CLINICAL APPLICATION OF NOVEL TECHNOLOGIES

NGS as a diagnostic tool in human diseases

BRFAA
BIOMEDICAL RESEARCH FOUNDATION
ACADEMY OF ATHENS

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HELLENIC REPUBLIC
National and Kapodistrian
University of Athens
EST. 1837

Talk Plot

Next Generation Sequencing – CV and future

Flavours of NGS

Applications in Medicine

1. **Oncology**
2. **Reproductive Medicine**
3. **Pathogen Genomics**
4. **Hematology**
5. **AI and NGS**
6. **Rheumatology**

History of Next Gen Sequencing

1st Generation

Sanger Sequencing

- Infer nt identity with dNTPs and visualize with capillary electrophoresis
- 500-1000 bp fragments
- Expensive, laborious, time consuming

Next Generation
(2nd Generation)

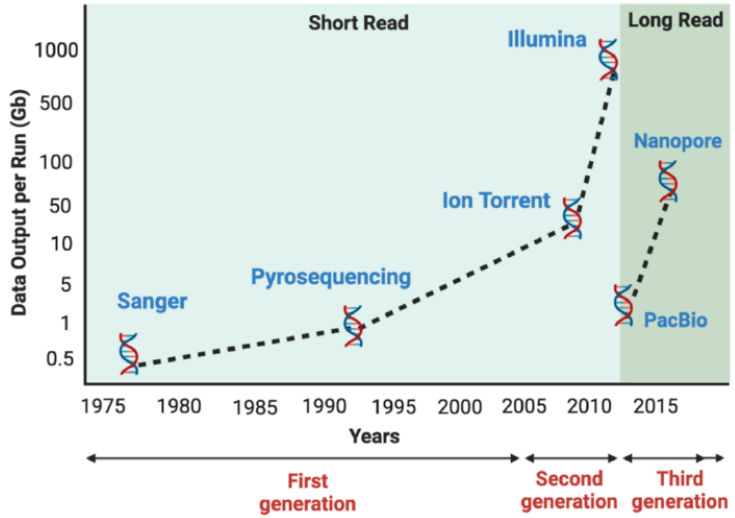
Massive Parallel Sequencing

- High throughput from the parallelization of millions of sequencing reactions
- ~50-500 bp fragments

3rd Generation

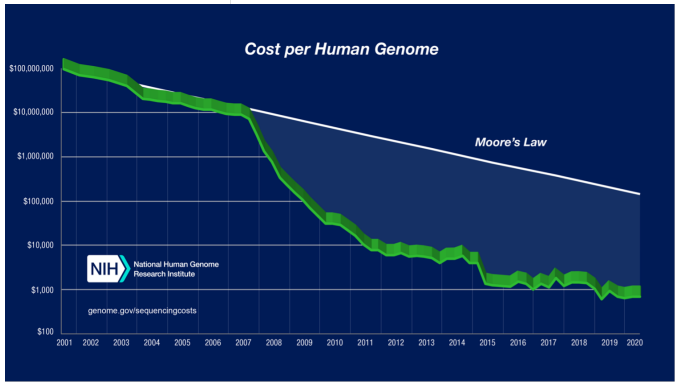
Single Molecule Real Time
Long Reads Sequencing

- Sequence native DNA and/or RNA in real time with single-molecule resolution
- Tens of kb fragments



Whole Genome Sequencing:
- Duration: <48 hrs
- Cost: < 2000 euros

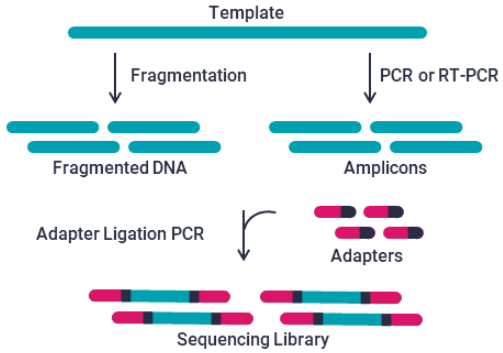
Whole Exome Sequencing:
- Cost: < 300 euros



Next Generation Sequencing Pipeline

STEP 1:
Extraction

STEP 2:
Library Prep



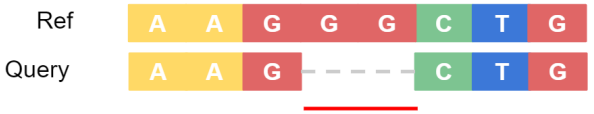
STEP 3:
Sequencing

STEP 4:
Analysis

Sequence Variants

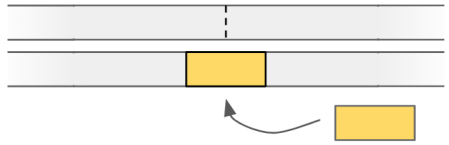
SNV (**S**ingle **N**ucleotide **V**ariant)

INDEL (**In**sertion or **Del**etion)

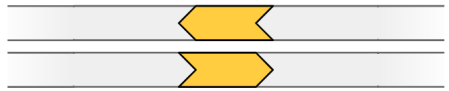


Structural Variants

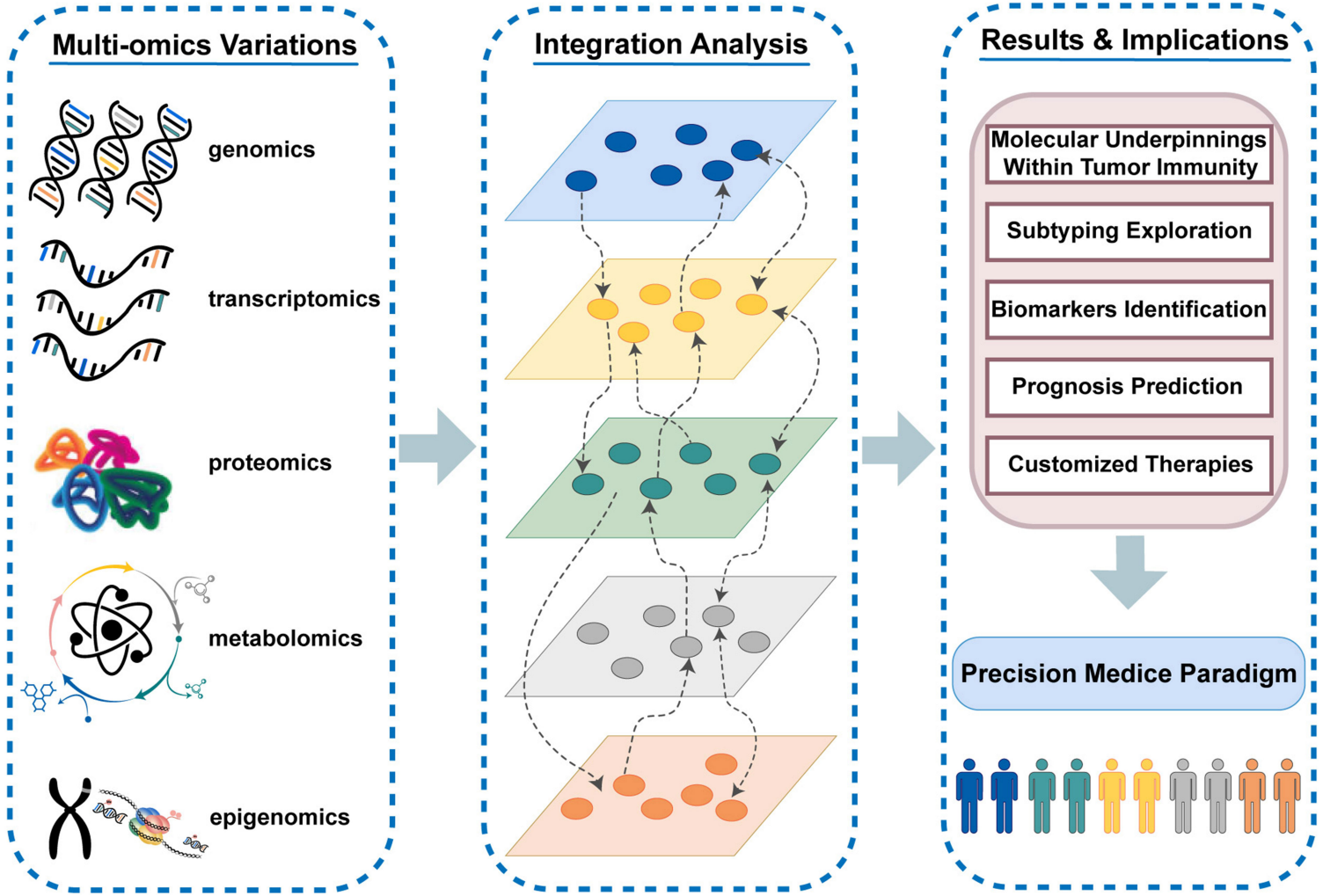
Insertion



Inversion



Multi – omics towards personalized medicine

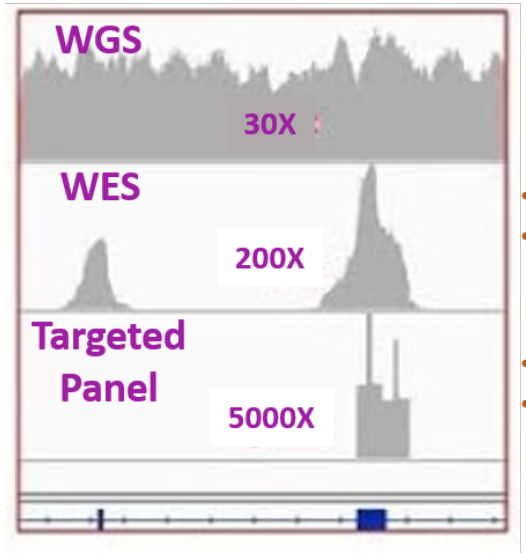


- Different layers of –omics describe the pathophysiology in a multi-parametric manner
- Need for advanced integration!
- Molecular Subtypes of the disease
- Precision Medicine = Personalized Medicine
- Dream or Sisyphean Struggle?

Chen S et al. Front. Immunol., 2022

Flavours of genome sequencing

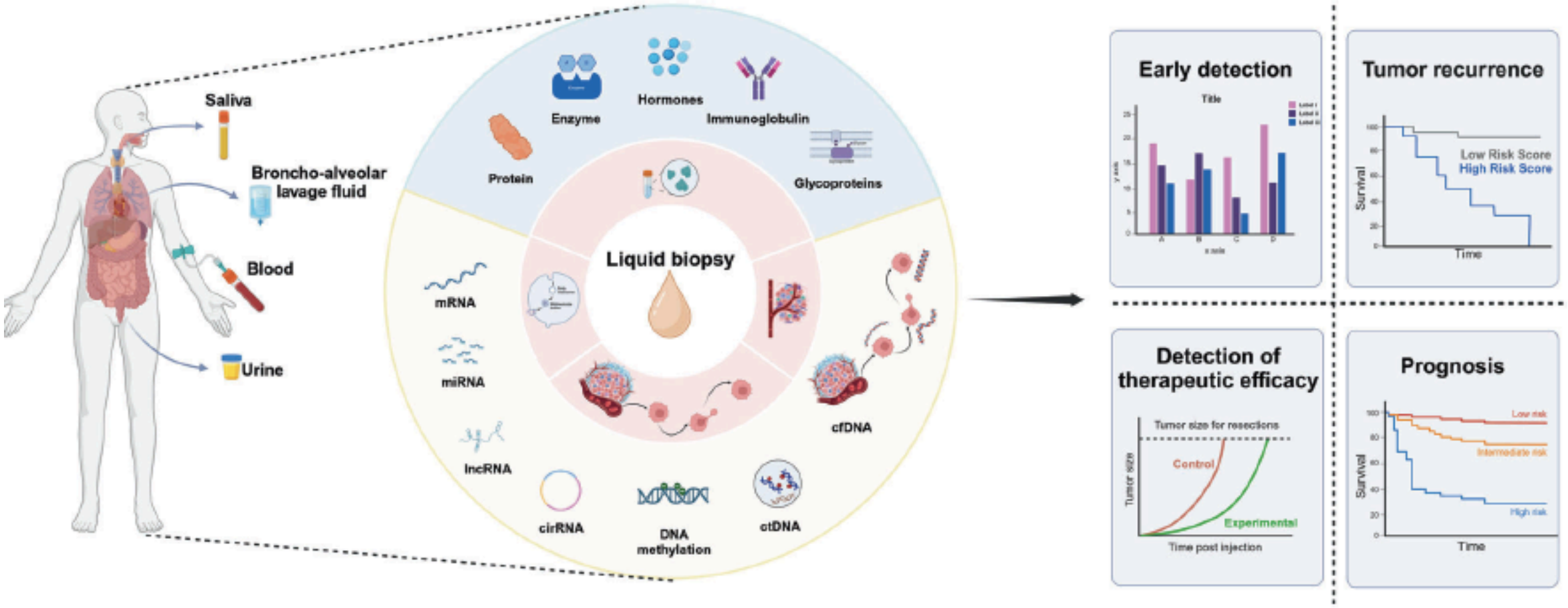
- **Whole Genome Sequencing:** every single bit, depending on depth, de novo assembly
- **Whole Exome Sequencing:** focus on protein-important events, cheaper, weak at regulatory events
- **Targeted Sequencing:** Evolution towards translational research, sequencing for less curious people



- 2% of genome
- 85% of disease causing variants

- Only targets of interest
- Seek specific disease causing variants

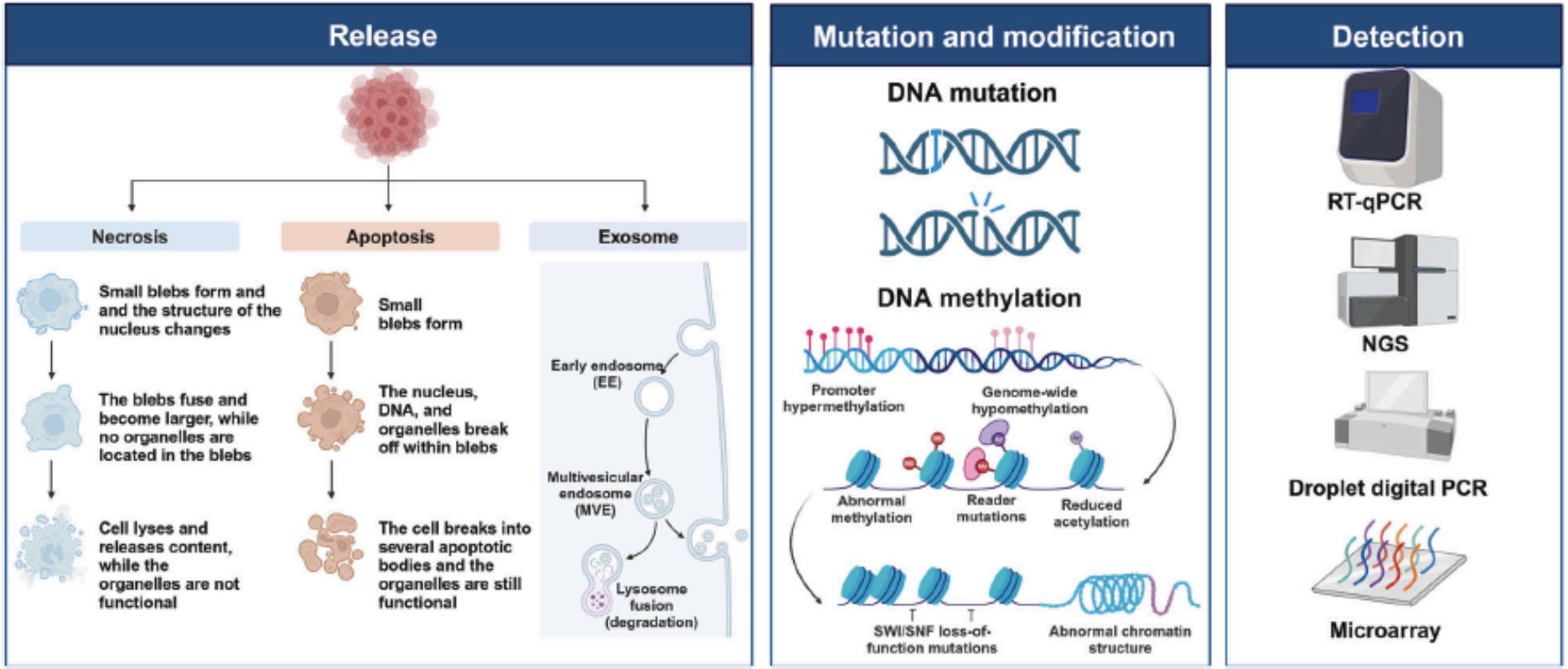
NGS in liquid biopsies



- Non invasive technique (vs tissue biopsy)
- Markers for early detection, efficacy, refractoriness, prognosis

Ma L. et al.Signal Transduction and Targeted Therapy (2024)

Cancer prognosis and detection through Liquid Biopsy



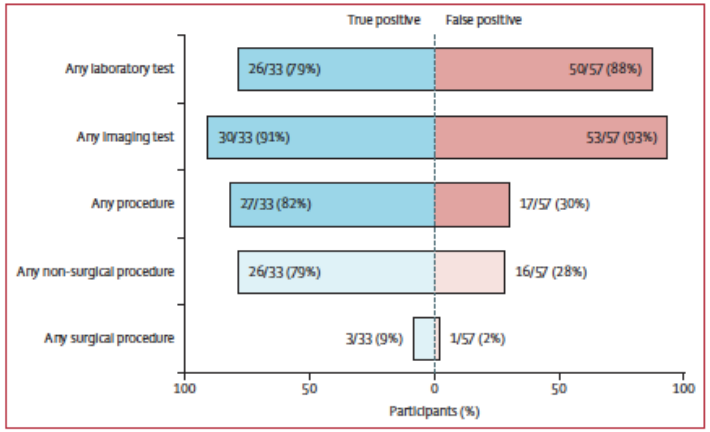
- ctDNA: secreted by tumor cells or released into the circulatory system during the apoptosis or necrosis.

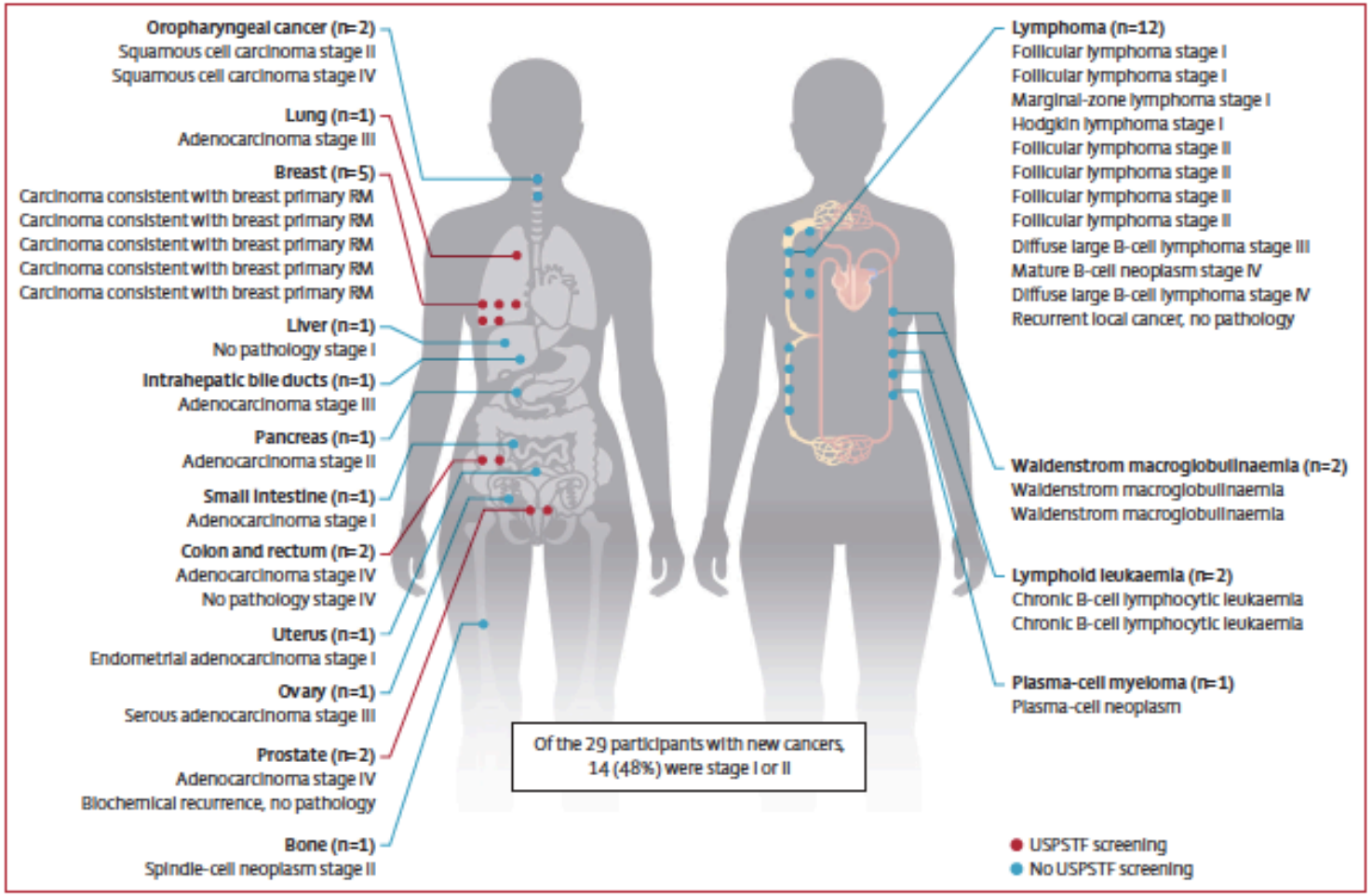
- Mutations and methylation of ctDNA used as detection indicators

Ma L. et al.Signal Transduction and Targeted Therapy (2024)

Multicancer early detection (MCED) from circulating cell-free DNA (cfDNA) → PATHFINDER

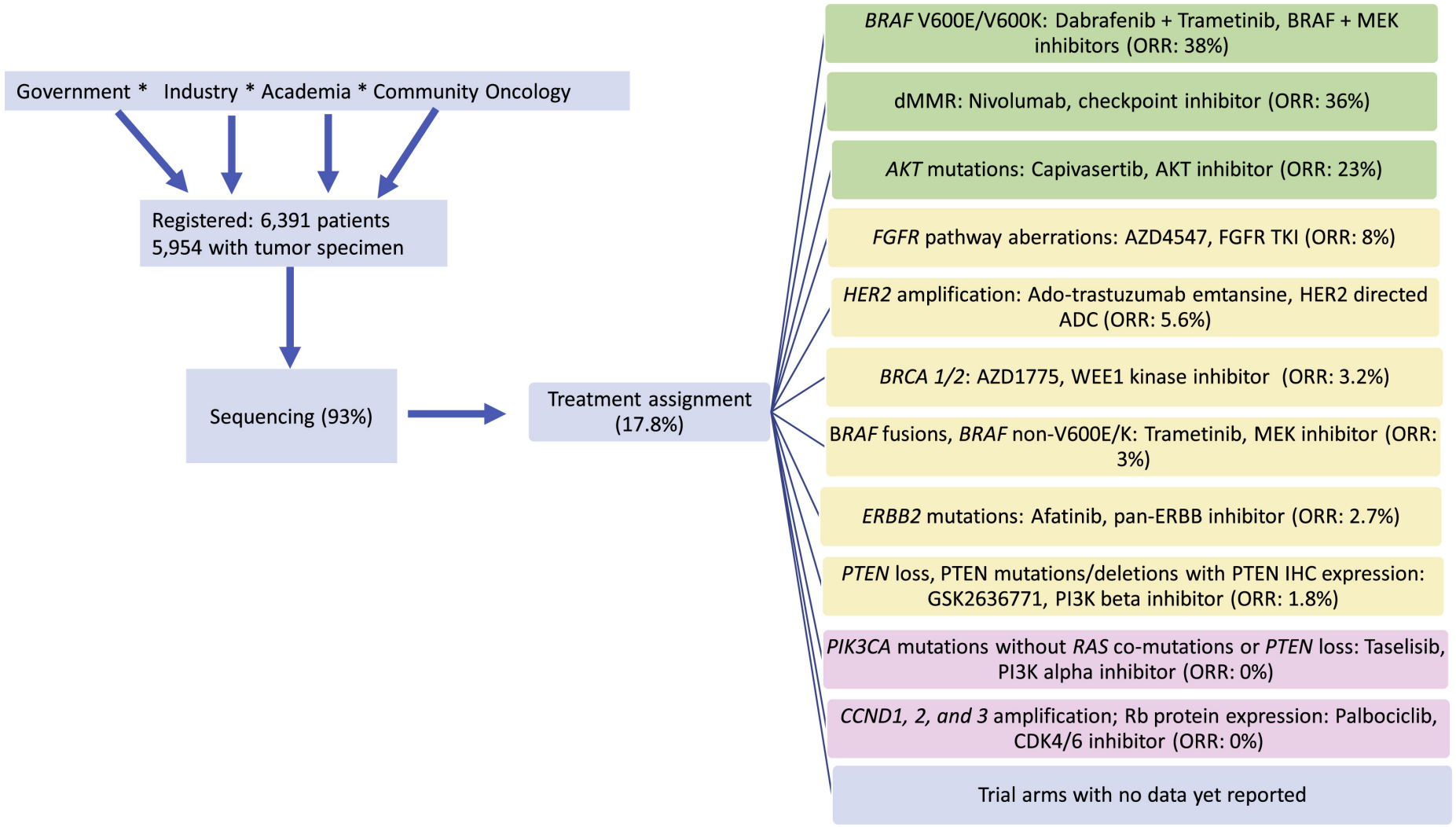
- Prospective cohort study to adults aged > 50 years without signs or symptoms of cancer → MCED testing (methylation pattern with NGS)
- **Primary outcome:** time to, and extent of, diagnostic testing required to confirm the presence or absence of cancer
- A cancer signal was detected in 92 where 35 participants were diagnosed with cancer (TPs) and 57 had no cancer diagnosis (FPs)





Schrag D. et al 2023 The Lancet

Precision Oncology

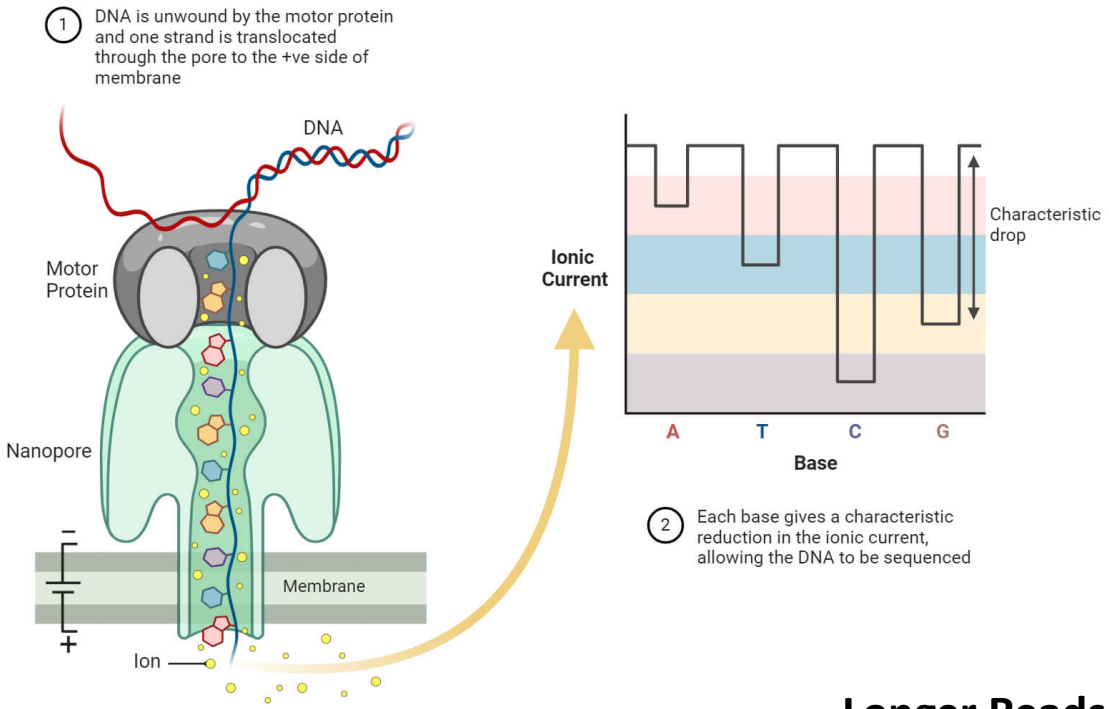


- NCI-MATCH (**Molecular Analysis for Therapy Choice**) trial
- Genomically driven, signal-seeking precision medicine
- Treatment-refractory malignant solid tumors
- 6,000 screened with a total of 1,593 patients assigned to 38 substudies.

O'Dwyer, P.J et al. Nat Med (2023)

3rd Generation Sequencing – Long Reads

Nanopore Sequencing Principle

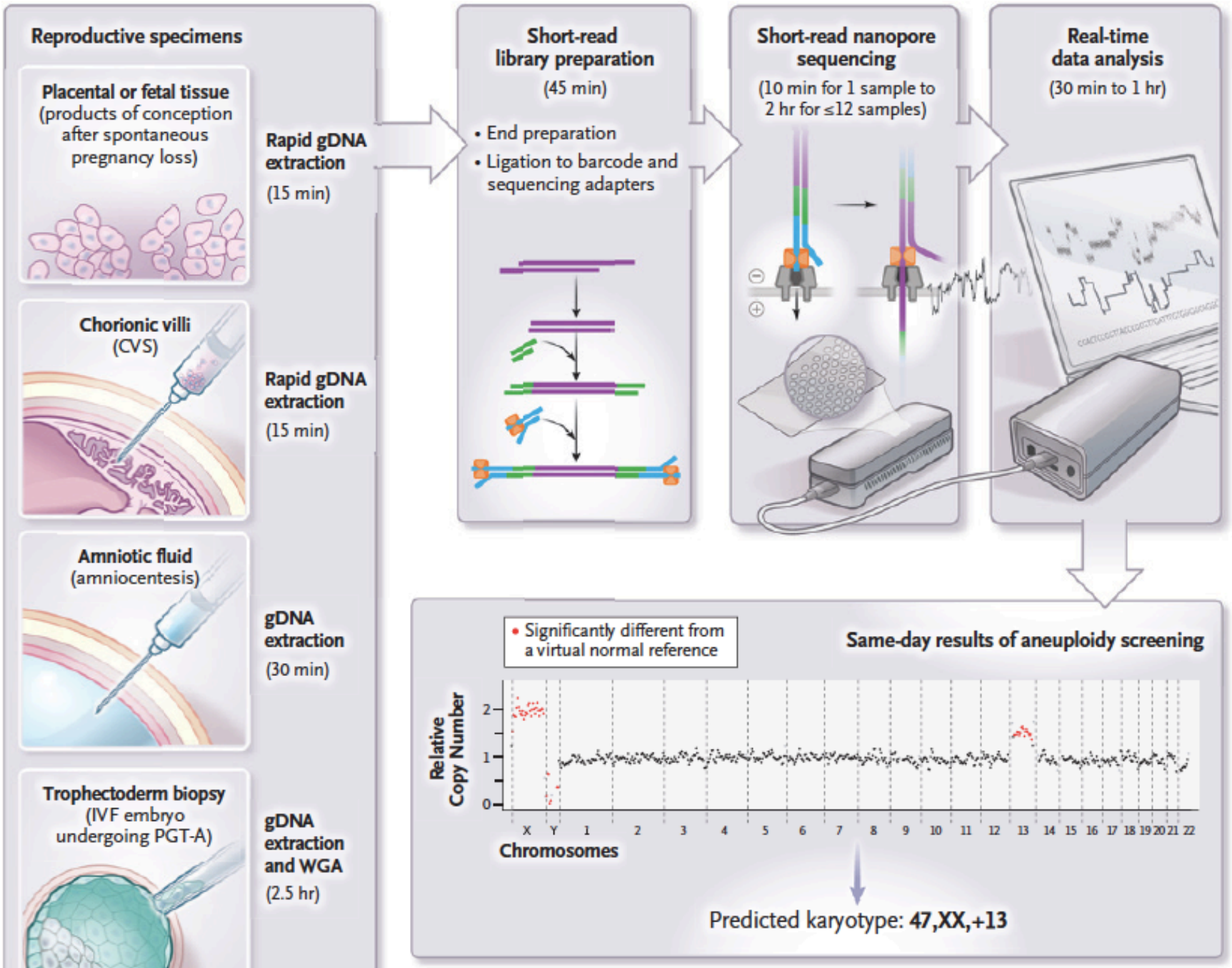


Longer Reads – 50 bp to 4 MB

Structural abnormalities (indels) and CNVs

Prone to errors but real FULL GENOME coverage

Short-read transpore rapid karyotyping (STORK)

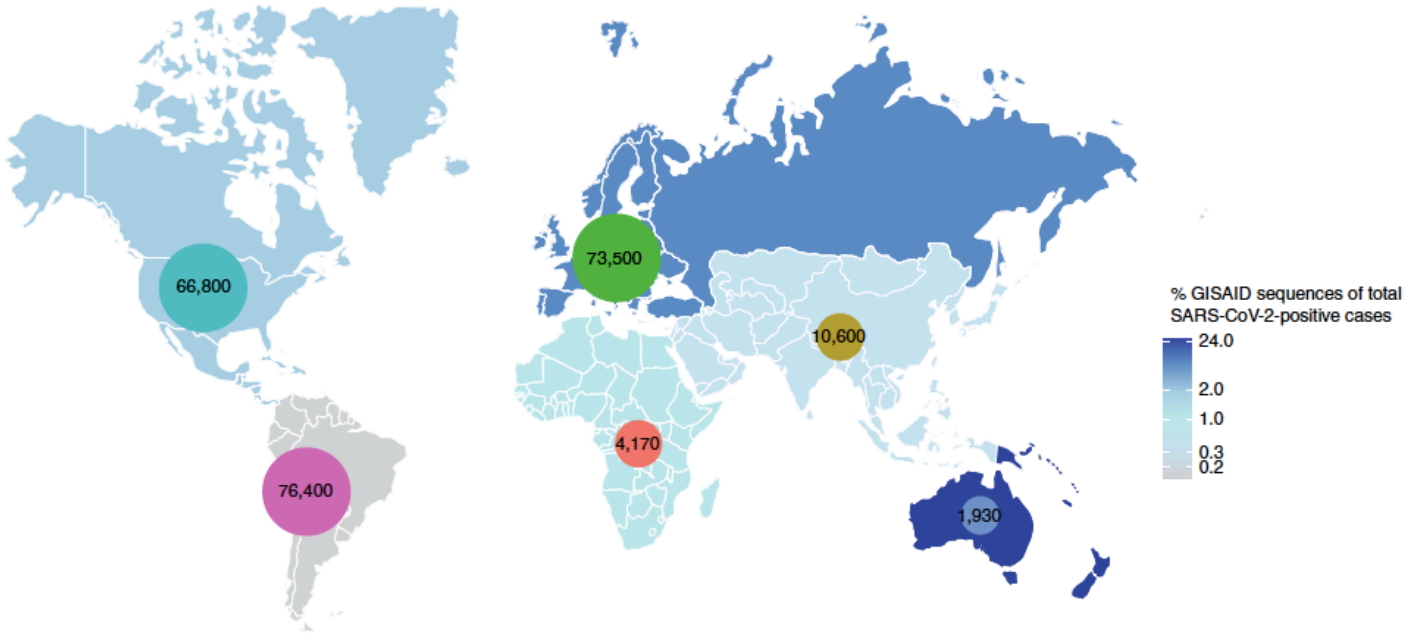


218 sequential, remnant, reproductive specimens of conception after spontaneous pregnancy loss

- Reduced cost,
- Same-day turnaround time
- Perform on-site
- Test for aneuploidy across all chromosomes

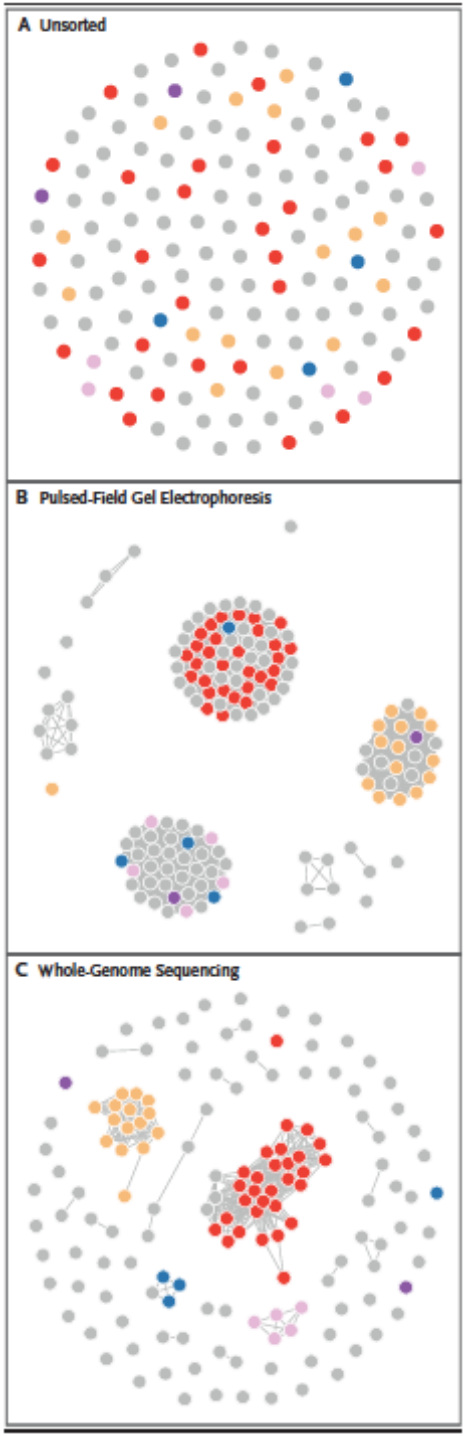
Wei S et al 2022 NEJM

Real – time molecular epidemiology



Molecular Surveillance = Genomic Monitoring

Escapees of therapy – Transmitabillity

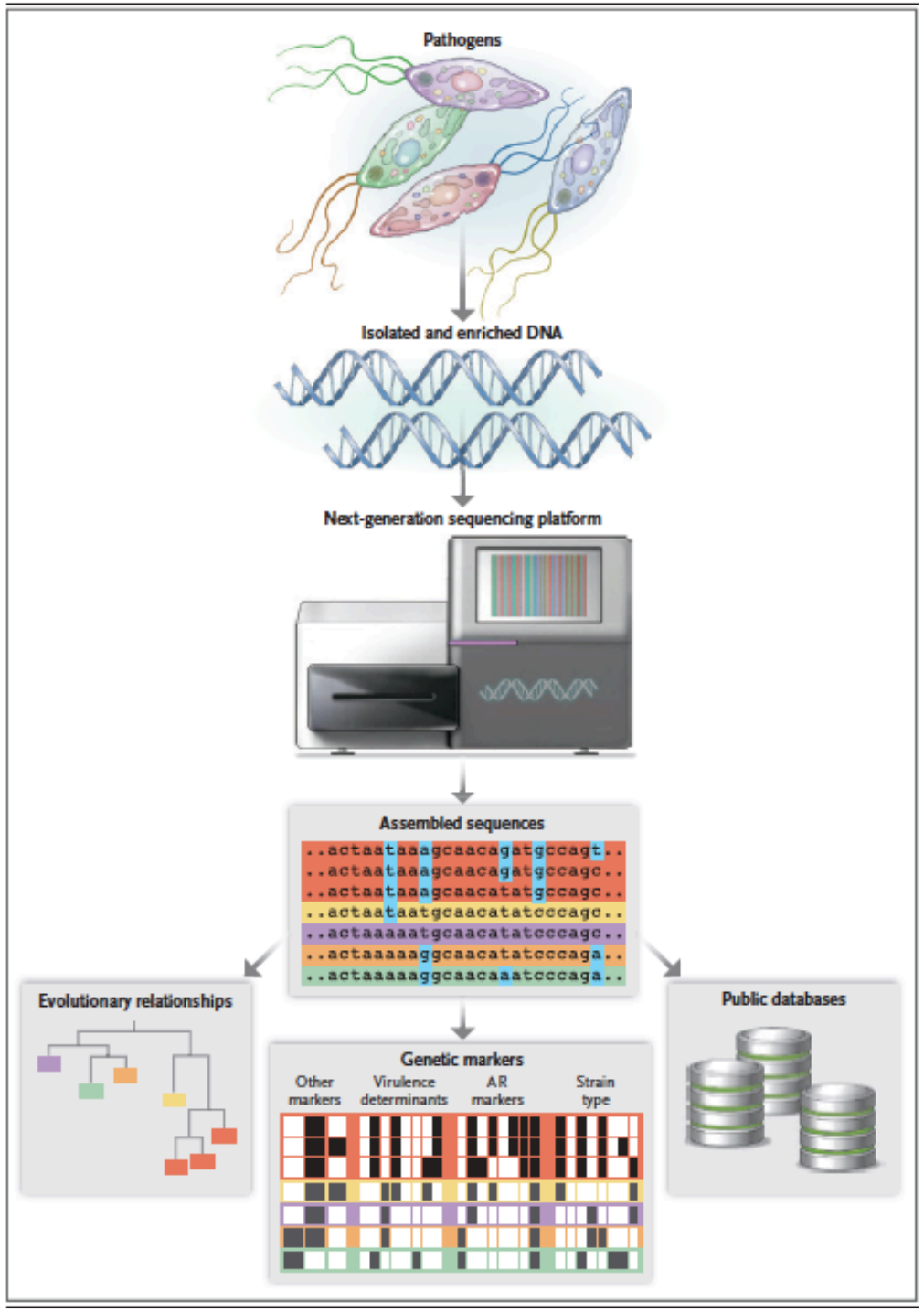


Pathogen Genomics in Public Health

- **Bacterial enteric illness**: improves detection of and response to outbreaks
- **Tuberculosis:** allows better targeting of interventions to stop transmission
- **Legionella:** provides a new tool to understand the ecology of the pathogen in water systems
- **Potential agents of bioterrorism**: allows for improved forensics

Culture-based system vs NGS from specimens
METAGENOMICS

Armstrong GL et al 2019 NEJM



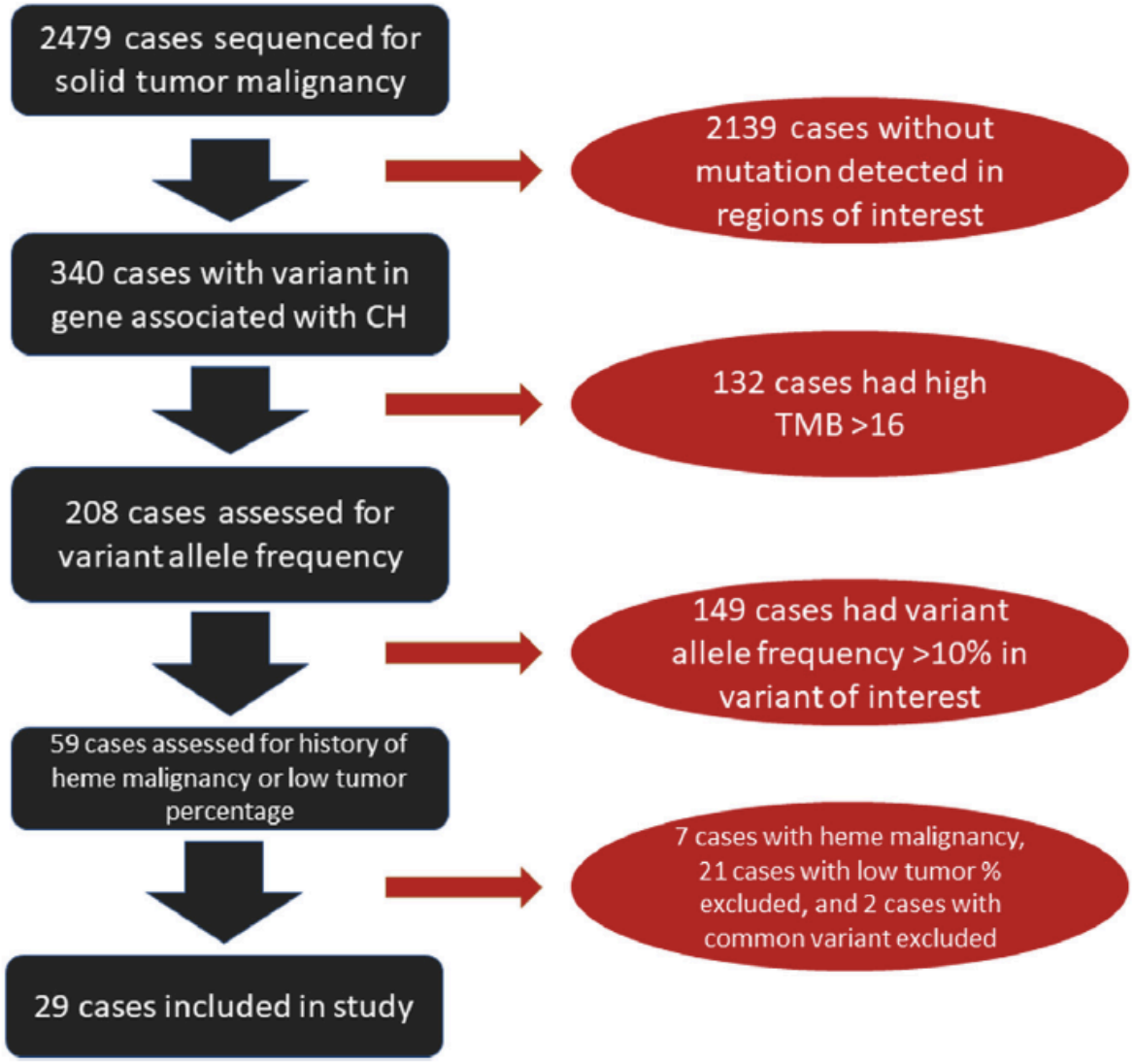
NGS in Hematologic Malignancies

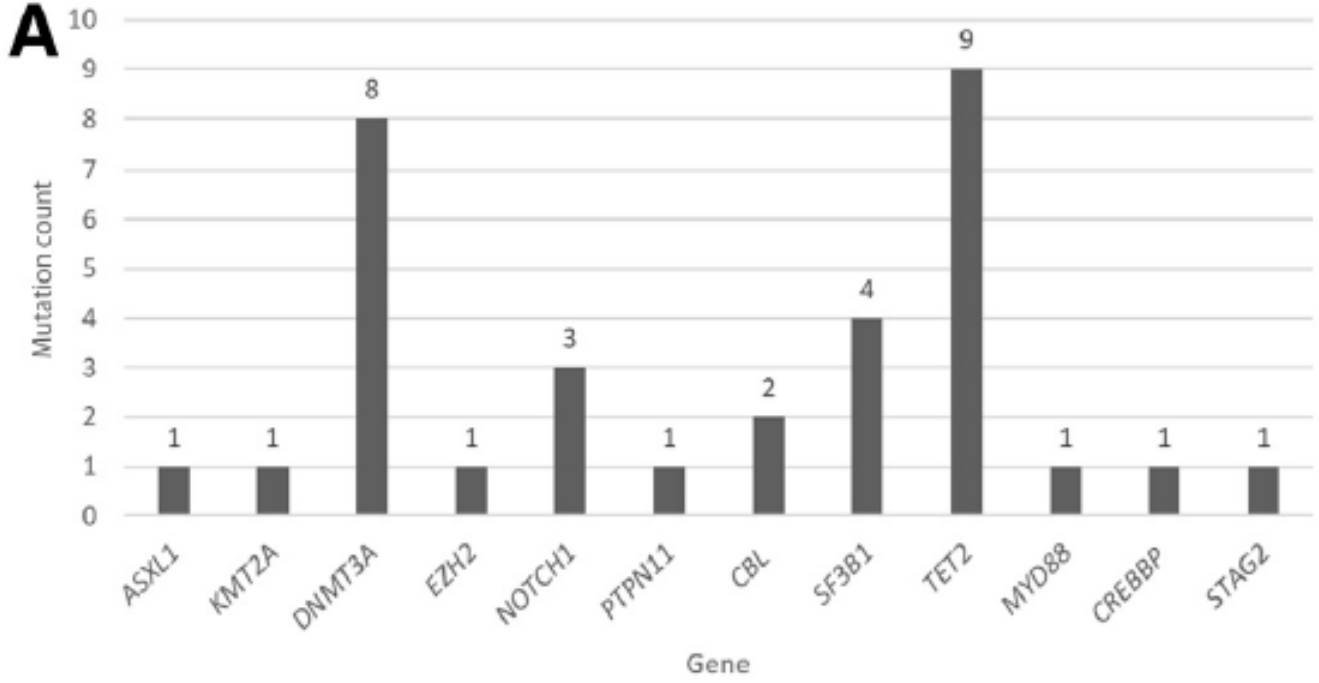
Table 1 Genetic variants detectable by next-generation sequencing assay and of clinical utility in hematologic malignancies

Diseases	Mutated genes	Fusions
Myeloid malignancies	*ABL1, ANKRD26, ASXL1*[a,b], *BCOR*[a,b], *BCORL1*[a], *BRCC3*[a], *CALR*[c], *CBL*[a], *CEBPA*[c], *CTCF*[a], *DDX41*[c], *DNMT3A*[a], *ETNK1*, *ETV6*[c], *EZH2*[b,c], *FLT3*[c,d], *GNAS*[a], *GNB1*[a], *IDH1*[a,c], *IDH2*[a,c], *JAK2*[a,c], *KIT*[c], *KMT2A*[d], *KRAS*[a,c], *MPL*[c], *NPM1*[c], *NRAS*[a,c], *PPM1D*[a], *PTPN11*[a,c], *RAD21*[c], *RUNX1*[c], *SETBP1*[a], *SF3B1*[a,b,c], *SH2B3*, *SRSF2*[a,b,c], *STAG2*[b,c], *TET2*[a], *TP53*[a,c], *U2AF1*[a,b,c], *WT1*[c], *ZBTB33*[a], *ZRSR2*[b]	*BCR::ABL1, CBFB::MYH11, DEK::NUP214, KMT2Ar, MECOMr, NUP98r, PML::RARA, RBM15::MRTFA, RUNX1::RUNX1T1,*
Lymphoid malignancies[e]	*BRAF, CXCR4, CYLD, DIS3, EGR1, FAM46C, FGFR3, HIST1H1E, ID3, IRF4, KRAS, LTB, MAX, MYD88, NRAS, PAX5, RB1, STAT3, STAT5B, TCF3, TP53, TRAF3*	*ABL1r*[f], *ABL2r*[f], *BCR::ABL1, CRLF2r*[f], *CSF1Rr*[f], *DGKHr*[f], *DUX4r, EPORr*[f], *ETV6::RUNX1, IGH::IL3,* other *IGHr, IL2RBr*[f], *JAK2r*[f], *KMT2Ar, MEF2Dr, MYCr, NTRK3r*[f], *NUTM1r, PAX5r, PGDFRBr*[f], *PTK2Br*[f], *TCF3::PBX1, TCF3::HLF, TSLPr*[f], *TYK2r*[f], *ZNF384r*

Cho YU et al 2024 Blood Research

Clonal Hematopoiesis (CHIP) Variants during Routine Solid Tumor Next-Generation Sequencing

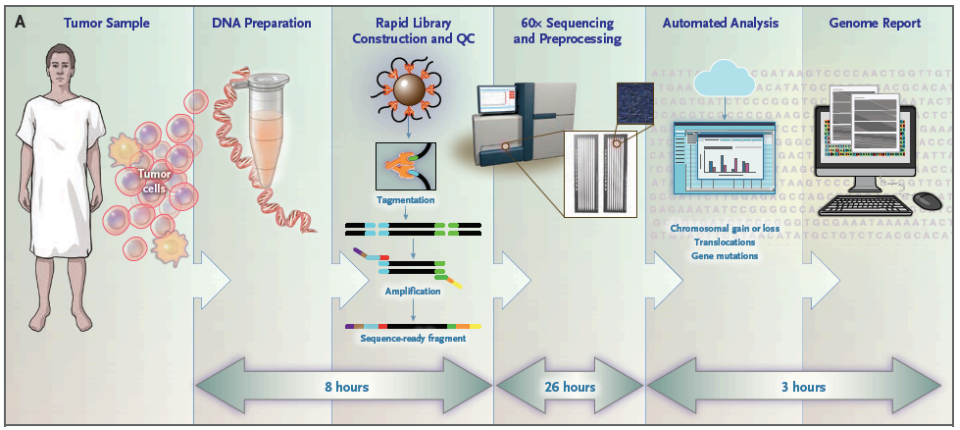


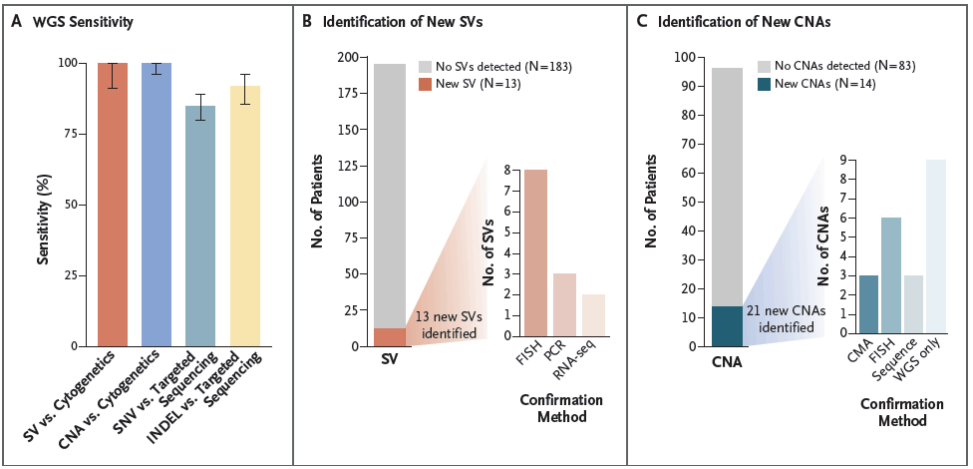


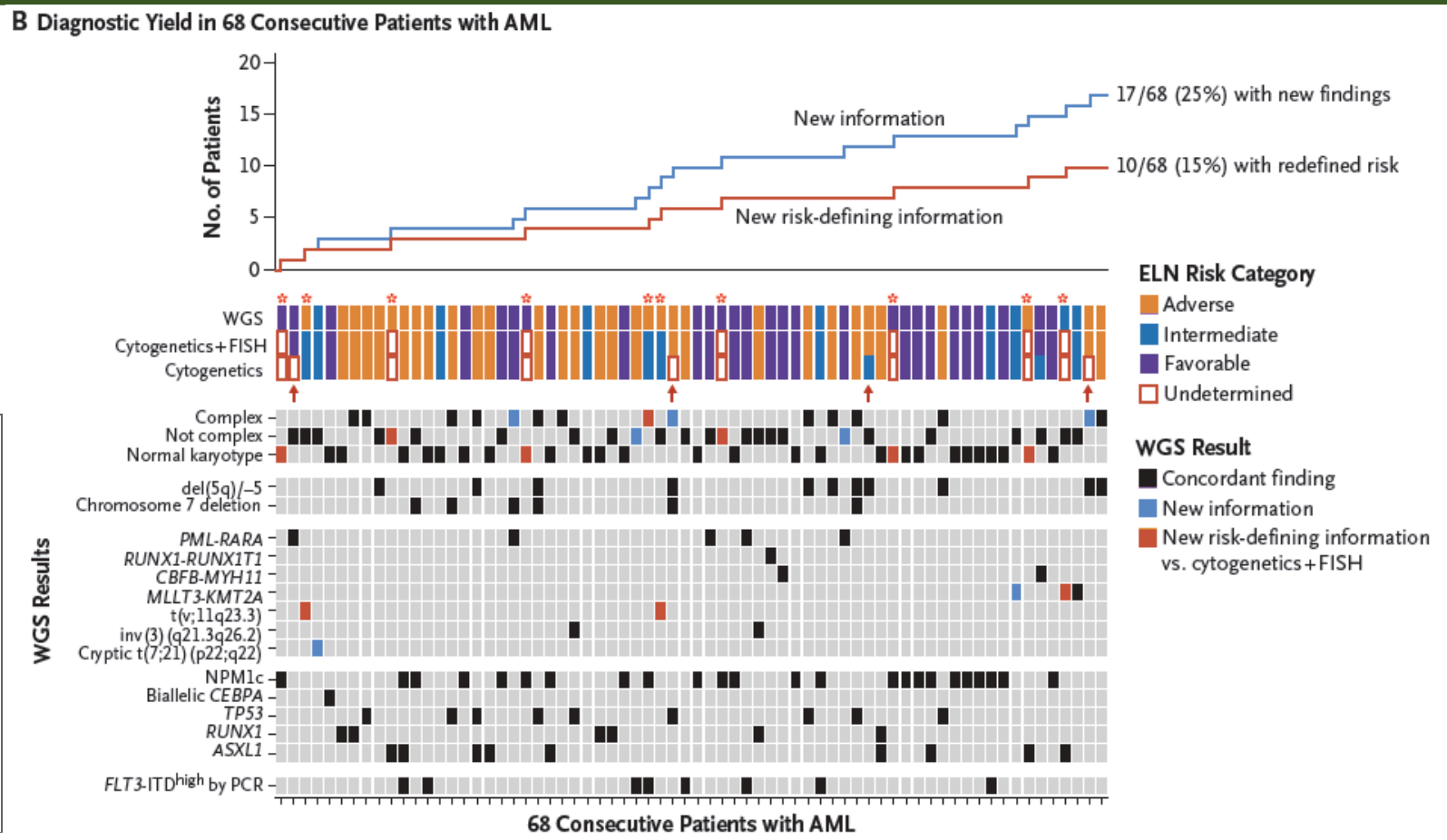
Clonal hematopoiesis (CH) and **clonal cytopenia of undetermined significance** (CCUS) show independent risk factors for **cardiovascular disease and myeloid malignancy**

Menon A et al 2024 J of Mol Diagn.

NGS for diagnosis of myeloid cancers

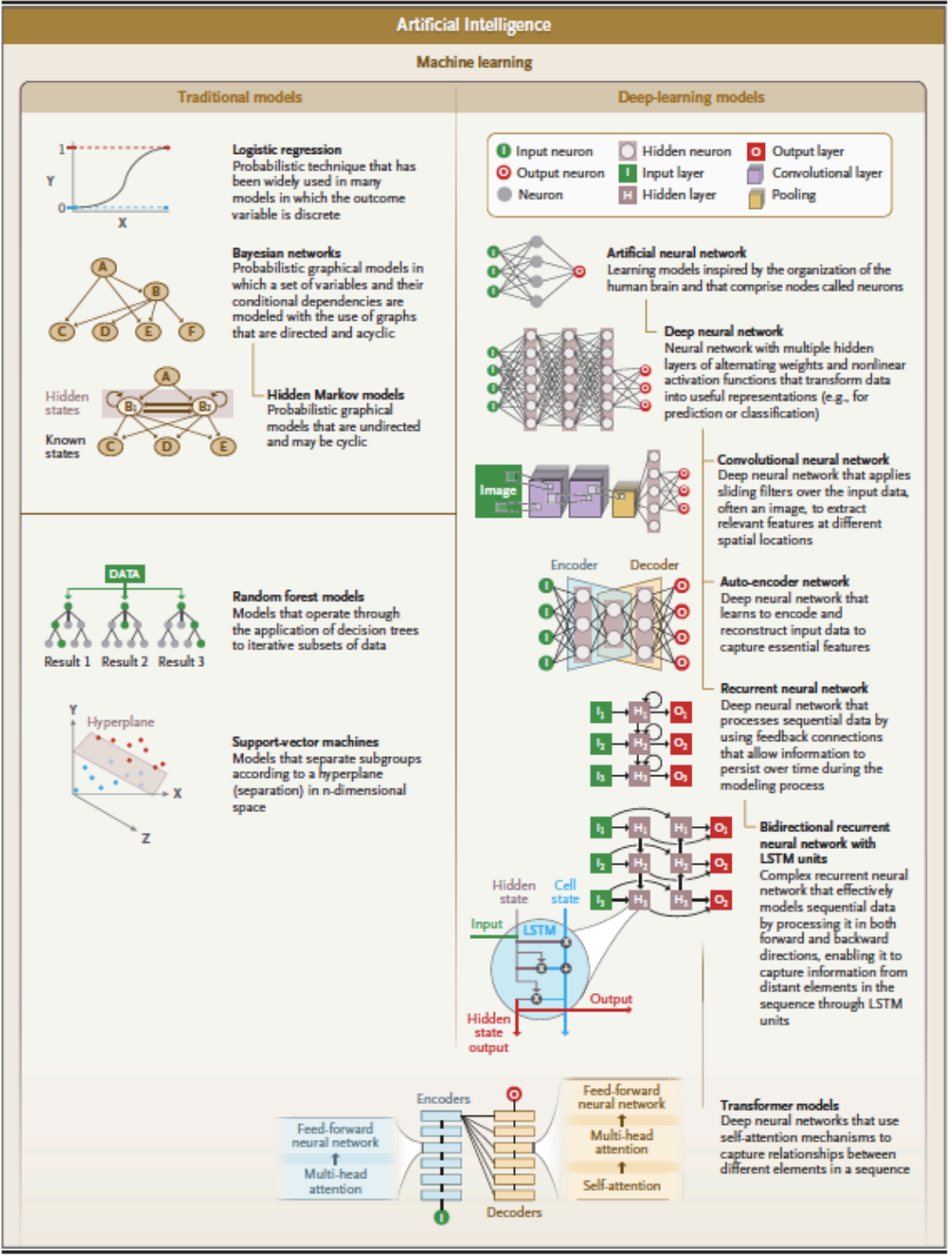






Whole-genome sequencing → rapid and accurate genomic profiling in patients with AML or MDS
Greater **diagnostic yield** than conventional cytogenetic analysis and more **efficient risk stratification**

Duncavage EJ et al 2021 NEJM

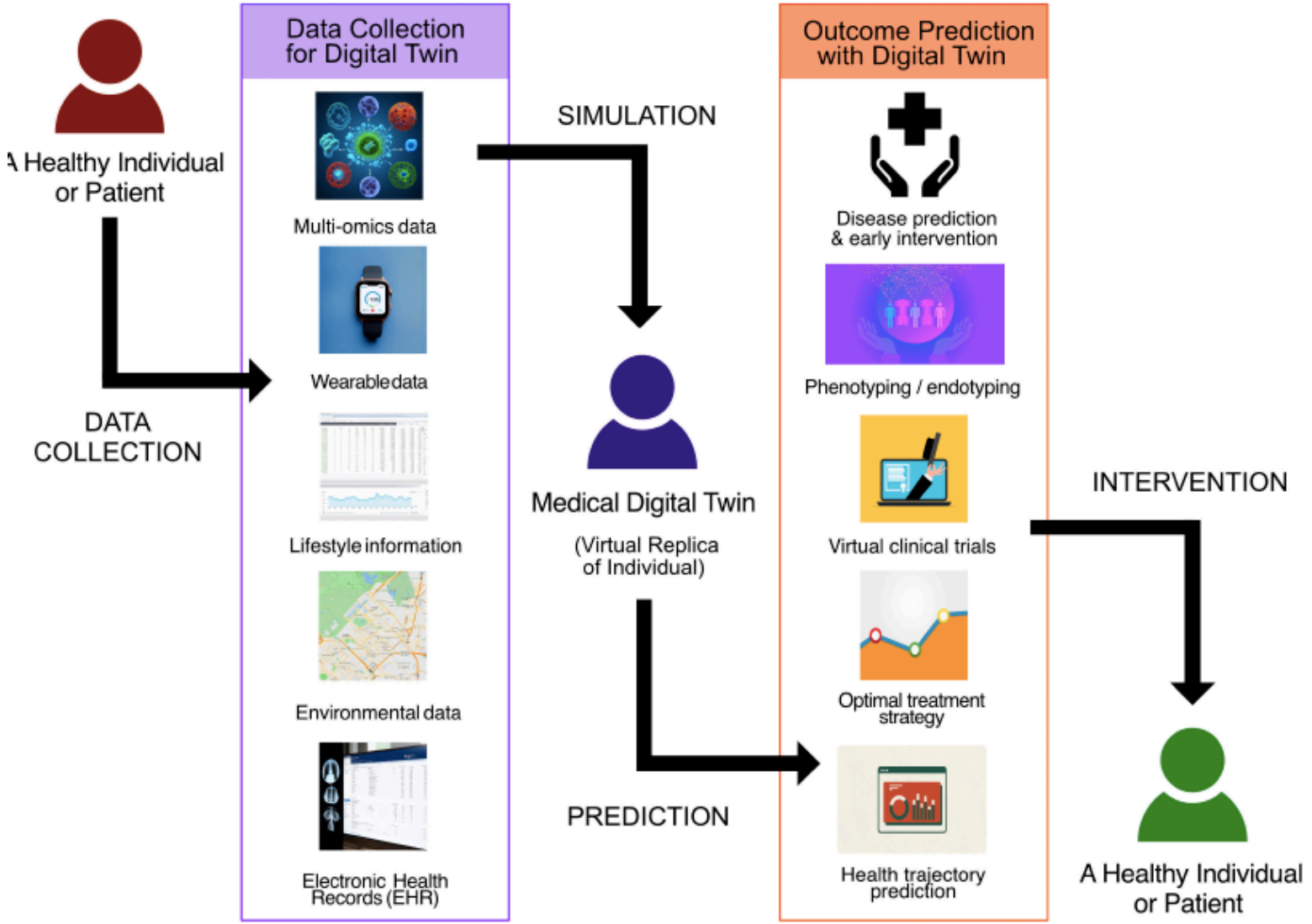


AI in Molecular Medicine

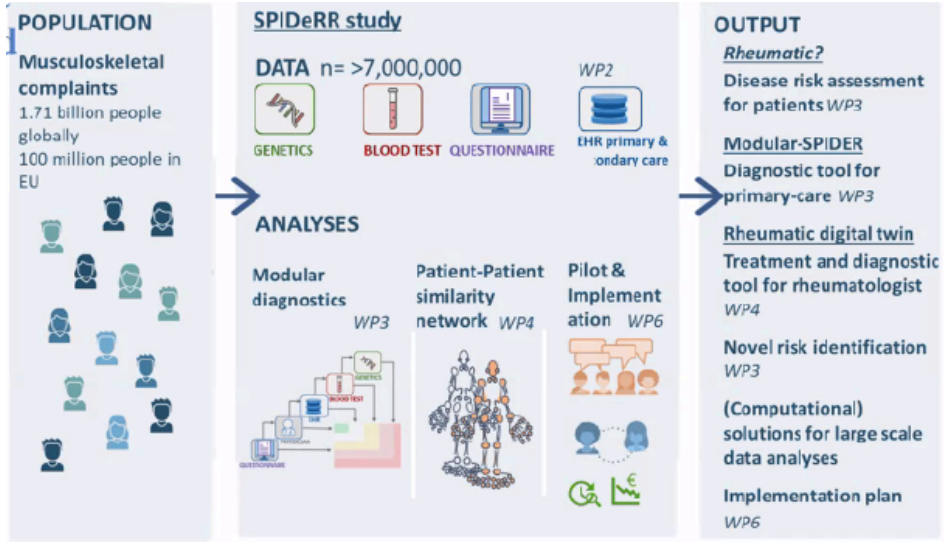
- Multi-omics data are complex before the description of pathophysiology
- Analyze down to abstract and low dimensity data
- Traditional and Deep Learning Methods
- Hidden motifs
- Accuracy in variant calling
- Prioritization of variants for rare diseases – Causal for Mendelians
- **Haplotype for clinical management**

Drazen JM et al 2023 NEJM

The idea of the Digital Twin



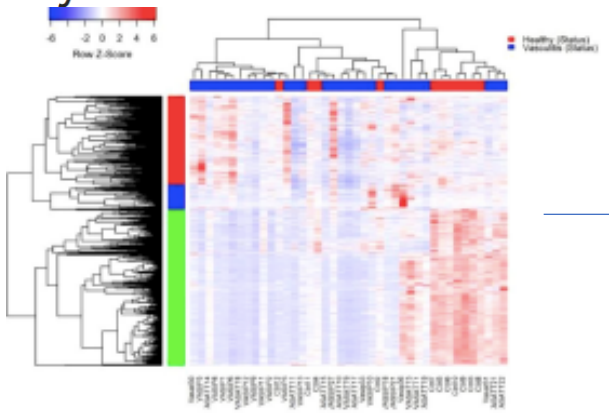
- AI training datasets concluding with next gen data
- **Predictive models to twin every new individual to a phenotype universe**
- Paradigm → Musculo-skeletal complaints

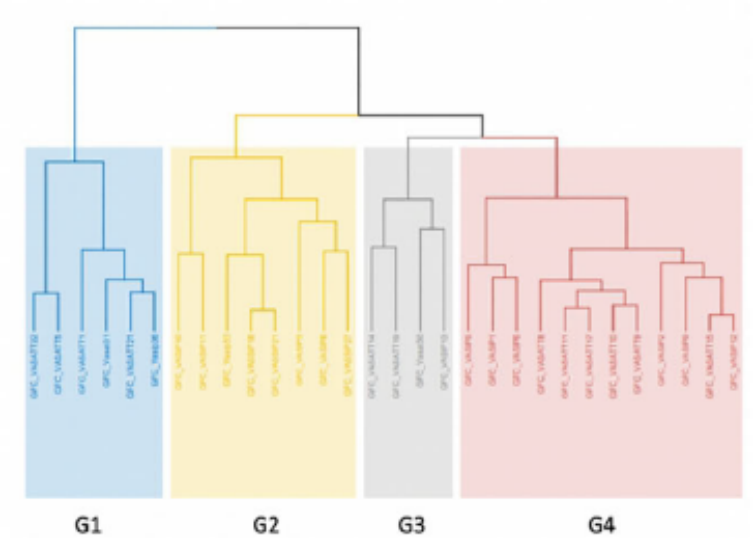


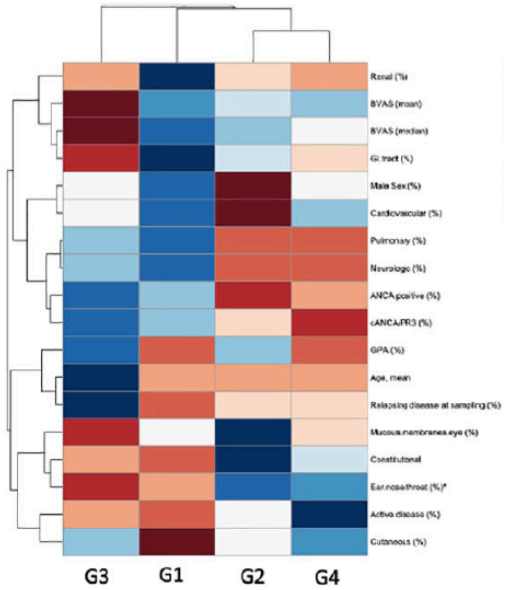
Tadao Ooka, JMA J. 2025

Whole Blood Transcriptome Profile establishes Molecular Endotypes of the Disease

The genomic landscape of ANCA-associated vasculitis: Distinct transcriptional signatures, molecular endotypes and comparison with systemic lupus erythematosus





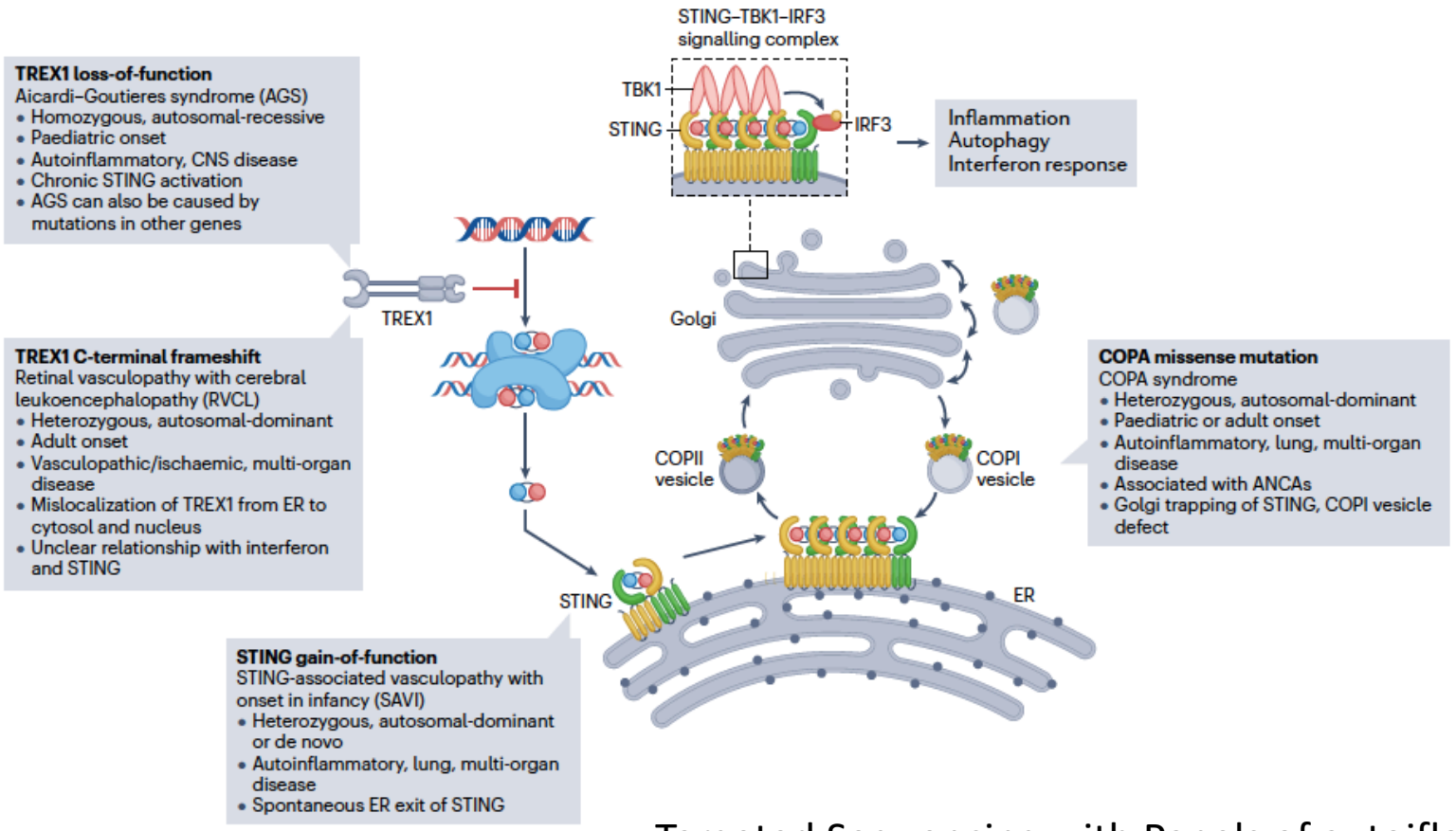


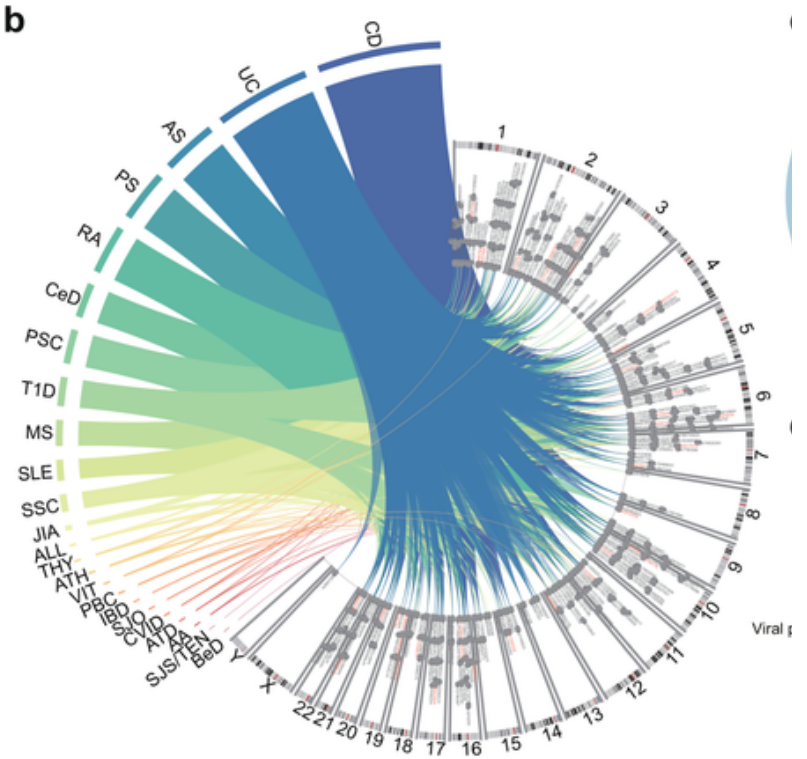
Unsupervised molecular taxonomy analysis → **4 endotypes** with neutrophil degranulation, aberrant metabolism and B-cell responses as potential mechanistic drivers.

Therapeutic insights directed towards B-cells, complement cascade, type I IFN or drug repurposing

Banos et al. 2023 Front Immunol

Rare inflammatory phenotypes





Targeted Sequencing with Panels of autoiflammatory/autoimmune entities

Enrichement through WGS/WES

Genotype – Phenotype Crosstalk

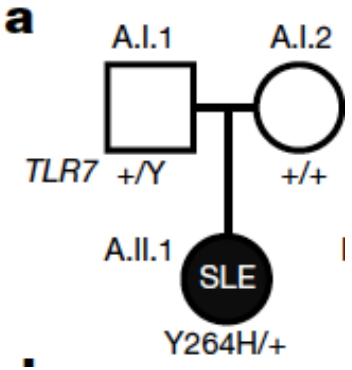
Donglin LT et al 2019 Nat Rev Rheum

Genetic variations of TLR7 drives SLE phenotype

Article

TLR7 gain-of-function genetic variation causes human lupus

- **TLR7 → sensor of degraded genetic material**
- **Case → Female from Spain with SLE diagnosis since 7th year of age**
- **Refractory ITP, high titer ANA/anti-dsDNA, low complement, arthralgias, choreia**
- **Past Therapies → RTX, GCs, AZA, MMF, ETN**

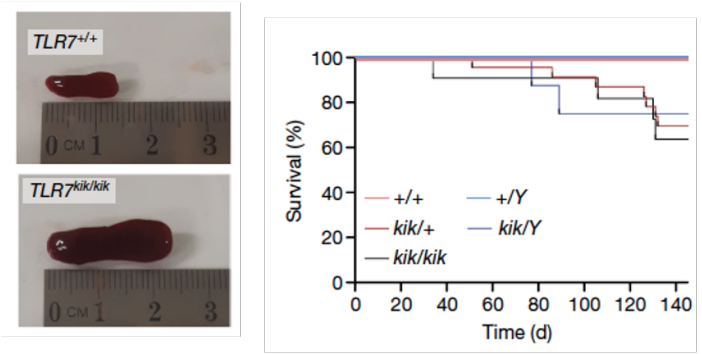


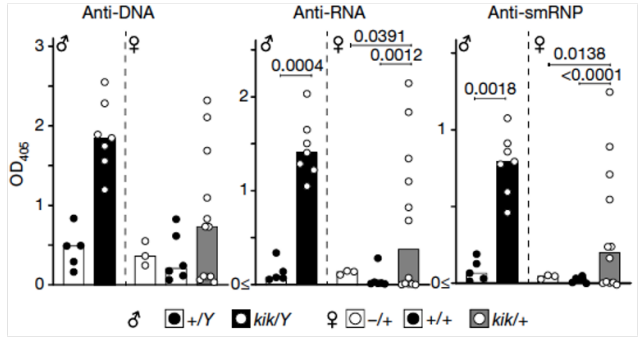
Whole exome sequencing -> De novo mutation

TLR7 p.Tyr264His (Y264H) missense variant (GOF)

Physicians do not want to hear about mice → **Insertion of mutated allele in mouse model with CrispR/Cas9**

Autoimmunity α





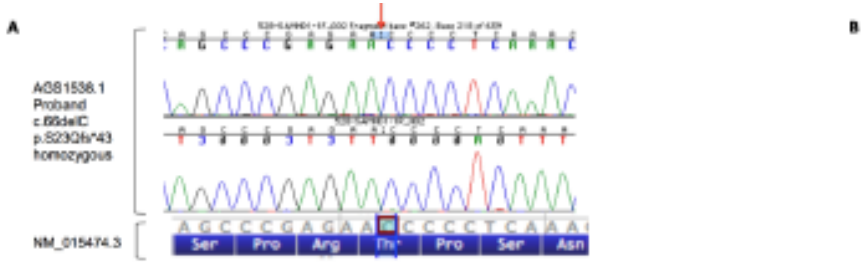
Increased GC B cells, ABC cells, pDCs, Tfh cells

Brown GJ. et al. 2022. **Nature**

Whole exome sequencing in rare pediatric cases

Increased interferon I signaling, DNA damage response and evidence of T-cell exhaustion in a patient with combined interferonopathy (Aicardi-Goutières Syndrome, AGS) and cohesinopathy (Cornelia de Lange Syndrome, CdLS)

Sorina Boiu[1,2,3,4], Nikolaos Paschalidis[5], George Sentis[6], Theodora Manolakou[6], Andrianos Nezos[7], Manolis Gialitakis[6], Maria Grigoriou[6], Erato Atsali[1], Melpomeni Giorgi[8], Argirios Ntinopoulos[9], Clio Mavragani[7], Periklis Makrythanasis[5,10,11], Dimitrios T. Boumpas[6,12] and Aggelos Banos[6]



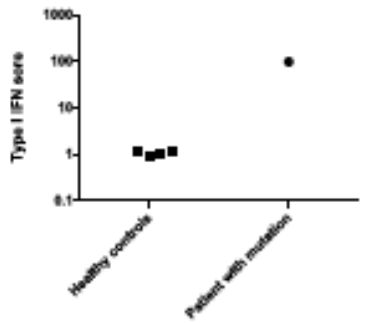
B

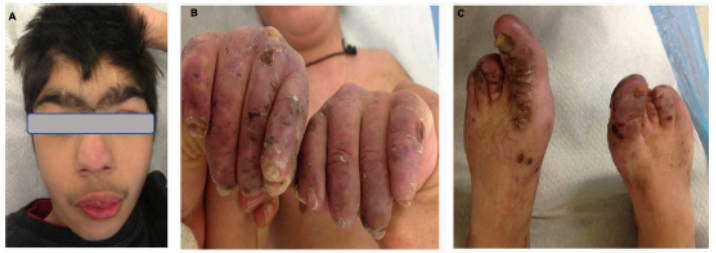
Whole-Exome Sequencing	
Reads	190,513,256
Mapped	187,891,382
Exonic Variants	23,626
Splicing Variants	1,304
Synonymous SNV	11,740
Nonsynonymous SNV	10,807
Stopgain	92
Stoploss	10
Frameshift deletion	114
Frameshift insertion 93	93
Nonframeshift deletion	196
Nonframeshift insertion	118

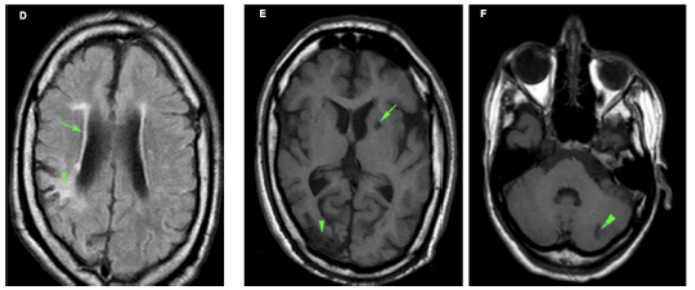
C

Chr	Start	End	Func	Gene	ExonicFunc	AAChange	Obs	Ref	Zyg	AD
chr20	35579980	35579981	exonic	SAMHD1	frameshift deletion	SAMHD1:NM_015474:exon1: c.69_67T	A	AG	hom	0-113
chrX	53400284	53400284	exonic	SMC1A	nonsynonymous SNV	SMC1A:NM_006306:exon22:	T	C	hom	1-155

D







- 20yo man → chilblain lesions, resorption of distal phalanges, somatic and psychomotor retardation, microcephaly, synophrys, hearing losing

- Whole exome sequencing → 2 genetic lesions (AGS/CdLS) exhibits distinctive features of genomic damage and interferon responses

Boiu et al. (2025) Pediatric Rheumatology

Take Home messages

- NGS is rapidly showing the potential to subsitute convevntional laboratory diagnostis
- Whole Genome Sequencing yields high accuracy information but still disconnected of clinical practice due to cost/logistics
- Precision Medicine is built on next generation sequencing – Milestone yet to come
- Artificial Intelligence is connecting the missing parts for big –omics data
- Therapeutic resolution for rare diseases


Thank you!