Diagnostic Genomics for Oncology

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UHN
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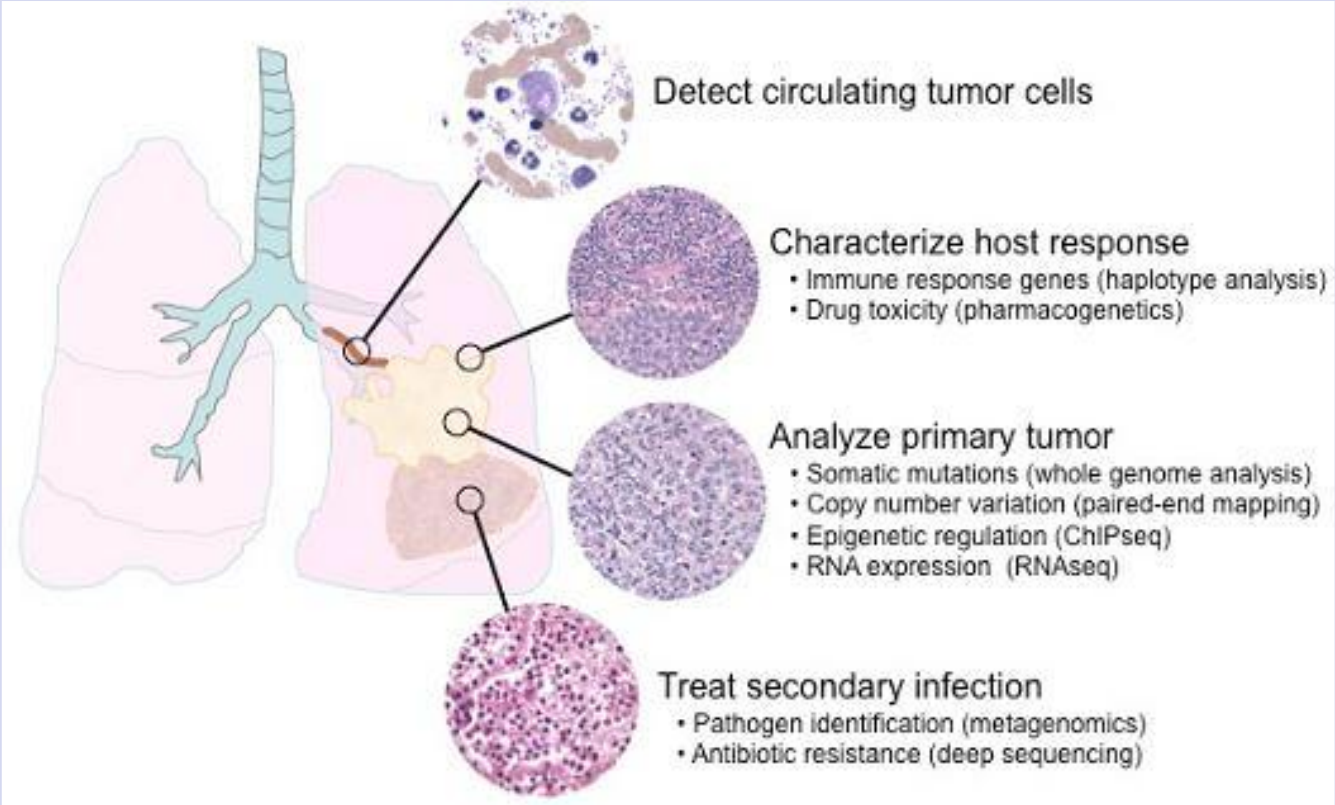
Diagnostic Genomics for Oncology

Research
Discovery

Clinical Validity
Clinical Utility

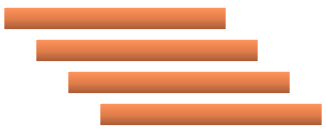
Patient Care



Anderson and Schrijver, Genes 1; 2010

Next Generation Sequencing for Diagnostic Genomics

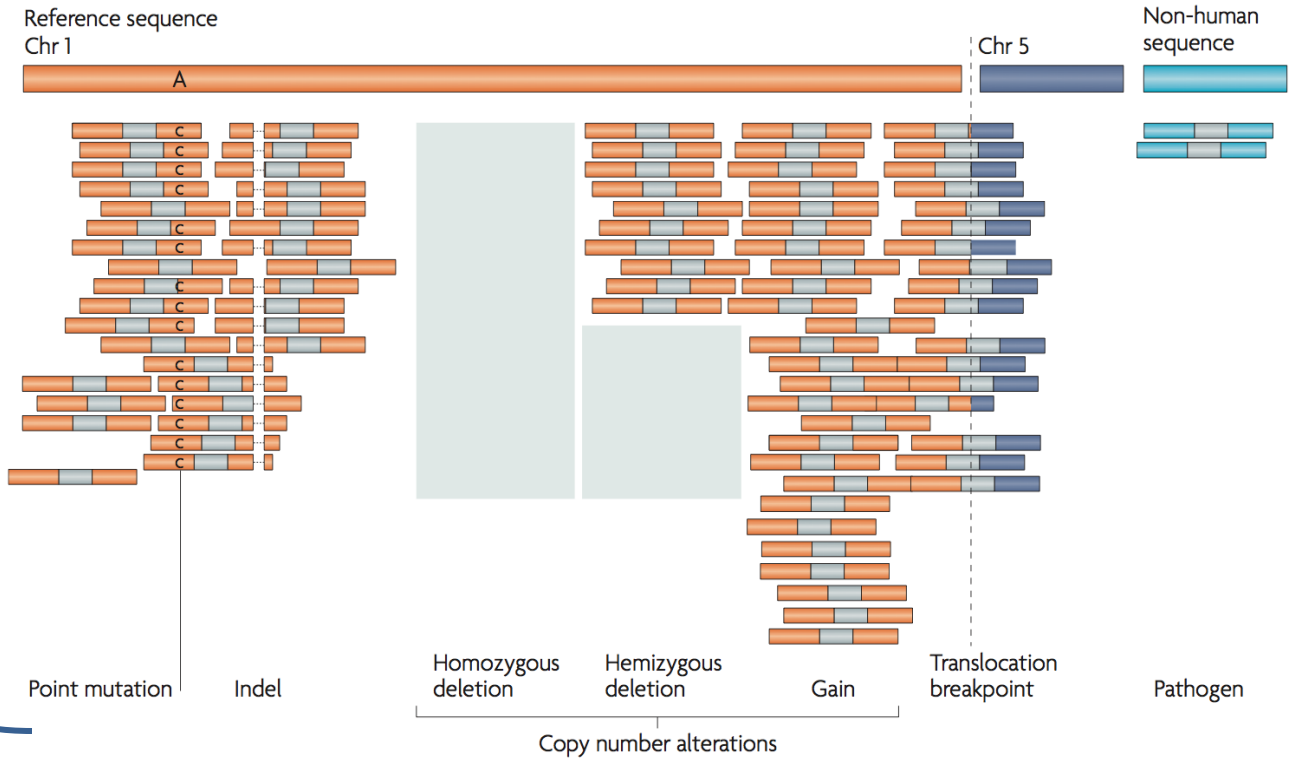
DNA or RNA



Library of fragments
(amplicon, hybridization)

NGS Sequencer

Alignment, analysis, variant filtering and interpretation



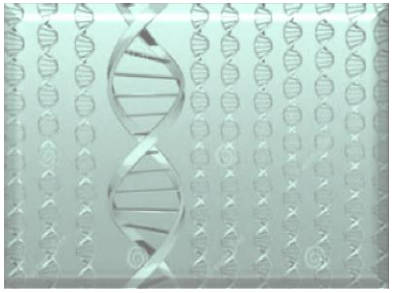
Meyerson, Gabriel, Getz. Nat Rev Genet. 2010

NGS presents new technical and analytic challenges for clinical tests

- Genomic complexity and design of the assay
  - What design best covers mutation spectrum?
  - Panel, ‘clinome’, exome, WGS? Other ‘omic’ tests?
- New data analysis and bioinformatics tools
- More variants with unclear disease effects
- Sample issues
  - Choices may be limited by small samples (FFPE)
  - Multiple tests to capture low quantity/quality samples
- Higher error rates and novel error modes
  - Less familiarity than with previous technical methods

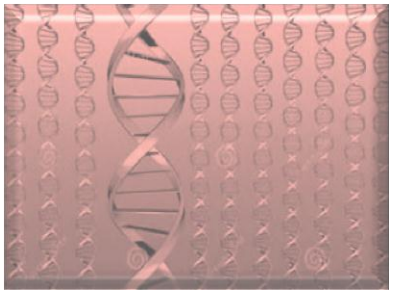
NGS Diagnostics for Oncology at UHN

Molecular Diagnostic Lab, Toronto General
Advanced Molecular Dx Lab, Princess Margaret



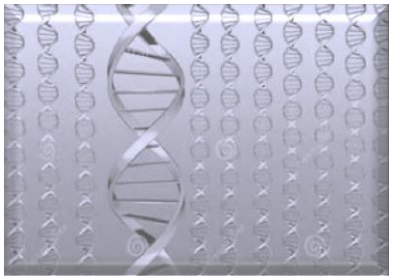
Solid Tumors

- 26 gene targeted panel *(TruSight Illumina)*
- 48-50 gene targeted panels *(TruSeq Illumina, AmpliSeq LifeTech)*
- 555 gene panel PMCC Hi5 *(Agilent SureSelect custom)*



Hematologic Malignancies

- 54 gene targeted panel *(TruSight Myeloid Illumina)*
- 555 gene panel PMCC Hi5 *(Agilent SureSelect custom)*



Inherited Cancers

- 52 gene PMCC Hereditary cancer panel *(Agilent SureSelect custom)*
- 555 gene panel PMCC Hi5 *(Agilent SureSelect custom)*

Next Generation Sequencing: Germline and Somatic Molecular Diagnostics

Germline

E.g. Inherited cancers

- Multigenic but single/few mutations per patient
- Variant frequency 50%
- Clinomes/Exomes/WGS for disorders with complex phenotypes
- Familial risk issues and need for genetic counselling

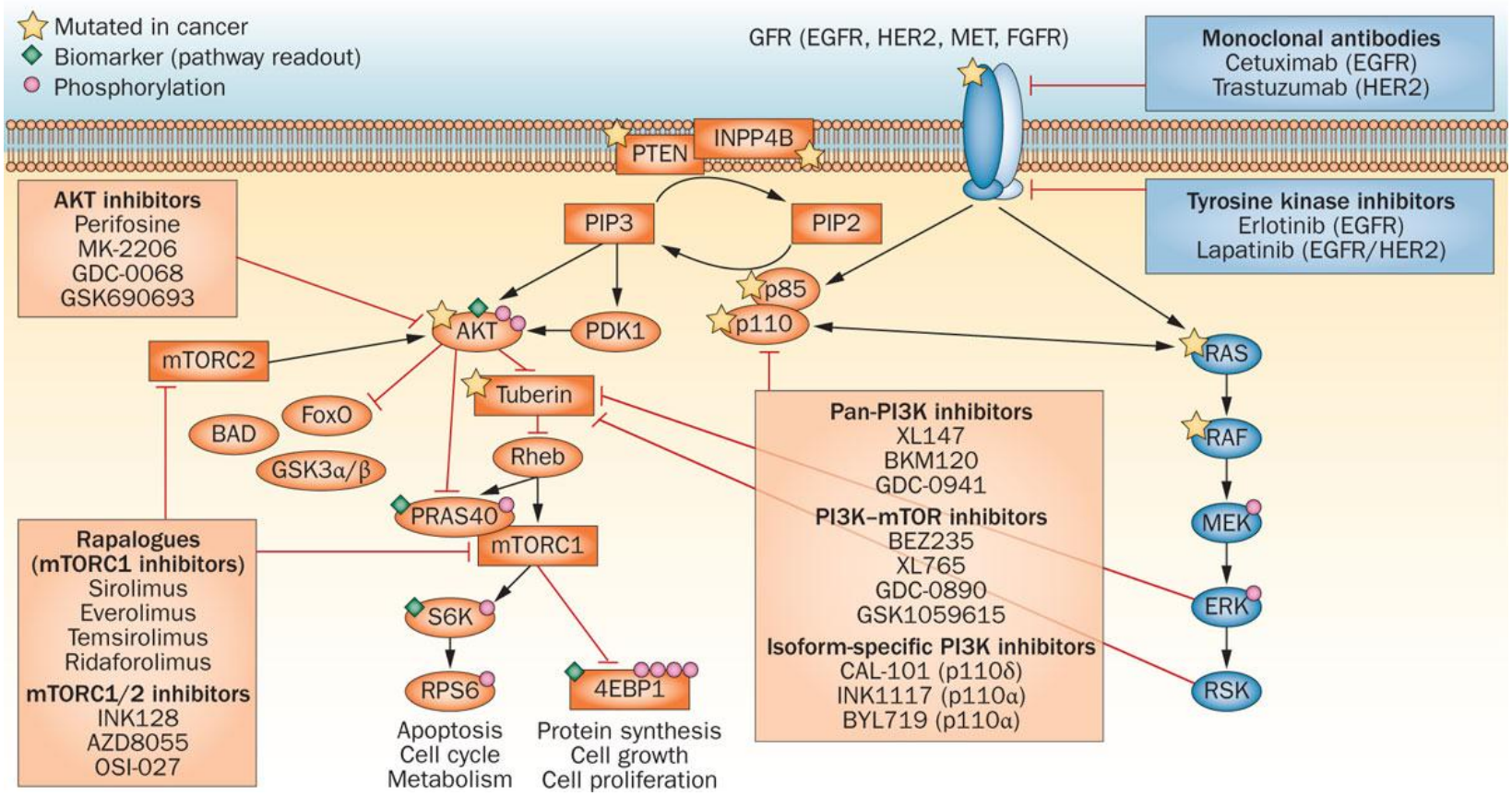
Somatic

E.g. Sporadic cancers

- Complex genetic alterations (SNV, CNV, expression, rearrangements, methylation)
- Variants freq. 1%-100%
- Tumors typically with known site of origin
- Actionability of variants for directing treatment
- No familial risk

A. Solid Tumor Molecular Diagnostics

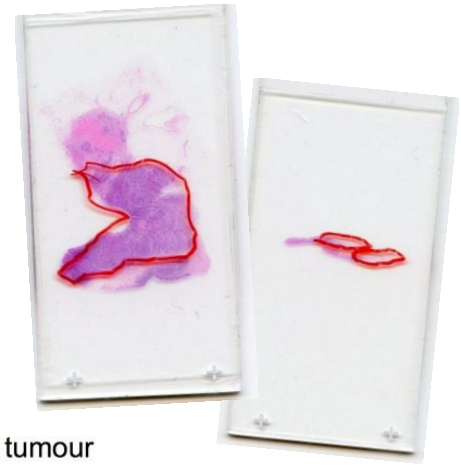
Gene Mutations and Drugs Targeting Cell Signaling Pathways

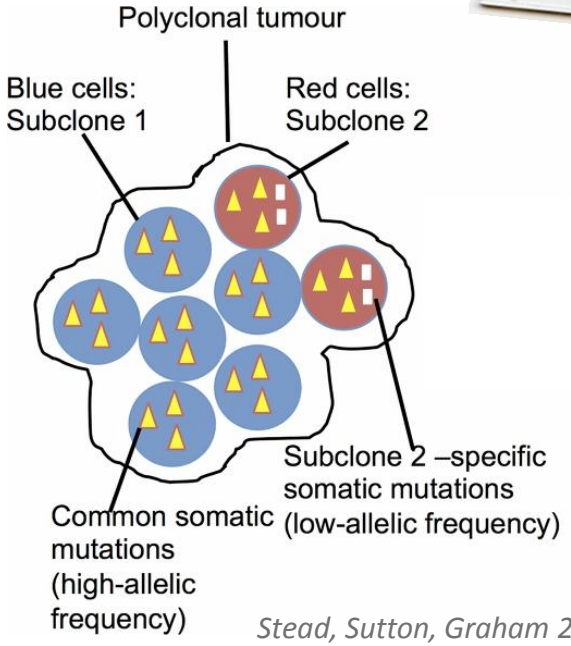


Rodon et al, 2013 Nat. Rev. Clin. Oncol.

A. Solid Tumor NGS Tests

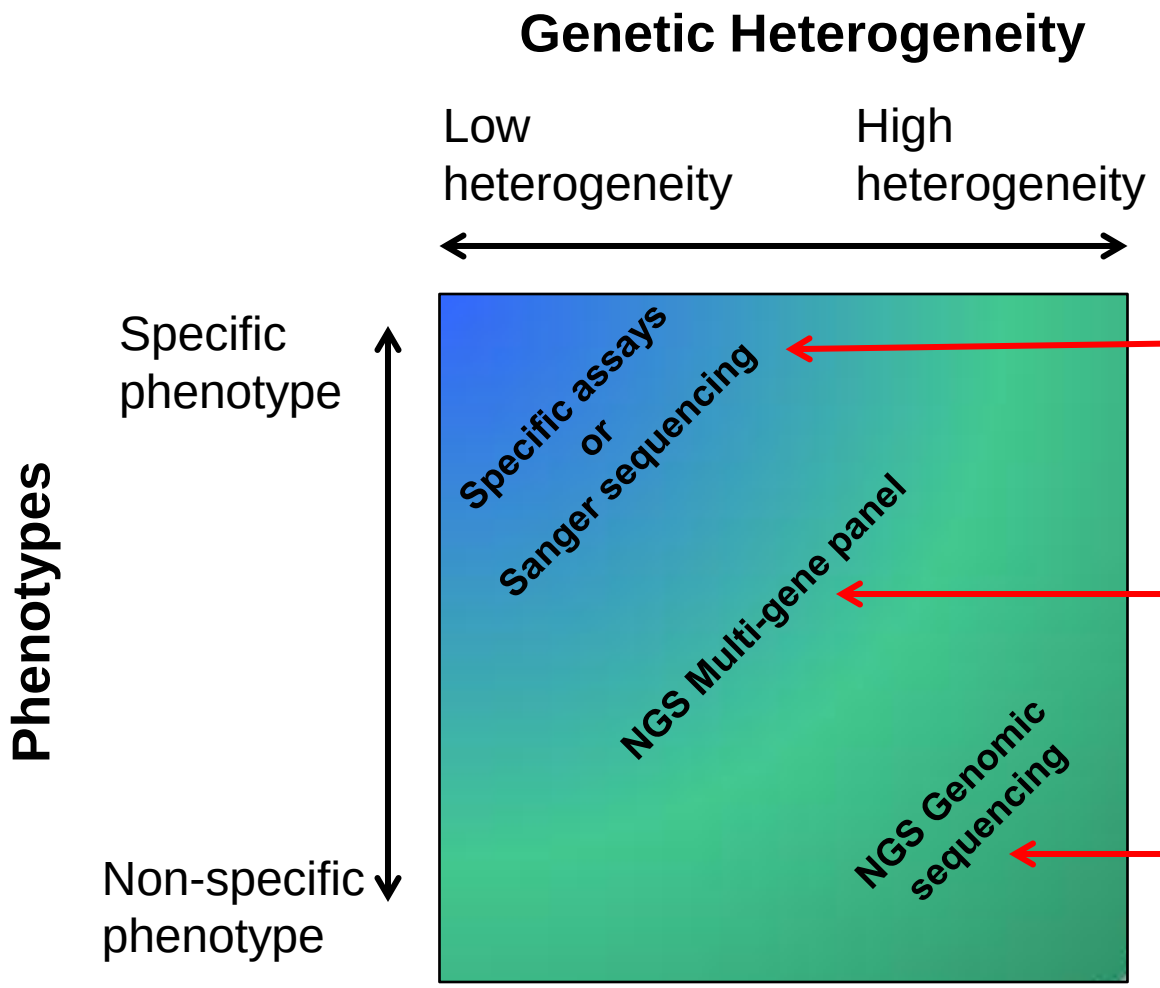
- Samples: Quality, quantity, pathology review
- Somatic allele frequency and detection of mutations
  - 0-100% mutant allele freq.
- Sequential single gene testing versus NGS panel simultaneous testing
- NGS panel content
- Heterogeneity
- Evolution of mutations





Stead, Sutton, Graham 2013, Hum Mutn 34

Clinical Tests and Heterogeneity



UHN Molecular Diagnostics Tests:

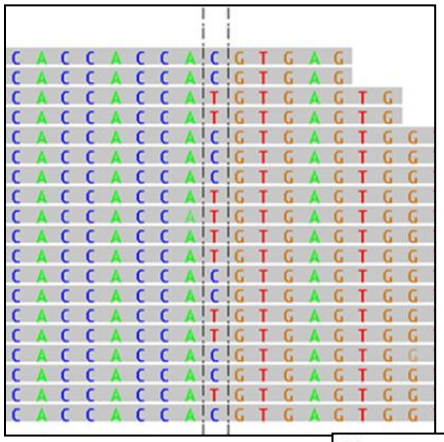
Gene-specific tests:
e.g. BRAF V600E
(non-NGS)

UHN Targeted NGS Gene Panels:
26-54 genes
Solid tumor, Myeloid, Hereditary

UHN Large NGS Gene Panel Hi5:
555 genes
SNV, CNV, SV

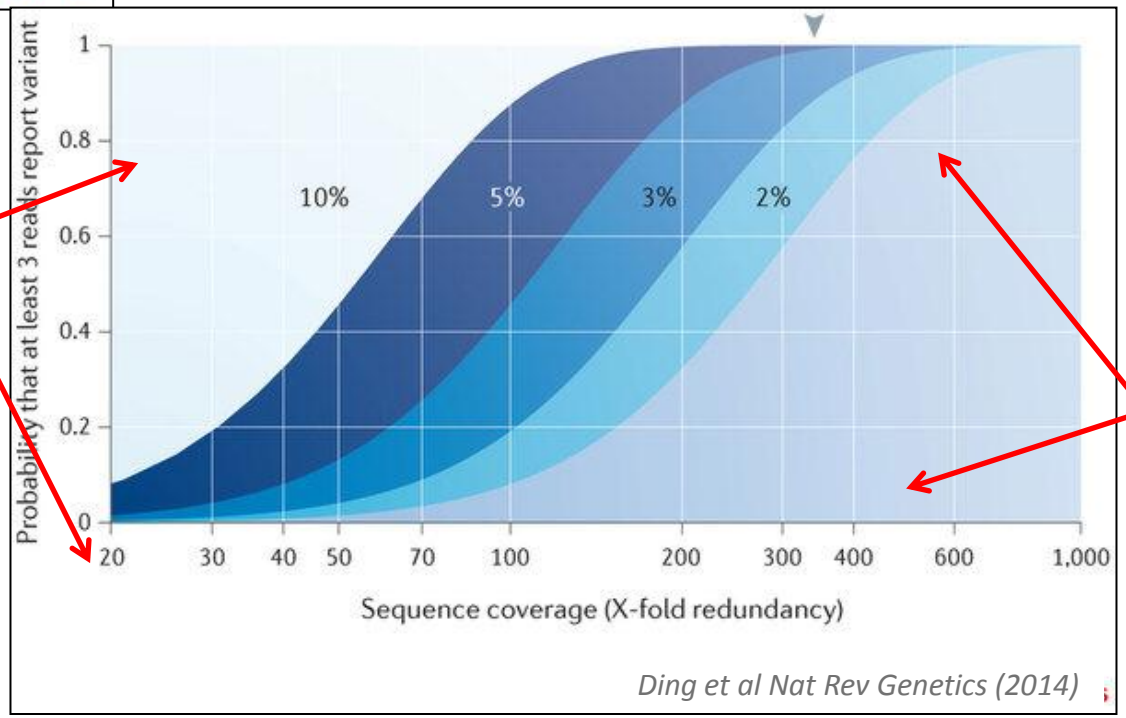
Boycott et al, CCMG, 2015

C>T variant



Design of clinical NGS test depends on required mutant allele frequency detection

Inherited Disease:
50%
Tests broad
But not deep
E.g. Exome with 20x read depth



Ding et al Nat Rev Genetics (2014)

Somatic Cancers:
Down to 5%
Tests deep
But not broad
e.g. Panel with 500x read depth

UHN Solid Tumor NGS Clinical Tests

FFPE Sample Quality/Quantity Dictates NGS Platform

FFPE Samples

80%

High Quantity (>10ng/ul)

75%

FFPE QC High Quality (ΔCt <3)

Illumina MiSeq NGS TruSeq

5%

FFPE QC Low Quality (ΔCt >3)

QC High Quality and >50ng/ul

Ion Proton NGS AmpliSeq

QC High Quality and <50ng/ul

Sequenom Solid Tumor

15%

Low Quantity (1-10ng/ul)

Ion Proton NGS AmpliSeq

5%

Very Low Quantity (<1ng/ul)

Insufficient

T Zhang et al, AMP Platform, 2014

Interpretation of variants from NGS*

Somatic tools

Does this variant impact patient management?

NCBI dbSNP

Exome Seq Project

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COSMIC

The Cancer Genome Atlas

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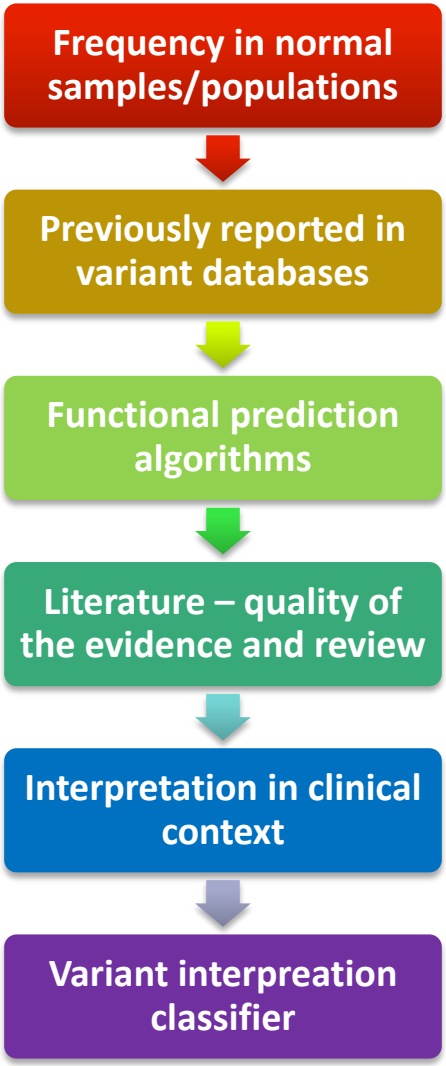
Locus specific DB

Human Gene Mutn DB

Leiden OVDB

In silico tools – SIFT, PolyPhen, Mutation Taster

Tumor site and histology



**Data in many databases, and as produced by in silico tools, can be wrong*

Evolution of variant classification guidelines:
Somatic Variants

	CLASS 1	CLASS 2	CLASS 3	CLASS 4	CLASS 5
Specific Variant is <u>Actionable</u>*:	*In Same Site*	*In Different Site*			
Other Variants in Same Gene are <u>Actionable</u>:			*In Same Site/ Histology*	*In Different Site/ Histology*	*Not Reported*
Variant <u>Frequency</u> in Site/Histology:	*Recurrent (>2% of cases)*	*Recurrent*	*Infrequent / Novel*	*Infrequent / Novel*	*Infrequent / Novel*
Variant Effect from <u>Prediction</u> Tools:			*3A: Affects Protein function* *3B: Unknown* *3C: Benign*	*4A: Affects Protein function* *4B: Unknown* *4C: Benign*	

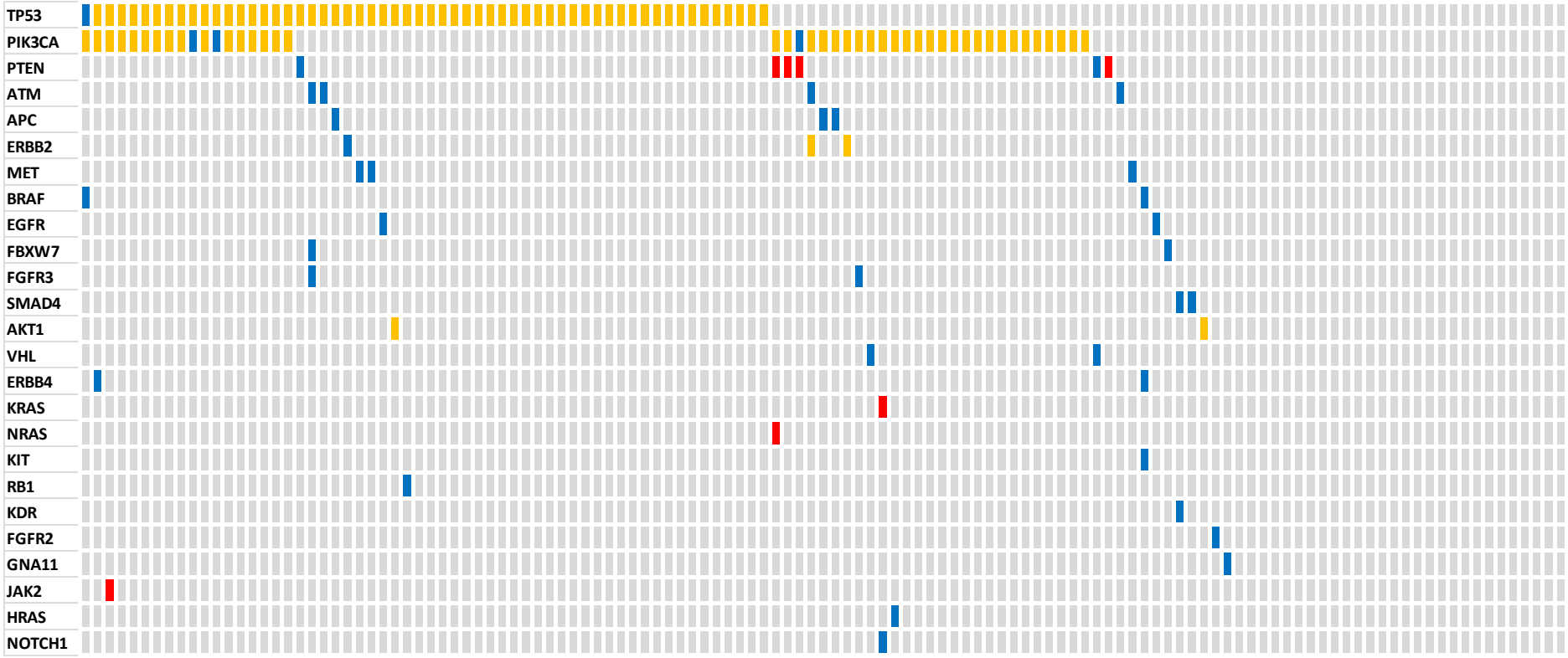
**actionable = druggable/predictive/prognostic/diagnostic*

M. Sukhai et al, Under revision

IMPACT/COMPACT Clinical Trial

(L Siu, P Bedard, S Kamel-Reid)

Breast Cancer, Variants by Classifier



N=121

Actionable – same site	Actionable – different site	Unknown Significance

Incidental Findings in Tumor Molecular Profiling

Variants unrelated to initial reason for testing *but* clinically actionable

Variants from NGS Testing of Tumor (Somatic and Germline)

Variants from NGS Testing of Blood (Germline)

May reveal germline mutations in cancer predisposition genes

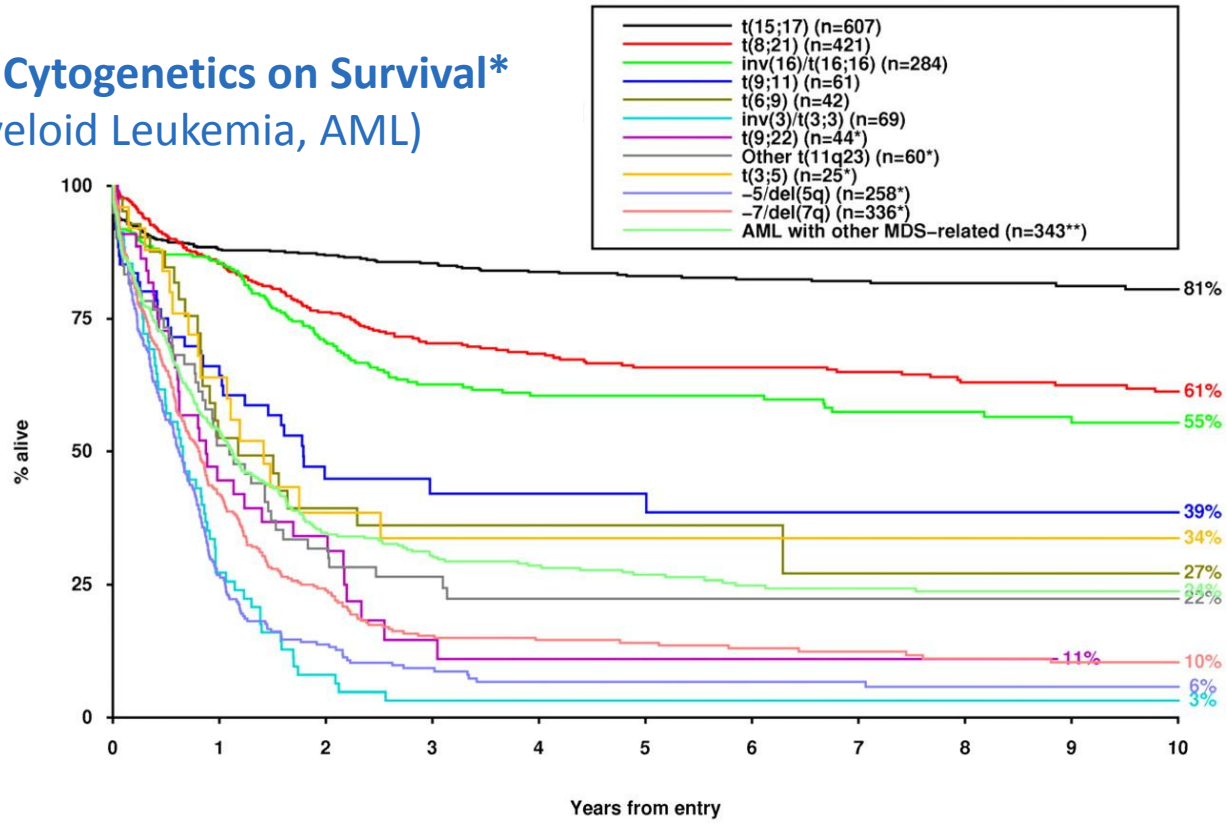
IMPACT/COMPACT trial: 2/1000 cases with pathogenic germline cancer predisposition mutation

Somatic Variants in Tumor from paired Tumor-Blood analysis

B. Hematologic Malignancies

Genetic abnormalities as indicators for management, response to therapy and survival

Impact of Cytogenetics on Survival*
(Acute Myeloid Leukemia, AML)



Favorable

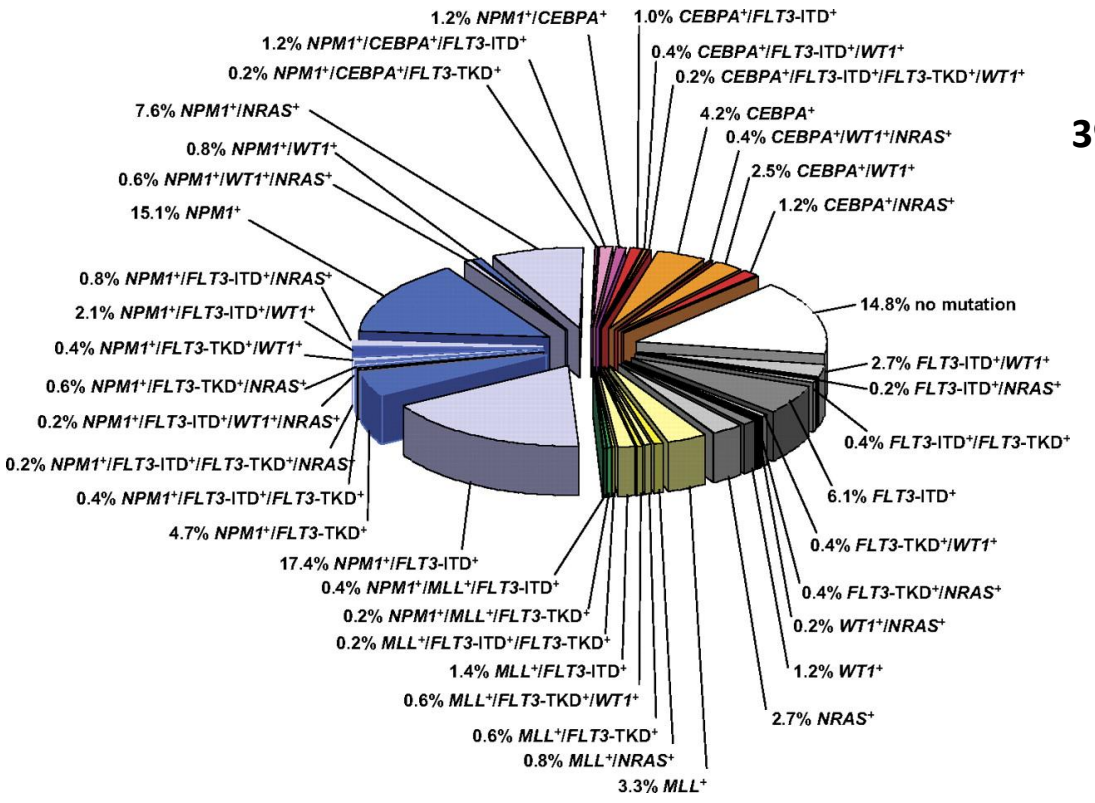
Intermediate

Unfavorable

* ~40% of AML is Cytogenetically Normal

Grimwade Blood 2010:116

Molecular Heterogeneity of Cytogenetically Normal AML



Dohner et al Blood 2010:115

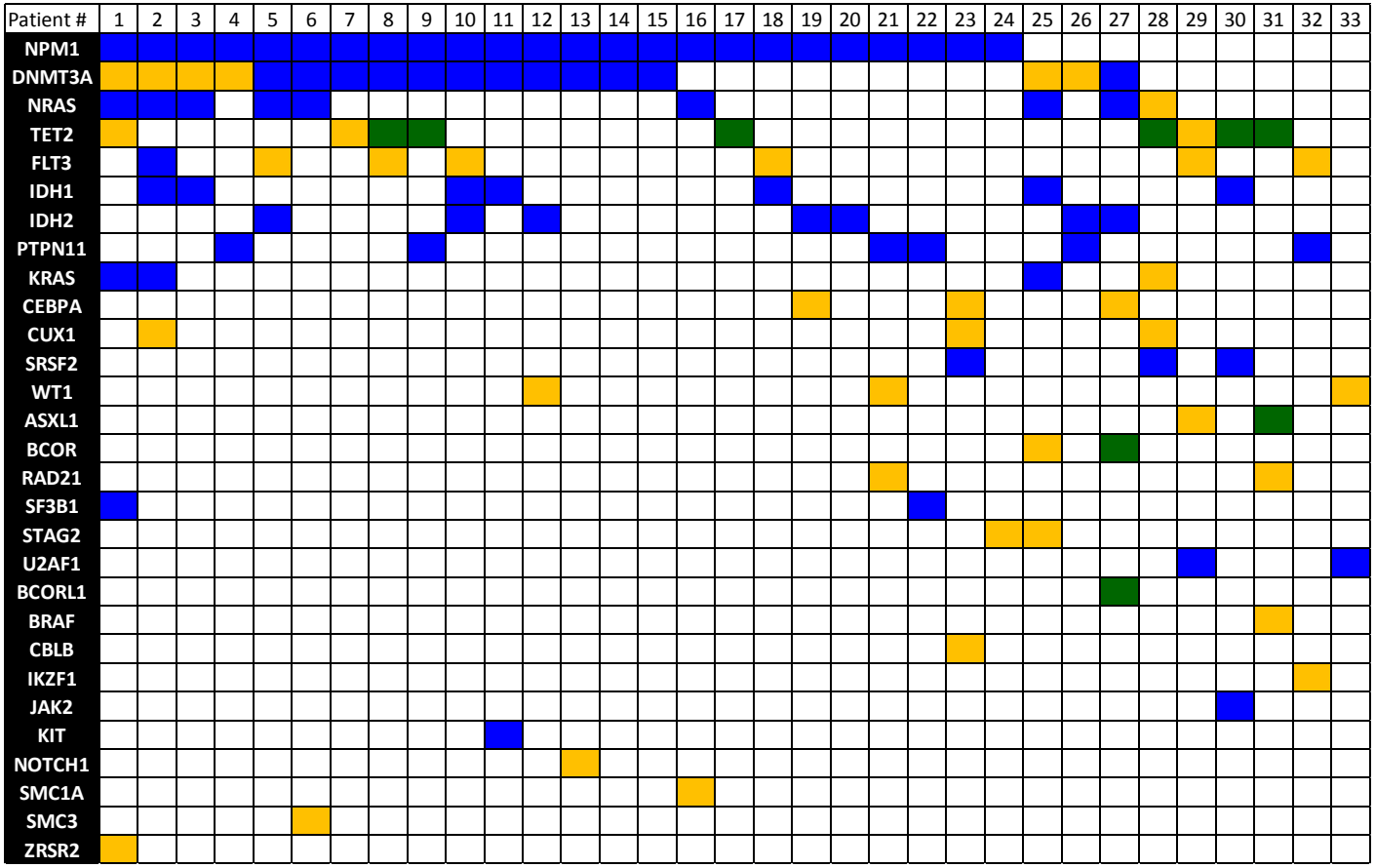
Myeloid NGS 54 gene Panel (Illumina TruSight)

39 genes – hotspot; 15 genes – complete

ABL1	DNMT3A	KDM6A	RUNX1
ASXL1	ETV6/TEL	KIT	SETBP1
ATRX	EZH2	KMT2A	SF3B1
BCOR	FBXW7	KRAS	SMC1A
BCORL1	FLT3	MPL	SMC3
BRAF	GATA1	MYD88	SRSF2
CALR	GATA2	NOTCH1	STAG2
CBL	GNAS	NPM1	TET2
CBLB	HRAS	NRAS	TP53
CBLC	IDH1	PDGFRA	U2AF1
CDKN2A	IDH2	PHF6	WT1
CEBPA	IKZF1	PTEN	ZRSR2
CSF3R	JAK2	PTPN11	
CUX1	JAK3	RAD21	

Molecular Classification of AML

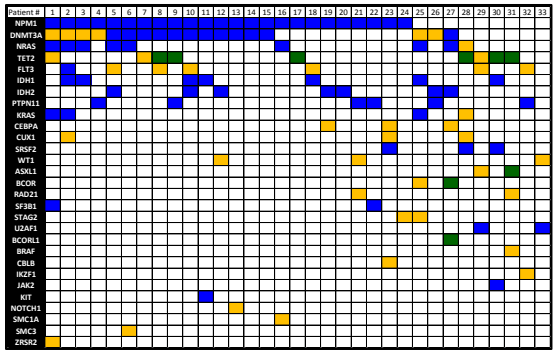
Illumina Myeloid Panel (N=33 cases)

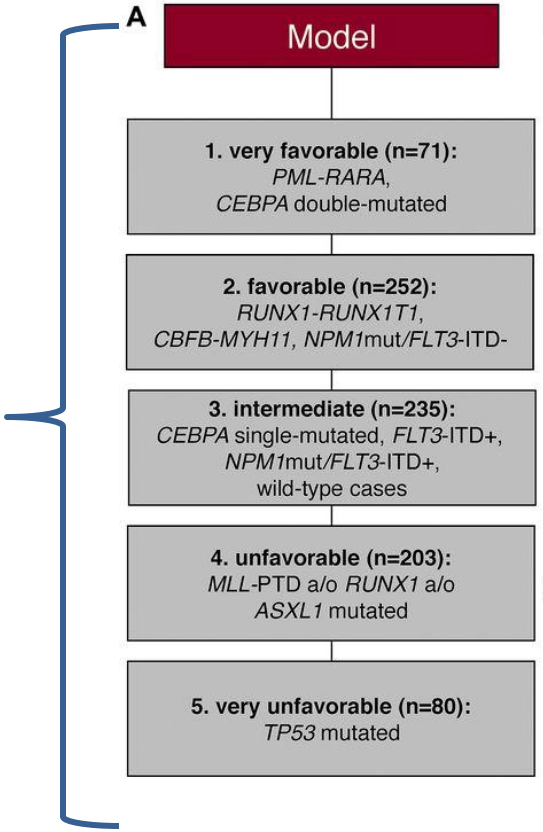


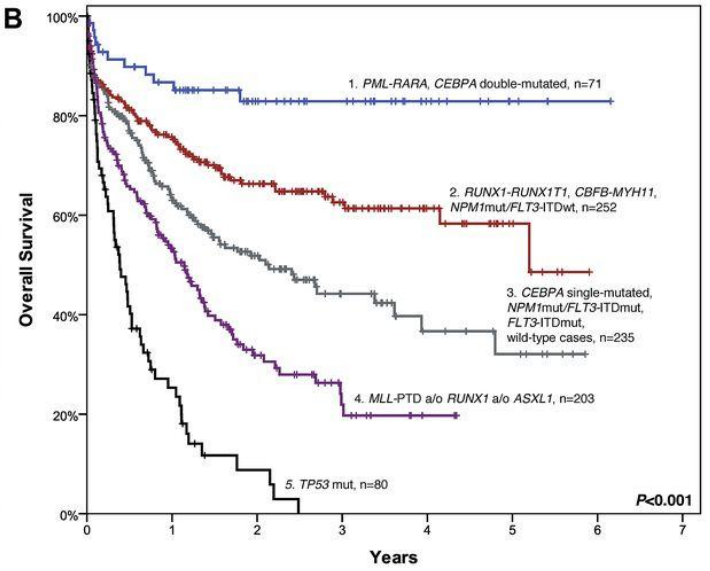
Sequenom and TMP-Detectable Variant | **Variant Detectable only by TMP** | **Multiple Variants in a Single Gene**

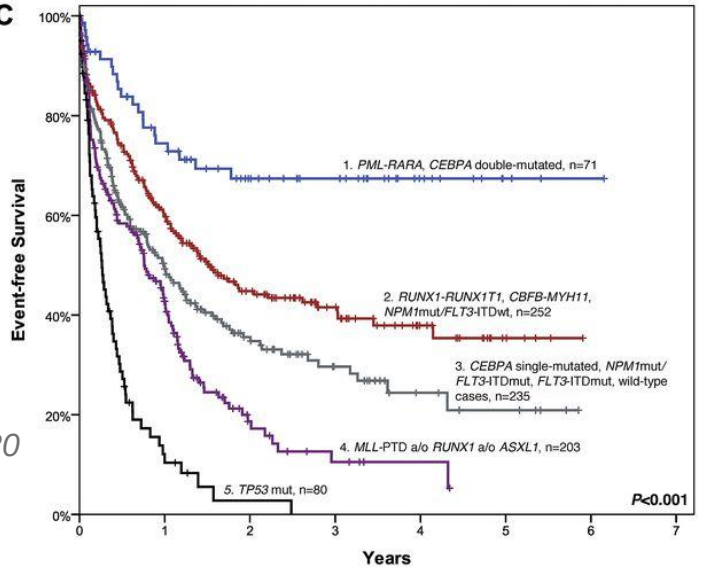
Courtesy Mahadeo Sukhai

AML Prognostic Model including Molecular



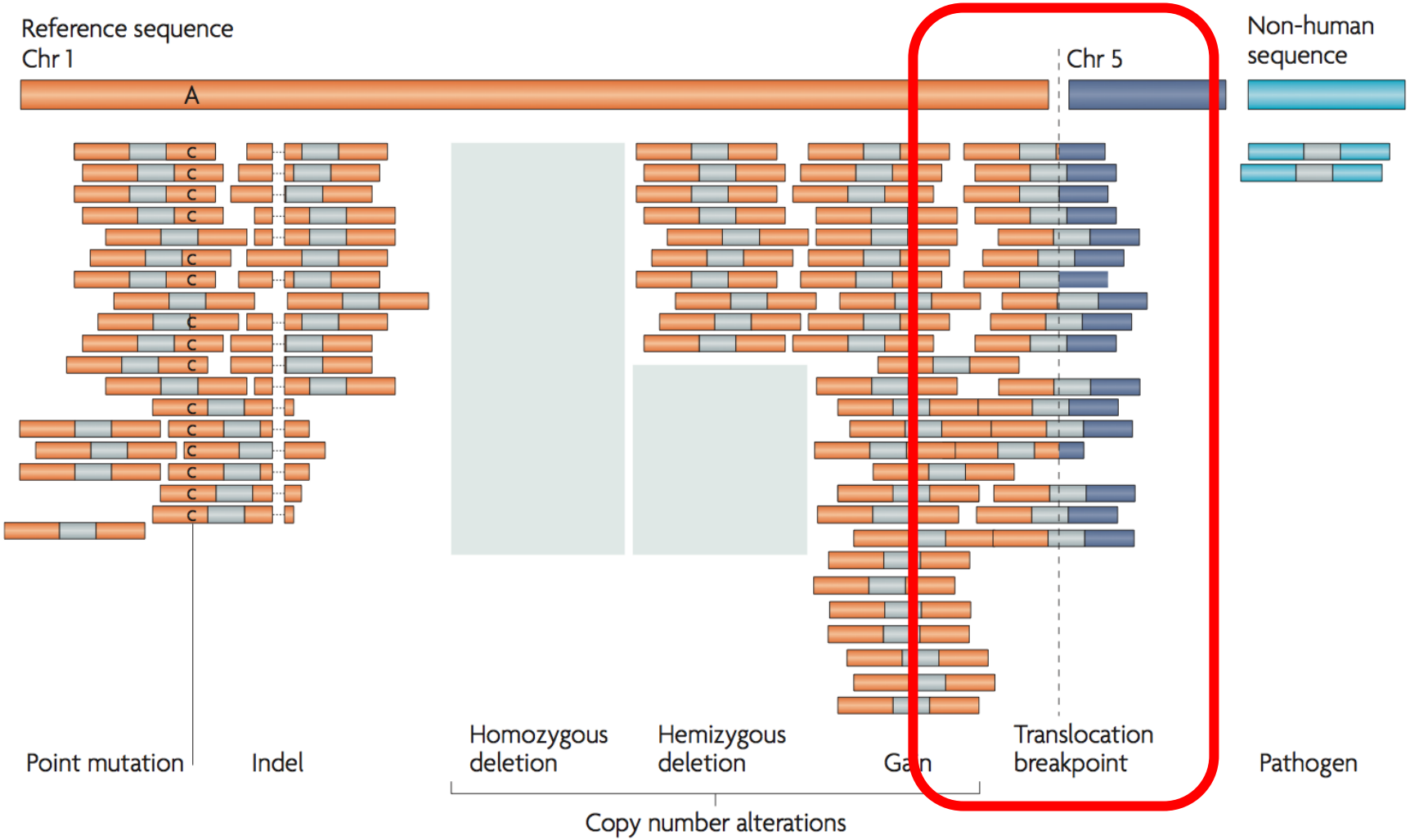






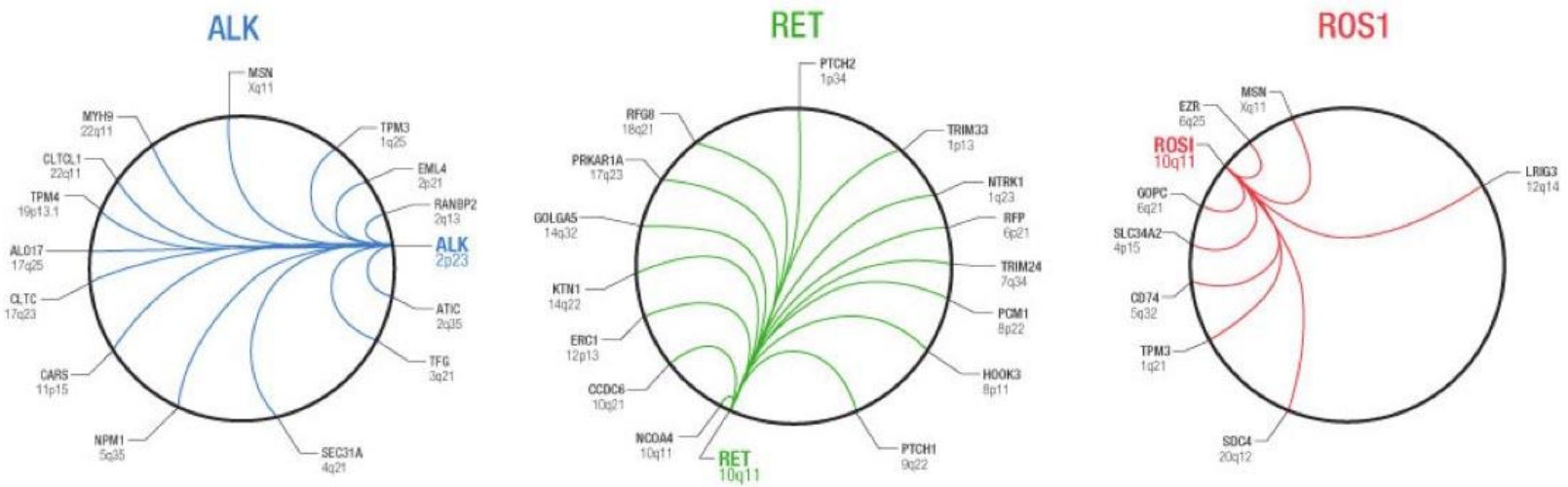
Grossmann V et al. Blood 2012;120
Patel et al. NEJM 2012; 366

Translocations in Hematologic Malignancies: Detection by NGS



Detection of Translocations from NGS

Archer™ ALK, RET, ROS1 NGS Translocation Panel

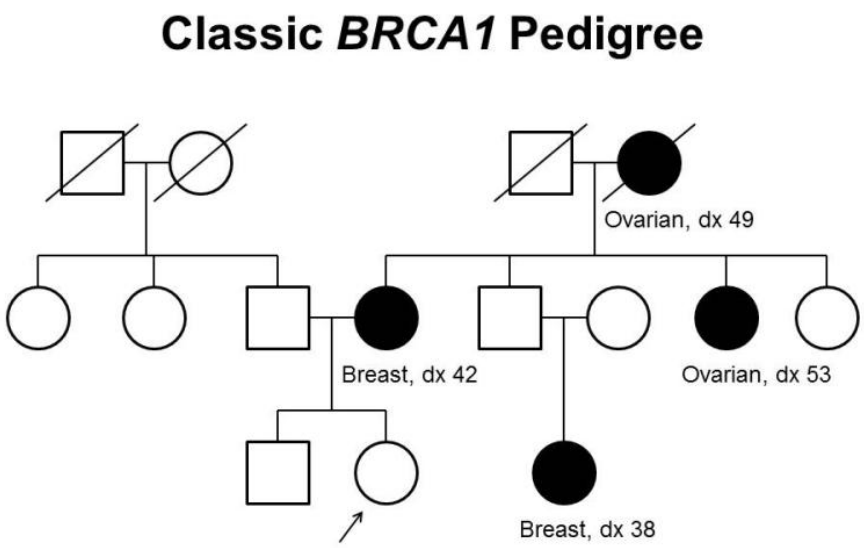


- Detection of known/novel *ALK, RET, ROS1* fusions using common exonic fusion breakpoint sequences as bait
- Informatics to determine the identity of the fusion partner

http://www.enzymatics.com/products/archer-fusionplex-alk-ret-ros1-panel-v2/

C. Inherited Cancer Testing

- Inherited germline mutations in cancer susceptibility gene
- Breast, ovarian, colon, other
- Issues:
  - Extent of genetic testing needed
  - Variant interpretations
  - Penetrance for new genes
  - Gene content of NGS gene panels
  - Genetic counselling and familial risk



www.cancer.gov

Interpretation of variants from NGS*

Somatic tools

Does this variant impact patient management?

NCBI dbSNP

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COSMIC

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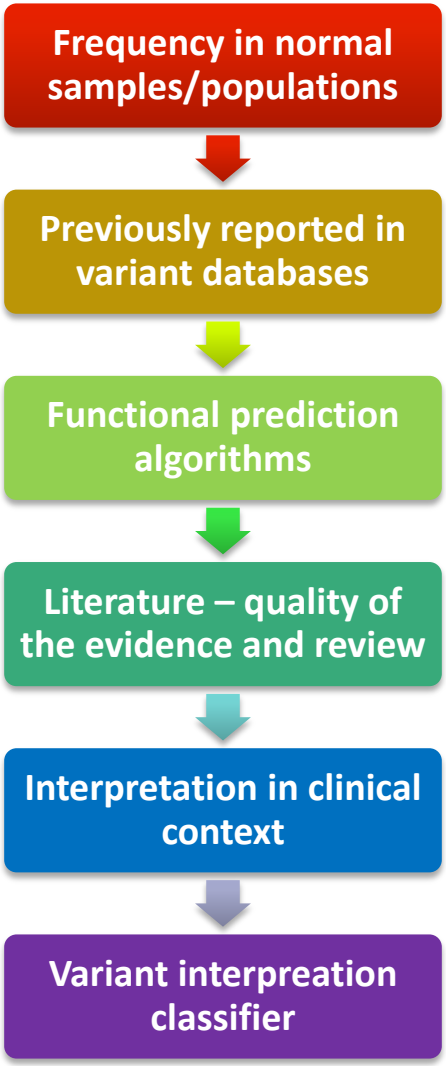
Locus specific DB

Human Gene Mutn DB

Leiden OVDB

In silico tools – SIFT, PolyPhen, Mutation Taster

Tumor site and histology



Germline tools

Is this a pathogenic mutation?

NCBI dbSNP

Exome Seq Project

1000 Genomes

Human Gene Mutn DB

Locus specific DB

Leiden OVDB

In silico tools – SIFT, PolyPhen, Mutation Taster

Familial segregation and pedigree analysis

Known familial variants

Cis/trans variants

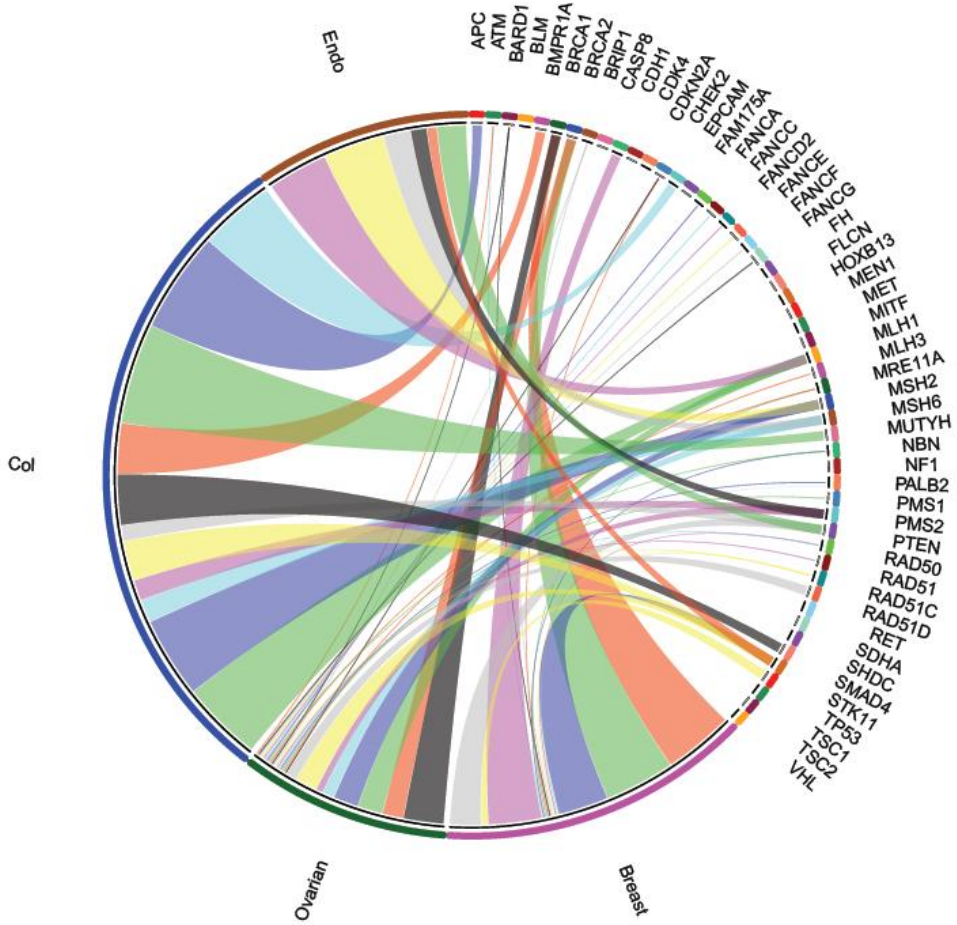
**Data in many databases, and as produced by in silico tools, can be wrong*

Hereditary Cancer NGS Panels
What genes should be included?

UHN Hereditary Cancer Panel

APC	CDKN2A	FLCN	NBN	SDHC
ATM	CHEK2	HOXB13	NF1	SMAD4
BARD1	EPCAM	MEN1	PALB2	STK11
BLM	FAM175A	MET	PMS1	TP53
BMPR1A	FANCA	MITF	PMS2	TSC1
BRCA1	FANCC	MLH1	PTEN	TSC2
BRCA2	FANCD2	MLH3	RAD50	VHL
BRIP1	FANCE	MRE11A	RAD51C	
CASP8	FANCF	MSH2	RAD51D	
CDH1	FANCG	MSH6	RET	
CDK4	FH	MUTYH	SDHA	

Homologous Recombination pathway genes
Mismatch Repair pathway genes
Genes included on commercially available panels



Courtesy Maksym Misyura

Variant Interpretation Classifications

Germline Variants

ACMG: **2008**

Pathogenic	Likely Pathogenic	Uncertain Significance (VUS)	Likely Benign	Benign

VUS?

ACMG: **2014**

Benign ⟷ **Pathogenic**

Strong **Supporting** | **Supporting** **Moderate** **Strong** **Very Strong**

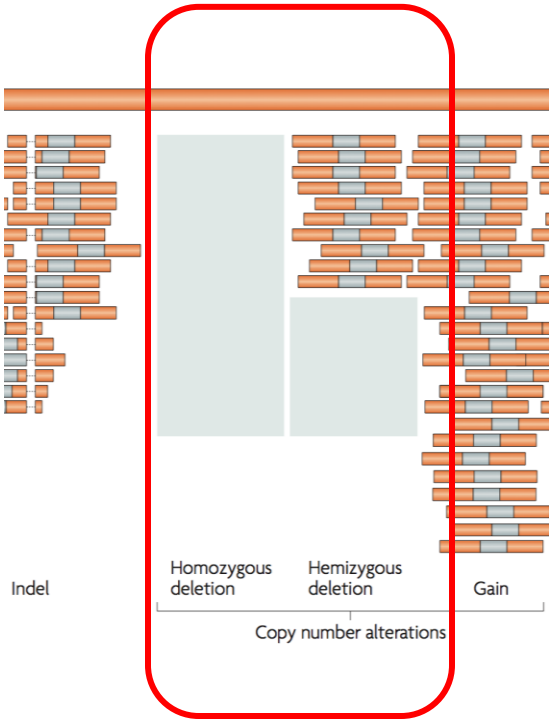
Gene Binning Strategy for Hereditary Panel Example: Ovarian Cancer

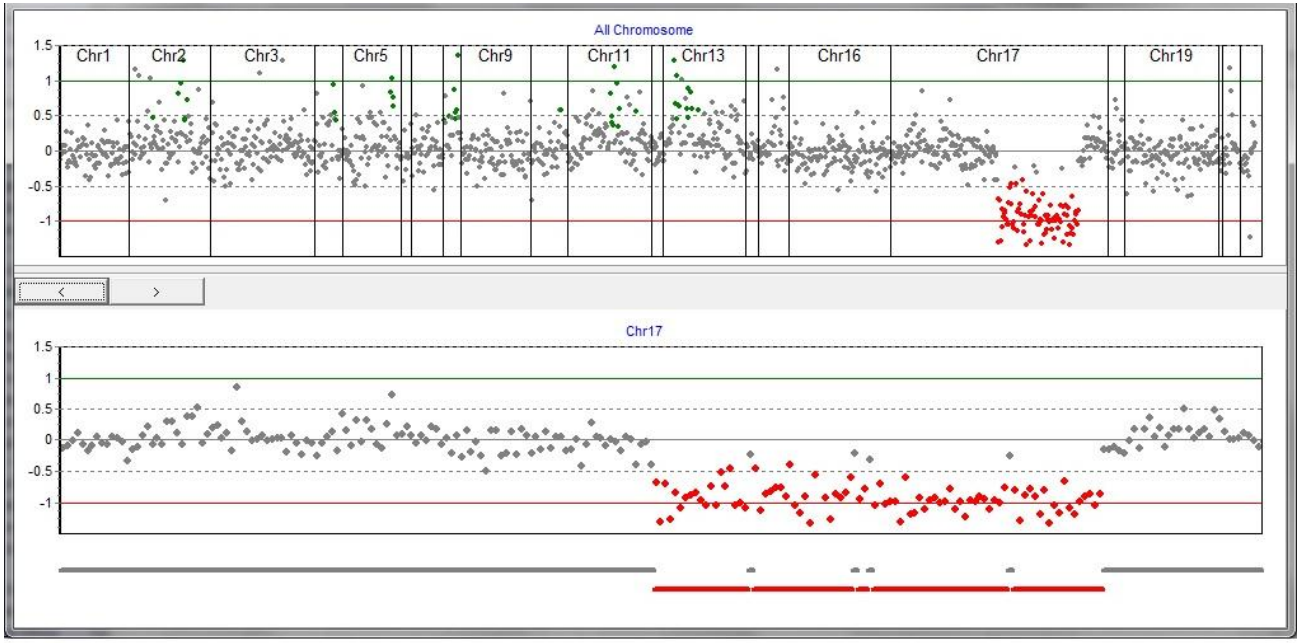
I *Associated with OvCa* *High Risk* *Actionable for OvCa*	II *Associated with OvCa* *Mod-low Risk* *Not Actionable for OvCa*	III *Associated with other Ca* *High Risk* *Actionable for other Ca*	IV *Associated with other Ca* *Mod-low Risk* *Not Actionable for other Ca*
BRCA1	MLH3	APC	HOXB13
BRCA2	PMS1	CDH1	CASP8
EPCAM	PALB2	TP53	MITF
MLH1	RAD50	PTEN	FAM175A
MSH2	RAD51	MEN1	
MSH6	RAD51C	FLCN	
PMS2	RAD51D	RET	
STK11	BRIP1	SMAD4	
	BARD1	BMPR1A	
	CHEK2	MUTYH	
	MRE11A	TSC1	
	NBN	TSC2	
	FANCA	VHL	
	FANCC	NF1	
	FANCD2	FH	
	FANCE	CDKN2A	
	FANCF	CDK4	
	FANCG	MET	
	ATM	SDHA	
		SHDC	
		BLM	

Report all pathogenic classes (very strong, strong, moderate, supporting) and include VUS

Report Pathogenic, very strong and strong categories only

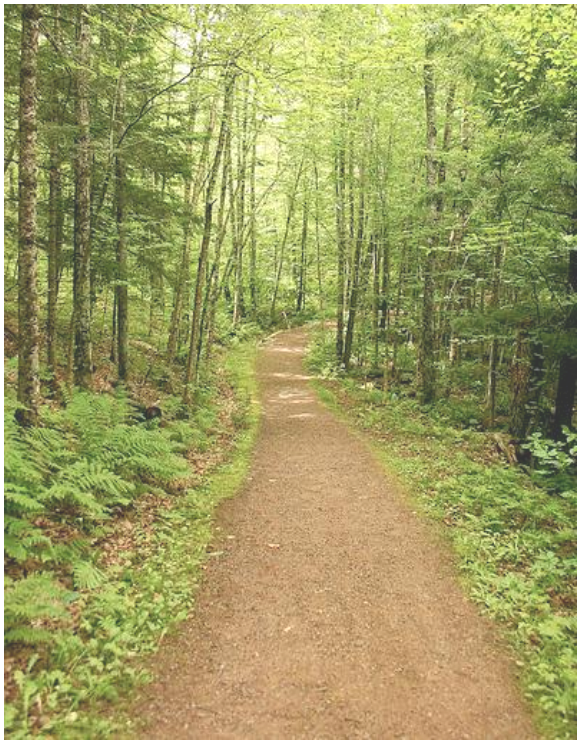
Copy Number Changes in Inherited Cancer: Detection by NGS





Heterozygous Deletion
BRCA1 Exons 1-16, C'some 17
(NextGene data analysis software)

The Path Forward



- Collaborations between clinical labs, researchers, clinicians are key to improving knowledge base for diagnostics and new NGS tests
- Sharing variants linked to clinical data and outcomes
- Sharing expertise and information to improve clinical molecular diagnostic tests and applications

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Jonette Martin
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PMH Foundation

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