Hartwig
Medical Foundation

Routine whole genome sequencing-based cancer diagnostics for precision medicine and research

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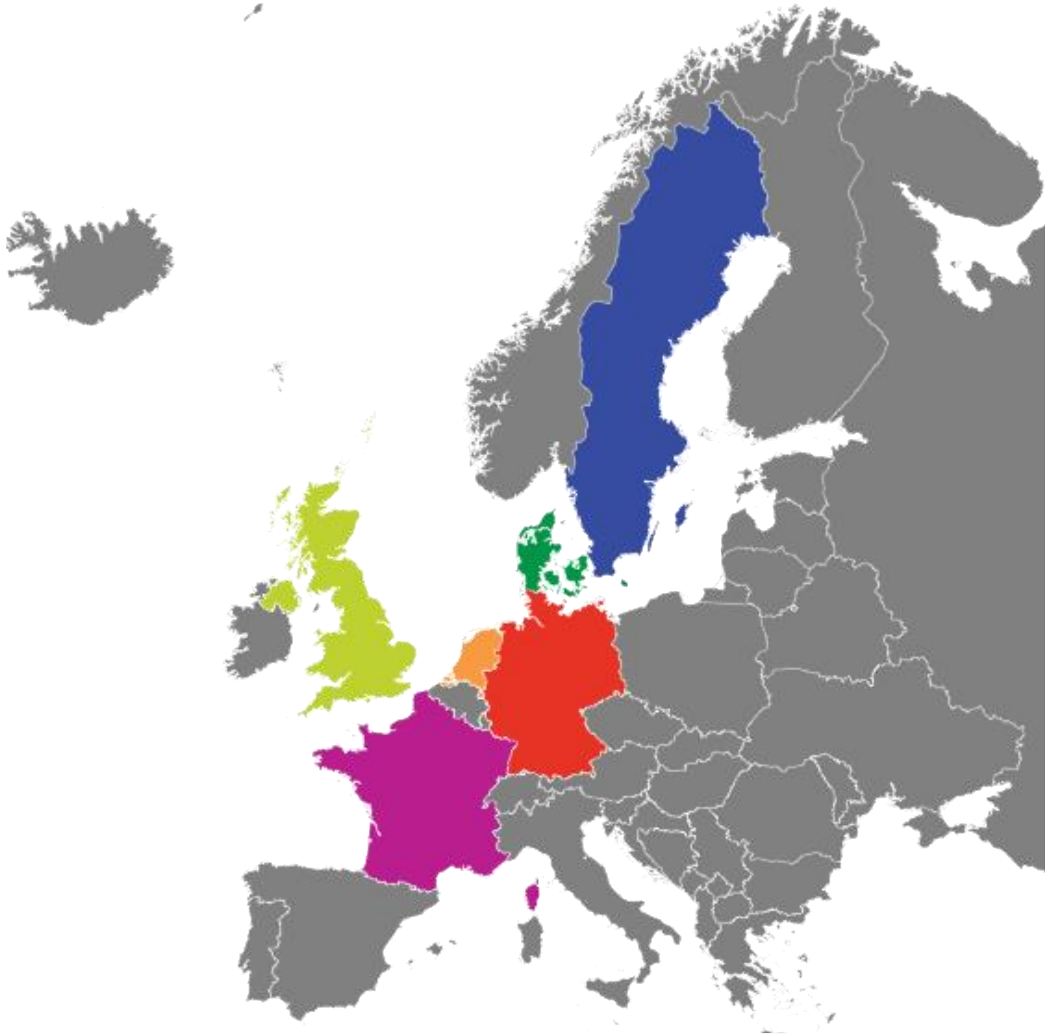
4. genomDE-Symposium 2025 Genommedizin, July 10, 2025

Europe is leading in national Clinical Cancer Genomics programs



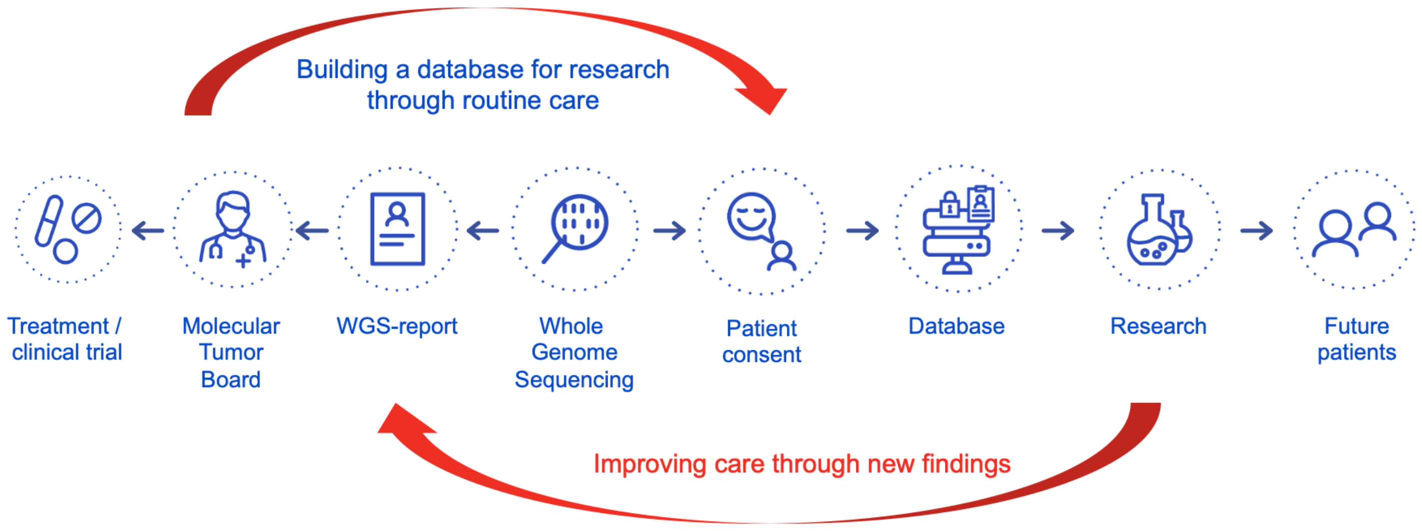
Countries have very different health care systems

Government-driven national programs
UK: NHS/Genomics England
FR: 2025 France Genomic Medicine Plan
DK: Danish National Genome Center
DE: GenomDE
(EU: 1+M Genome Project)

Bottom-up, government funded
SE: Genomic Medicine Sweden

Bottom-up, philantropy
NL: Hartwig Medical Foundation

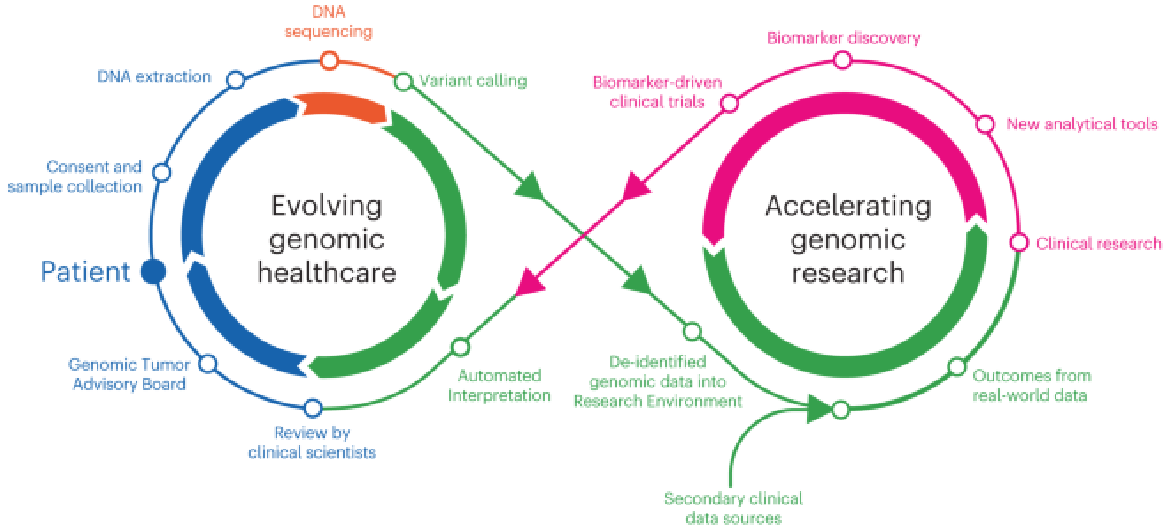
The learning care/innovation cycle – for better care



Hartwig Medical OncoAct

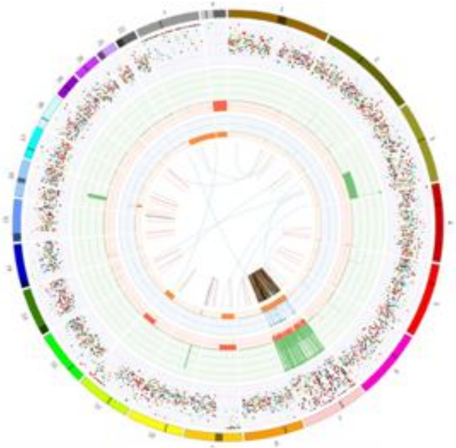
Hartwig Medical Database

Genomics England



WGS can uncover a wealth of relevant information

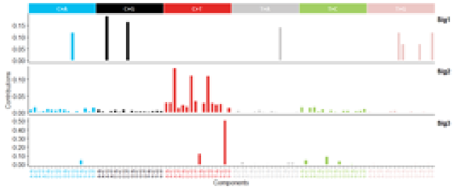
To inform medical specialists on clinical actionability and relevance for cancer patients

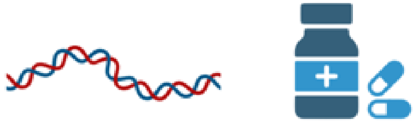


Cancer drivers from genome-wide variant calling
- small and structural variants
- purity and ploidy
  - bi-allelic, subclonal, driver vs passenger
- optimized for diagnostic cancer genes

Viral presence

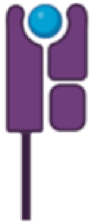
Complex biomarkers
- DNA repair status (MSI, HRD)
- mutational signatures





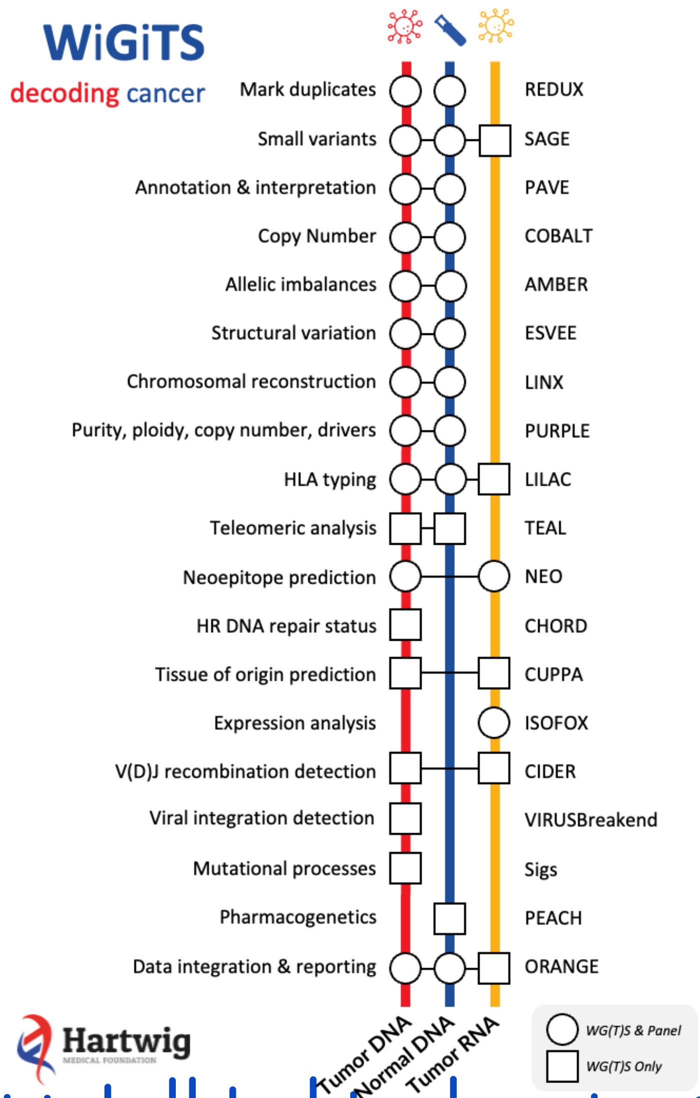
Germline information
- cancer predisposition
- pharmacogenetics

Tissue of origin and Tumor (sub)typing



HLA typing and neoepitopes
- immunotherapy
- vaccination

Hartwig WiGiTS: comprehensive, efficient and flexible data analysis



Multi-platform support

Fast, inexpensive & runnable anywhere

Open source software

(Stephan Watts, Oliver Hofmann, UniMelb)

Universal NGS Pipeline

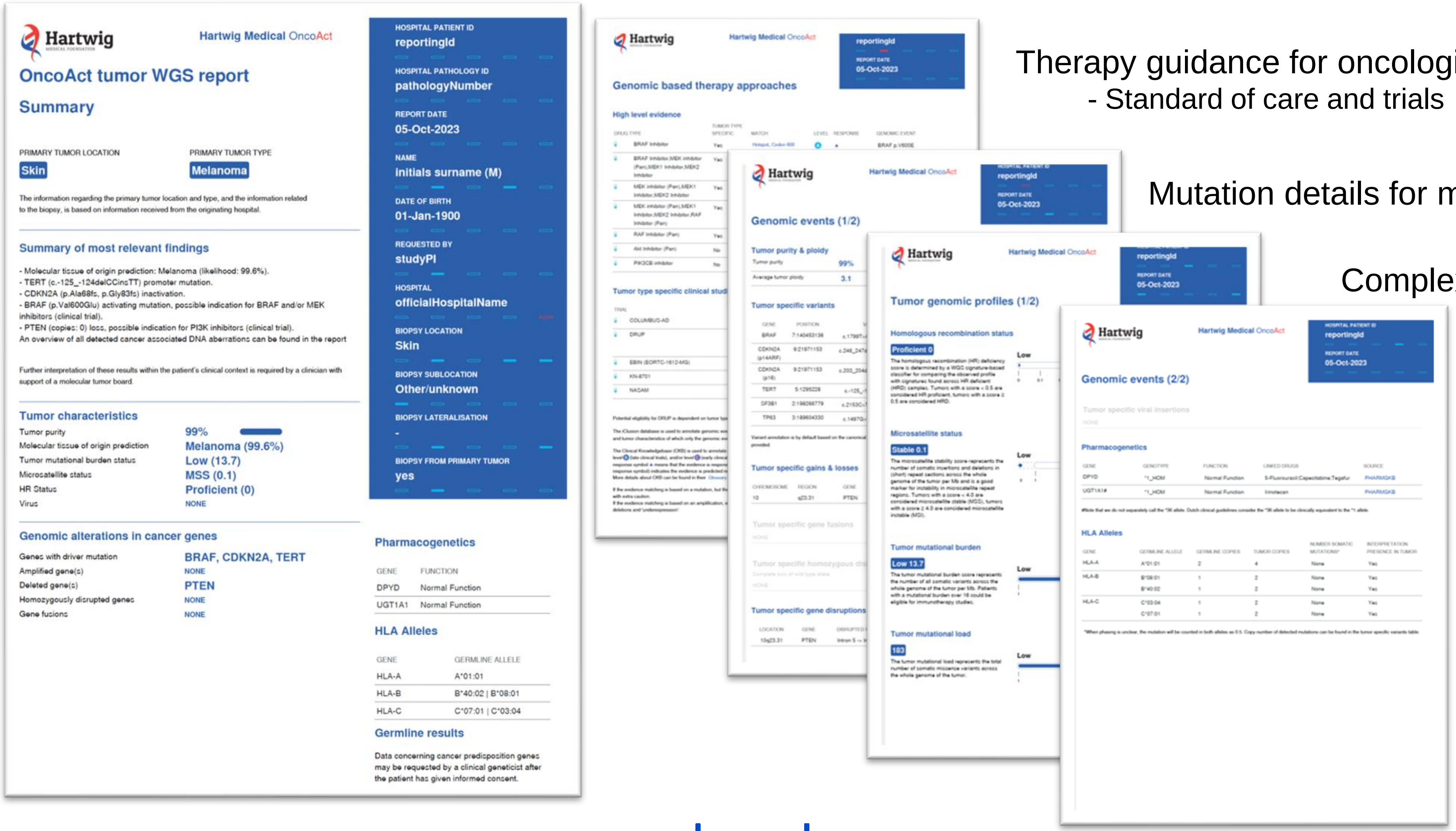
Panel, WES, WGS
Paired and tumor-only
Tissue (FF, FFPE) and cfDNA

Longitudinal patient analysis

Multimodal (DNA, RNA, ctDNA)
Disease monitoring

https://github.com/hartwigmedical/
https://nf-co.re/oncoanalyser/

Fully automated (draft) patient report generation


Hartwig

Hartwig Medical OncoAct

OncoAct tumor WGS report

Summary

PRIMARY TUMOR LOCATION	PRIMARY TUMOR TYPE
Skin	Melanoma

The information regarding the primary tumor location and type, and the information related to the biopsy, is based on information received from the originating hospital.

HOSPITAL PATIENT ID	reportingId
HOSPITAL PATHOLOGY ID	pathologyNumber
REPORT DATE	05-Oct-2023
NAME	initials surname (M)
DATE OF BIRTH	01-Jan-1900
REQUESTED BY	studyPI
HOSPITAL	officialHospitalName
BIOPSY LOCATION	Skin
BIOPSY SUBLOCATION	Other/unknown
BIOPSY LATERALISATION	-
BIOPSY FROM PRIMARY TUMOR	yes

Summary of most relevant findings

- Molecular tissue of origin prediction: Melanoma (likelihood: 99.6%).
- TERT (c.-125_-124delCCinsTT) promoter mutation.
- CDKN2A (p.Ala68fs, p.Gly83fs) inactivation.
- BRAF (p.Val600Glu) activating mutation, possible indication for BRAF and/or MEK inhibitors (clinical trial).
- PTEN (copies: 0) loss, possible indication for PI3K inhibitors (clinical trial).

An overview of all detected cancer associated DNA aberrations can be found in the report

Further interpretation of these results within the patient's clinical context is required by a clinician with support of a molecular tumor board.

Tumor characteristics

Tumor purity	99%
Molecular tissue of origin prediction	Melanoma (99.6%)
Tumor mutational burden status	Low (13.7)
Microsatellite status	MSS (0.1)
HR Status	Proficient (0)
Virus	NONE

Genomic alterations in cancer genes

Genes with driver mutation	BRAF, CDKN2A, TERT
Amplified gene(s)	NONE
Deleted gene(s)	PTEN
Homozygously disrupted genes	NONE
Gene fusions	NONE

Pharmacogenetics

GENE	FUNCTION
DPYD	Normal Function
UGT1A1	Normal Function

HLA Alleles

GENE	GERMLINE ALLELE
HLA-A	A*01:01
HLA-B	B*40:02 | B*08:01
HLA-C	C*07:01 | C*03:04

Germline results

Data concerning cancer predisposition genes may be requested by a clinical geneticist after the patient has given informed consent.

Genomic based therapy approaches

High level evidence

Tumor type specific clinical studies

Genomic events (1/2)

Tumor purity & ploidy

Tumor specific variants

Tumor specific gains & losses

Tumor specific gene fusions

Tumor specific homozygous disruptions

Tumor specific gene disruptions

Tumor genomic profiles (1/2)

Homologous recombination status

Proficient 0

Microsatellite status

Stable 0.1

Tumor mutational burden

Low 13.7

Tumor mutational load

183

Genomic events (2/2)

Tumor specific viral insertions

Pharmacogenetics

HLA Alleles

Therapy guidance for oncologist
- Standard of care and trials

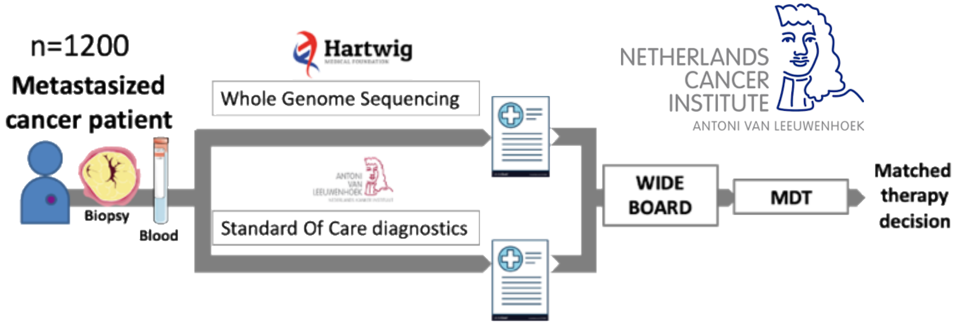
Mutation details for molecular pathologist / MTB

Complex biomarkers

Pharmacogenetics
Germline mutations
HLA typing

Demonstrating clinical validity and utility

Clinical Implementation Study



- feasibility: TAT of 9 days, success rate 70%
- validity: >99.6% concordance SOC
- utility: additional findings in 60%

Journal of Pathology
J Pathol October 2022; **258:** 179–188
Published online 3 August 2022 in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.5988

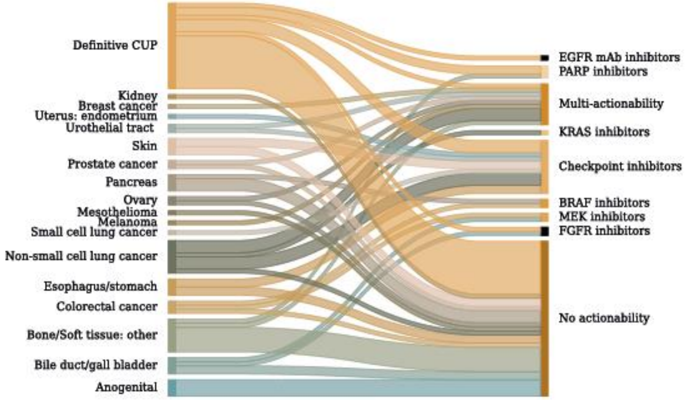
ORIGINAL ARTICLE

Feasibility of whole-genome sequencing-based tumor diagnostics in routine pathology practice

Kris G Samsom[1†], Luuk J Schipper[2,3†], Paul Roepman[4], Linda JW Bosch[1], Ferry Lalezari[5], Elisabeth G Klompenhouwer[5], Adrianus J de Langen[6], Tineke E Buffart[7], Immy Riethorst[4], Lieke Schoenmaker[4], Daoin Schout[1], Vincent van der Noort[8], Jose G van den Berg[1], Ewart de Bruijn[4], Jacobus JM van der Hoeven[4], Hans van Snellenberg[4], Lizet E van der Kolk[9], Edwin Cuppen[3,4,10], Emile E Voest[2,3,11], Gerrit A Meijer[1‡*] and Kim Monkhorst[1‡]

Cancer of Unknown Primary

- algorithm development (database)
- proof-of-concept clinical value



Resolved diagnosis 67%
Potentially actionable 50%

ESMO OPEN
ORIGINAL RESEARCH

Complete genomic characterization in patients with cancer of unknown primary origin in routine diagnostics

L. J. Schipper[1,2†], K. G. Samsom[3†], P. Snaebjornsson[3], T. Battaglia[1], L. J. W. Bosch[3], F. Lalezari[4], P. Priestley[5], C. Shale[5], A. J. van den Broek[6], N. Jacobs[6], P. Roepman[6], J. J. M. van der Hoeven[6], N. Steeghs[7], M. A. Vollebergh[8], S. Marchetti[7], E. Cuppen[2,6,9], G. A. Meijer[3], E. E. Voest[1,2,8] & K. Monkhorst[3*]

Volume 7 ■ Issue 6 ■ 2022

Missie Tumor Onbekend

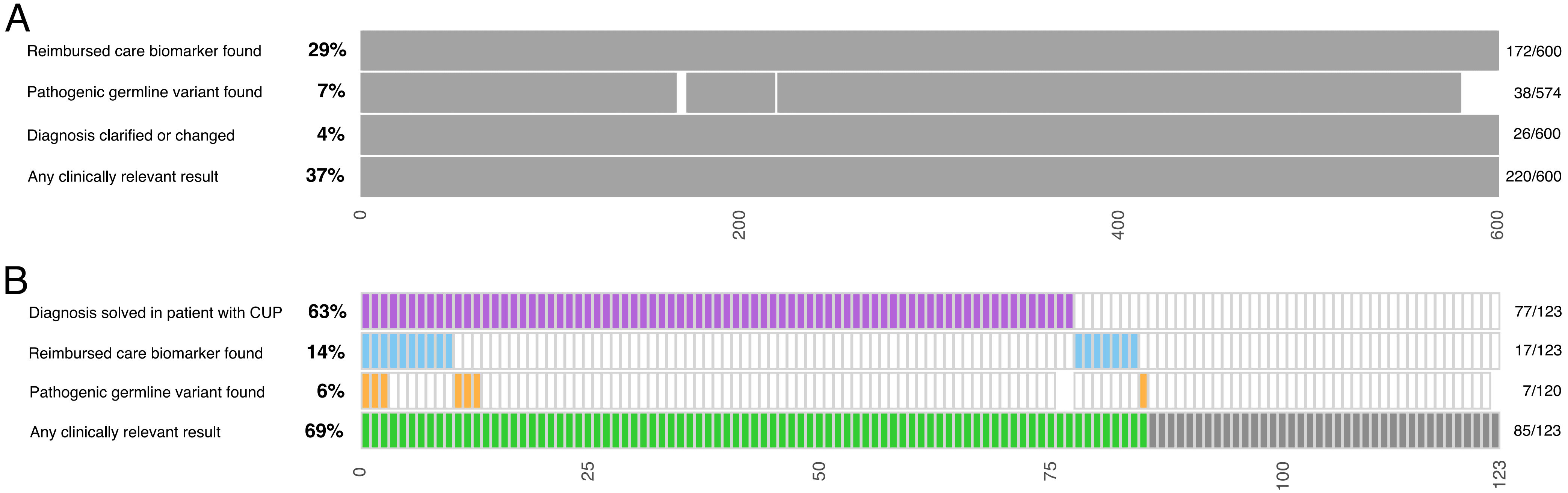
2021: Use of WGS for routine diagnostics in NKI-AvL

2021: CUP is first reimbursement indication for WGS

Real-world evidence (n = 888) confirms study results

Routine use in a comprehensive cancer center (NKI-AvL 2021-2023)
Solid tumors, mostly metastatic

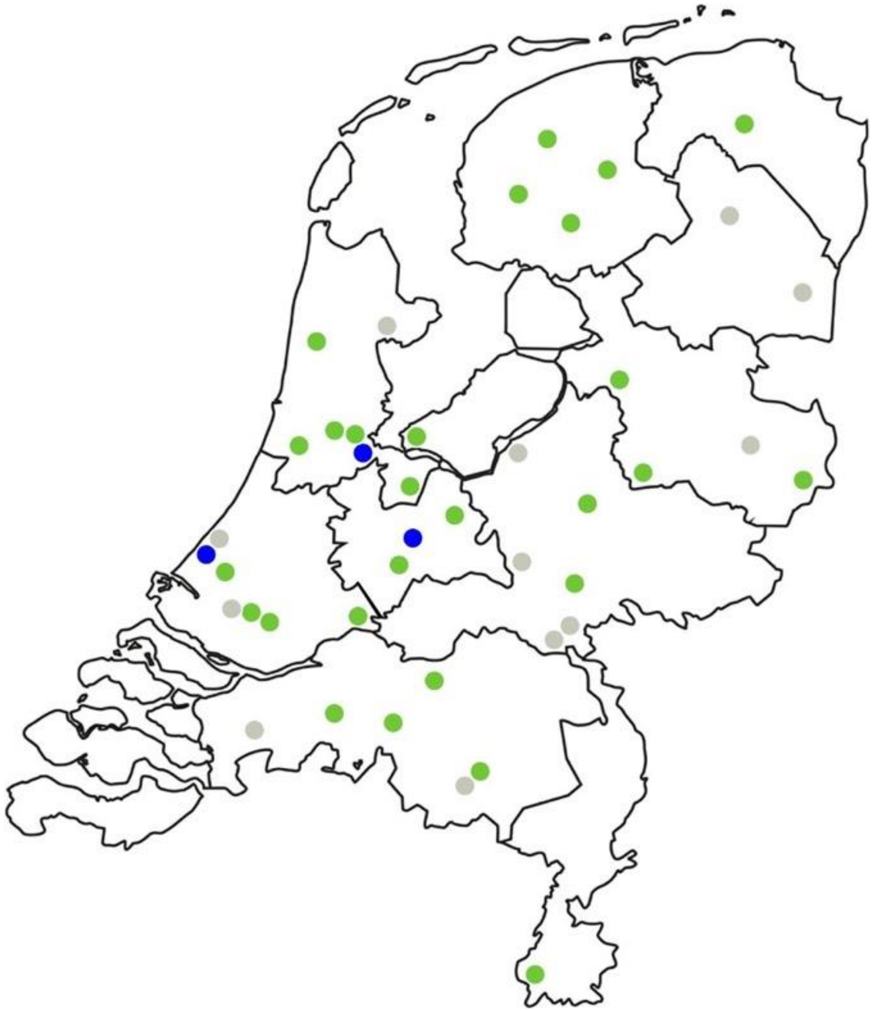
Success rate 89%; turnaround time average 6 days



Clinical consequences of WGS-based diagnostics in 42% of patients
plus identification of experimental therapy options in >50% of patients (mostly drug-repurposing)

Jeffrey van Putten, Kim Monkhorst; unpublished data

Diagnostic use of WGS for oncology in the Netherlands



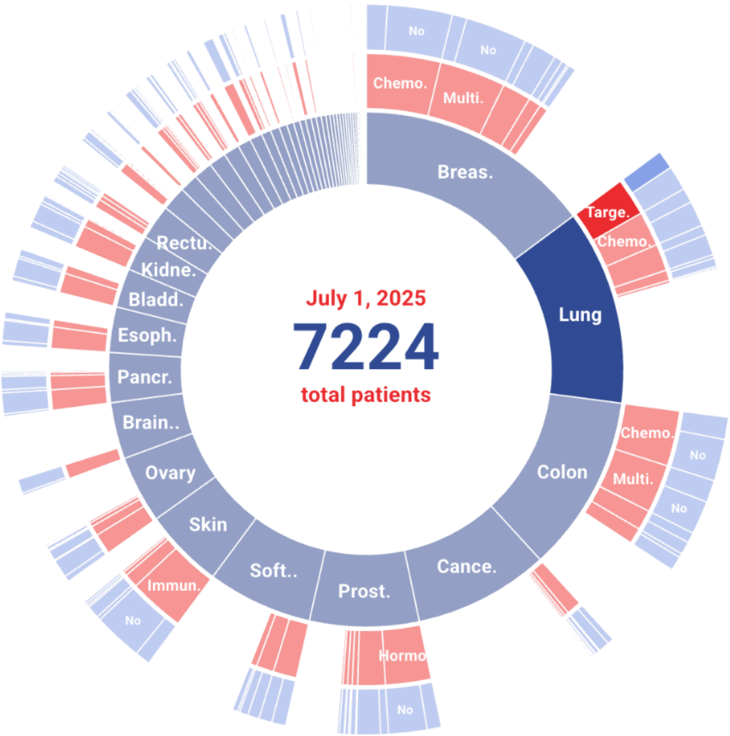
- Service level agreements for WGS-based diagnostics with **~30 out of 71 hospitals** in The Netherlands
- Databasing agreement including **patient consent** (opt-in) with most
- Enabling **WGS-based diagnostics** for:
  - **Routine** all patients (comprehensive cancer center NKI-AvL)
  - Cancer of Unknown Primary in **guidelines** and reimbursed)
  - **Last resort**: Phase 1 unit (EMC)
  - **Projects**: GENAYA, OPTIC (CRC), GLOW (GBM) (all hospitals)

~1,100 patients per year (steadily growing)

June 24, 2025: Dutch health care authorities (ZIN) approved WGS reimbursement for all patients that are in sufficient conditions to undergo treatment

⇒ driven by pan-cancer treatments like NTRK inhibitors

Data reuse is enabled through Hartwig database



Includes clinical metadata: patient, tumor, treatment

˜60% with matching RNA-seq

Access controlled

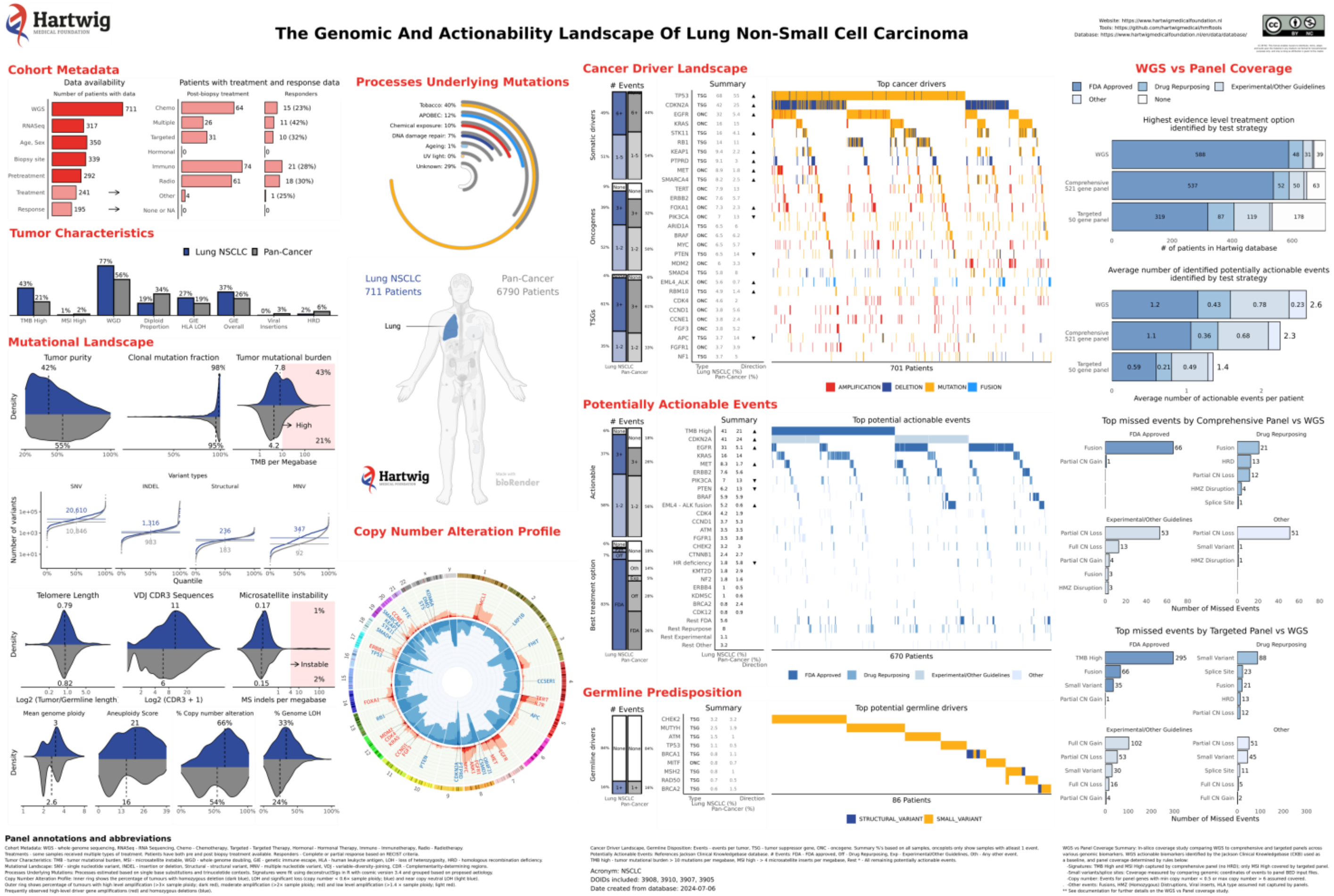
- Free
- Use for ‘common good’
- Raw and analysed data
- Google cloud

Catalog

Request data

https://catalog.hartwigmedicalfoundation.nl
https://www.hartwigmedicalfoundation.nl/en/data/data-access-request/

Cancer vignettes illustrate richness of data



Based on WiGiTs output

Available under CC-BY

- education
- statistical data mining

>80 cancer types

https://www.hartwigmedicalfoundation.nl/en/data/vignettes/

Robust GDPR-proof procedure for international data sharing

Data Access Procedure ensures that the 'Licensed Data' is only shared when:

- Countries guarantee strong **data protection**
- **Academic institutes** only (non-profit)
- Researchers with proven **track record**
- **Data minimization perspective**

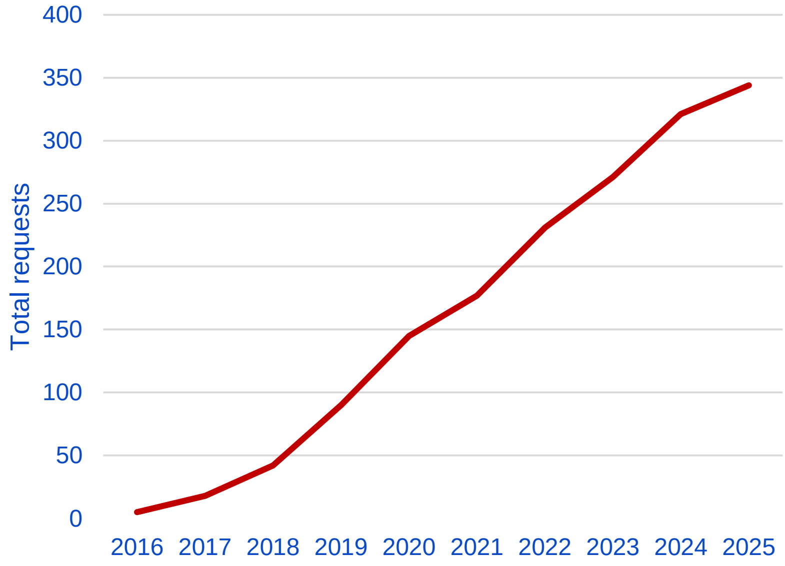
All 'anonimized' data (raw and analyzed) is made available through GCP **cloud** (institutional authentication).

Downloads are possible; user is **contractually** bound for having the right **security** measures in place and is legally **liable** for misuse and data leaks (requires additional paperwork for non-EU countries)

Phase 1: Internal scientific and legal assessment
↓
Phase 2: (external) scientific assessment
↓
Phase 3: Data Access Board
↓
Phase 4: Final decision
↓
Phase 5: License agreement
↓
Phase 6: Sharing the data

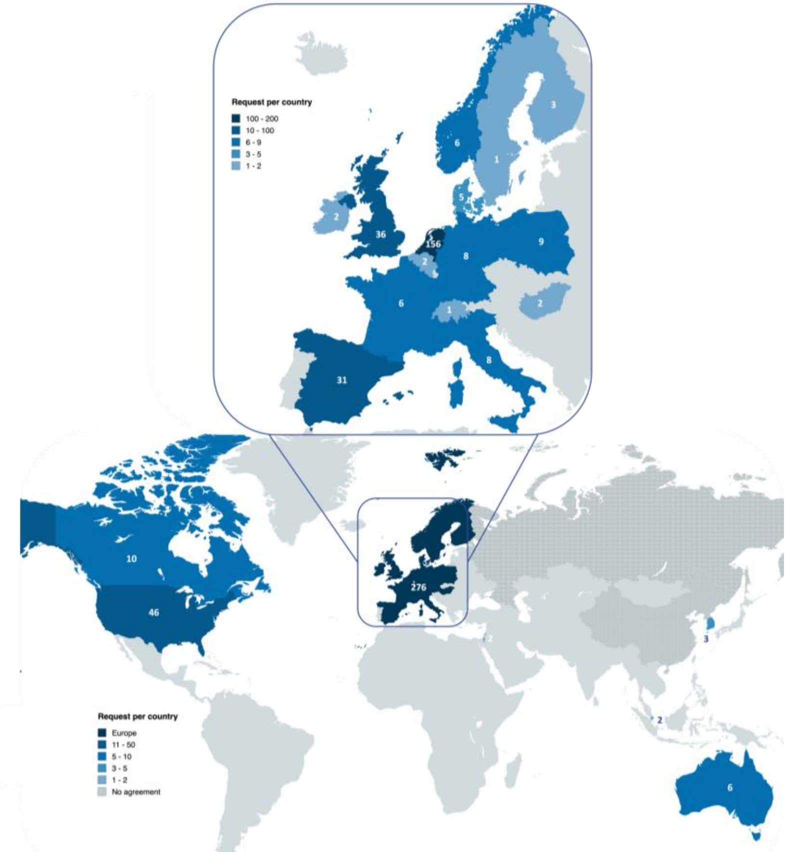
Global data requests & impactful publications

Data Access Requests
~50 per year

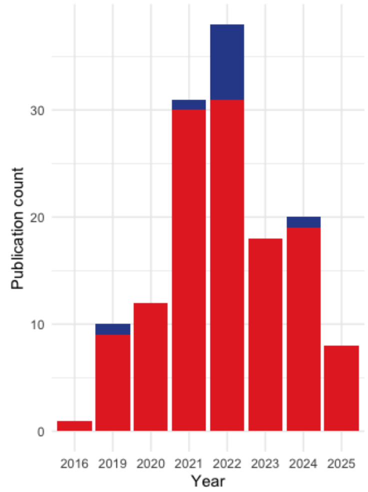


Global use

DPIA for non-EU countries required

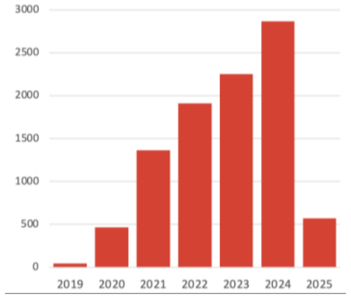


Publications



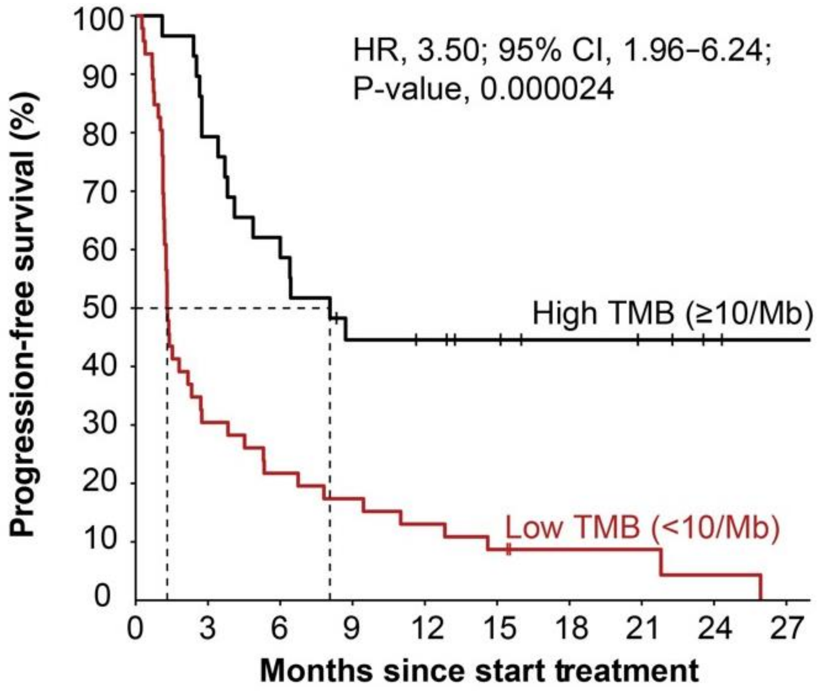
Citations

	All	Since 2020
Citations	9544	9453
h-index	44	44
i10-index	85	85



https://www.hartwigmedicalfoundation.nl/en/data/research-and-science/datarequests/

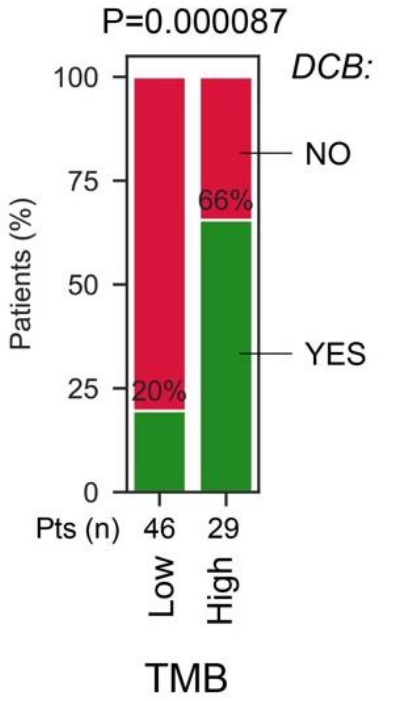
Learning care system in action: (non) response biomarkers



No. at Risk

Low TMB	29	23	18	12	11	9	7	5	3	2
High TMB	46	14	10	8	6	4	2	2	1	0

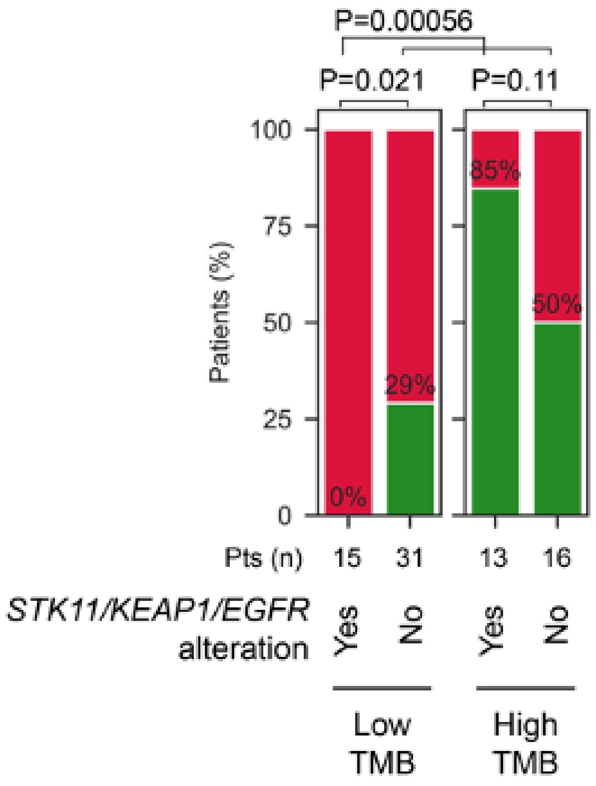
Clinical utility?



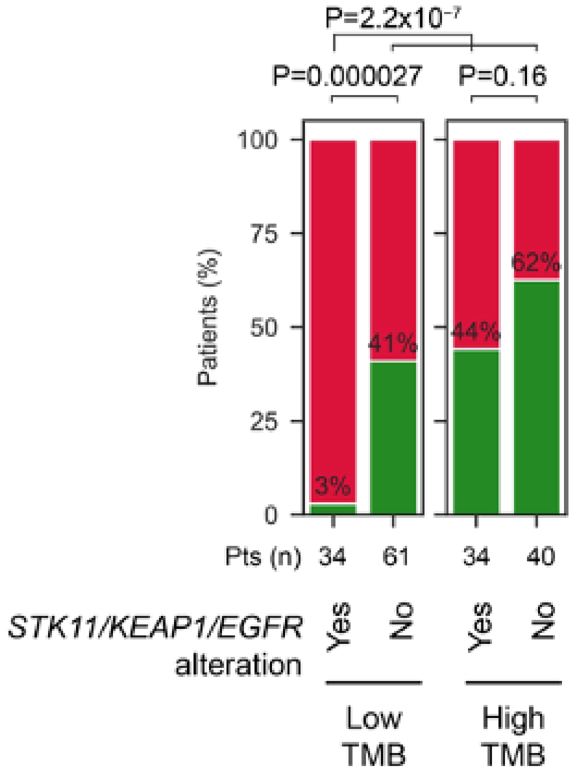
Combining genomic biomarkers to guide immunotherapy in non-small cell lung cancer
Van de Haar, Mankor, Clinical Cancer Research

Learning care system in action: (non) response biomarkers

Refinement



Confirmation

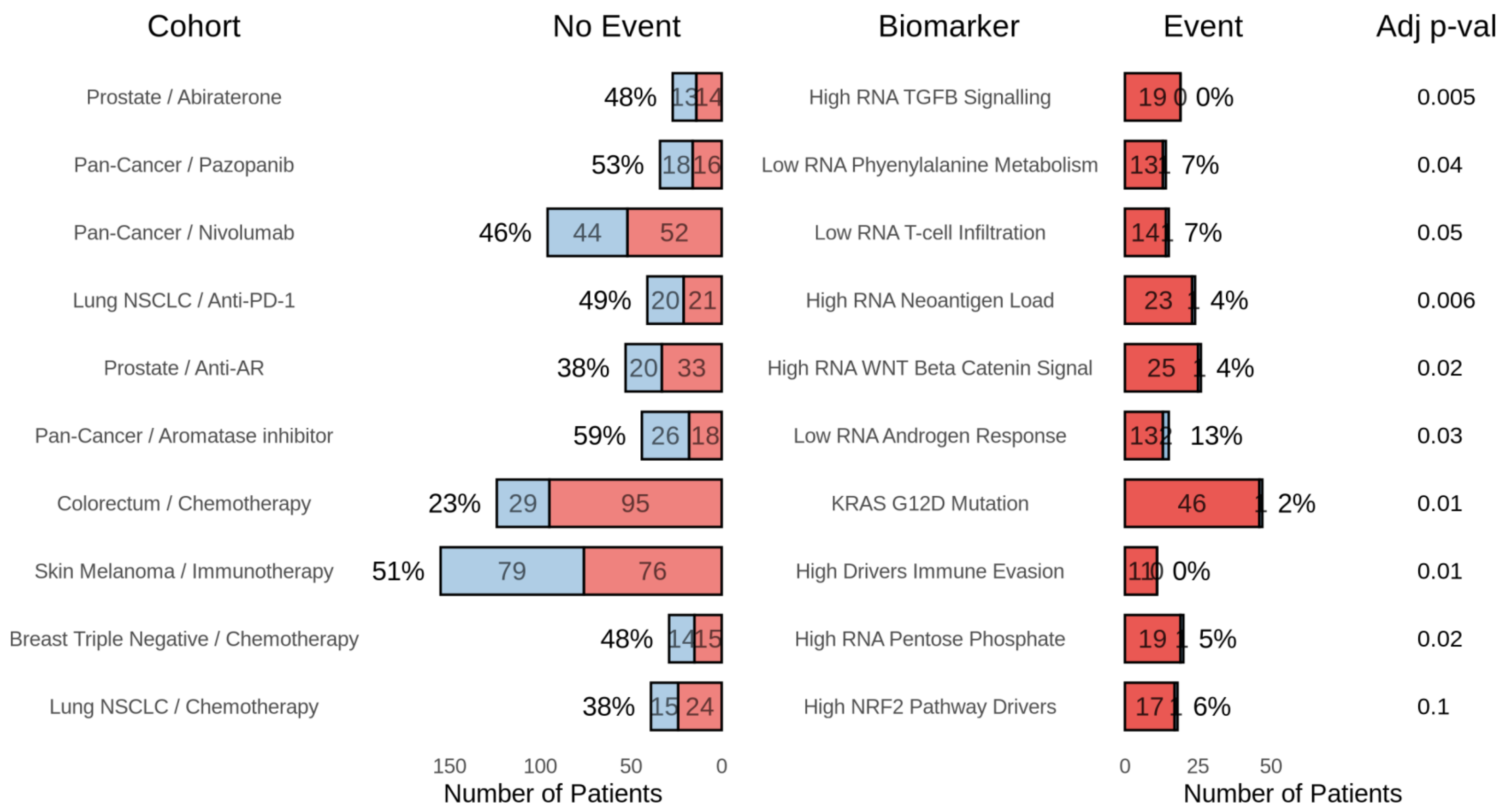


Costs societal impact

- 20%, treatment could potentially be omitted*
- Treatment cost savings ~€ >1.5 M
- Screening all patients by WGS € <0.5 M
- **Net saving of € 1 M**

**Prospective study required*

Can we do this systematically?



Interesting leads (many derived from RNA data) that make sense from a biological perspective
But statistically underpowerd when applying multi-testing correction!

Joseph Usset, Francisco Martinez-Jimenez; unpublished data

Hartwig Medical Foundation

What will the future look like?

1) From fat genomics data to real big data

- Expand WGS data resources by orders of magnitude

Image source: https://www.independentforlonger.com/economic-development-basics/global-partnerships/

International collaboration is key!!

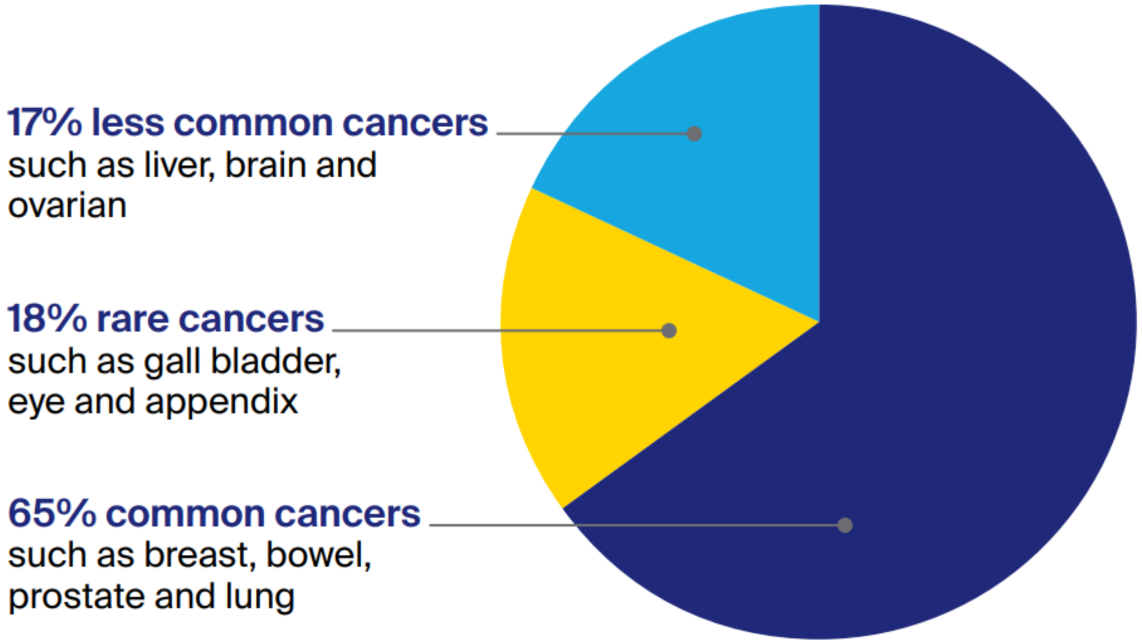
- connecting similar resources world-wide
- global network based on coalition of the willing
- uniformly analyzed genomics data
- standardized clinical data
- trusted authentication and federated access mechanisms

Ongoing exploratory collaboration projects

- Genomics England (cross-validation)
- DKFZ/NCT Germany (rare cancers)
- UMCCR, Melbourne Australia
- 2025 France Genomic Medicine Plan (CUP)

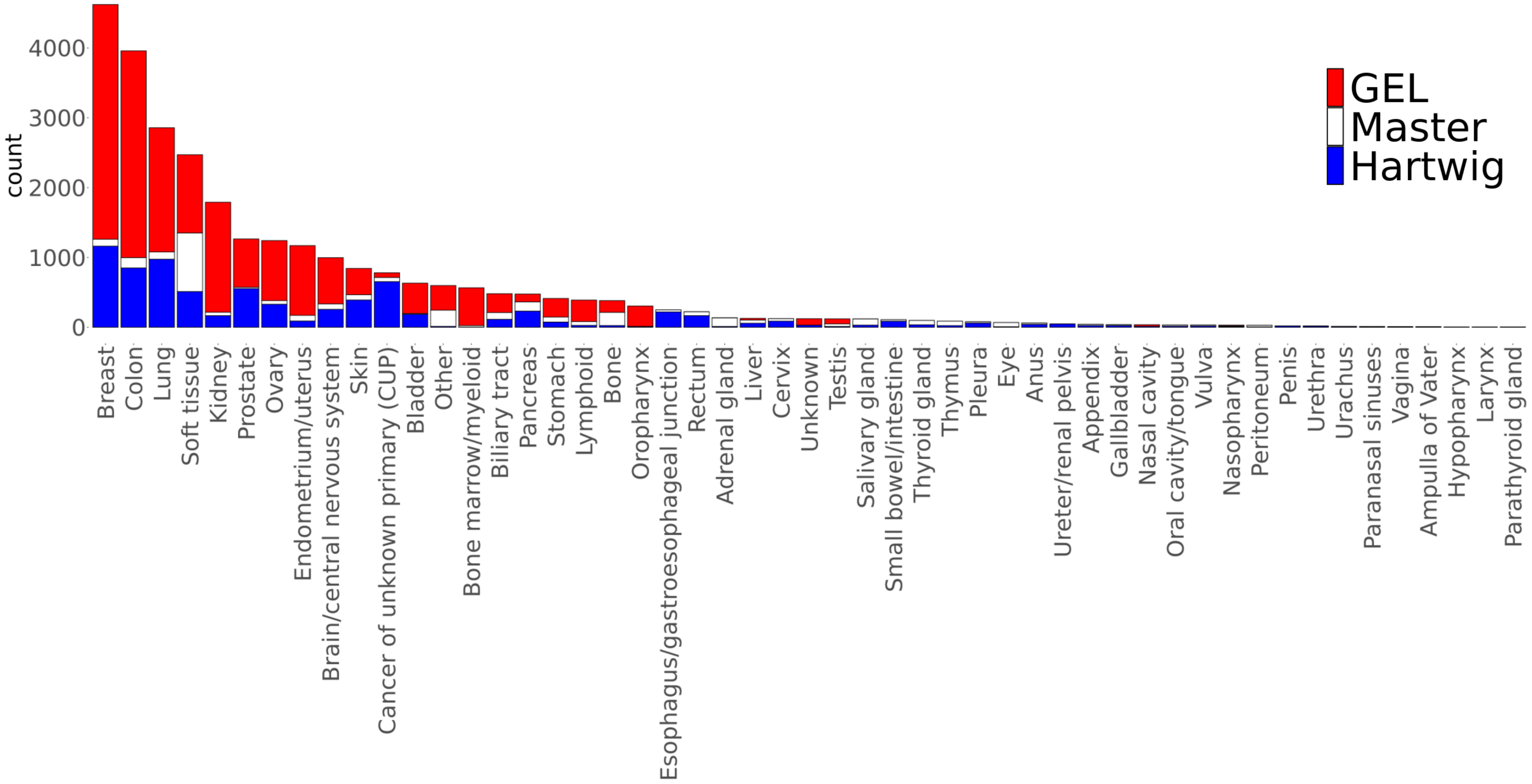
1) From fat genomics data to real big data

- Expand WGS data resources by orders of magnitude
- Specific focus on rare cancers to address unmet needs

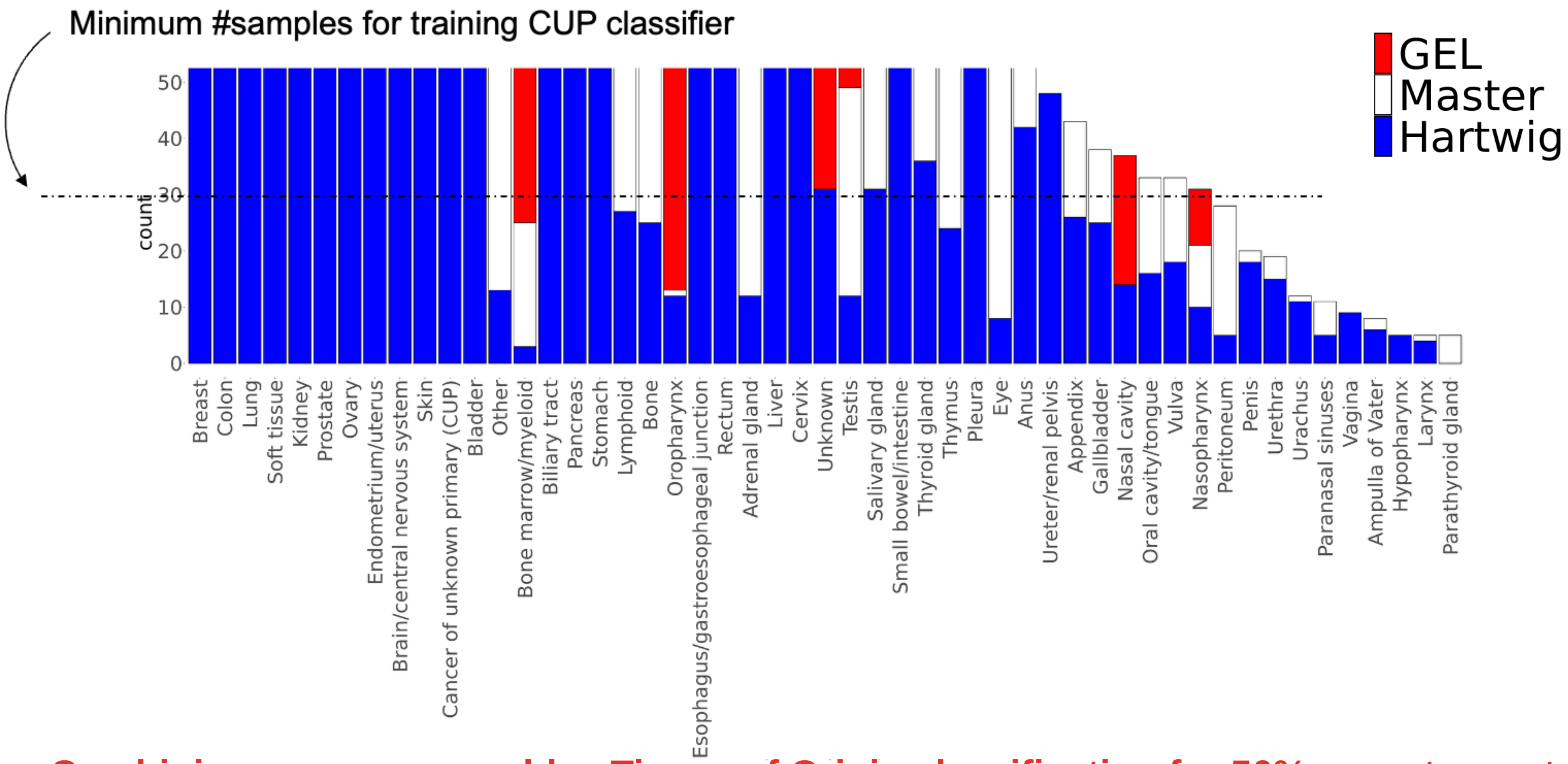


Source: https://www.cancervic.org.au/cancer-information/rare-and-less-common-cancers/overview.html

Combined patient counts for Hartwig, GEL and NCT Master



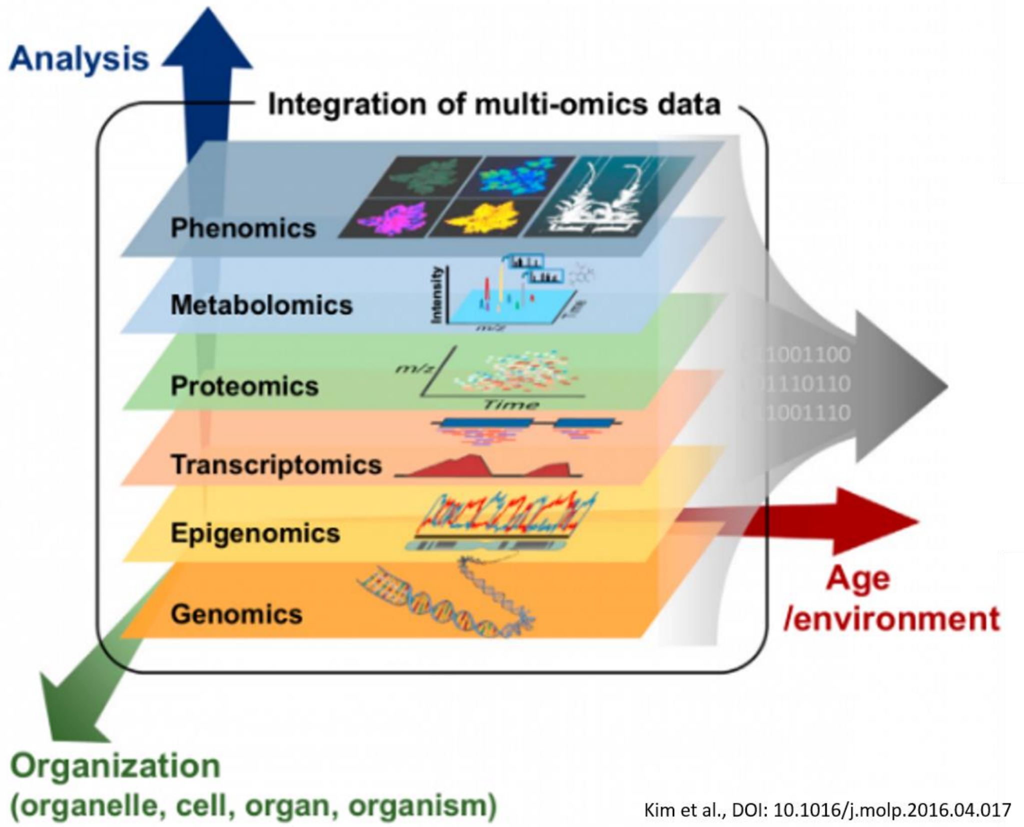
Merging resources today already boosts abilities



Combining resources enables Tissue of Origin classification for 50% more tumor types

1) From fat genomics data to real big data

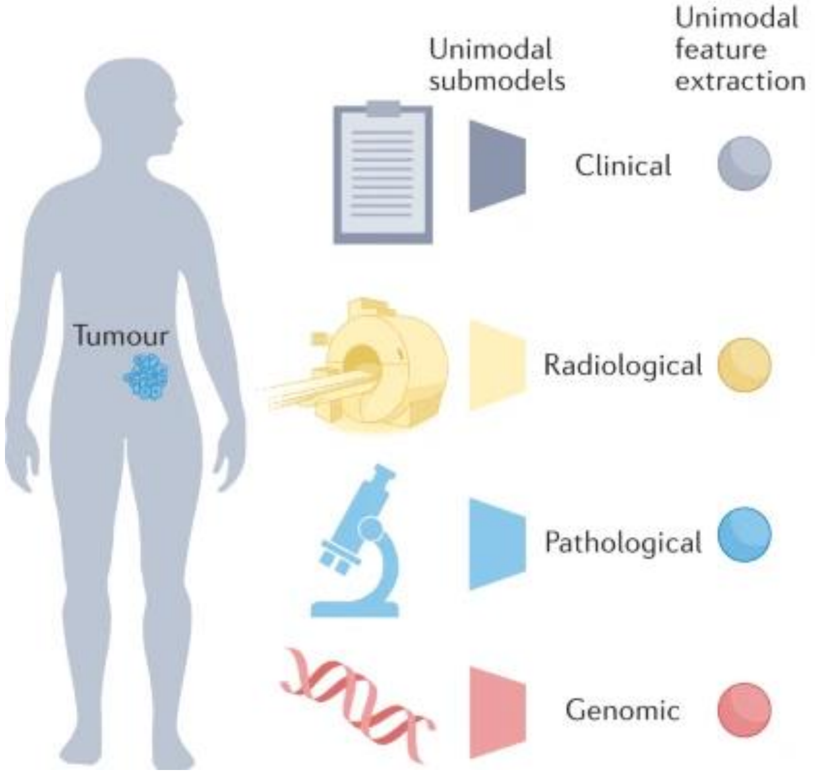
- Expand WGS data resources by orders of magnitude
- Specific focus on rare cancers to address unmet needs
- Extend characterization with multi-omics approaches



Kim et al., DOI: 10.1016/j.molp.2016.04.017

1) From fat genomics data to real big data

- Expand WGS data resources by orders of magnitude
- Specific focus on rare cancers to address unmet needs
- Extend characterization with multi-omics approaches
- Integrate multimodal data sources
    - imaging, histopathology, etc (EHDS)
    - model systems, (patient-derived) cell lines, organoids



Source: Nature Reviews Cancer volume 22, pages114–126 (2022)

1) From fat genomics data to real big data

- Expand WGS data resources by orders of magnitude
- Specific focus on rare cancers to address unmet needs
- Extend characterization with multi-omics approaches
- Integrate multimodal data sources
- Capitalize on AI developments to discover relevant patterns and biomarkers

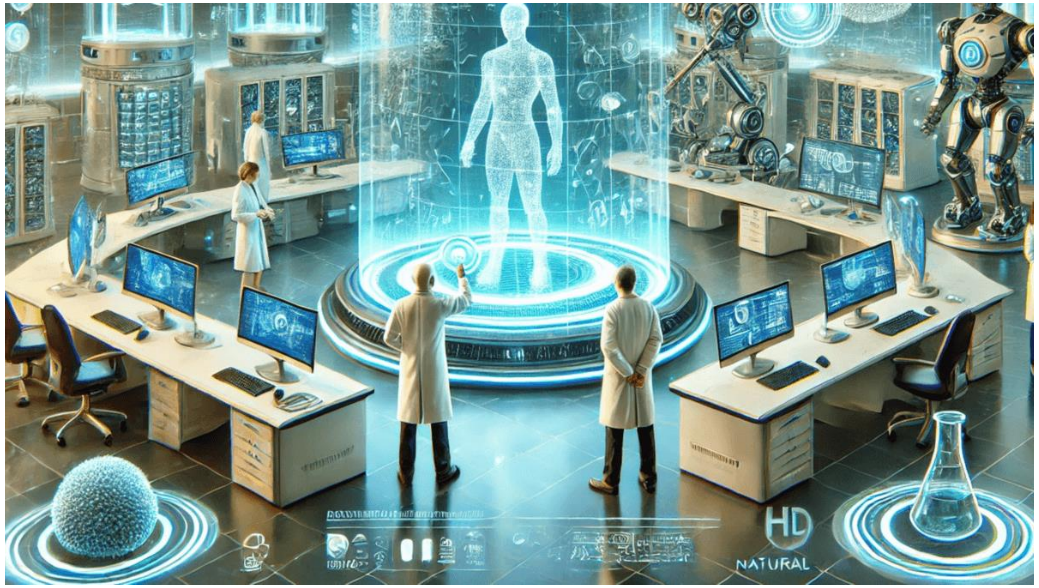
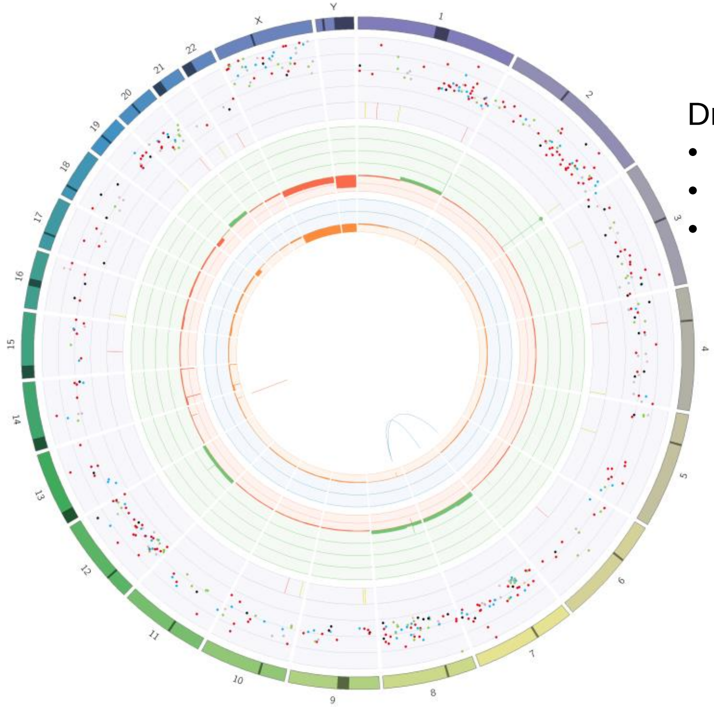


Image source: https://www.elucidata.io/blog/cancer-research-in-the-age-of-ai-the-next-frontier

Why aren’t we massively implementing cancer WGS?

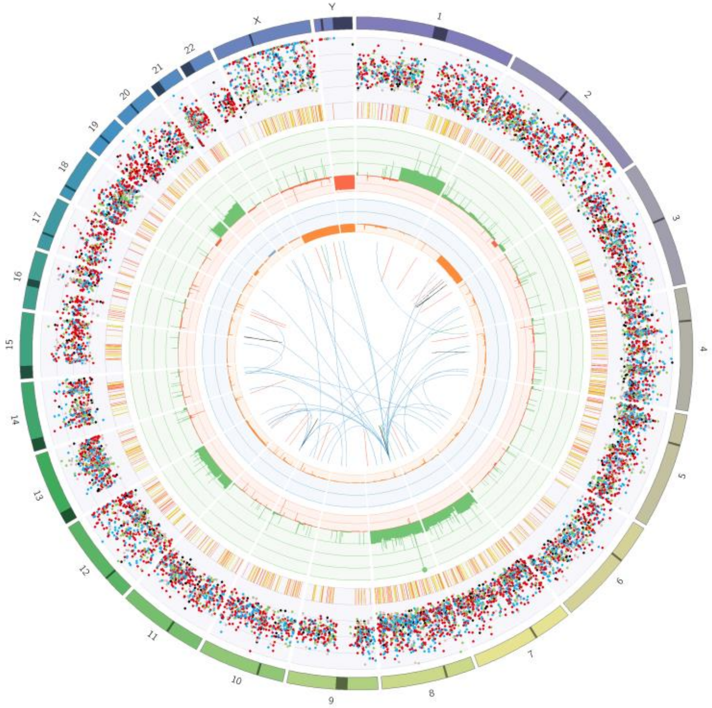
1) high quality WGS for care AND research requires high quality tissue input

Fresh



Drivers:
- KRAS G12V
- PIK3CA N345K
- TCF7L2 R420Q

FFPE



Drivers:
- APC M1211fs
- APC G1312fs
- **KRAS G12V**
- **PIK3CA N345K**
- TP53 G266E
- TCF7L2 P353L
- **TCF7L2 R420Q**
- SOX9 V306fs
- ARID1A P1436fs
- JAK2 N1109Y
- MED12 Y283F
- TNFAIP3 C478Y
- FLT1 R281*

DNA sequencing and FFPE are not friends!

Technically easy to mitigate, but culturally very challenging

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

- **Radiology**: collect 4 biopsies: almost always possible

- Biopsy evaluation in **central sectioning room**:
  - **Diagnostic request**: best biopsy for FFPE; rest for FF (or RNAlater)
  - **Molecular request**: all biopsies for FF (or RNAlater)

- **PrestoChill**: rapid freezing followed by sectioning, microscopic evaluation, and macrodissection of tumor-containing part of biopsy for DNA/RNA isolation

Transformation to non-FFPE (FF or RNA-later) also needed for maximizing potential of **other emerging technologies**, like single cell, proteomics, functional screens, organoids

nature protocols https://doi.org/10.1038/s41596-023-00933-5

Protocol Check for updates

Optimized whole-genome sequencing workflow for tumor diagnostics in routine pathology practice

Kris G. Samsom[1,11], Linda J. W. Bosch[1,11], Luuk J. Schipper[2,3], Daoin Schout[1], Paul Roepman[4], Mirjam C. Boelens[1], Ferry Lalezari[5], Elisabeth G. Klompenhouwer[5], Adrianus J. de Langen[6], Tineke E. Buffart[7], Berit M. H. van Linder[1], Kelly van Deventer[1], Kay van den Burg[1], Unga Unmehopa[1], Efraim H. Rosenberg[1], Roelof Koster[1], Frans B. L. Hogervorst[1], José G. van den Berg[1], Immy Riethorst[4], Lieke Schoenmaker[4], Daphne van Beek[4], Ewart de Bruijn[4], Jacobus J. M. van der Hoeven[4], Hans van Snellenberg[4], Lizet E. van der Kolk[8], Edwin Cuppen[3,4,9], Emile E. Voest[2,3,10], Gerrit A. Meijer[1] & Kim Monkhorst[1]

Nature Protocols | Volume 19 | March 2024 | 700–726 700

Why aren’t we massively implementing cancer WGS?

1) high quality WGS for care AND research requires high quality tissue input

non-FFPE (FF or RNA-later)

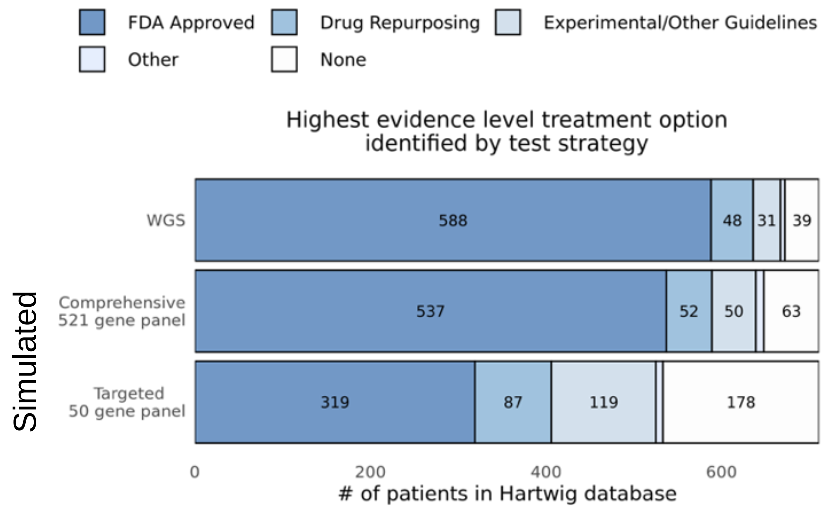
2) panel NGS is cheaper than WGS

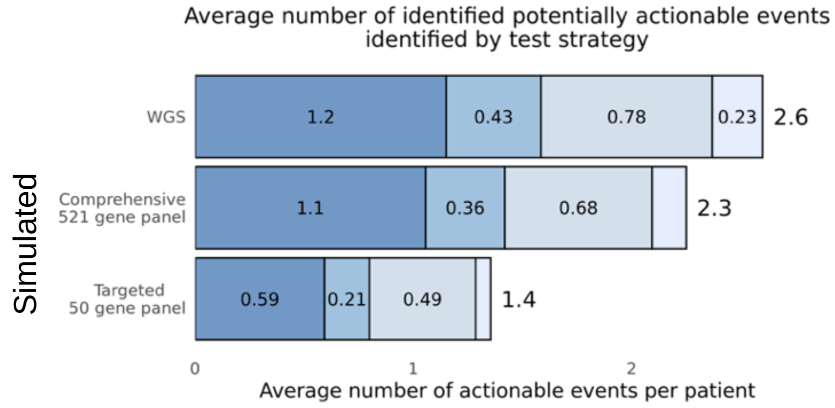
WGS: 2,000 – 3,000 Euro/Dollar

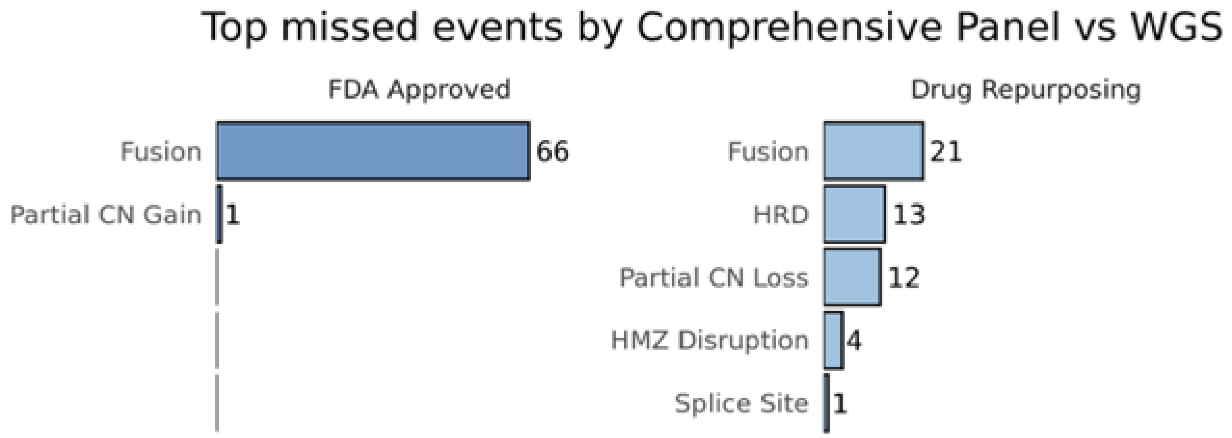
Large panel like TSO500: 750 – 1,000 Euro/Dollar

But is this really true?

Non-small cell lung cancer (NSCLC; n=711 patients)





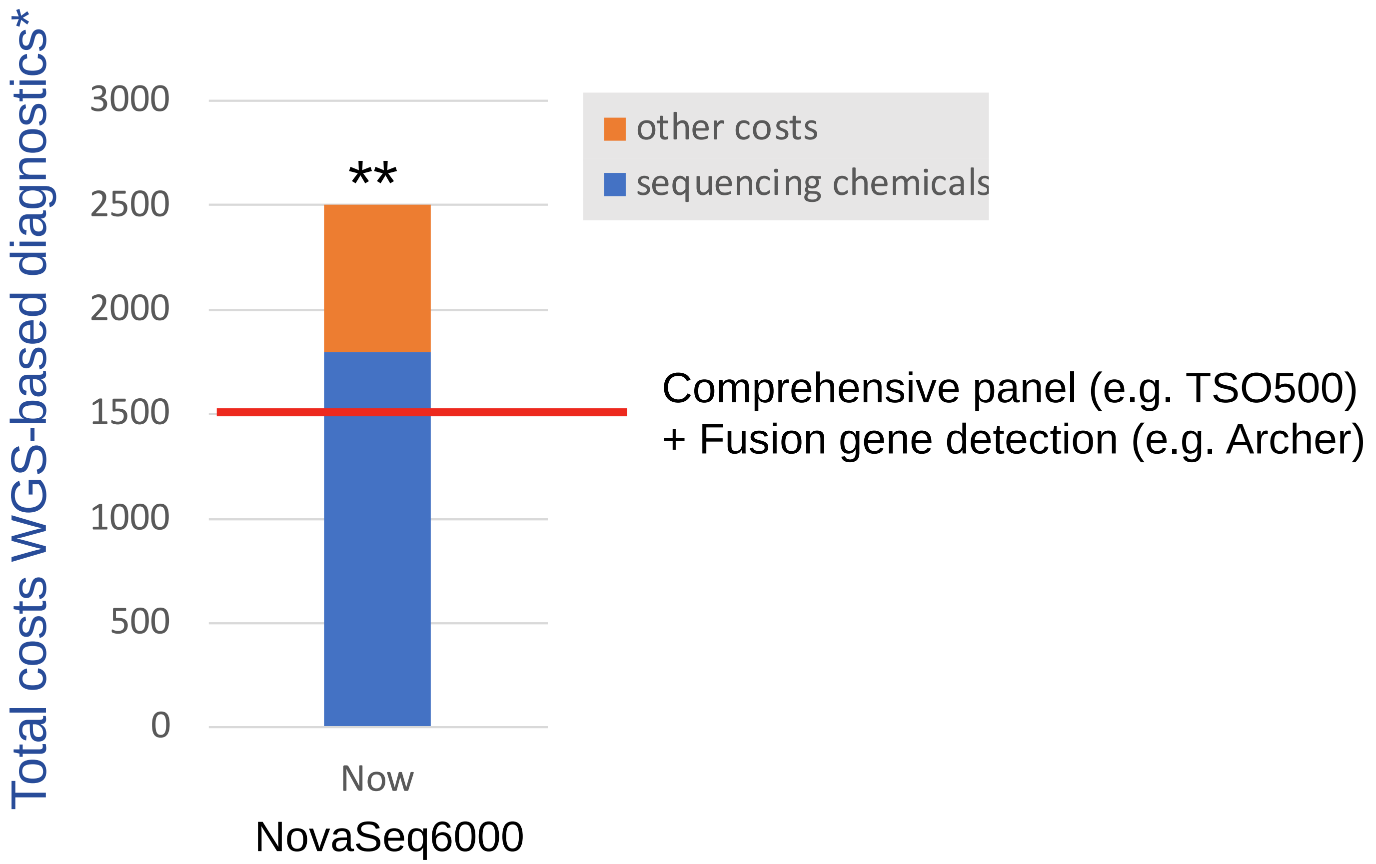


Fusion gene detection requires an additional assay on top of standard comprehensive panel

So does Homologous Recombination Deficiency (HRD) detection

~10% of relevant actionable events are missed

Sequencing: reagents main determinant of test costs



** based on 4 genome equivalents (tumor-normal pairs, 90x vs 30x)*
*** update numbers for 2022, calculated as described in Pasmans et al., Expert Rev Pharmacoecon Outcomes Res (2021) Jun;21(3):413-414.*

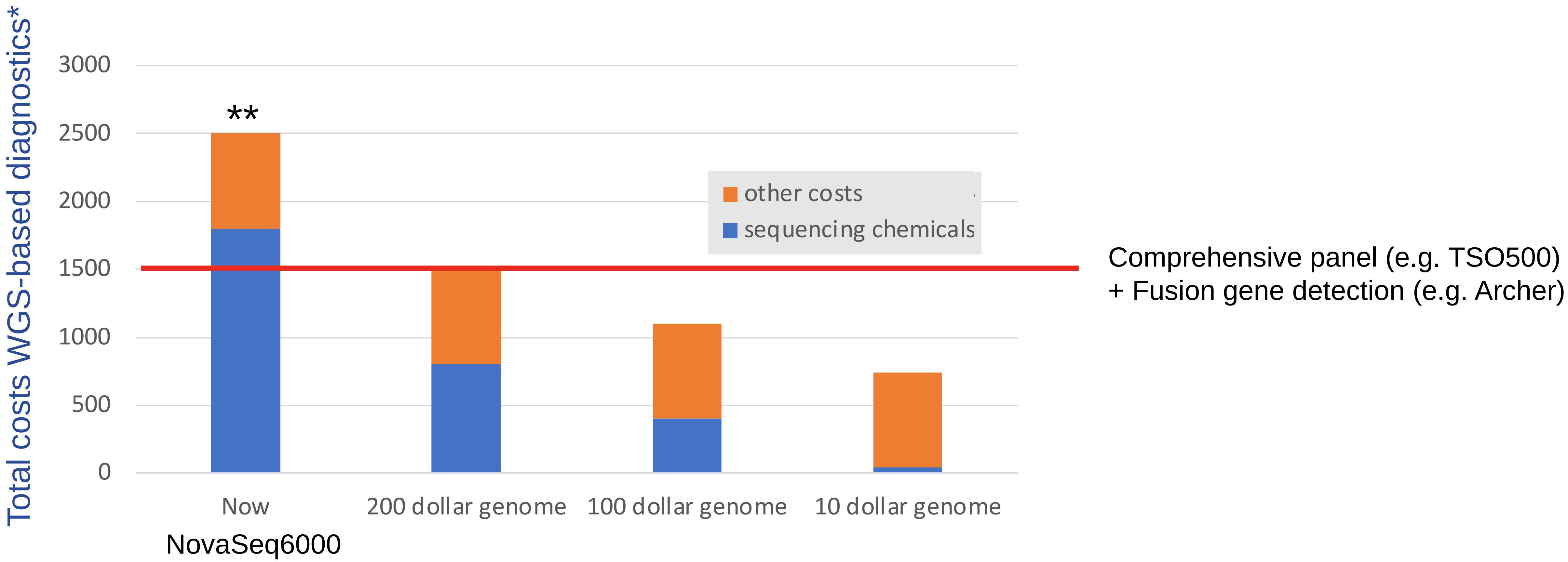
Expert Review of Pharmacoeconomics & Outcomes Research

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ierp20

Micro-costing diagnostics in oncology: from single-gene testing to whole- genome sequencing

Clémence T. B. Pasmans, Bastiaan B. J. Tops, Elisabeth M. P. Steeghs, Veerle M. H. Coupé, Katrien Grünberg, Eiko K de Jong, Ed M. D. Schuuring, Stefan M. Willems, Marjolijn J. L. Ligtenberg, Valesca P. Retèl, Hans van Snellenberg, Ewart de Bruijn, Edwin Cuppen & Geert W. J. Frederix

Sequencing: reagents main determinant of test costs



** based on 4 genome equivalents (tumor-normal pairs, 90x vs 30x)*
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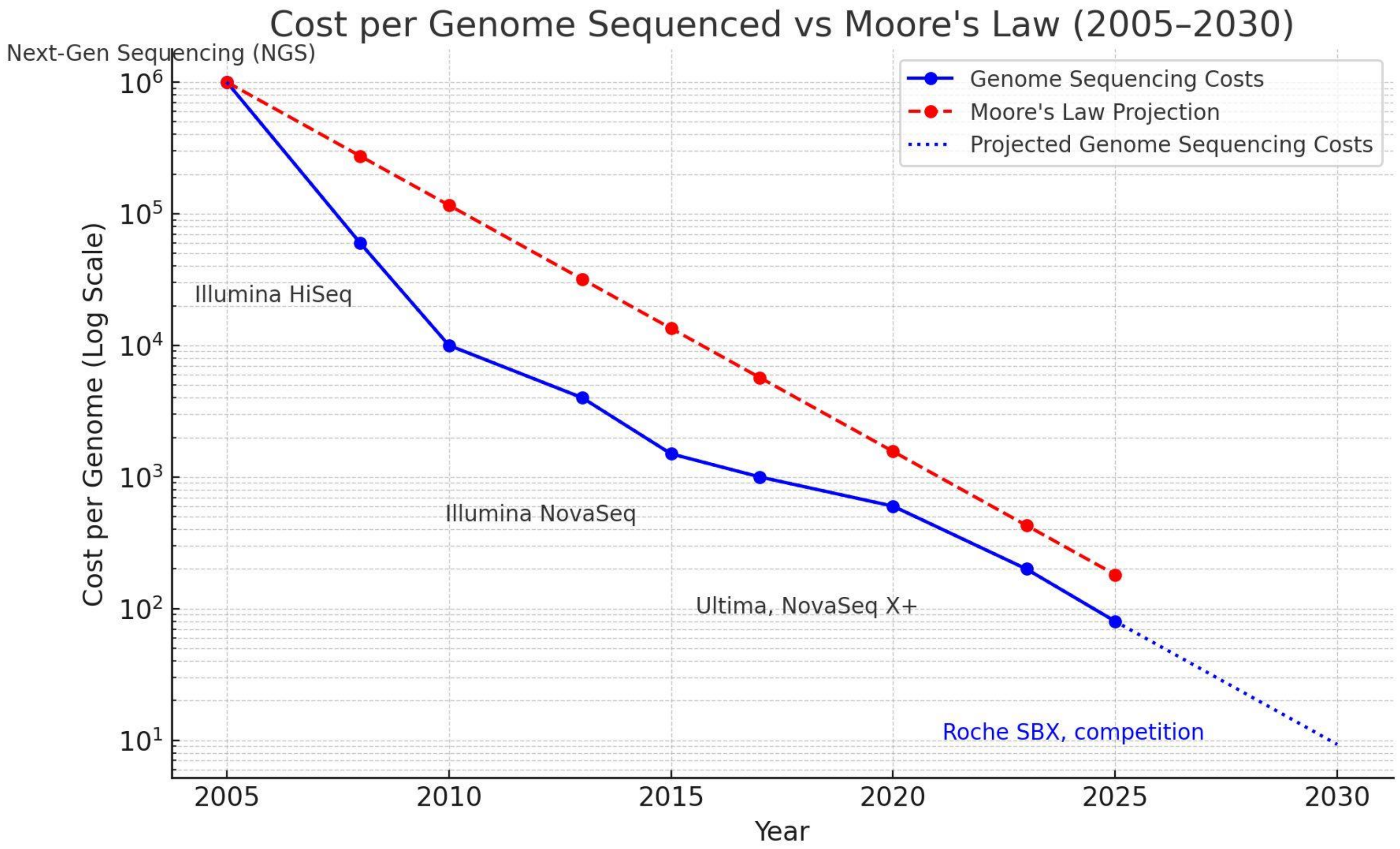
2) Technological innovations: Cheaper and faster short-read WGS



Sources: NIH Genome Sequencing Program, Illumina press releases, Ultima Genomics announcements, Roche investor presentations

March 19, 2025

Andy Watson • 1st
CEO & Board Member | Advisor
Book an appointment
9h •

Are You Ready for the $10 Genome?
The $10 genome is on the horizon, and it's set to shake up biology and healthcare in ways we've only dreamed of. This isn't just an incremental improvement – it's a seismic shift that will redefine what's possible.

2) Technological innovations: Cheaper and faster short-read WGS

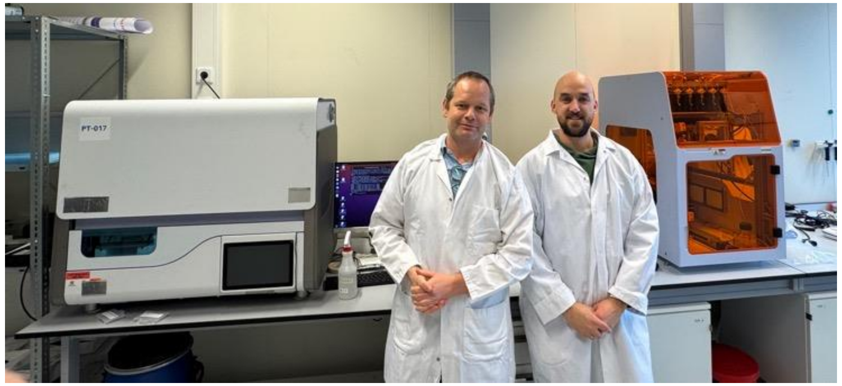
illumina | ULTIMA GENOMICS | Roche

Illumina NovaSeq6000/X+
Next generation SBS
Reagent cost reduction ˜50%

$250 genome on 25B chip

Ultima Genomics UG100
Flow-based sequencing on wafers
Installed at Hartwig early 2024

$80-100 genome
Highly sensitive ctDNA detection using ppmSeq

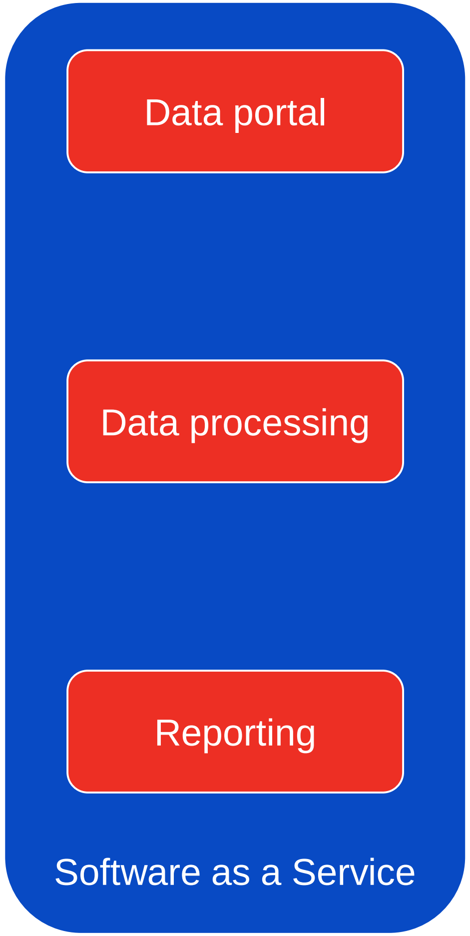


Roche Axelios (prototype)
Nanopore sequencing by expansion (SBX)
Alpha testing at Hartwig early 2025
Commercial launch summer 2026
$??
4 hour run time

- **Hartwig WiGiTS data analysis tools are being made compatible for all three platforms**
- **Preliminary conclusions: all platforms are suited for WGS-based cancer diagnostics**

3) Software as a Service for data analysis, annotation and interpretation

Hospitals often lack IT infrastructure and expertise to handle, analyse and interpret WGS data

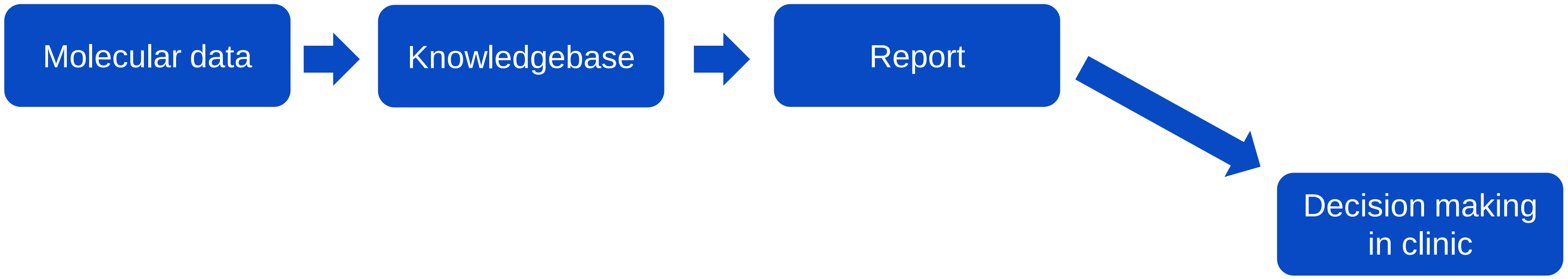


- Authenticated login (hospital-controlled)
- Upload data from sequencer
- Sample overview
- Interactive reports
- Download data

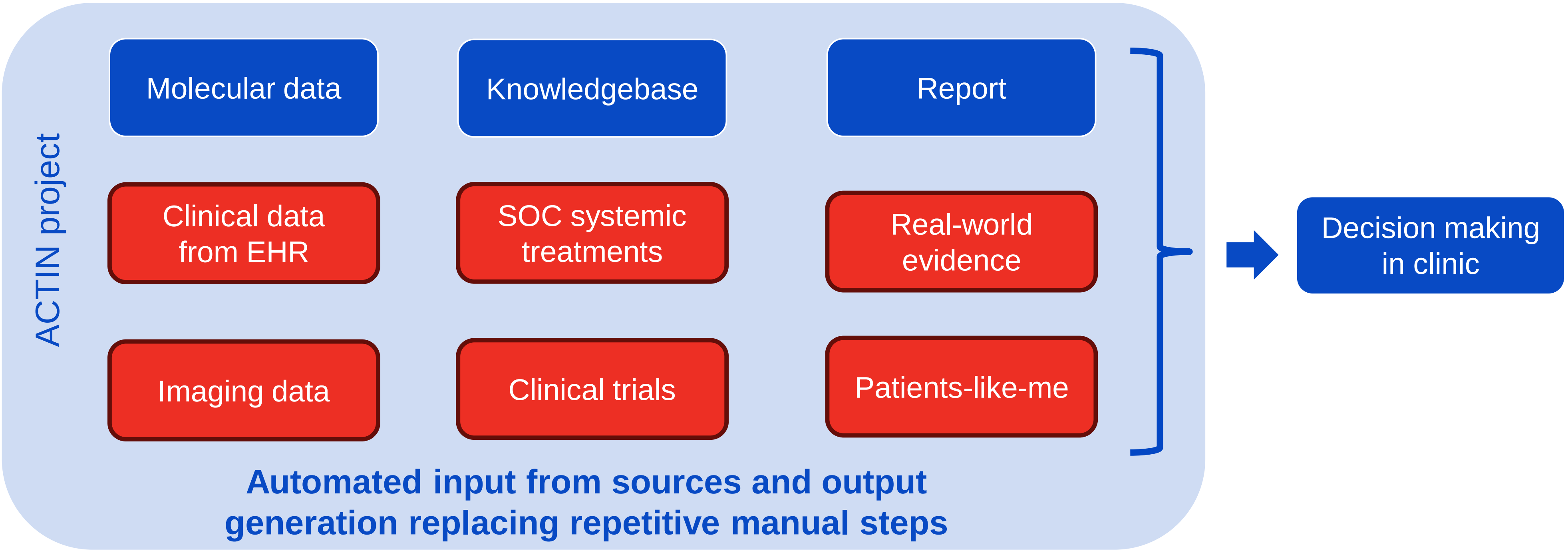
- Powered by **Hartwig WiGiTS**
- Sequencing platform agnostic
- Tumor/normal WGS, Tumor-only WGS, panel

- **CE-IVD** approved OncoAct
- Empowered by **Hartwig database**

4) Improving data-driven cancer decision support systems



4) Improving data-driven cancer decision support systems



Beyond molecular data

Beyond targeted treatments

Beyond clinical study evidence

4) Improving data-driven cancer decision support systems

Hartwig OncoActin (under development)

Standard of care options considered potentially eligible

Treatment	Literature efficacy evidence	Real-world efficacy evidence	Warnings
FOLFOXIRI-B	TRIBE2 **PFS:** 12.0 months (95% CI: 11.1-12.9) **OS:** 27.4 months (95% CI: 23.7-30.0)	**PFS:** 8.8 months, IQR: 5.4 **OS:** 17.6 months, IQR: 21.3	
PEMBROLIZUMAB	KEYNOTE-177 **PFS:** 16.5 months (95% CI: 5.2-32.4) **OS:** NA	**PFS:** 8.3 months, IQR: 13.2 **OS:** NaN months	MSI status undetermined
FOLFOX-B	TRIBE2 **PFS:** 9.8 months (95% CI: 9.0-10.5) **OS:** 22.5 months (95% CI: 20.7-24.8)	**PFS:** 7.2 months, IQR: 6.4 **OS:** 13.8 months, IQR: 16.5	

Expected outcomes

- Clinical Trial
- Real World Data
- Patients-like-me

Erasmus MC trials that are open and potentially eligible (2 cohorts from 2 trials)

Trial	Cohort	Molecular	Warnings
MEC 16-758 DRUP (Phase 2)	Talazoparib	ATM c.5762G>A splice	Inactivation event(s) ATM c.5762G>A splice for ATM but event(s) are not biallelic
MEC 23-0391 SAR445877 (Phase 1/2)	Part 2 Dose expansion Cohort D: Infiltrated "hot" tumors		No head and neck cancer, kidney cancer, liver cancer, lung non-small cell carcinoma, melanoma or urinary bladder cancer

Better trial matching

- Inclusion/Exclusion criteria
- Automated data collection from EHR
- Local and (inter)national
- Better context

5) Improving therapy and disease progression monitoring

Tumor-informed (WGS) ctDNA monitoring

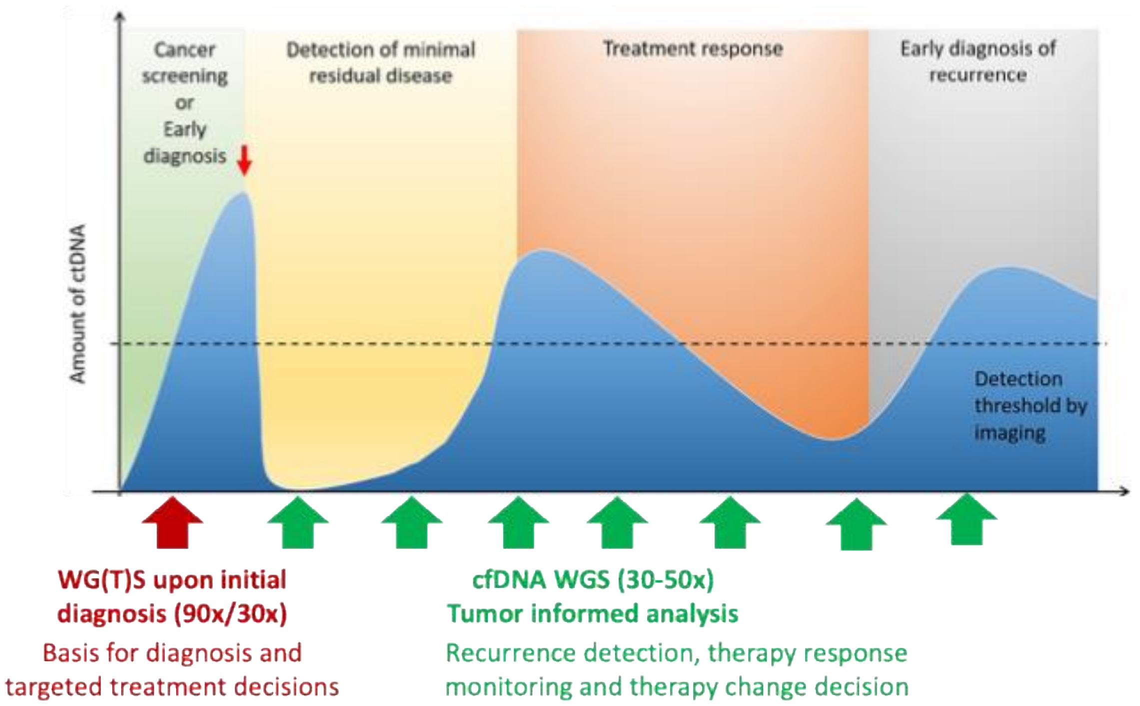
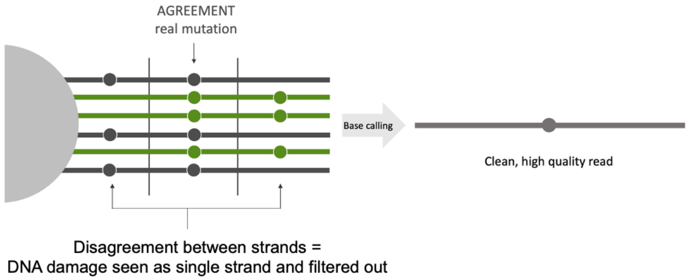


Image source: Journal of Human Genetics volume 66, 909–926 (2021)

Paired-plus minus sequencing (ppmSeqTM) on **Ultima UG100**



- Very low raw read error rate phred 50-60
- average 7x coverage per 1ng input
- > 30x WGS coverage with 1 blood tube

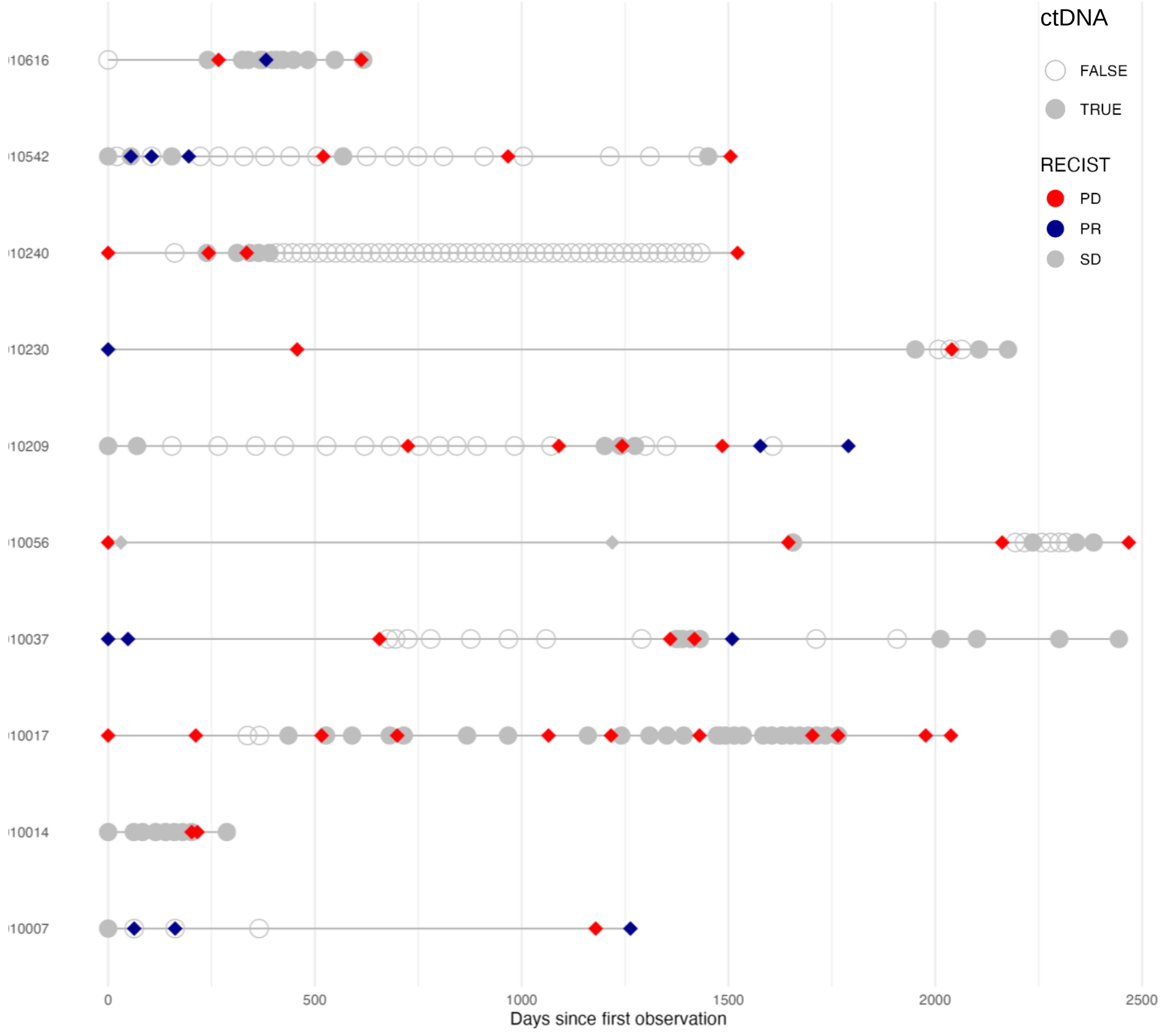
- **1-size-fits-all test**
  - WGS diagnostics required
  - Presence of sufficient somatic mutations
- **Ultrasensitive** detection limit of 0.001-0.01%

Pilot study – retrospective longitudinal sampling in NSCLC

- **Collaboration with NKI**
  - WGS-based diagnostics already routine care

NETHERLANDS CANCER INSTITUTE
ANTONI VAN LEEUWENHOEK

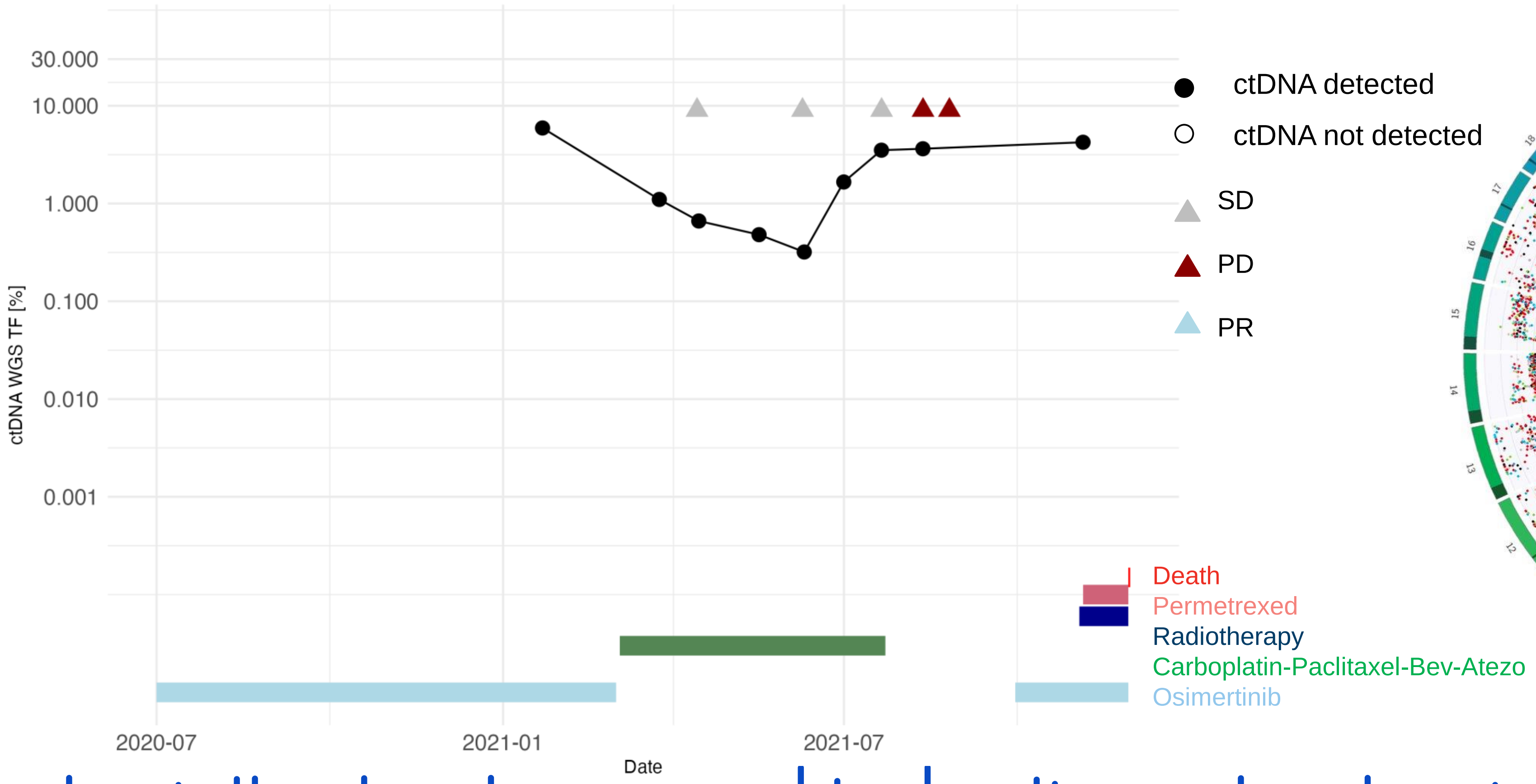
- **Retrospective study cohort**
  - 10 metastatic NSCLC patients
  - 192 plasma samples
- **ctDNA detection**
  - >=30X coverage on Ultima UG100
  - Data analysis using WISP tool (part of Hartwig WiGiTS)
- **Results**
  - 190 out of 192 successful processed
  - Lowest ctDNA fraction detected was 43ppm

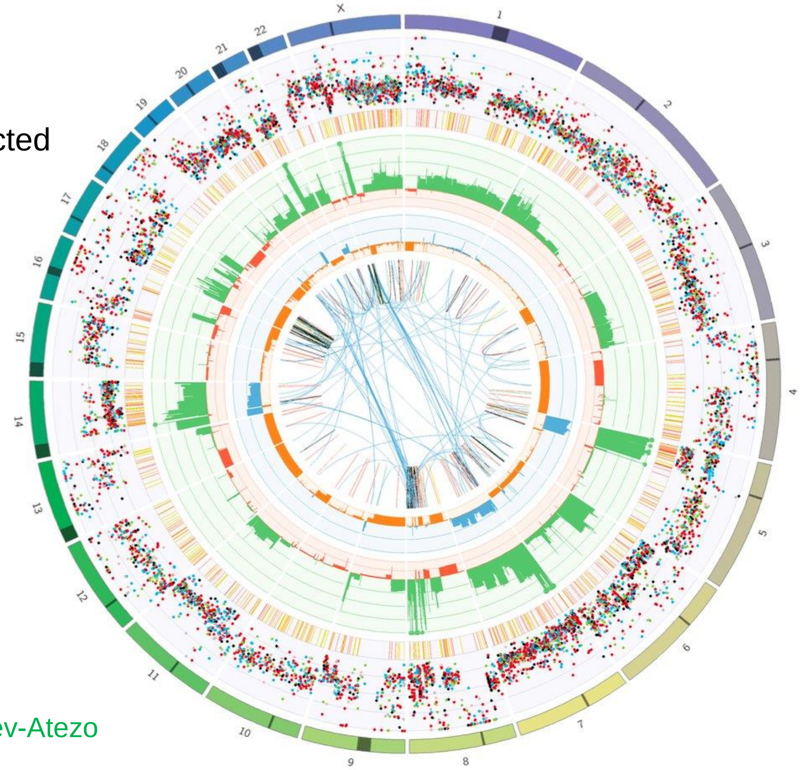


Noe, Monkhorst, van der Pol, unpublished data

Case study patient 1

- ~14 000 somatic variants in tissue
- ctDNA tumor fraction never decreased, similar to what was observed in the clinical data
- 2x increase between June-August 2021 prior to progression detected in September 2021

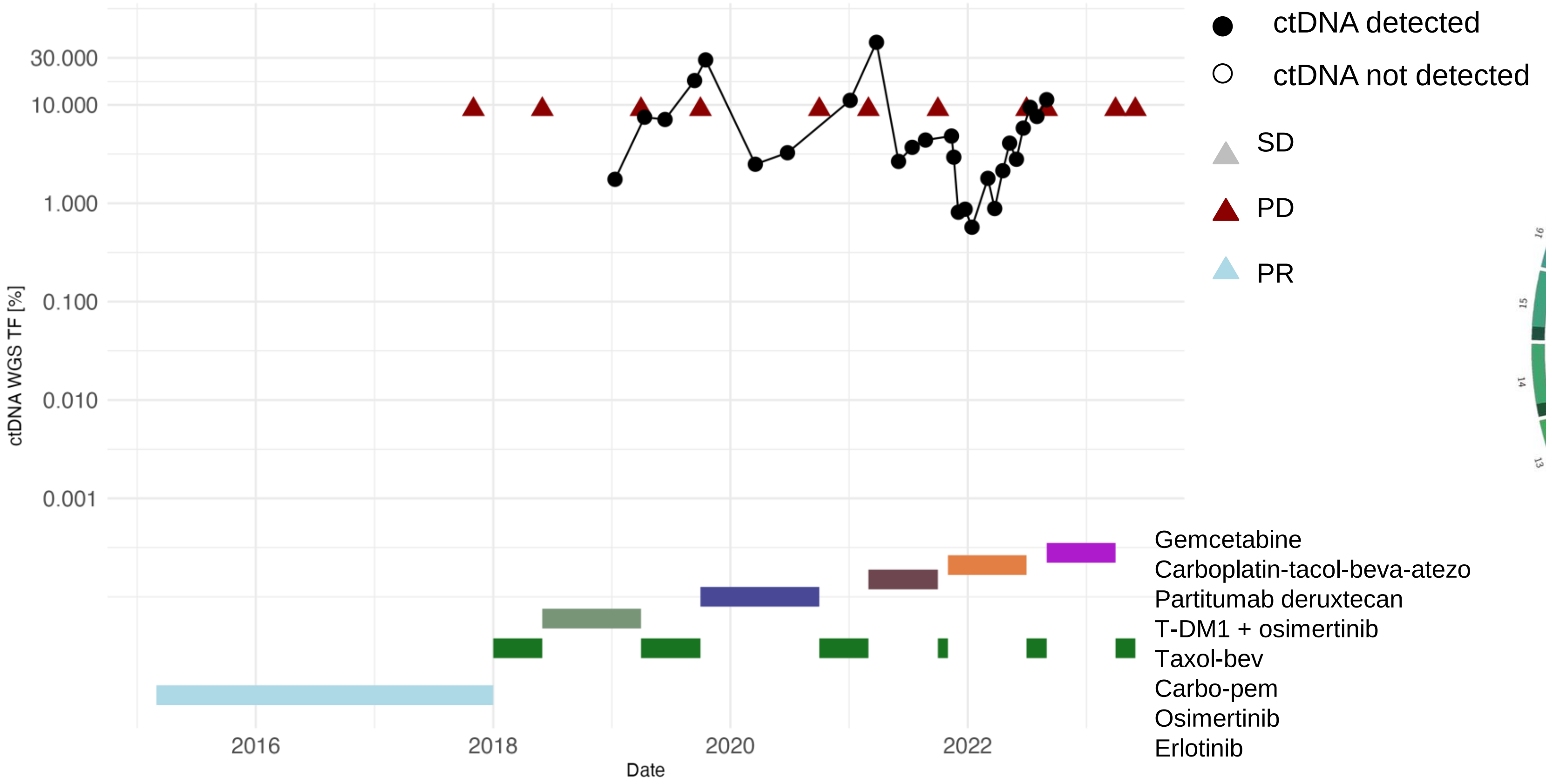


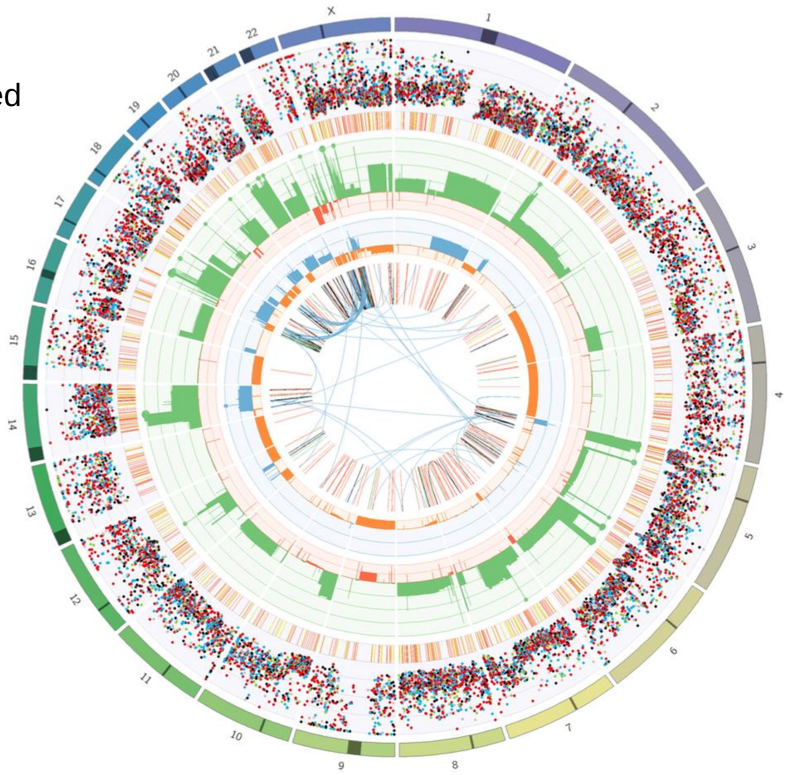


Noe, Monkhorst, van der Pol, unpublished data

Case study patient 2

- ~16 500 somatic variants in tissue
- High ctDNA tumor fraction at times (43%)
- ctDNA tumor fraction increase prior to progression





Noe, Monkhorst, van der Pol, unpublished data

Summary

- **Routine WGS-based cancer diagnostics**
  - Is feasible
  - Has value for todays patient care
  - Is getting cheaper with emerging technologies
  - Enables versatile disease monitoring

- **Data reuse**
  - Is feasible, including international data sharing
  - Enables scientific and biomarker discovery
  - Improves tomorrows patient care
  - Contributes to health care system sustainability
  - Is boosted by international collaboration

Acknowledgements

All patients and families

Hartwig NL – AUS – CAN – ES

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Joris vd Haar
Emile Voest

Support

ULTIMA GENOMICS
illumina
KWF
Missie Tumor Onbekend
Roche

Hartwig
Medical Foundation

The right treatment for each cancer patient

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