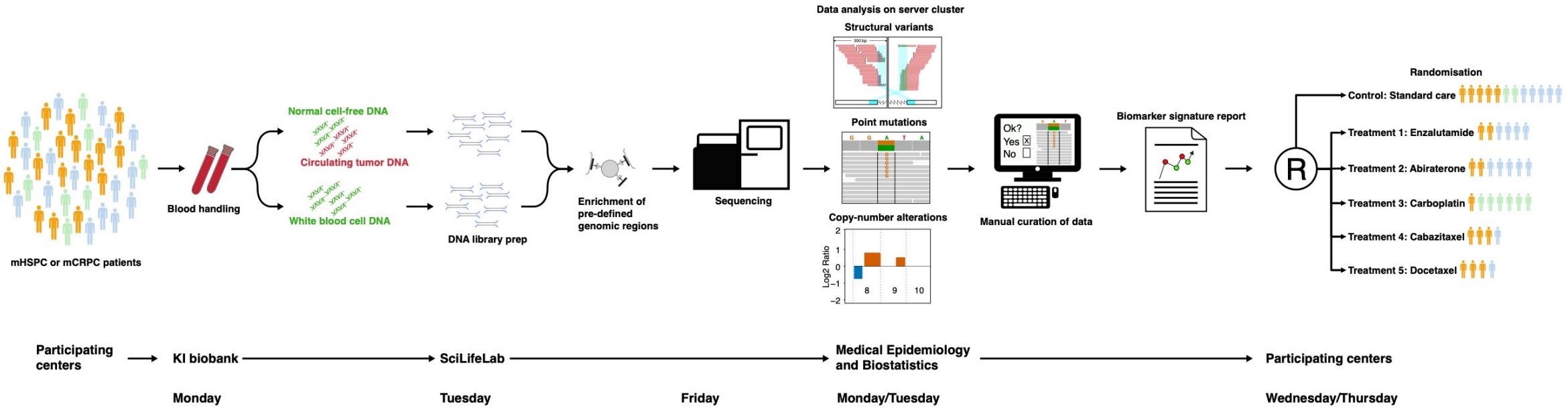
- Team of experts recommend against cfDNA-only testing for prostate cancer.

Summary so far ..

- Cancer DNA fraction determines which variant types that may be detected.
- Circulating tumor DNA is a direct representation of the metastatic lesions where Individual metastasis contribute to a small fraction of the total ctDNA.
- Cell-free DNA sequencing should be performed with simultaneous germline DNA sequencing to minimize the risk of false positive variants originating from the white blood cells.

Genomic profiling process for ProBio

From blood draw to biomarker signature report



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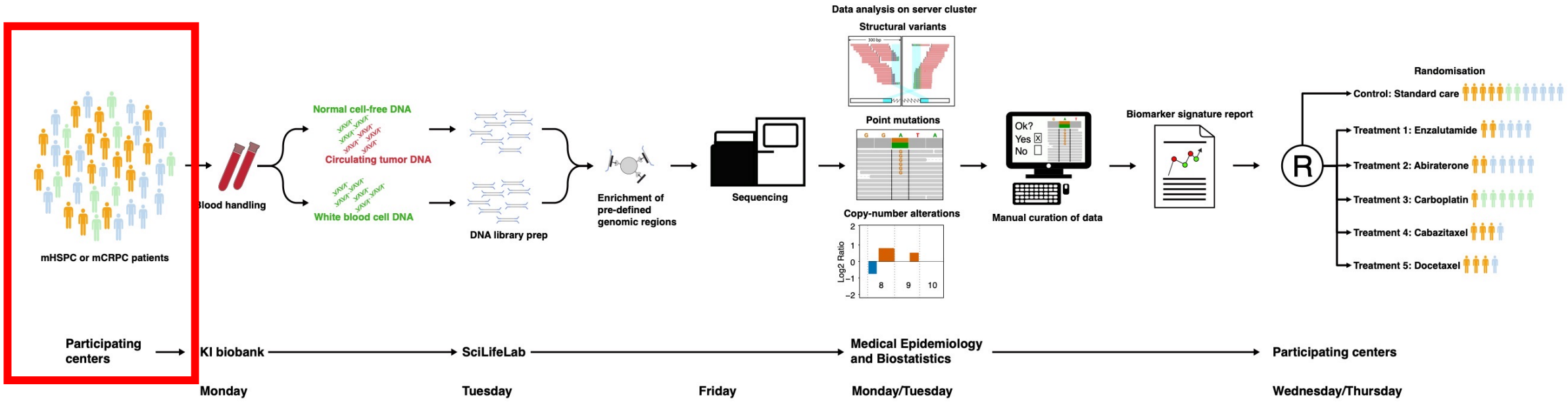

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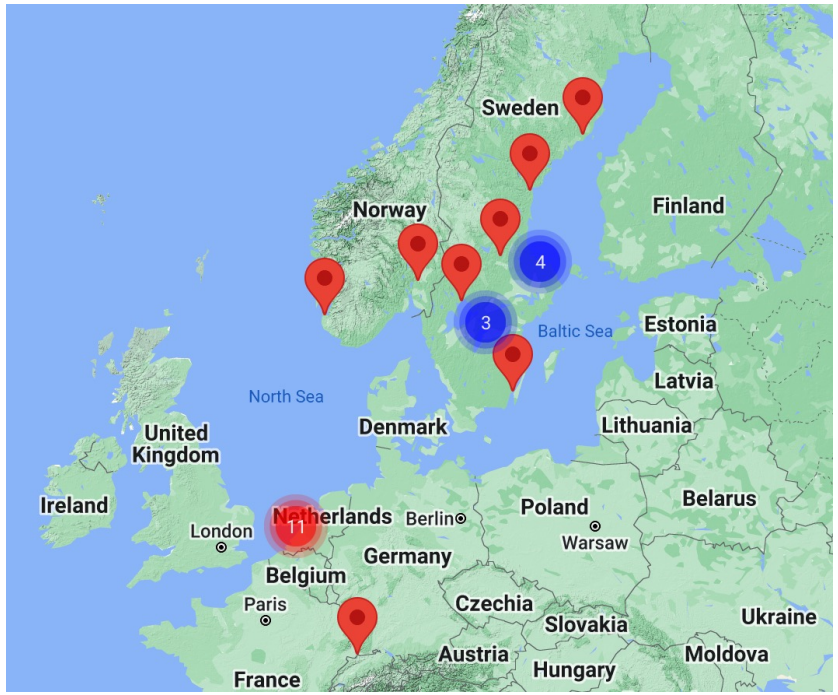
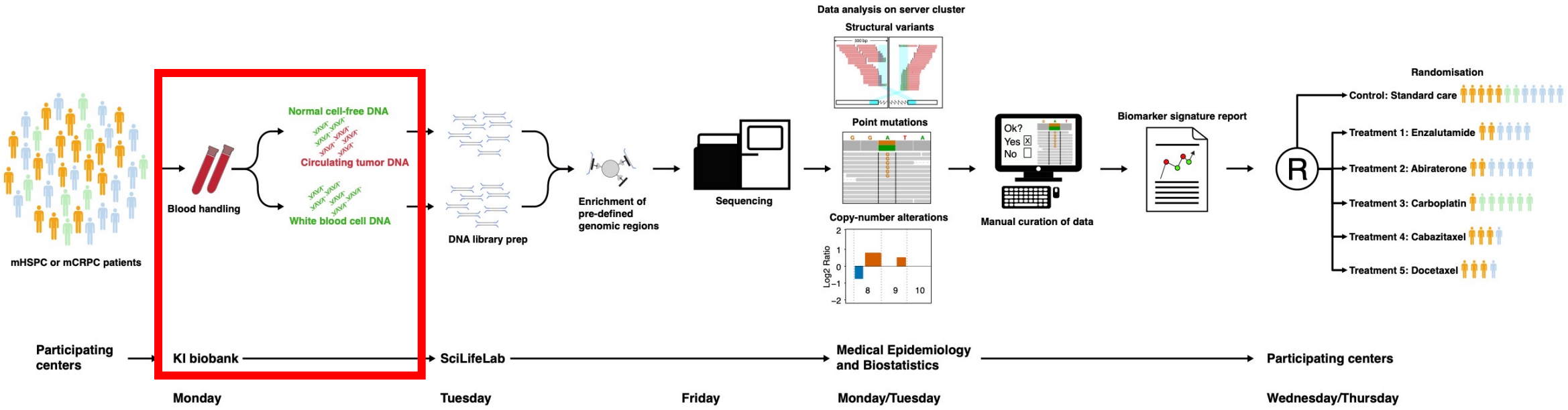
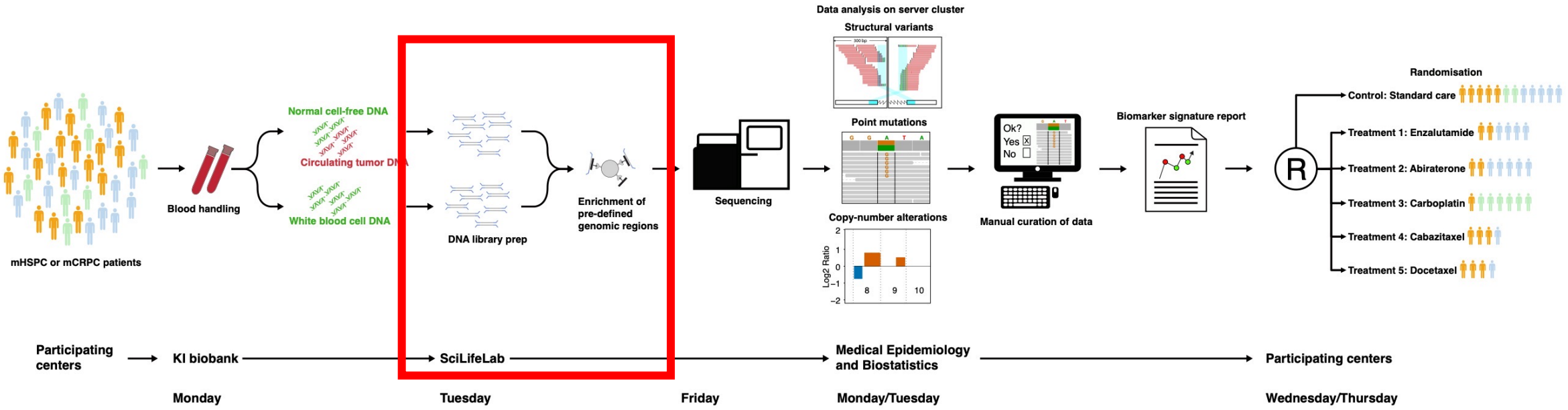


- Inclusion and blood draw.
  - Friday afternoon – Friday before lunch.
    - Sweden/Norway/Switzerland.
      - Ship Streck tubes to KI biobank.
    - Belgium.
      - Ship plasma and whole blood OR extracted DNA to KI biobank.

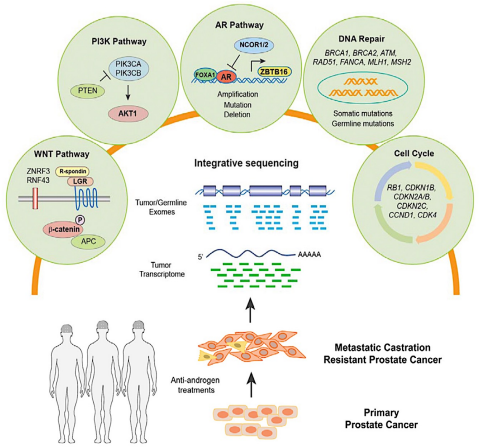
From blood draw to biomarker signature report

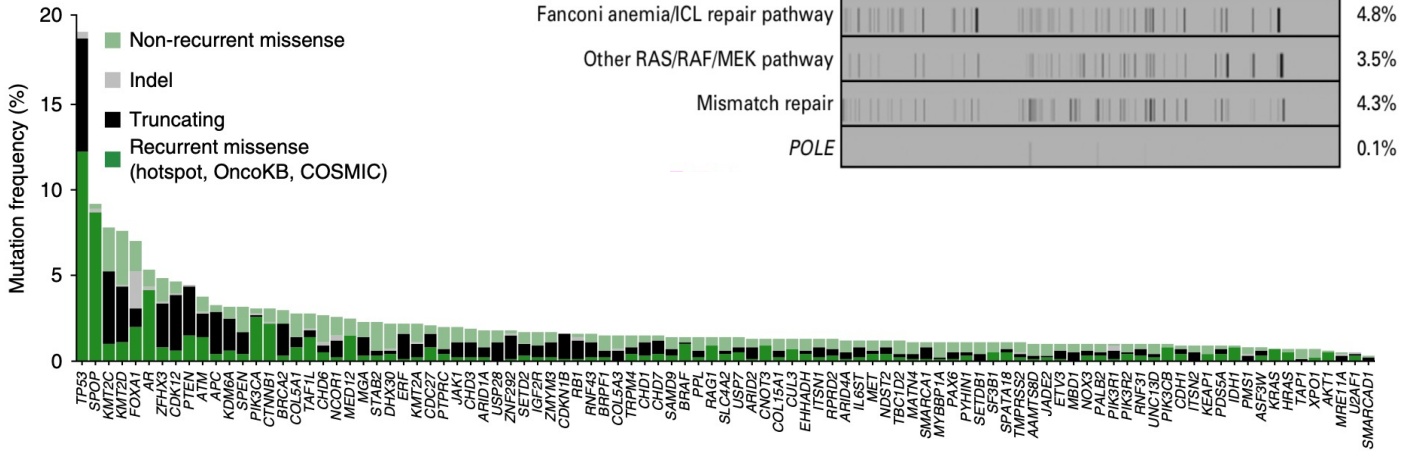


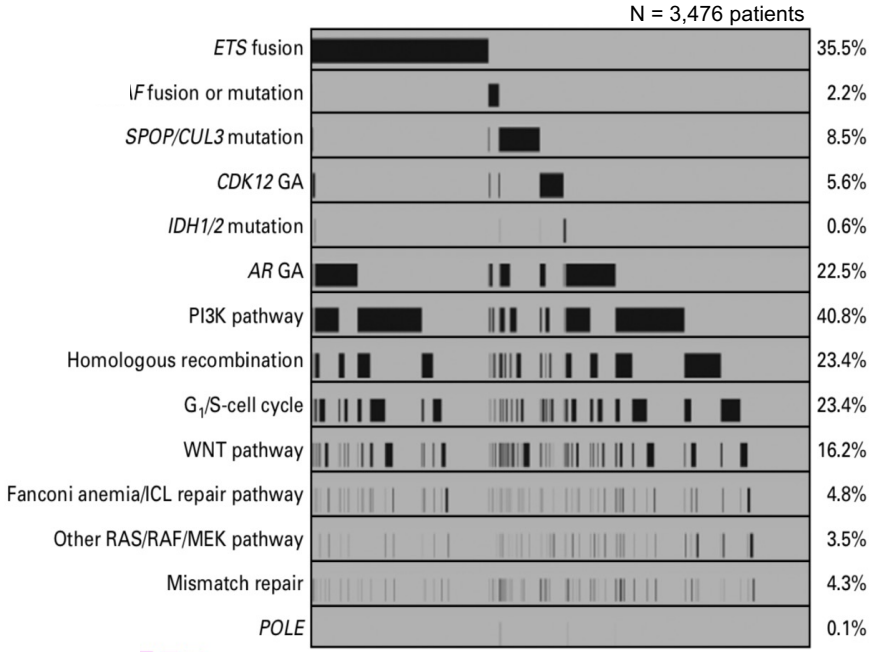
From blood draw to biomarker signature report

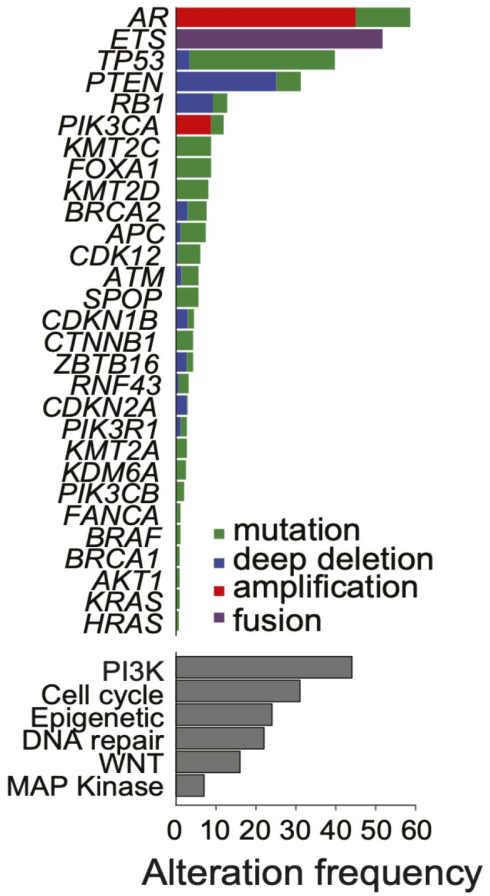


Inventory of already published data



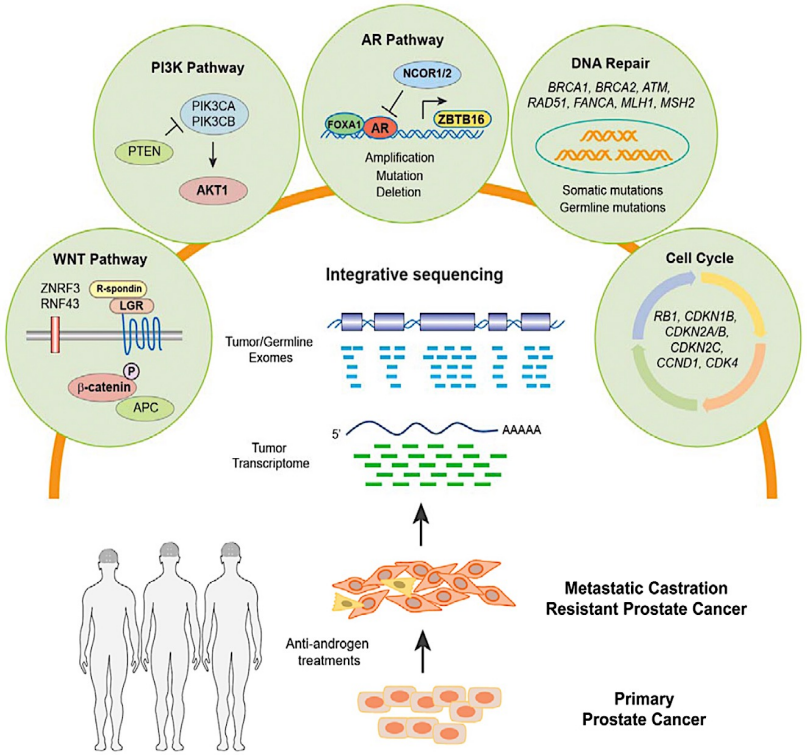






Robinson et al, Cell, 2015; Armenia et al, Nat Gen, 2019; Chung et al, JCO PO, 2019; Abida et al, PNAS, 2019

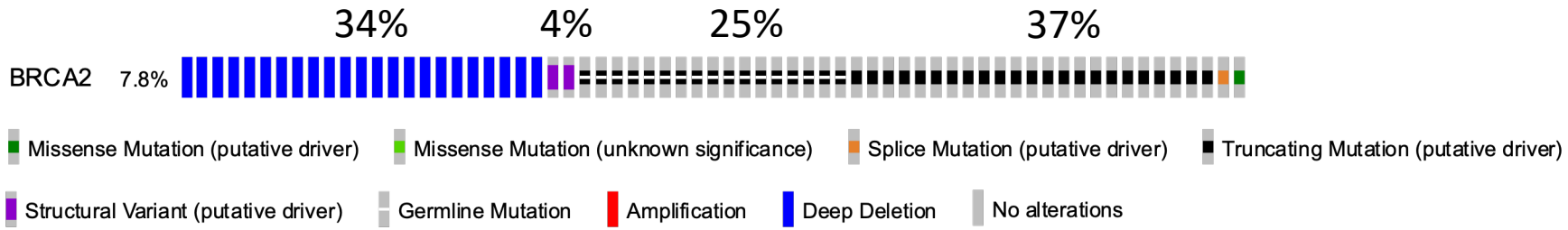
Special care taken for the biomarkers investigated in ProBio



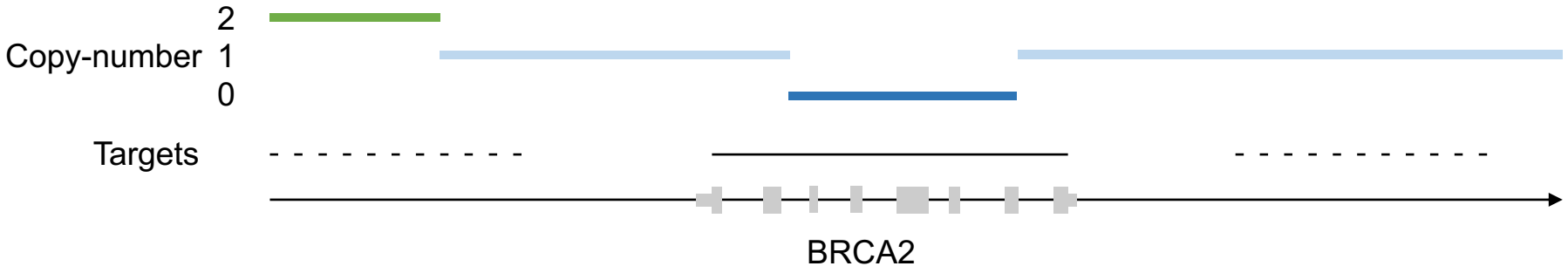
Biomarker	Therapy	Example
Variants of *TP53* & the *Androgen Receptor (AR)*	ARS inhibitors	Abiraterone Enzalutamide
Genomic defects in DNA repair genes (DRD) or homologous recombination repair deficiency (HRD)	PARP inhibitors Platinum-based CT	Olaparib Carboplatin
TMPRSS2-ERG fusion	Taxane-based CT	Docetaxel
Alterations in the PI3K-AKT-mTOR signaling pathway	AKT inhibitors	Ipatasertib Capivasertib
Etc.		

Robinson et al, Cell, 2015

Tailored BRCA2 design



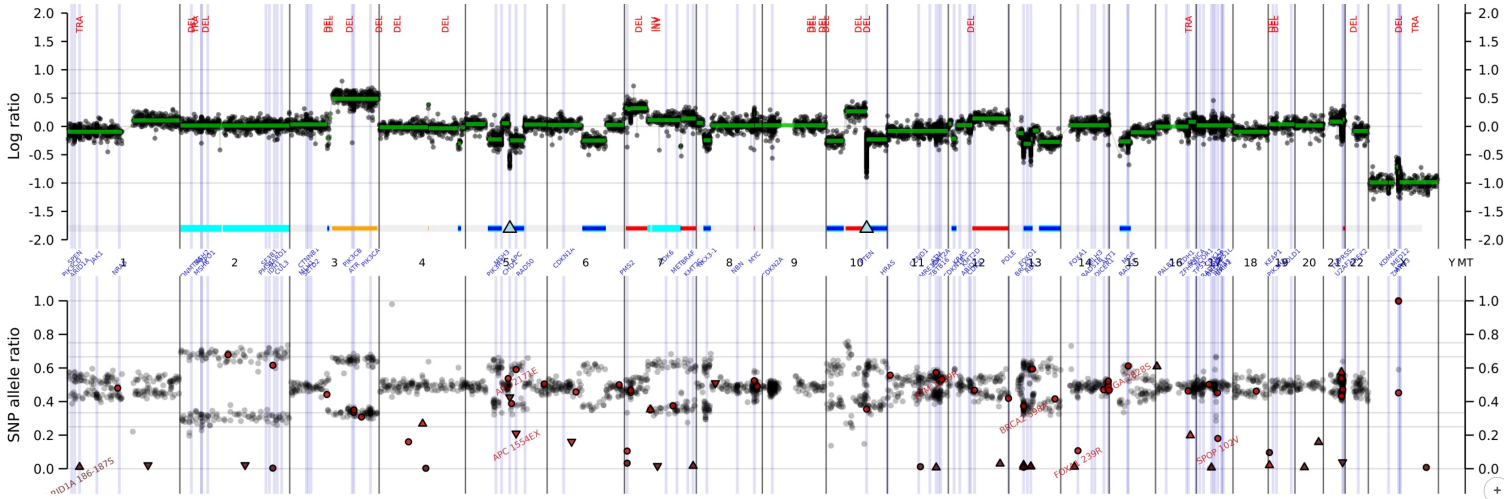
To maximize sensitivity, entire gene-body is targeted + control regions.



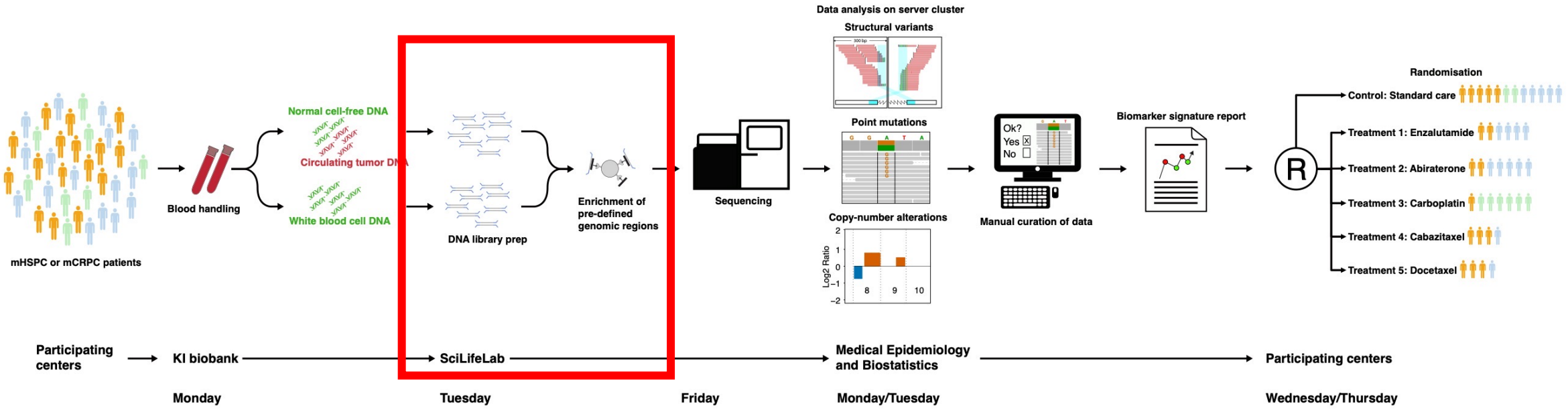
The ProBio assay

ProBio panel = Custom ctDNA panel for metastatic prostate cancer (1.34 Mb).

- Mutations in 79 genes.
  - Limit of detection: ≥0.005
- Structural rearrangements in 7 genes.
  - Limit of detection: ≥0.005
- SNP backbone for genome-wide copy-number alterations.
- Homozygous deletions, for selected genes:
  - Limit of detection: 0.20 -> ~0.05
- Microsatellites to infer MSI.
- Tumor Mutational Burden (TMB) estimation.

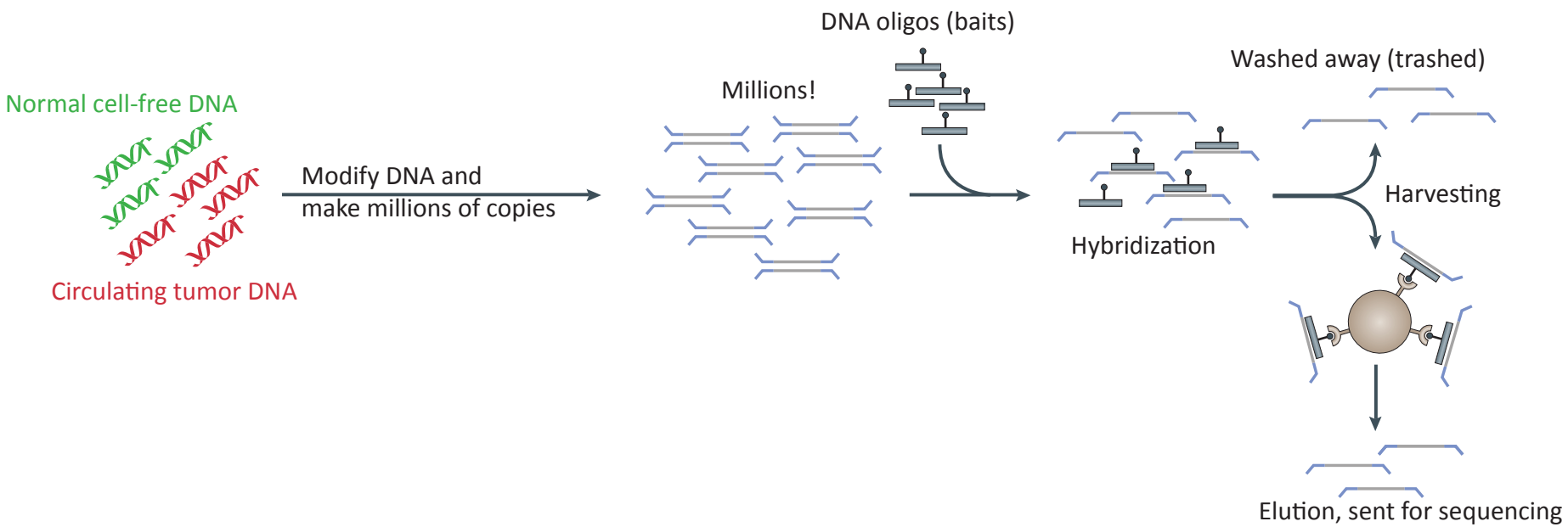


From blood draw to biomarker signature report



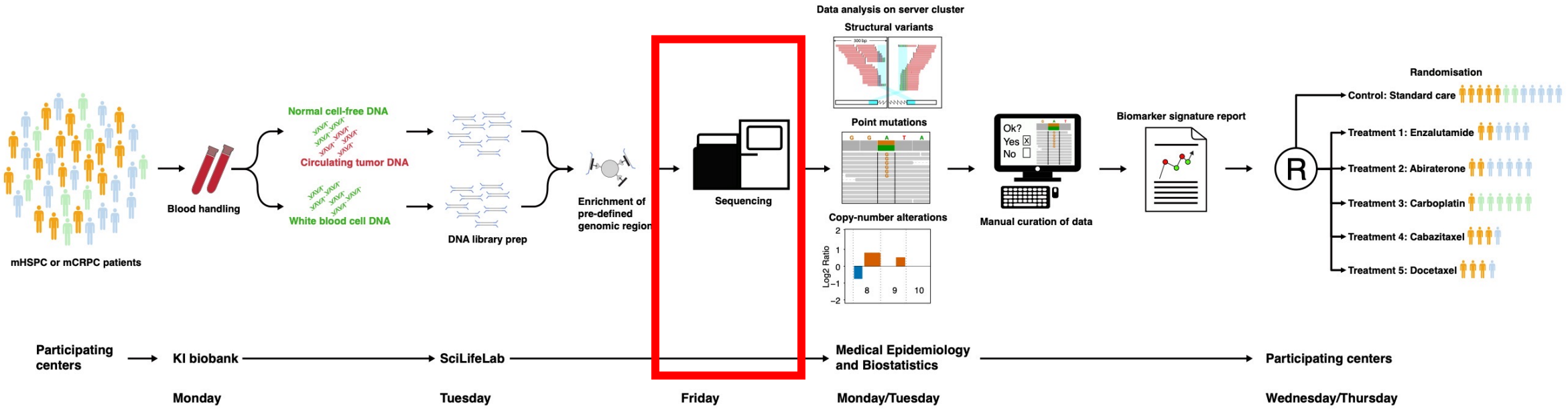
Targeted or panel-based sequencing in ProBio

- In-solution hybridization-based capture/targeted sequencing/panel sequencing.



Adopted from: Advances in understanding cancer genomes through second-generation sequencing, Nat Rev Gen 2010

From blood draw to biomarker signature report



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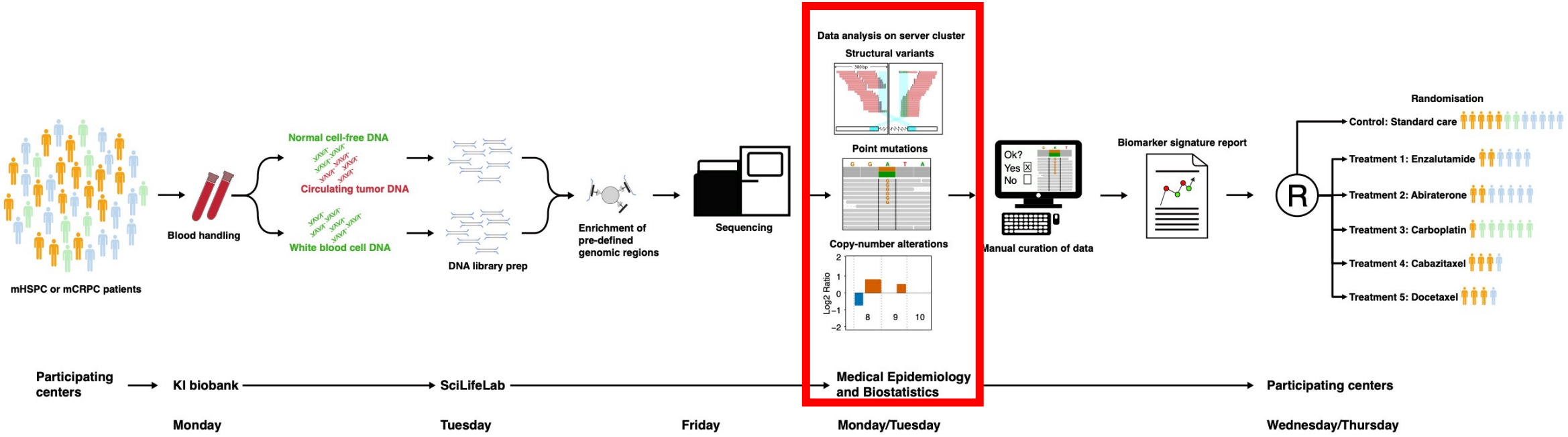
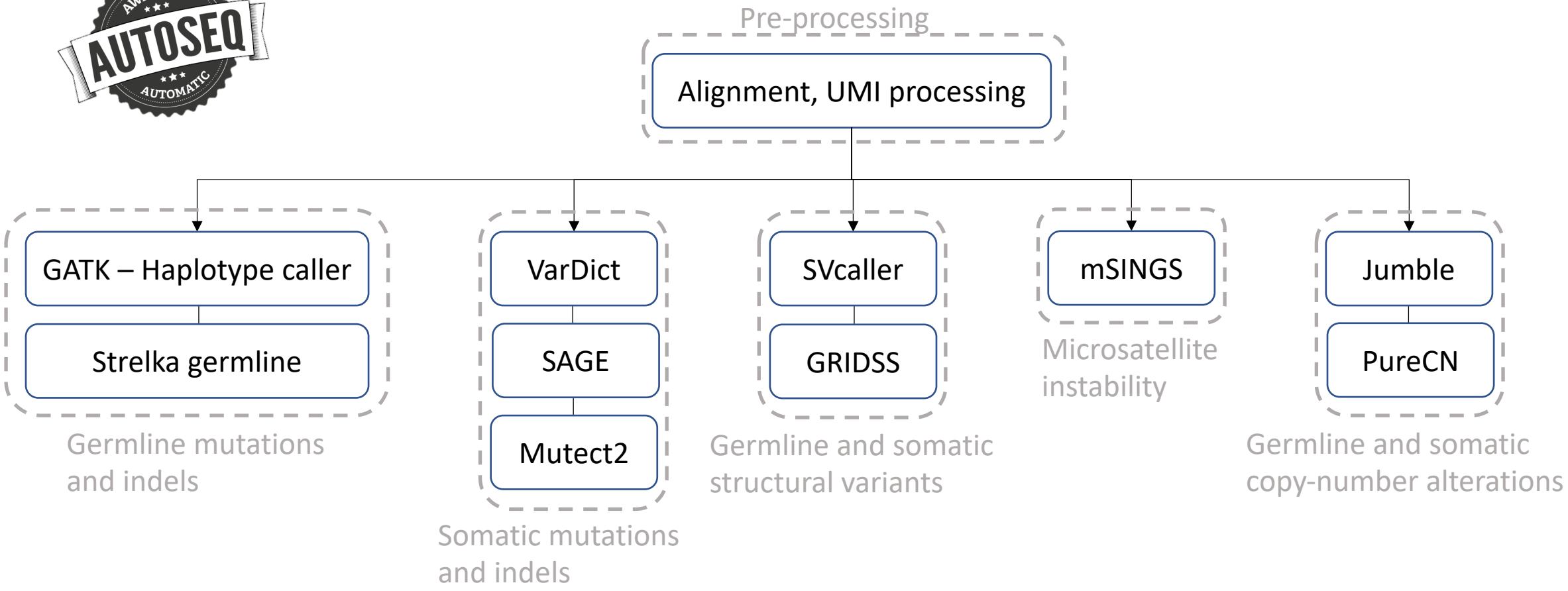
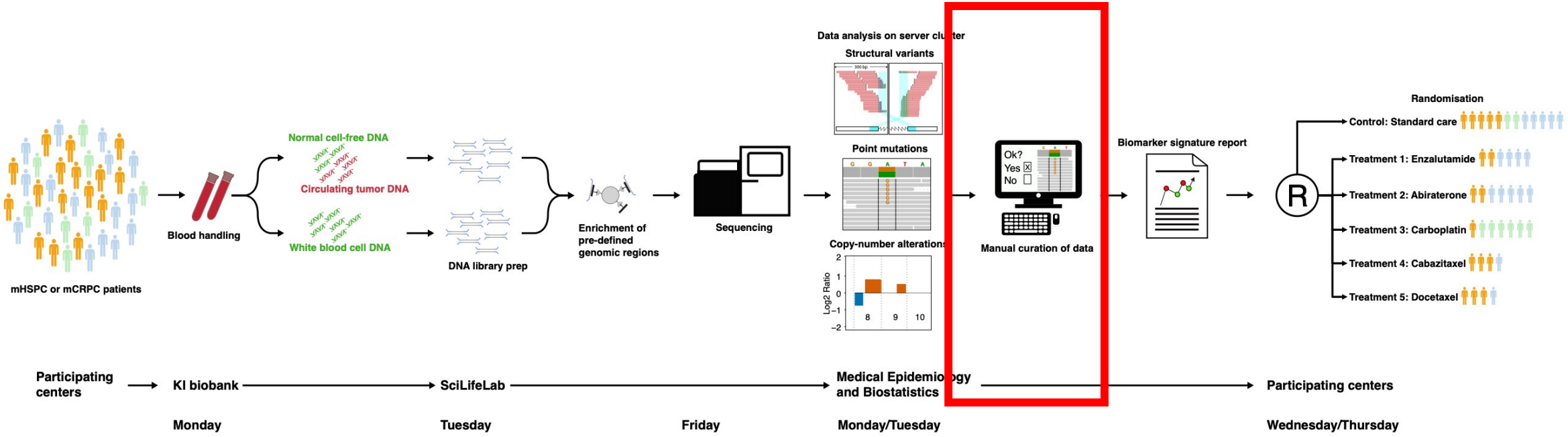


AutoSeq Pipeline

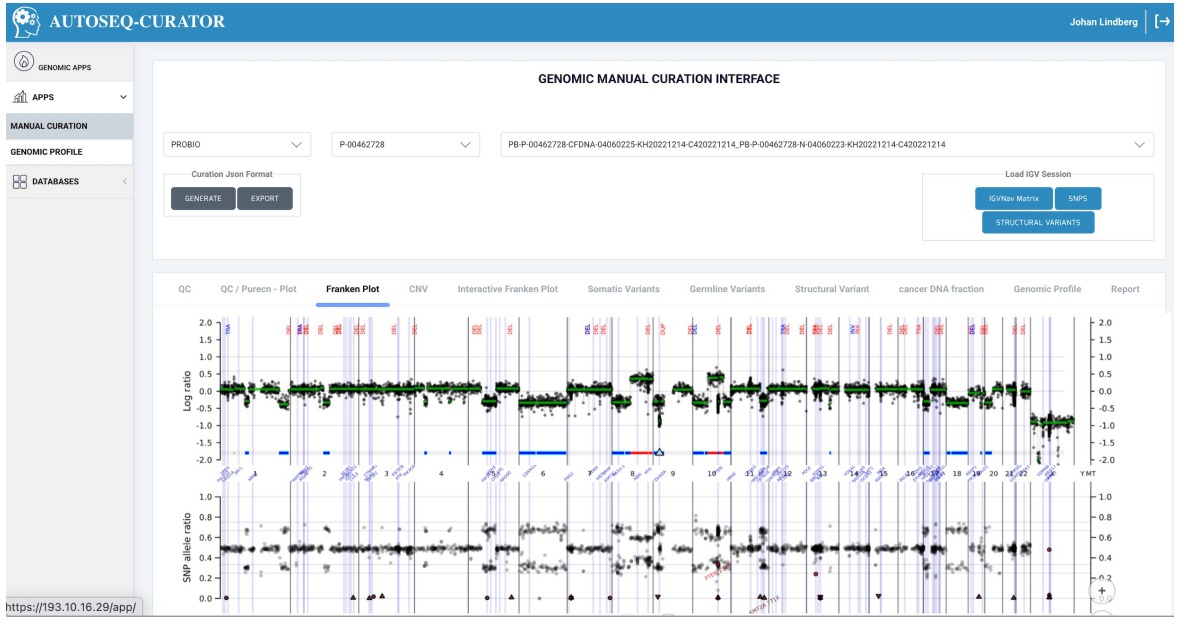


From blood draw to biomarker signature report



Curation (examination) of the results

Curation interface



Everything available by a click of a button.

SOP in google cloud

SOP AUTOSEQ001: Manual evaluation of targeted sequencing data, performed by in solution hybridization-based capture and processed by the AutoSeq pipeline

1. Authors and reviewers

	Name and function	Signature	Date
Drafted by	Johan Lindberg, Senior researcher.	JL	2019-01-08
Evaluated by	Rebecka Bergström, Bioinformatician.	RB	2019-01-08
Updated by	Johan Lindberg	JL	2021-06-21
Updated by	Johan Lindberg	JL	2022-08-01
Approved by			

2. Version history

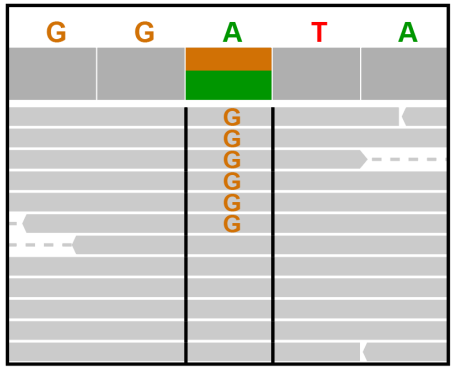
Version	v1.0
Valid from	
SOP code	AUTOSEQ001

3. Modifications to protocol

Date	Description modifications

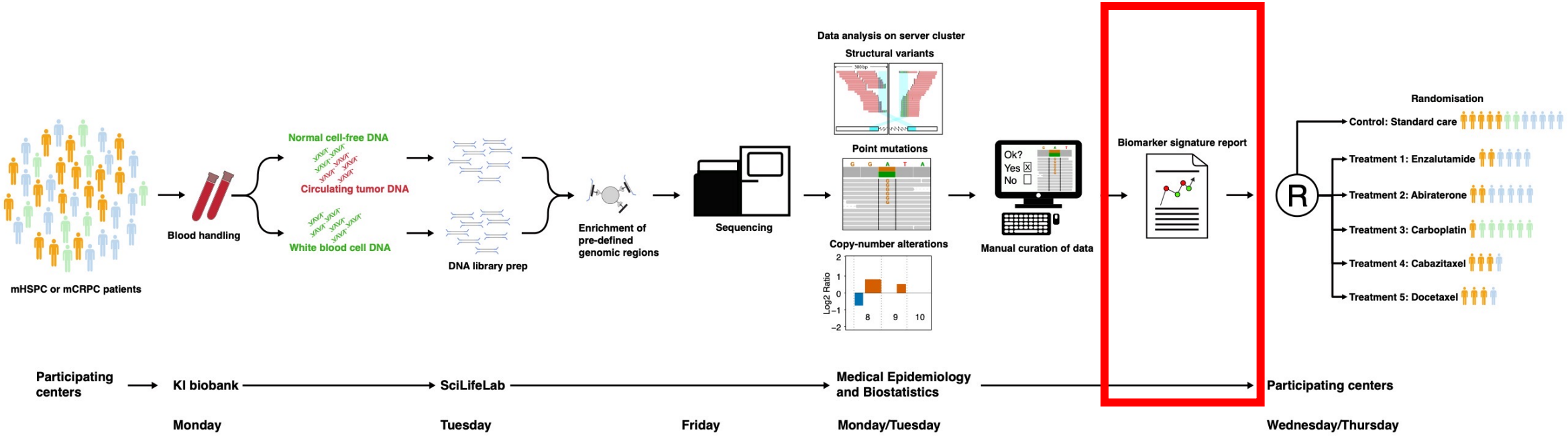
v1.0 PROBIO000XXX

Variant assessment



Curation took 1 – 4 hours before the development of the curation interface.
Now it takes 15 – 30 min.

From blood draw to biomarker signature report



Pre-specificed biomarker signatures

Biomarker signature	Gene(s)	Alteration type	Comment	Detailed info
ARA	Androgen receptor	Mutation, regardless of clonality. Only the mutations in this list qualifies.	Hotspot mutation	L702H
			Hotspot mutation	V716M
			Hotspot mutation	W742C
			Hotspot mutation	W742L
			Hotspot mutation	H875Y
			Hotspot mutation	F877L
			Hotspot mutation	T878A
			Hotspot mutation	T878S
			Hotspot mutation	F877L
			Hotspot mutation	T878A
			Hotspot mutation	M896T
		Genomic structural rearrangement, regardless of clonality.	See figure 1	See figure 1
		Copy-number alteration.	See figure 2	See figure 2

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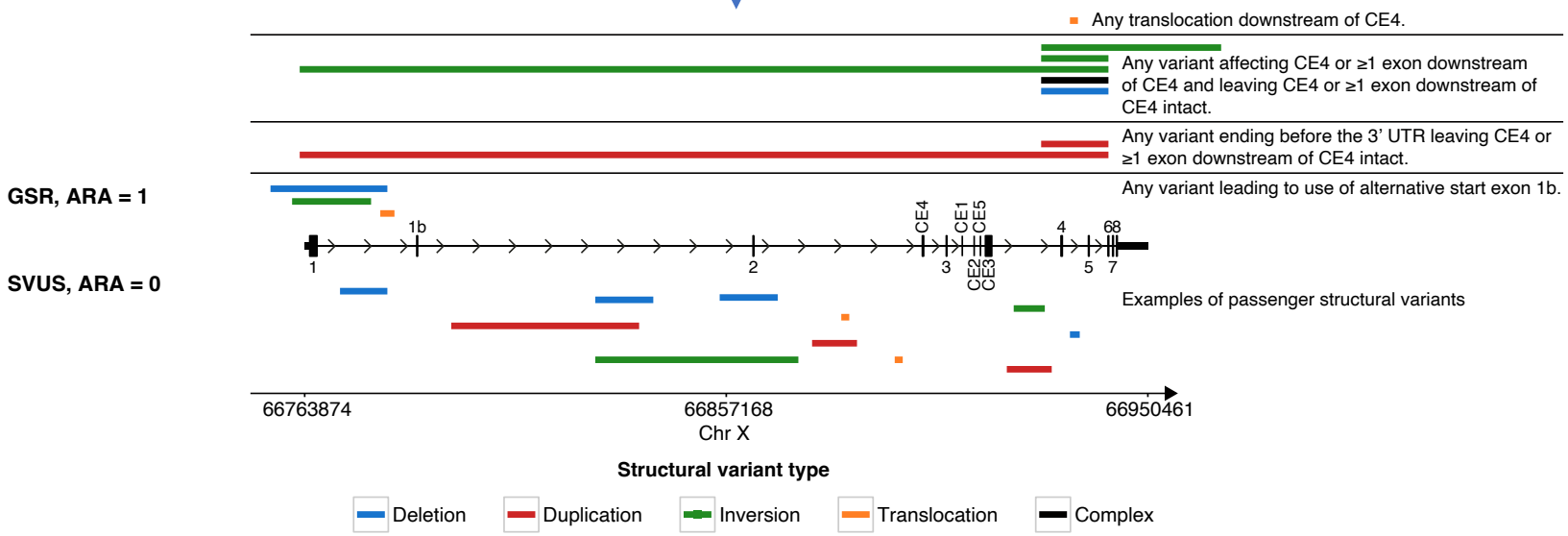
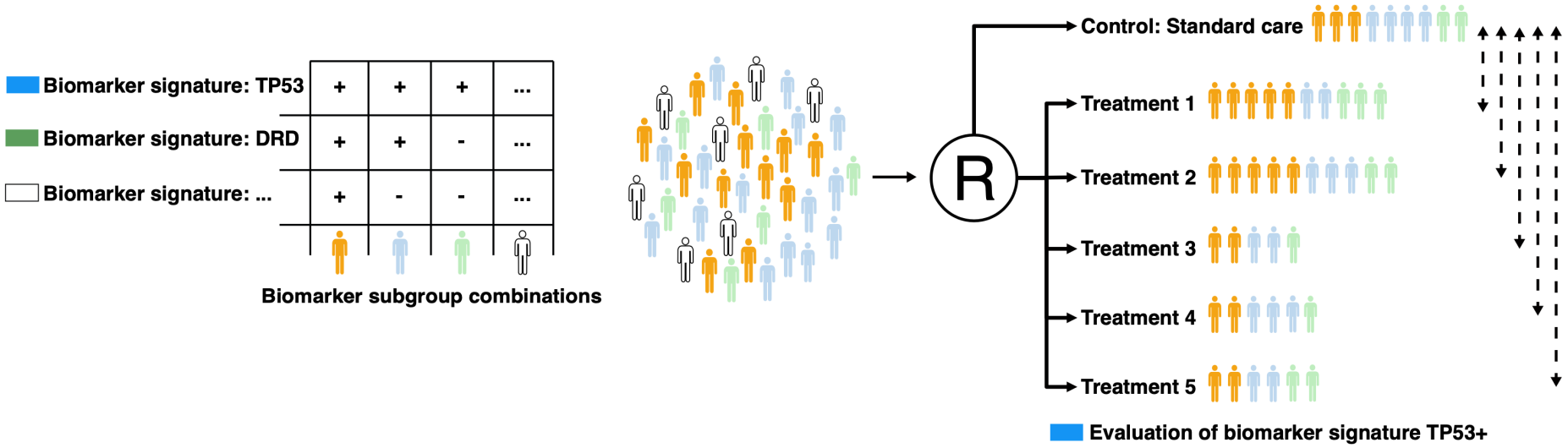
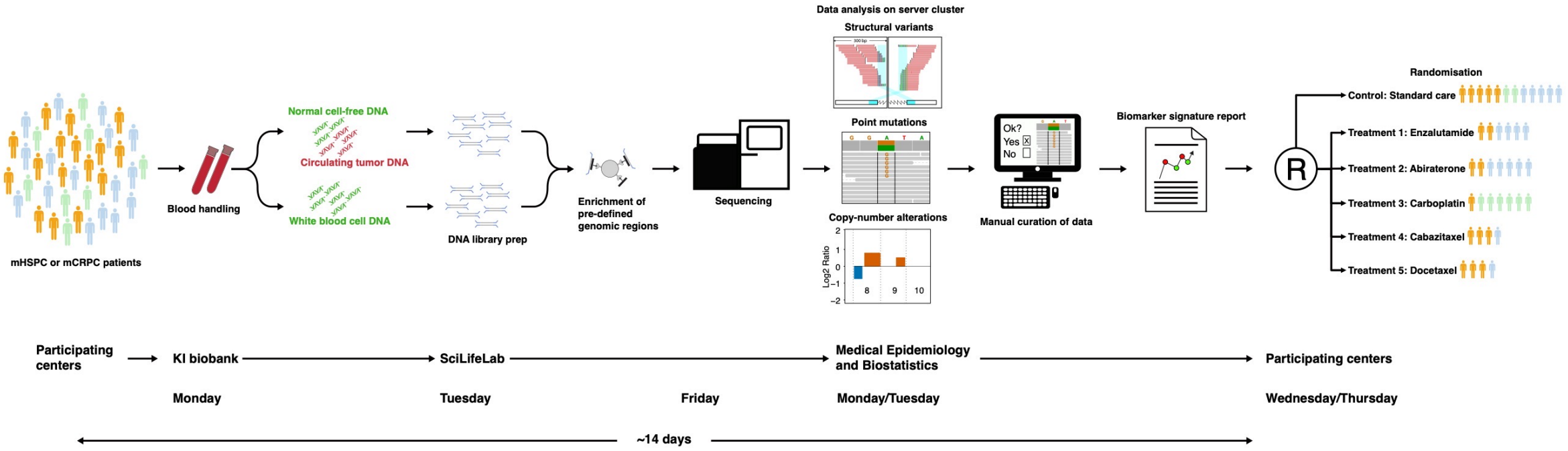


Biomarker signatures - biomarker subgroup combinations

- Multiple molecular biomarkers with predictive potential.
  - Biomarker = “biomarker signature”.
  - Each patient has a “biomarker subgroup combination”.
  - ARA, TP53, T-E, DRD, HRD, PI3K



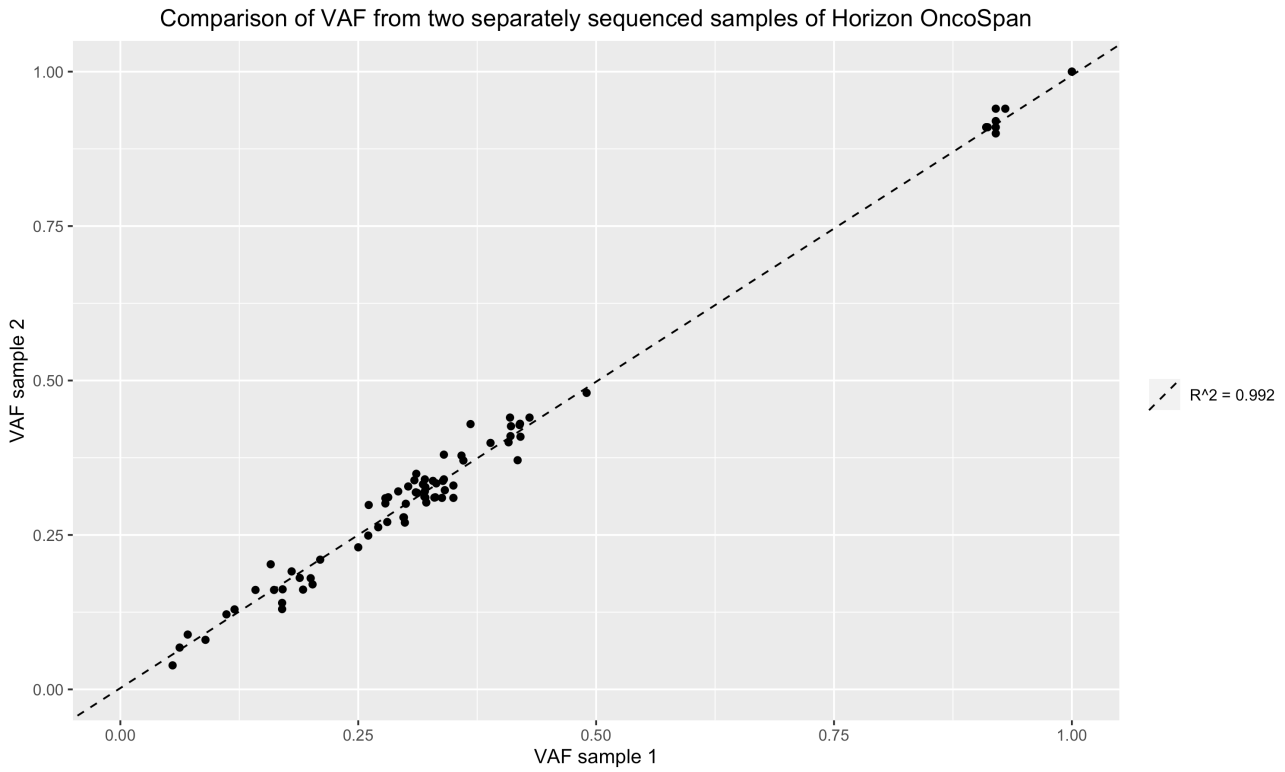
From blood draw to biomarker signature report



- Ways to speed up turn-over:
  - Additional sequencing starts / week.
  - Automated Sunday sequencing data delivery.

Validation of the ProBio assay

- Validation using commercial reference samples.
- Limited value.
  - No homozygous deletions.
  - Lack relevant structural variants.



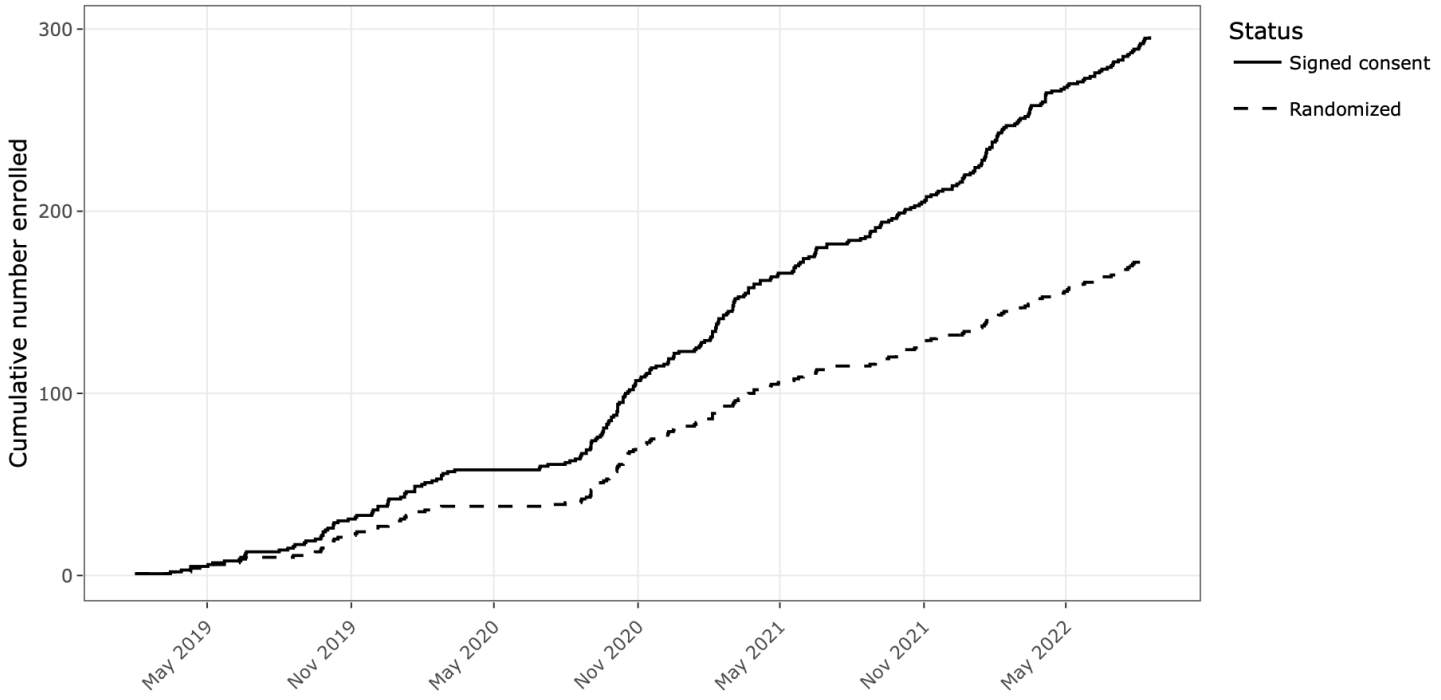
Detected 97/98 variants.
Missed one in a repeat region.

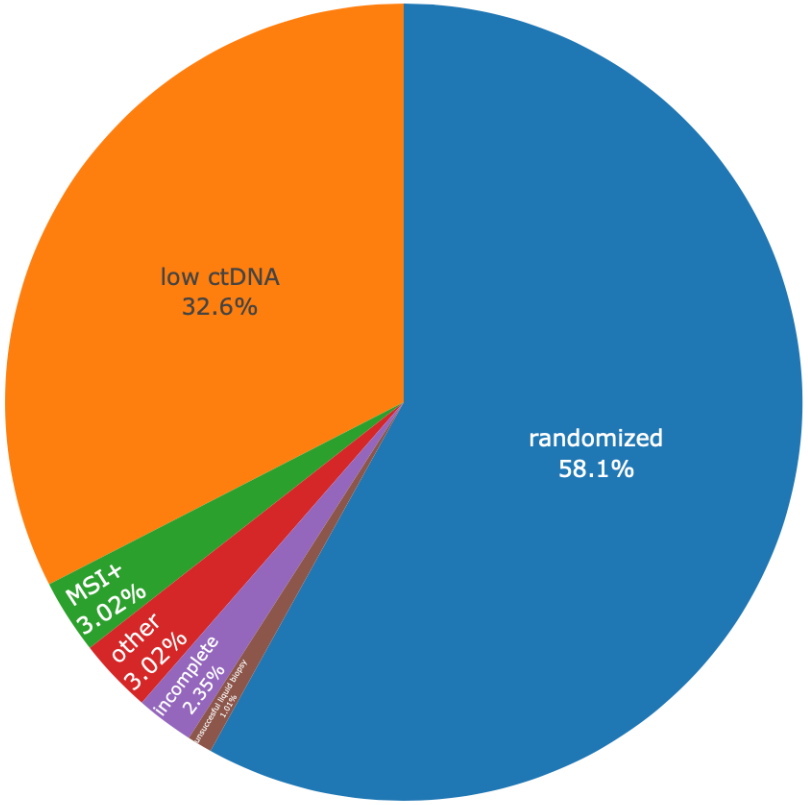
Validation of the ProBio assay

- Contacted by a commercial assay provider, asked to be their external validation lab.
- 244 blinded samples analysed.
- Challenges:
  - Limited cfDNA input.
  - Our current assay is broader but not as sensitive.
  - Completely different technology.
    - Lab-setup.
    - Target regions.
    - Bioinformatic pipeline.
- Concordance is very high.
  - 100% for homozygous deletions.

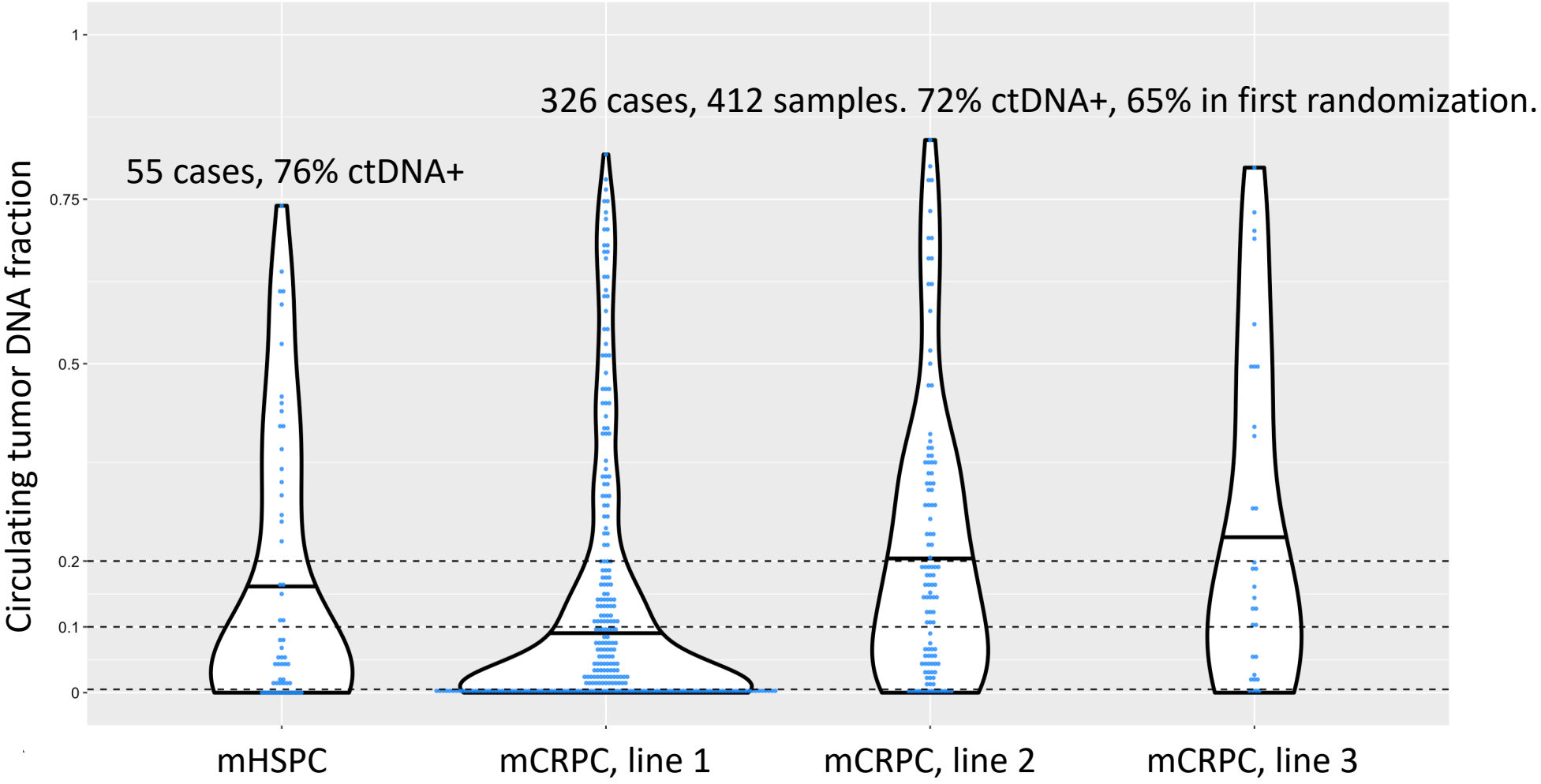
Low tumor burden cases – a challenge

mCRPC, 20220825





Low tumor burden cases – a challenge



ctDNA-negatives from other studies

- Annala et al, Cancer Discovery 2018.
  - mCRPC.
  - 43% ctDNA- at a sensitivity of ≥0.02.
- Grail, Nature Medicine 2019.
  - mCRPC.
  - 5% ctDNA- at a sensitivity of ≥0.001.

Circulating Tumor DNA Genomics Correlate with Resistance to Abiraterone and Enzalutamide in Prostate Cancer

Matti Annala[1,2], Gillian Vandekerkhove[1], Daniel Khalaf[3], Sinja Taavitsainen[2], Kevin Beja[1], Evan W. Warner[1], Katherine Sunderland[3], Christian Kollmannsberger[3], Bernhard J. Eigl[3], Daygen Finch[4], Conrad D. Oja[5], Joanna Vergidis[6], Muhammad Zulfiqar[7], Arun A. Azad[8], Matti Nykter[2], Martin E. Gleave[1], Alexander W. Wyatt[1], and Kim N. Chi[1,3]

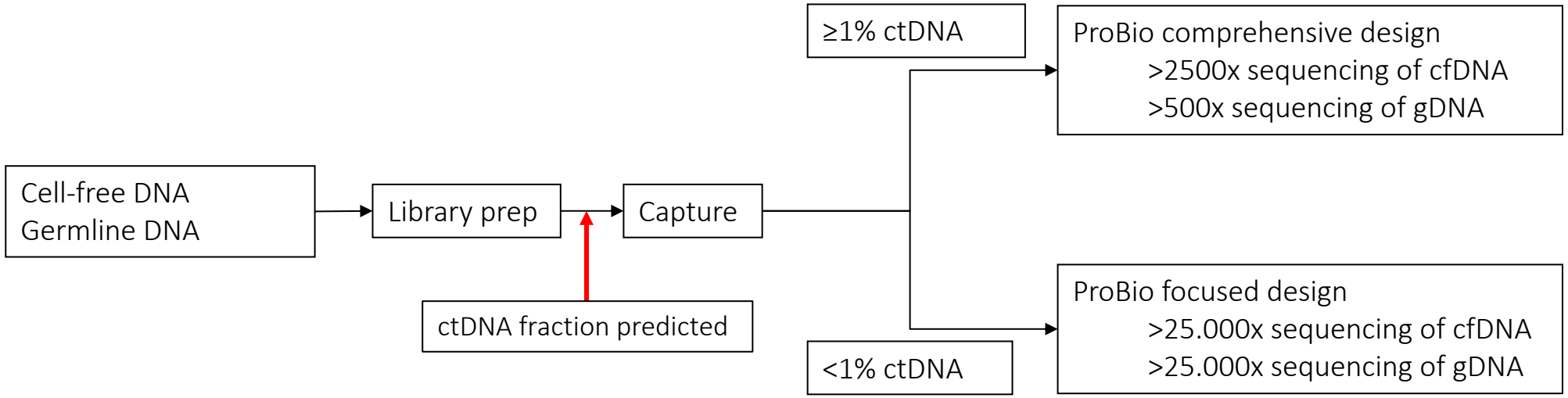
nature medicine
ARTICLES
https://doi.org/10.1038/s41591-019-0652-7

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

Pedram Razavi[1,2,13*], Bob T. Li[1,13], David N. Brown[3,13], Byoungsok Jung[4], Earl Hubbell[4], Ronglai Shen[5], Wassim Abida[1], Krishna Juluru[6], Ino De Bruijn[7], Chenlu Hou[4], Oliver Venn[4], Raymond Lim[3], Aseem Anand[1], Tara Maddala[4], Sante Gnerre[4], Ravi Vijaya Satya[4], Qinwen Liu[4], Ling Shen[4], Nicholas Eattock[4], Jeanne Yue[4], Alexander W. Blocker[4,9], Mark Lee[4,10], Amy Sehnert[4,11], Hui Xu[4], Megan P. Hall[4], Angie Santiago-Zayas[1], William F. Novotny[4,12], James M. Isbell[8], Valerie W. Rusch[8], George Plitas[8], Alexandra S. Heerdt[8], Marc Ladanyi[3], David M. Hyman[1], David R. Jones[8], Monica Morrow[8], Gregory J. Riely[1], Howard I. Scher[1], Charles M. Rudin[1], Mark E. Robson[1], Luis A. Diaz Jr.[1], David B. Solit[1,2,7], Alexander M. Aravanis[4] and Jorge S. Reis-Filho[2,3*]

An attempt to increase the fraction of ctDNA+ cases

- ctDNA negative cases drive cost.
  - Aim for 0.001 limit of detection with new assay.
  - Much smaller design = less information.
- Assay development.
  - Before sequencing apply cfDNA concentration, LDH, ALP, PSA, metastasis info to predict ctDNA fraction.
  - ctDNA fraction cut-off depends on classifier performance.
- Small assay will soon be introduced.

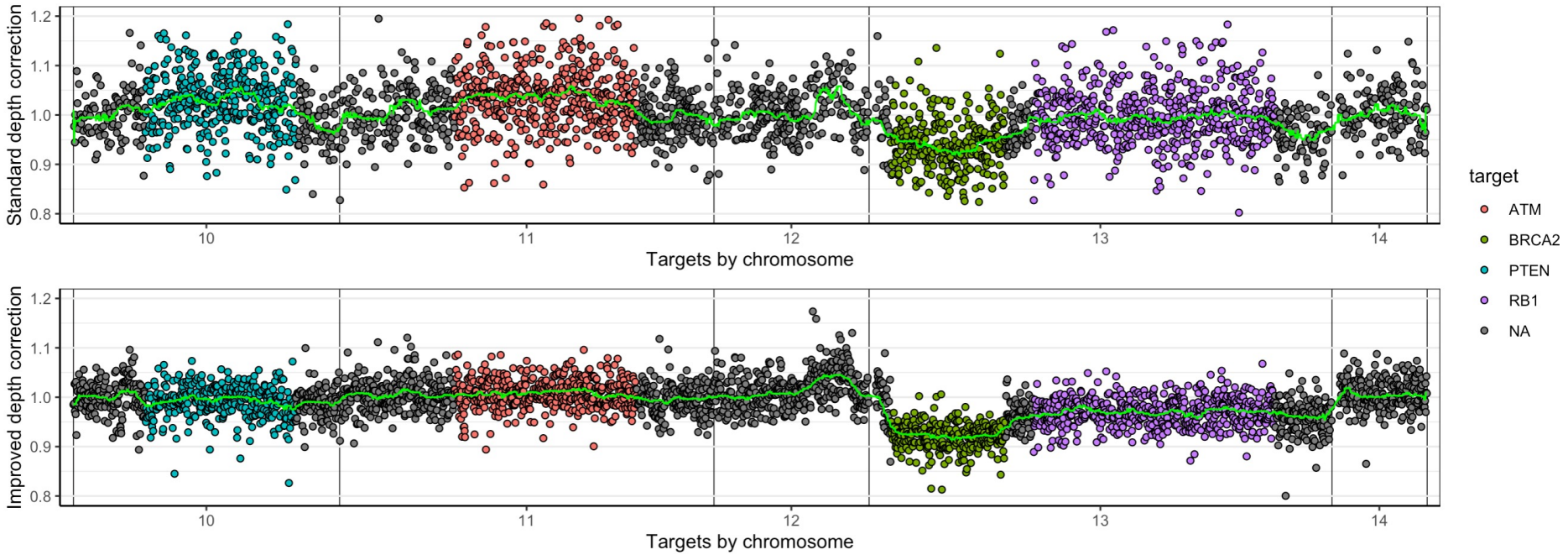


Jumble – new algorithm for copy-number analysis

- Using truth-set from the orthogonal validation project.
- Example case:
  - 41% noise reduction compared to CNV-kit.
  - Purity ~0.10.

CNVkit

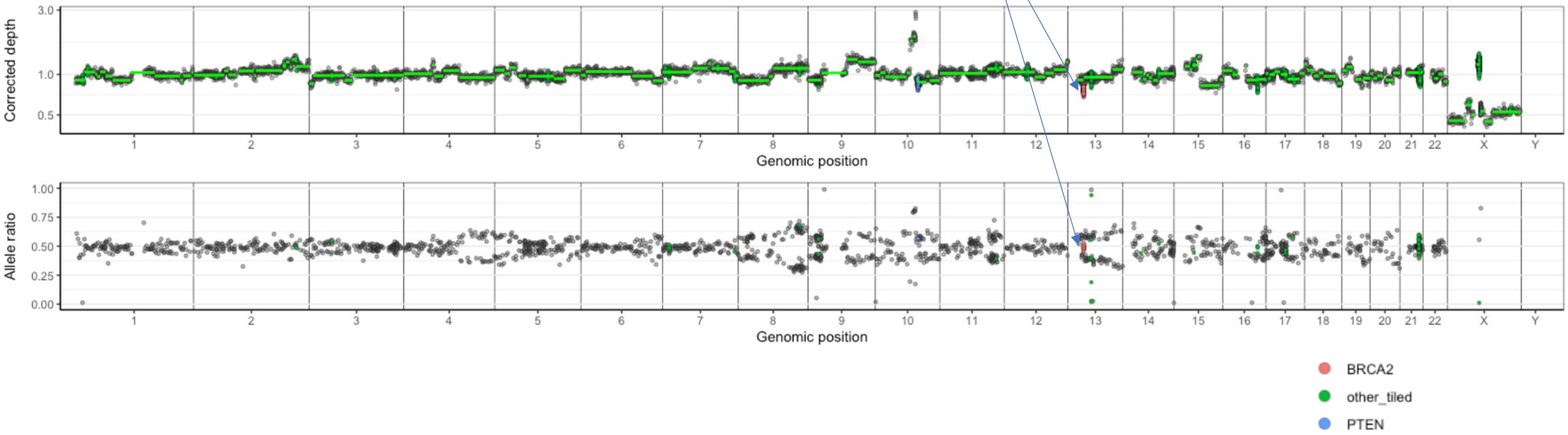
Jumble



Jumble – preliminary results

BRCA2 homozygous deletion

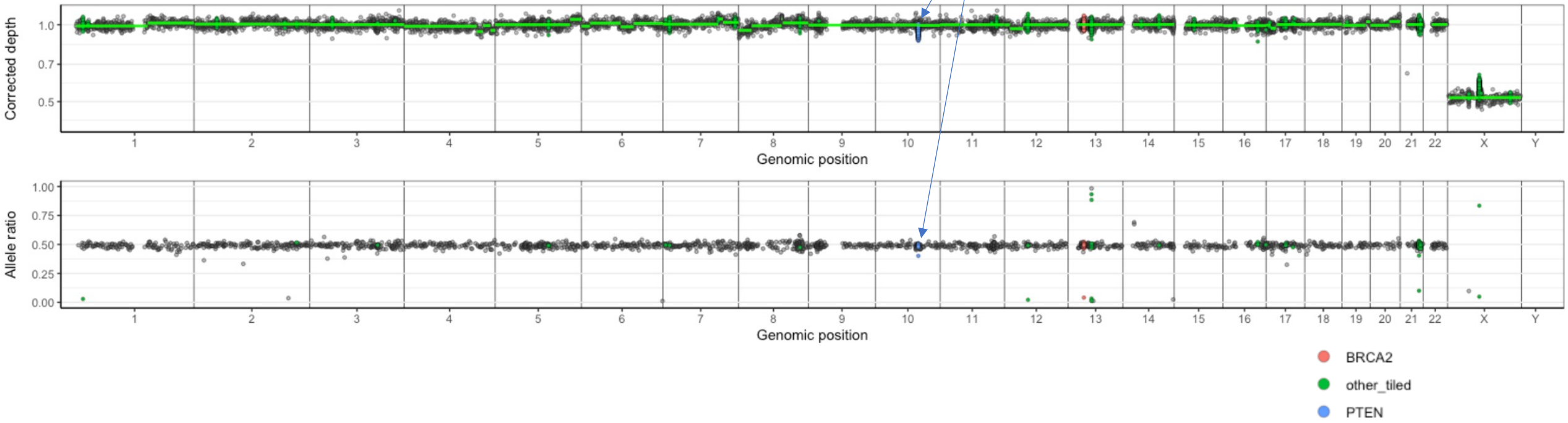
~10% purity



Jumble – preliminary results

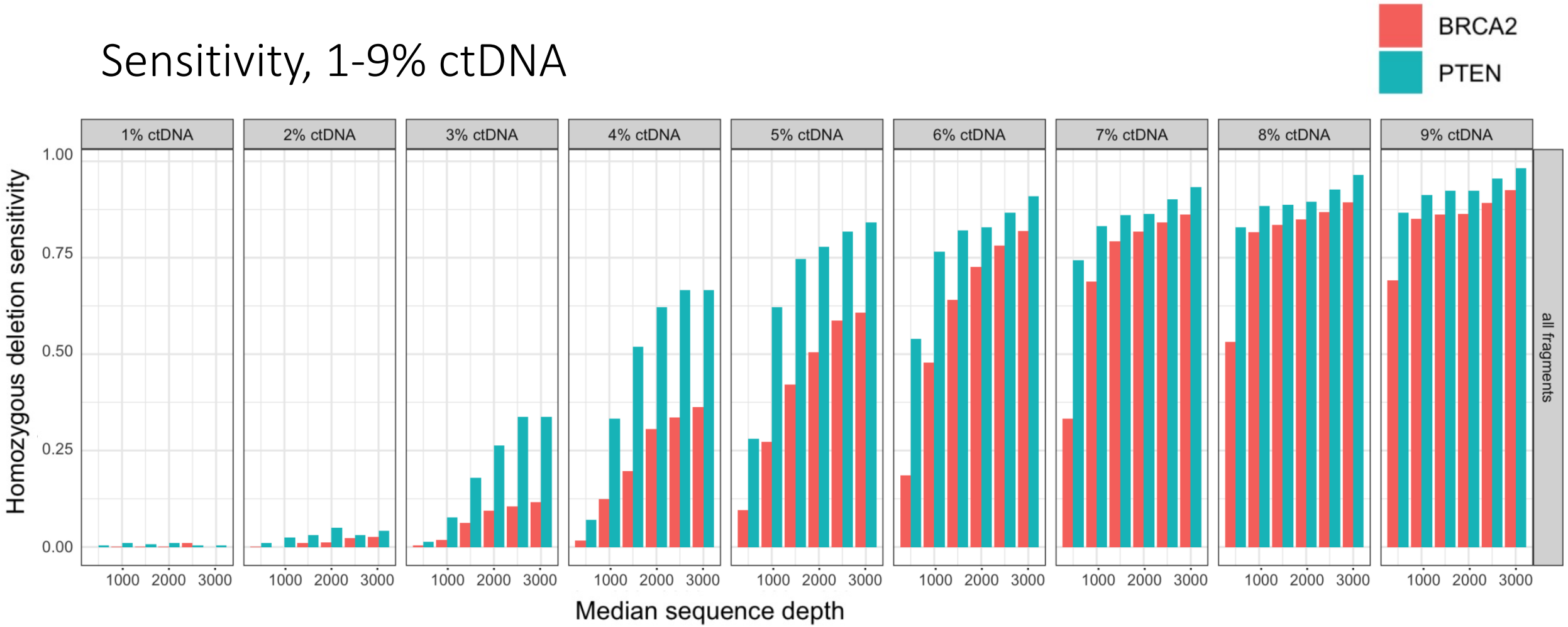
PTEN homozygous deletion

~5% purity

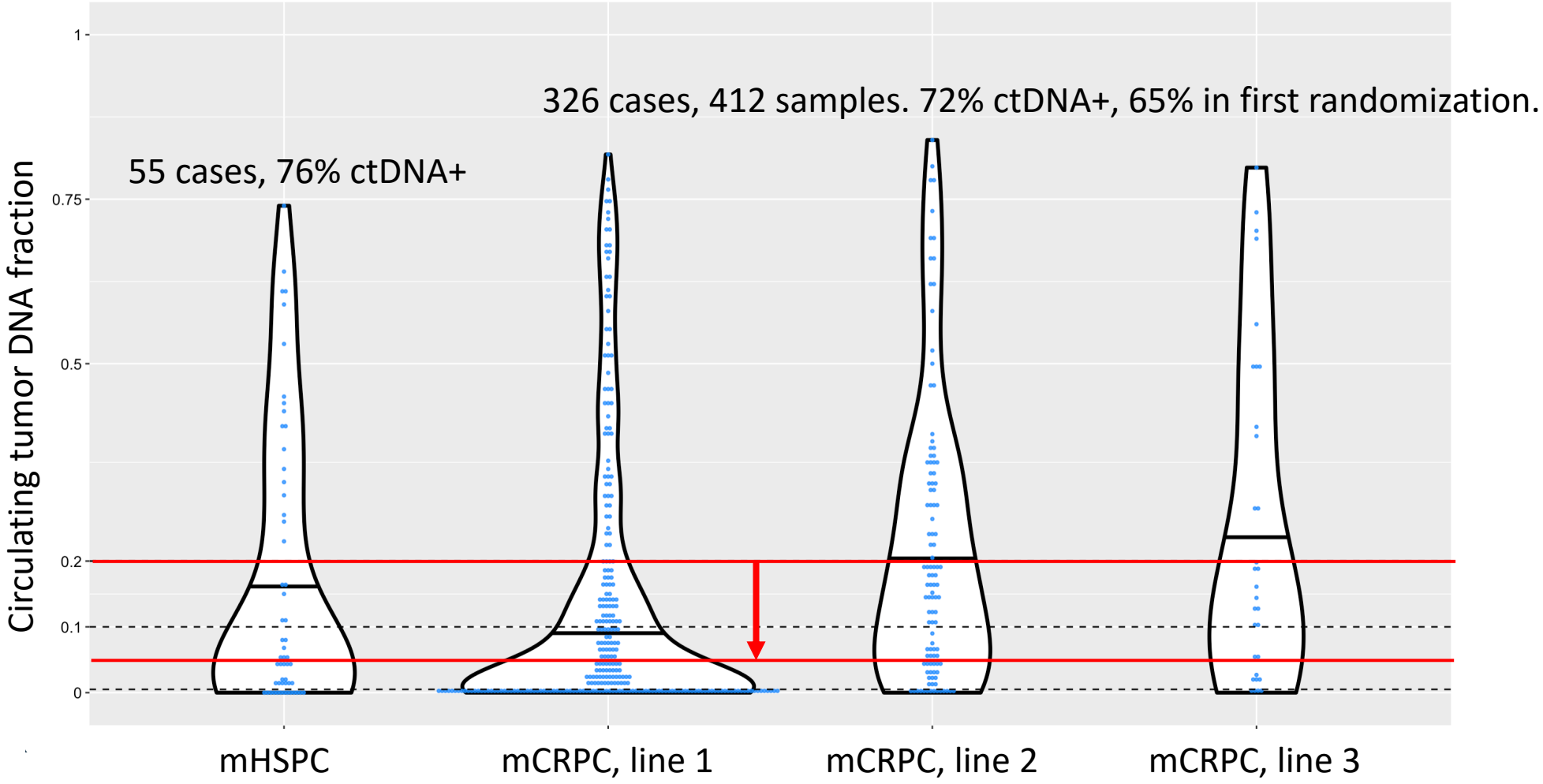


Jumble – preliminary results

Sensitivity, 1-9% ctDNA

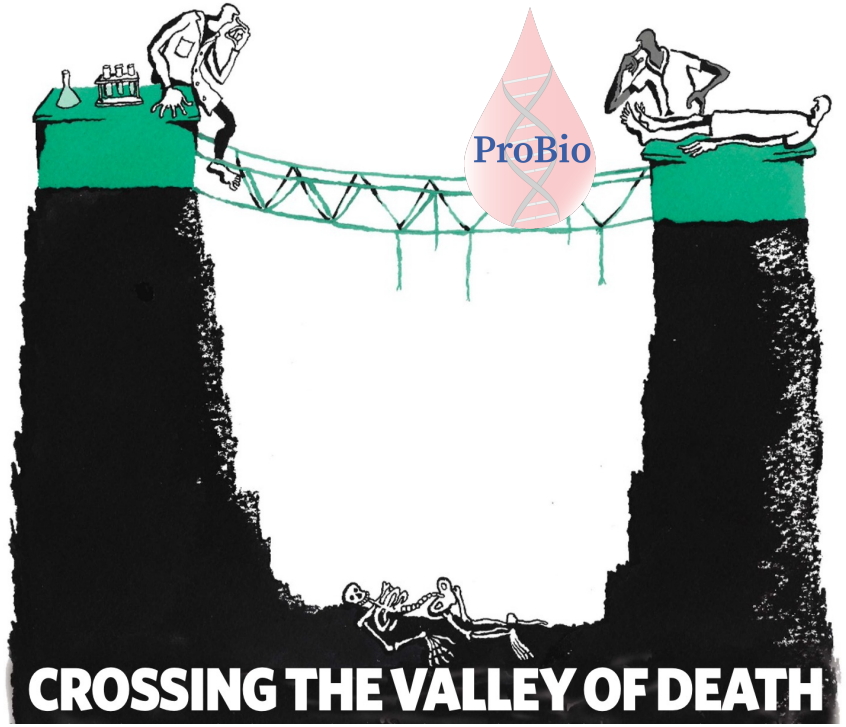


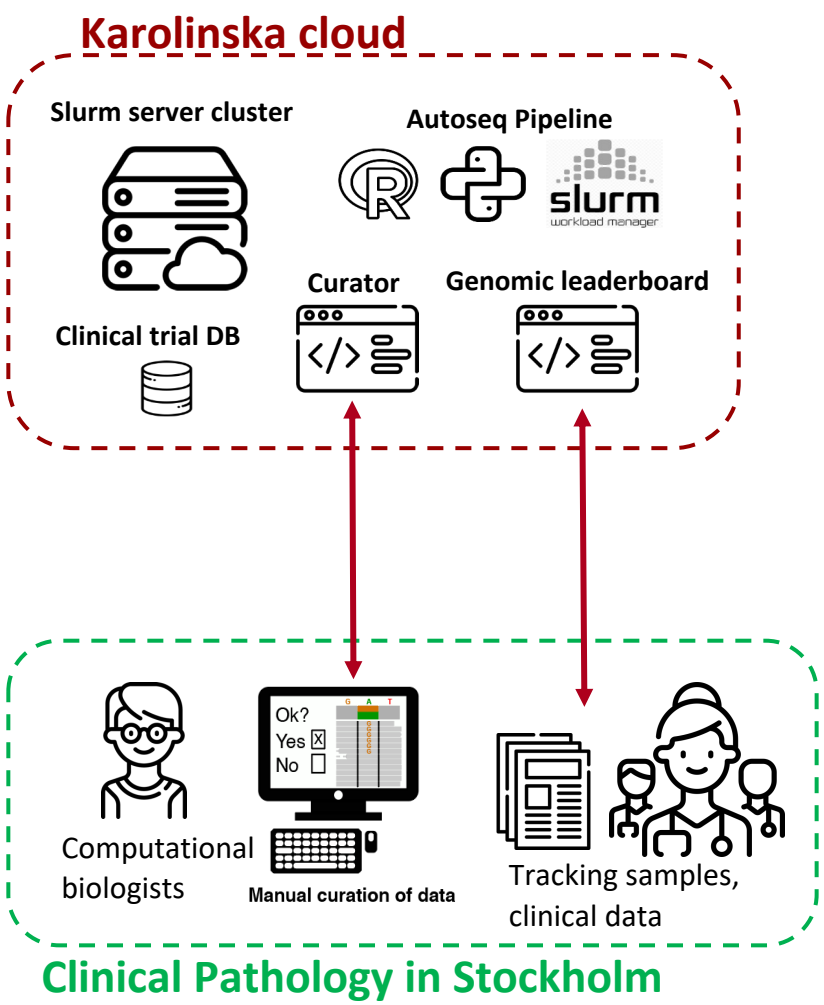
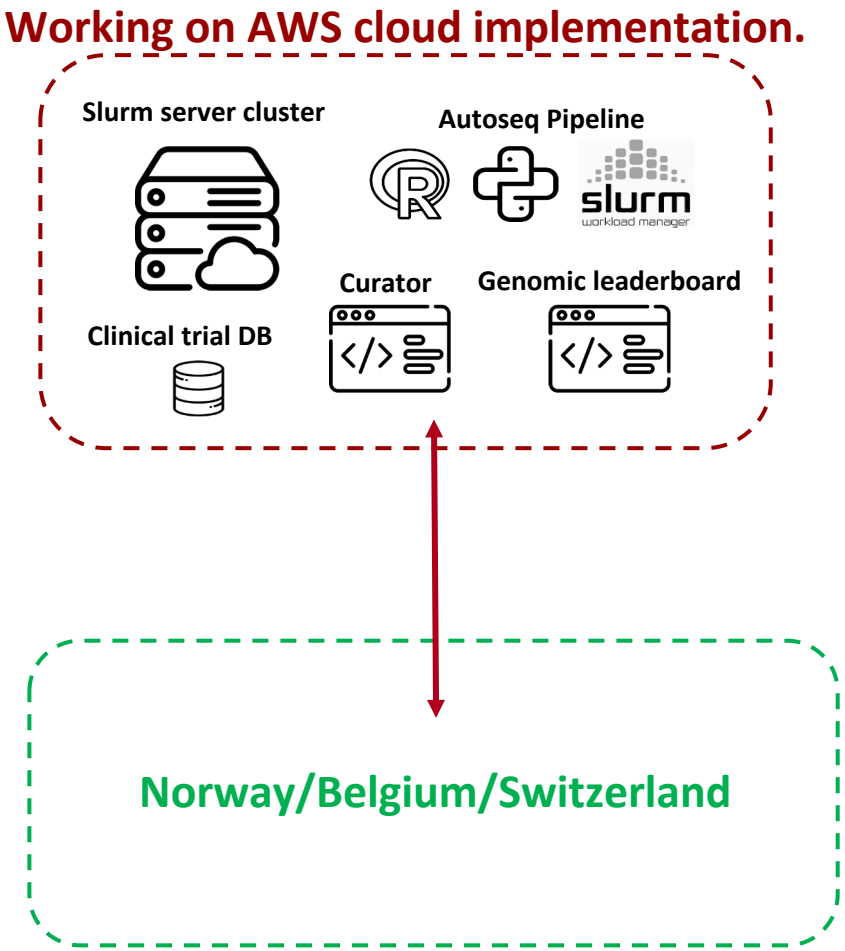
Low tumor burden cases – a challenge



Clinical implementation at Clinical Pathology (K)

- Olaparib approval in Sweden.
- Implementation of our assay/software solution through the iPCM project.
- Online in January.



Translational news feature, Nature, 2008.

Cloud ready software