IX SIMPOSIO

Grupo Cooperativo Español de Citogenética Hematológica

Avances de las técnicas citogenéticas y moleculares en el diagnóstico de las hemopatías malignas

Organiza:

GCECGH

SEHH Sociedad Española de Hematología y Hemoterapia

FEHH Fundación Española de Hematología y Hemoterapia

Aplicación del mapeo óptico del genoma en neoplasias hematológicas

Adam C. Smith, PhD, FCCMG, FACMG, erCLG

Director,
Cancer Cytogenetics Laboratory
Laboratory Medicine Program, University Health Network

Assistant Professor,
Department of Laboratory Medicine and Pathobiology
University of Toronto

www.gcecgh.org

#gcecgh

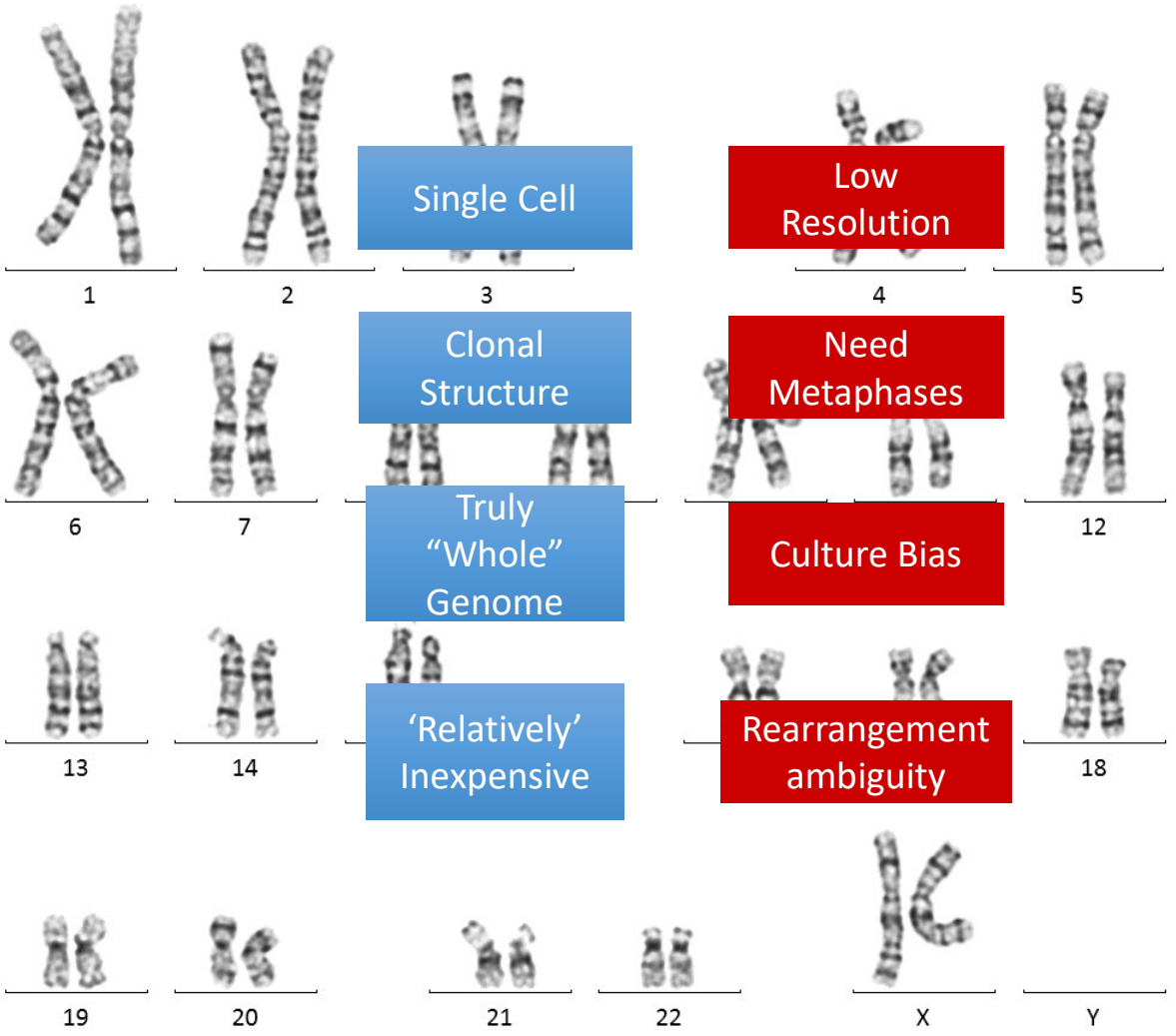
Presenter Financial Disclosure

I have the following financial relationships to report within the past 24 months.

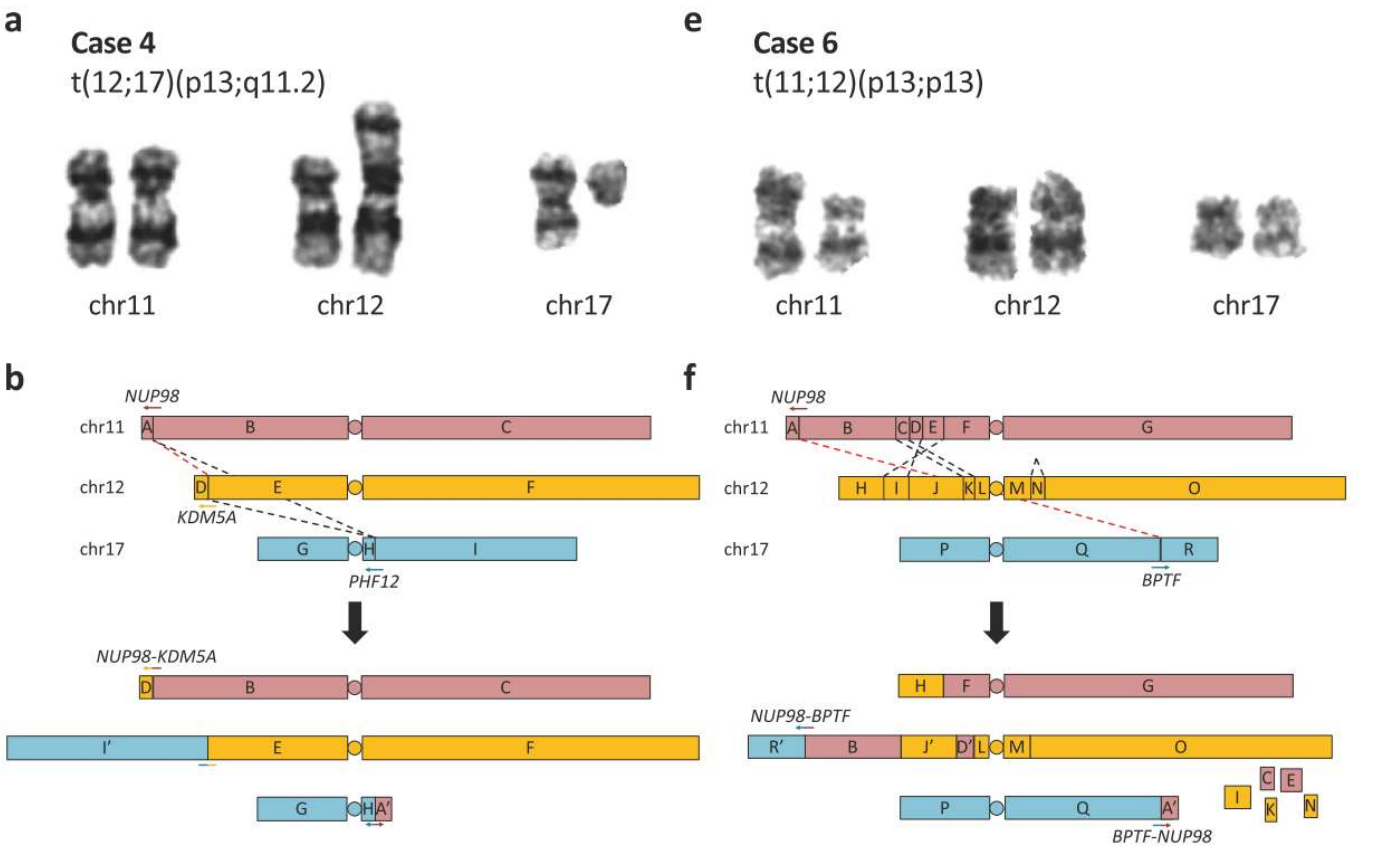
Name of Company	Nature of Relationship	Current Status
Bionano Genomics	Personal Financial Interest	On-going
Bionano Genomics	Travel Support	On-going
Pfizer Canada	Advisory Board Member	Activities Completed

The G-banded Karyotype

The “original” Whole Genome…from telomere to telomere for more than 40 years



Urgent clinical need for technologies with higher resolution that can detect clinically relevant abnormalities that can also detect variant, cryptic and complex SVs.



Kim et al. (2020). Cryptic genomic lesions in adverse-risk acute myeloid leukemia identified by integrated whole genome and transcriptome sequencing. Leukemia, 34(1), 306–311.

Towards a Technology Agnostic Classification

© American College of Medical Genetics and Genomics
ACMG TECHNICAL STANDARD | Genetics inMedicine

Technical laboratory standards for interpretation and reporting of acquired copy-number abnormalities and copy-neutral loss of heterozygosity in neoplastic disorders: a joint consensus recommendation from the American College of Medical Genetics and Genomics (ACMG) and the Cancer Genomics Consortium (CGC)

Fady M. Mikhail, MD, PhD[1], Jaclyn A. Biegel, PhD[2], Linda D. Cooley, MD, MBA[3], Adrian M. Dubuc, PhD[4], Betsy Hirsch, PhD[5], Vanessa L. Horner, PhD[6], Scott Newman, PhD[7], Lina Shao, MD, PhD[8], Daynna J. Wolff, PhD[9] and Gordana Raca, MD, PhD[2]

The Journal of Molecular Diagnostics, Vol. 19, No. 1, January 2017
ELSEVIER
the Journal of Molecular Diagnostics
jmd.amjpathol.org

SPECIAL ARTICLE

Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists

Marilyn M. Li,*† Michael Datto,*‡ Eric J. Duncavage,*§ Shashikant Kulkarni,*¶ Neal I. Lindeman,*‖ Somak Roy,*,** Apostolia M. Tsimberidou,*†† Cindy L. Vnencak-Jones,*‡‡ Daynna J. Wolff,*§§ Anas Younes,*¶¶ and Marina N. Nikiforova*,**

CLINICAL

TECHNICAL

blood
Special Report
Check for updates

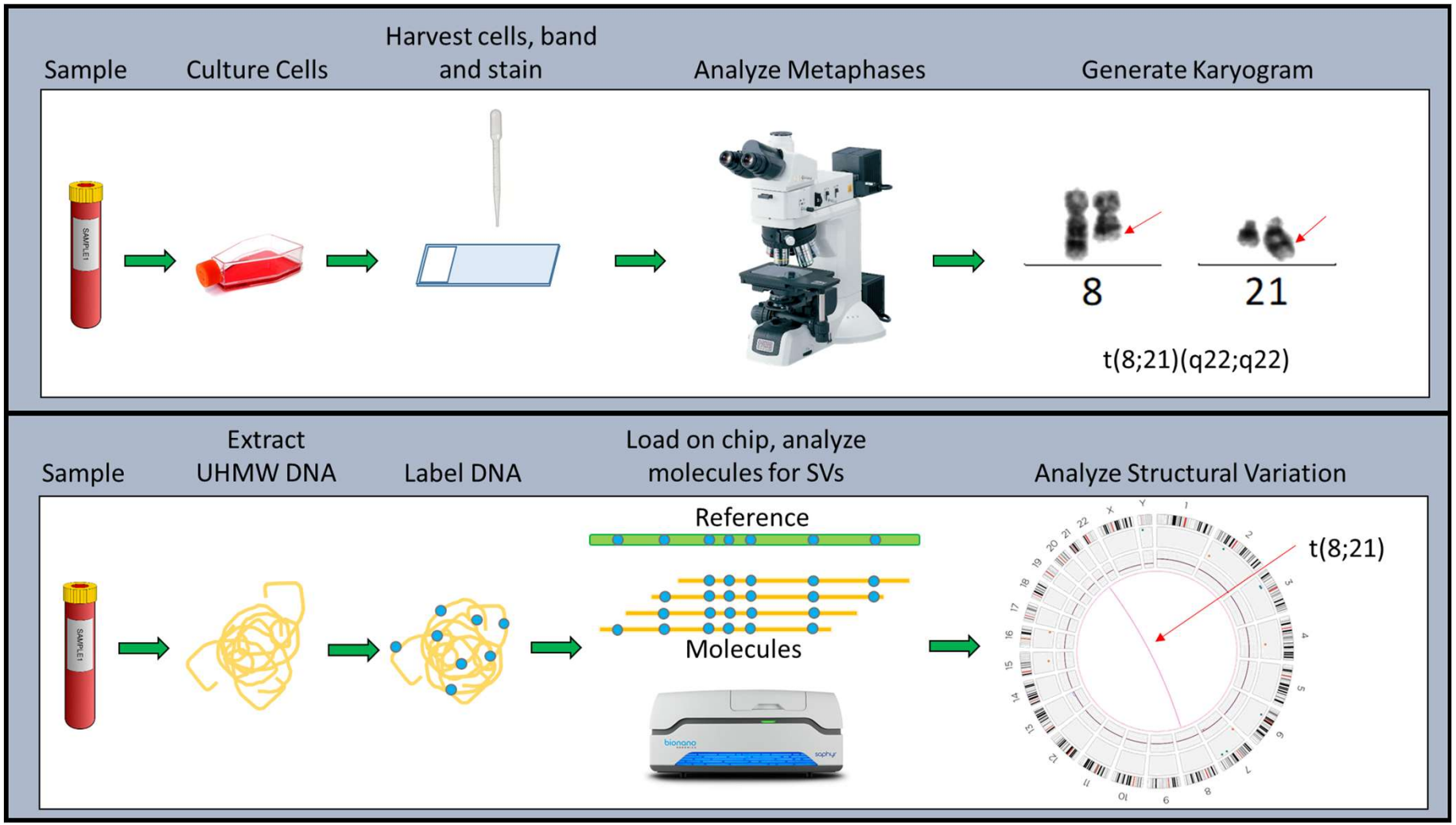
Guiding the global evolution of cytogenetic testing for hematologic malignancies

Yassmine M. N. Akkari,[1] Linda B. Baughn,[2] Adrian M. Dubuc,[3] Adam C. Smith,[4] Mar Mallo,[5] Paola Dal Cin,[3] Maria Diez Campelo,[6] Marta S. Gallego,[7] Isabel Granada Font,[8] Detlef T. Haase,[9] Brigitte Schlegelberger,[10] Irma Slavutsky,[11] Cristina Mecucci,[12] Ross L. Levine,[13] Robert P. Hasserjian,[14] Francesc Solé,[5] Brynn Levy,[15] and Xinjie Xu[2]

FINANCIAL

LOGISTICAL

What is Optical Genome Mapping?



Smith et al., American Journal of Hematology (2022)

Table 1. Resolution and Limit of Detection of Karyotyping versus Optical Genome Mapping

Structural Variant Class	Karyotype	Optical Genome Mapping			
	Lower Limit of Detection (LLOD)	Rare Variant Assembly (300x)[1] LLOD	Increase in Resolution Compared to Karyotype	*De novo* assembly (300x)[1] LLOD	Increase in Resolution Compared to Karyotype
Unbalanced SV (insertion, duplication, deletion)	~10,000 kbp[2]	5 kbp	2000x	0.5 kbp	20000x
Translocation	~10,000 kbp	>70 kbp[3]	140x	> 50 kbp	200x
Inversion	~10,000 kbp	100 kbp	100x	50 kbp	200x
SV Lower Limit of Detection	~14%[4] cell level analysis (~7% VAF)	5%		15-25%	
Ploidy Change (triploidy, tetraploidy)	~14%	currently not detectable		currently not detectable[5]	

Smith et al., American Journal of Hematology (2022)

A Framework for OGM

Why do we need it?

- Official guidelines can take years to be developed (judging by similar implementations, e.g. microarray)
- Experience of early-adopters can help other labs implement more quickly by taking advantage of collective international experience
- A more standardized global implementation – to help with uniform interpretation

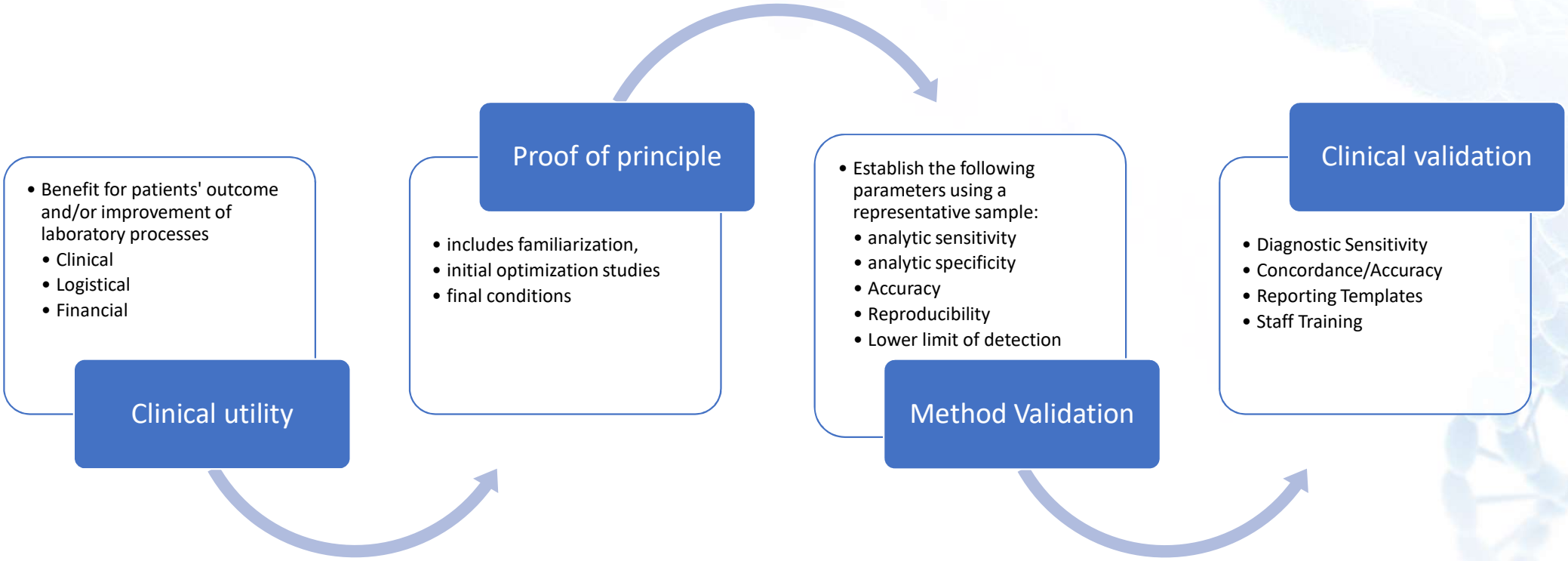
  - 3 Sections:
    - A) Validation
    - B) Quality Control
    - C) Analysis and Interpretation

An OGM Roadmap

International Working Group for OGM in Hematologic Malignancies

Name	Institution	Title
Adam Smith , PhD, FCCMG	University Health Network, Toronto	Director, Cancer Cytogenetics Laboratory
Rashmi Kanagal-Shamanna, MD	MD Anderson Cancer	Director, Microarray Facility
Barbara Dewaele, Phd	UZ Leuven	Manager, Genetics Lab for Hematological Malignancies
Katrina Rack, Phd	UZ Leuven	Manager, Genetics Lab for Hematological Malignancies
Alex Hoischen, Phd	Radboud UMC	Research PI
Kornelia Neveling, Phd	Radboud UMC	Biologist, Genome Diagnostics
Marian Stevens-Kroef, PhD	Radboud UMC	Clinical Laboratory Geneticist, Dept of Human Genetics
Gordana Raca, MD, PhD, FACMG	Children's Hospital Los Angeles	Director, Clinical Cytogenomics Laboratory
Brynn Levy, MSc, PhD, FACMG	Columbia University Medical Center	Director, Clinical Cytogenomics Laboratory
Ravindra Kolhe , MD, PhD	Augusta University	Director, Georgia Esoteric & Molecular Laboratory
Blanca Espinet, PhD	Hospital del Mar, Barcelona	Director, Molecular Cytogenetics Laboratory
Anna Puiggros, PhD	Hospital del Mar, Barcelona	Biotechnologist, Molecular Cytogenetics Laboratory
Francesc Sole, PhD	Carreras Leukemia Research Institute	Research Principal Investigator
Mar Mallo, PhD	Carreras Leukemia Research Institute	Research Principal Investigator
Tuomo Mantere, PhD	University of Oulu, Finland	Postdoctoral Researcher
Nikhil Sahajpal, PhD	Greenwood Genetic Centre/Augusta University, USA	ABMG LGG Fellow
Alka Chaubey, PhD, FACMG	Bionano Genomics, USA	Chief Medical Officer
Alex Hastie, Phd	Bionano Genomics, USA	Vice President of Clinical Affairs

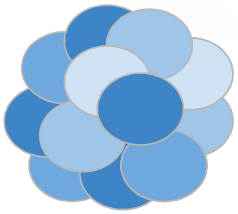
Phases for Validation



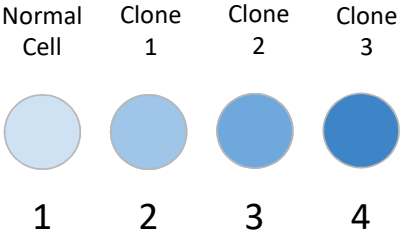
REFERENCE	COHORT SIZE	CLINICAL REFERRAL	NUMBER OF ABNORMALITIES INCLUDED (SOC)	CONCORDANCE WITH CYTOGENETICS RESULTS	OGM ADDITIONAL FINDINGS
Radboud University Neveling et al., 2020	48	AML, MDS, CML, CLL, ALL, MM, MPN, T-PLL, LYBM	112	100%	18 potential gene fusions absent from COSMIC database. 26 insertions/deletions overlapping with well-established cancer genes
Cancer Genomics Consortium Levy et al., 2020	100	AML	NA	100%	3 translocations, 1 inversion, 2 deletions and 1 derivative chromosome
CHU Amiens Lestringant et al., 2021	10	B and T ALL	78	97%*	4 fusions, 6 deletions, 2 gains, 1 duplication, 3 complex chromosomal rearrangements
Johns Hopkins University Stinnett et al. 2021	5	Leukemia/Lymphoma and Solid Tumors	30	100% KT/FISH 100% CMA >10% VAF **	71 additional calls (7.7% involving cancer genes)
University Hospital Olomouc Kriegova et al. 2021	11	Multiple myeloma	NA	98%	
Augusta, Emory Sahajpal et al. 2022	69	CLL, AML, MDS, MM, lymphoma, PCM, CML, ET and others	164	99%	OGM detected chromosomal aberrations missed by karyotyping and FISH in 35 cases
Hannover Luhmann et al. 2021	12	Ped. ALL	NA	~98%†	Many new and unknown SVs including gene fusion of JAK2 and NPAT
Ruhr University Bochum Gerding et al 2022	27	AML and MDS	NA	~93%	In 67% of cases karyotype was clarified by OGM leading to re-classification of risk score in some cases
University Hospital – Essen Suttorp et al 2022	24	Ped. AML	NA	~87%***	OGM detected a total of 32 additional with clinical relevance. No change to risk stratification in 19/20 by OGM with 1 case moved to high risk (5%).
University Hospital – Leuven Rack et al. 2022	41	B and T ALL	24	~96%	Only 24/34 cases correctly classified by SOC techniques while 33/34 classified by OGM (30% increase in classification!)
M.D. Anderson Yang et al., 2022	101	MDS	194	99%	OGM identified 224 cryptic, clinically significant SVs in 34% of pts.
Paris –Necker/Cochin Balducci et al., 2022	68	MDS/AML	130	100%††	OGM revealed clinically relevant SVs missed by SOC in 33% (9/27) and 54% (22/41) of the MDS and AML respectively.”
TOTAL	**516**	**VARIOUS**	**>700**	**>99%**	

EVIDENCE OF CLINICAL UTILITY

OGM versus Karyotype: A comment about making "quantitative comparisons"

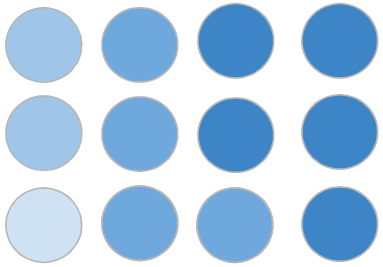


Collection of normal and tumour cells



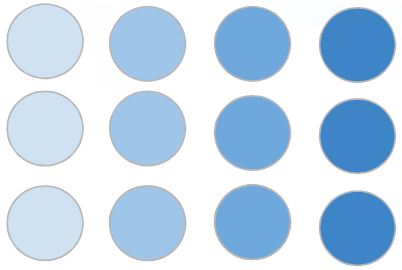
Mitotic Potential

Clone 1 < Clone 2 < Clone 3



KARYOTYPE RESULT

Clone 1 = Clone 2 = Clone 3

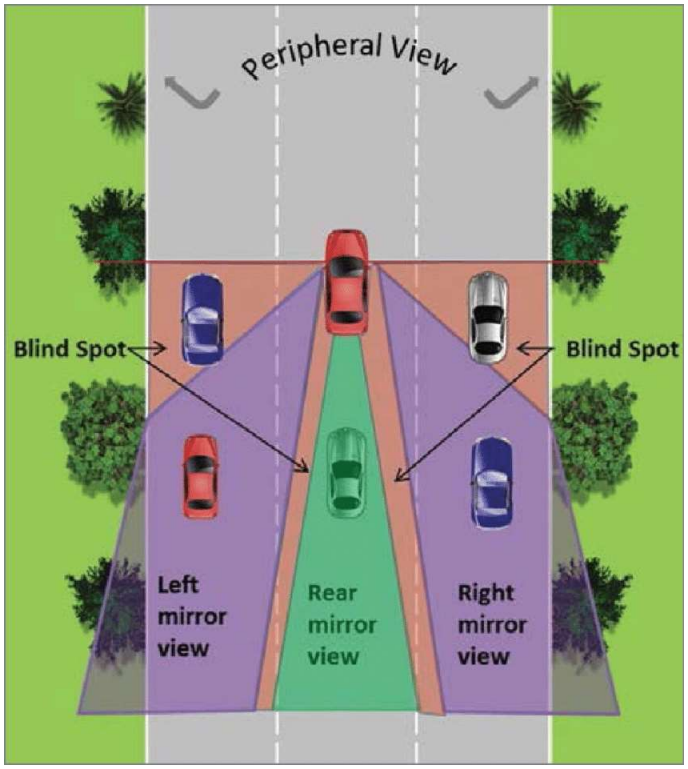


OGM/FISH*/NGS RESULT

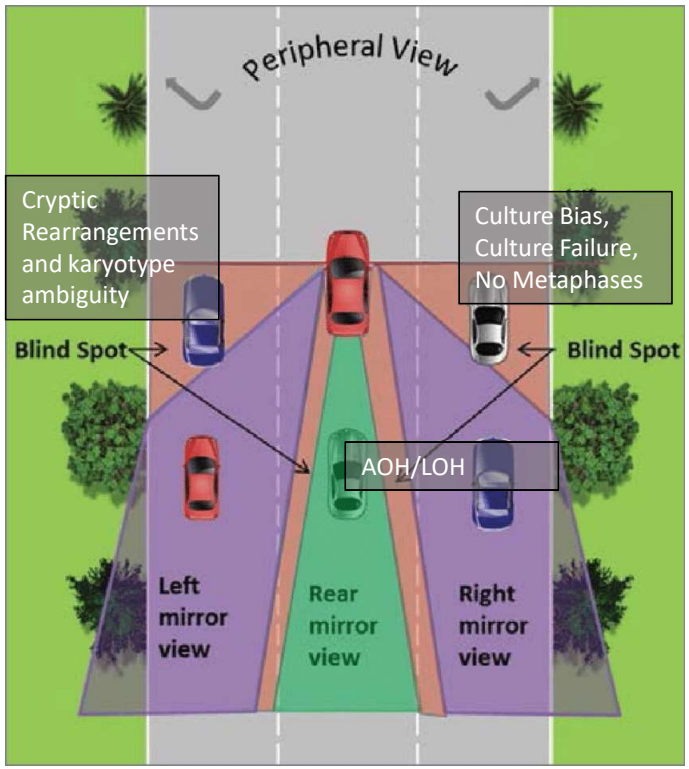
*potentially subject to smaller culture bias

Blindspot Analysis:
OGM is not a karyotype…it has advantages and disadvantages – like any technology

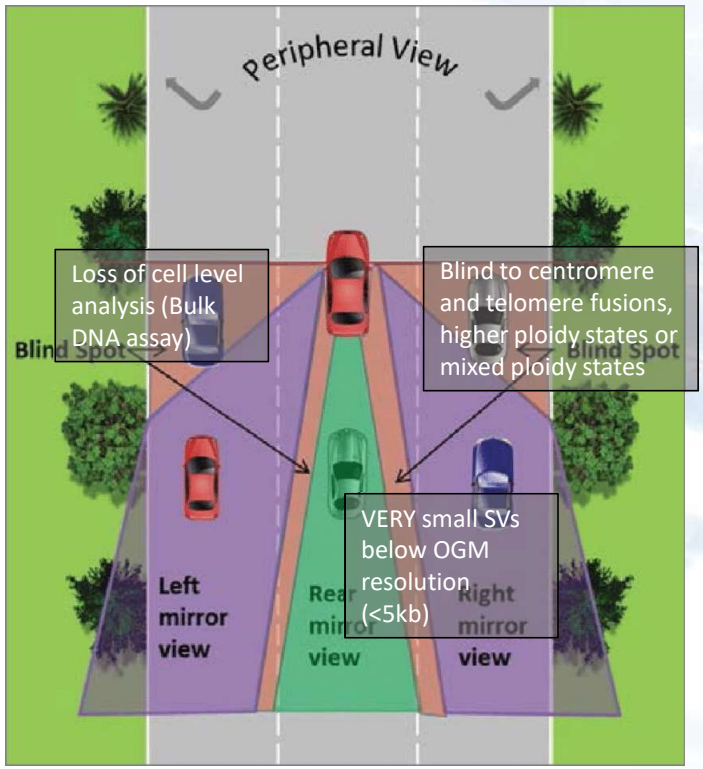
DRIVING



CONVENTIONAL BANDING ANALYSIS

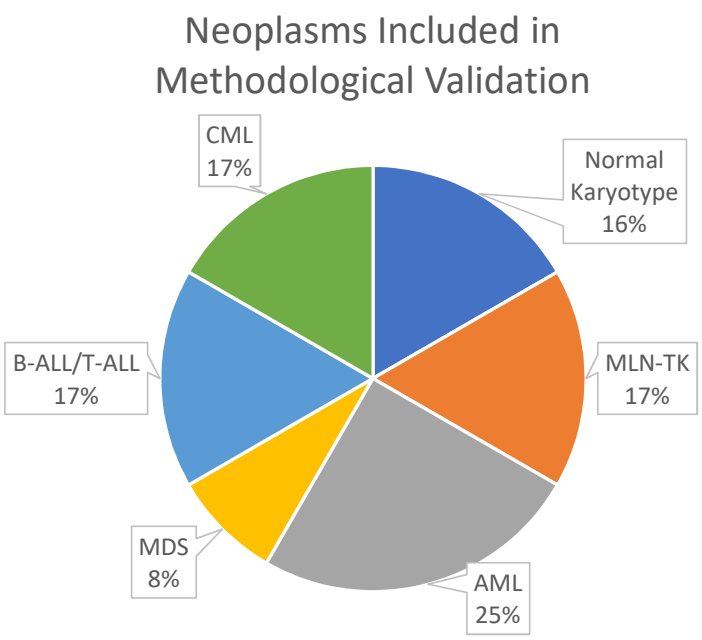


OGM



Methodological Validation ≥ 59 samples

Jennings et al. (2017). Guidelines for Validation of Next-Generation Sequencing–Based Oncology Panels: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. Journal of Molecular Diagnostics, 19(3), 341–365.



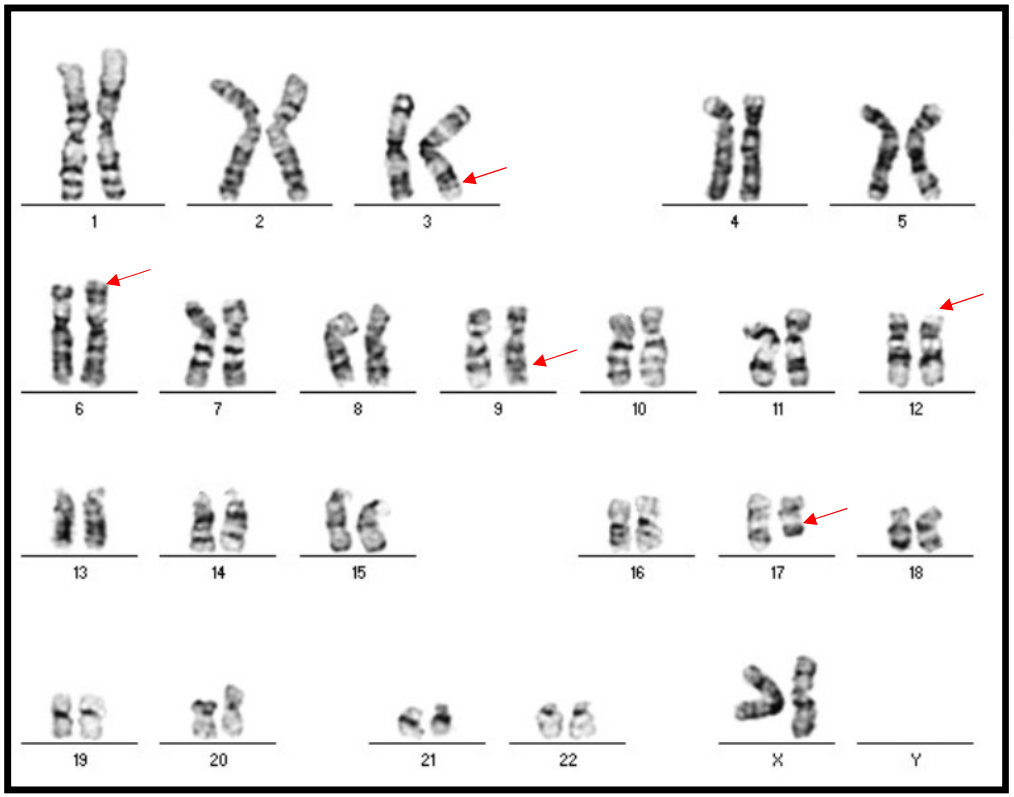
OGM Assay Performance Comparison to Standard of Care Testing (60 Patients)

Parameter	Calculation	Assay Value
Sensitivity/ Positive percentage agreement	TP/(TP+FN)	96%
Specificity/ Negative percentage agreement	TN/ (TN+FP)	100%
Positive predictive value (PPV)	TP/ (TP+FP)	100%
Negative predictive value (NPV)	TN/ (TN+FN)	76%
Accuracy (Concordance)	TP+TN/All Results	96%

Changes in Diagnosis, Prognosis and Reduction in Ancillary Testing

Criteria	Result
OGM Result Changed Diagnosis	14%
OGM Result Changed Prognosis	14%
Cases where SOC Ancillary Studies Required	**48%**

A Part of Clinical Utility – Reducing Ancillary Testing



ORIGINAL KARYOTYPE
46,XX,t(3;6;9;12;17)(q26.2;p23;q34.3;p13;q23)[20]

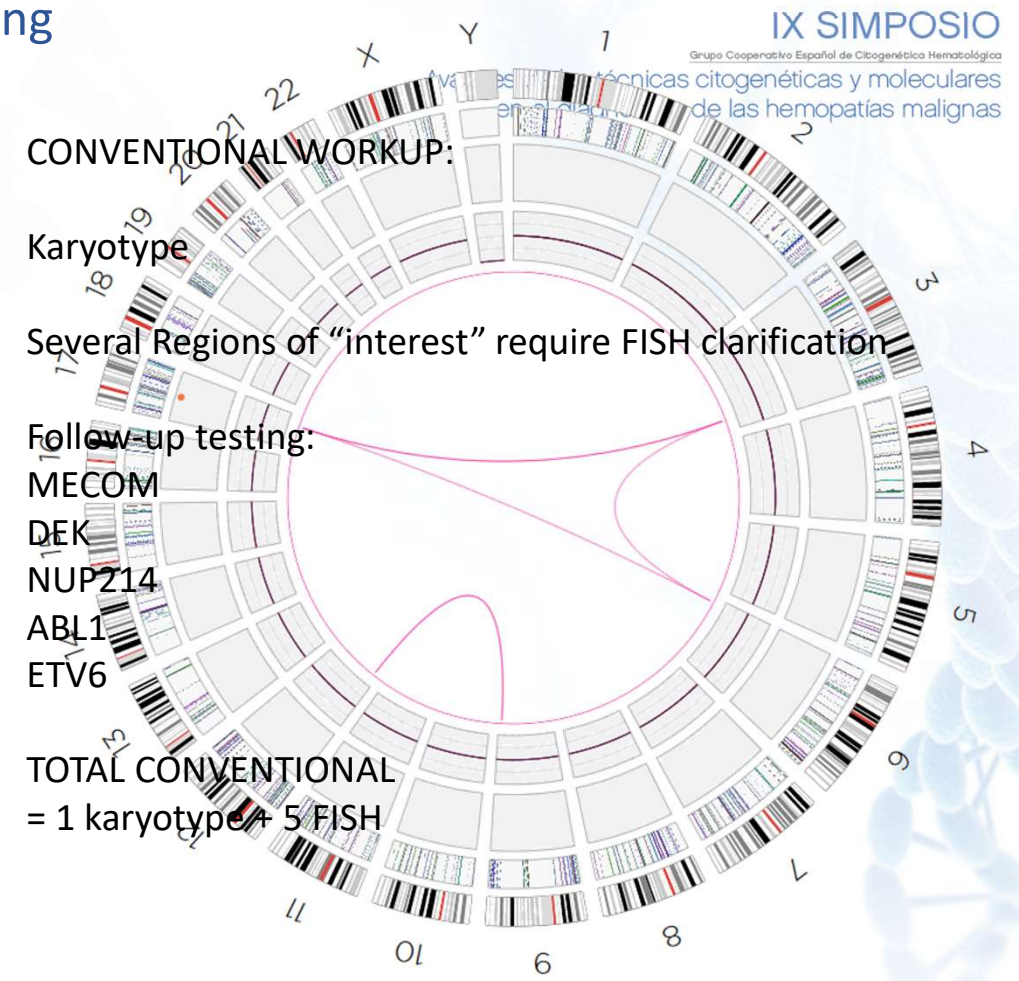
CONVENTIONAL WORKUP:

Karyotype

Several Regions of "interest" require FISH clarification

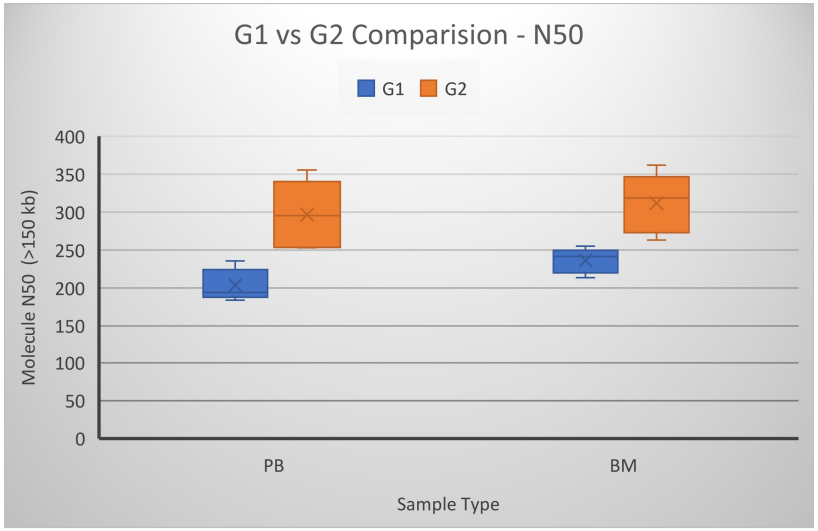
Follow-up testing:
MECOM
DEK
NUP214
ABL1
ETV6

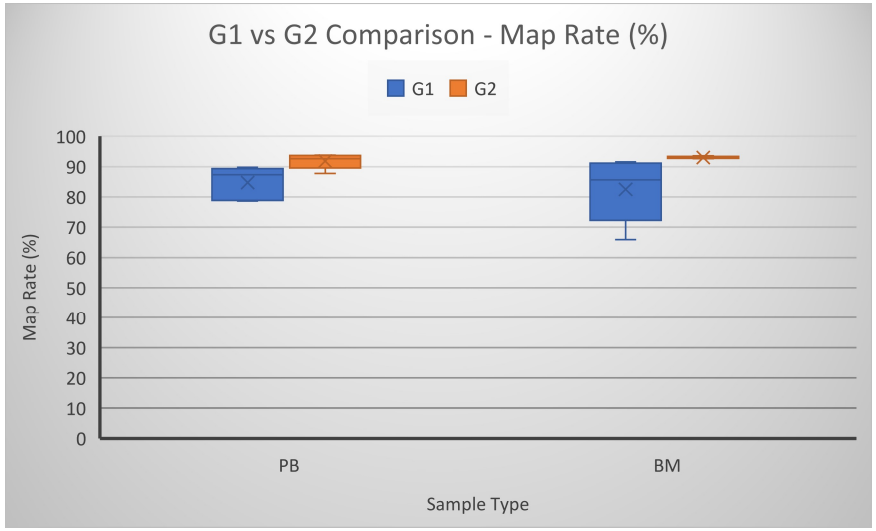
TOTAL CONVENTIONAL
= 1 karyotype + 5 FISH

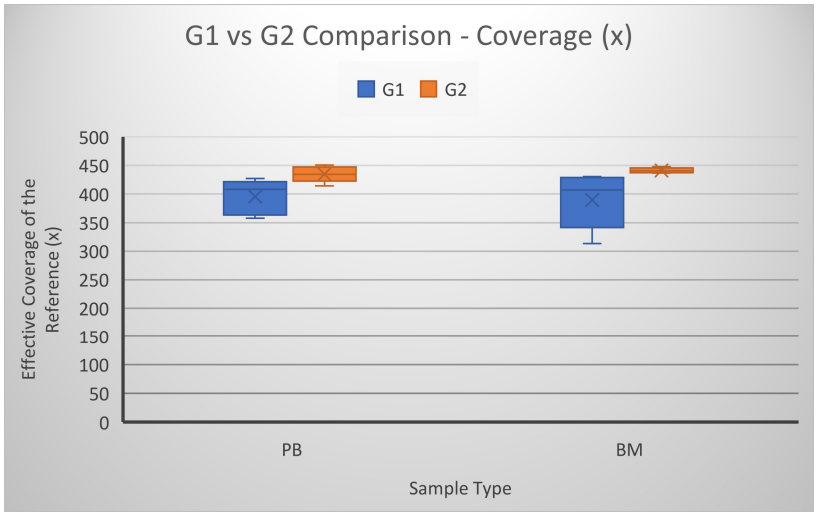


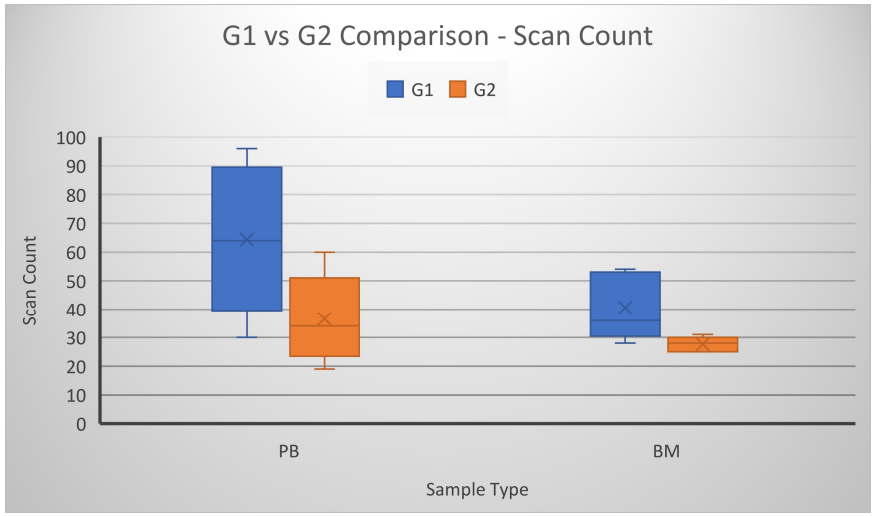
KARYOTYPE AFTER OGM
46,XX,t(3;5;17)(MSI2::MECOM),t(9;12)(ETV6::ABL1)
VAF 0.45-0.48

G1 Versus G2 Chemistry Comparison









ELN AML Risk Stratification - 2017 versus 2022

Table 5. 2017 ELN risk stratification by genetics

Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}†
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}†
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)*
	−5 or del(5q); −7; −17/abn(17p)
	Complex karyotype,§ monosomal karyotype‖
	Wild-type *NPM1* and *FLT3*-ITD^{high}†
	Mutated *RUNX1*¶
	Mutated *ASXL1*¶
	Mutated *TP53*#

Table 6. 2022 European LeukemiaNet (ELN) risk classification by genetics at initial diagnosis[a]

Risk Category[b]	Genetic Abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*[b,c] • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*[b,c] • Mutated *NPM1*[b,d] without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*[e]
Intermediate	• Mutated *NPM1*[b,d] with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD • t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*[b,f] • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23;q34.1)/*DEK*::*NUP214* • t(v;11q23.3)/*KMT2A*-rearranged[g] • t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • t(8;16)(p11;p13)/*KAT6A*::*CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*, *MECOM(EVI1)* • t(3q26.2;v)/*MECOM(EVI1)*-rearranged • -5 or del(5q); -7; -17/abn(17p) • Complex karyotype,[h] monosomal karyotype[i] • Mutated *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*[j] • Mutated *TP53*[k]

The devil is in the "supplemental table 6" details…

REVIEW ARTICLE | JUNE 29, 2022

International Consensus Classification of Myeloid Neoplasms and Acute Leukemia: Integrating Morphological, Clinical, and Genomic Data

Daniel A. Arber ✉, Attilio Orazi, Robert P. Hasserjian, Michael J. Borowitz, Katherine R Calvo, Hans Michael Kvasnicka, Sa A. Wang, Adam Bagg, Tiziano Barbui, Susan Branford, Carlos E. Bueso-Ramos, Jorge Cortes, Paola Dal Cin, Courtney D. DiNardo, Hervé Dombret, Eric J Duncavage, Benjamin L. Ebert, Elihu Estey, Fabio Facchetti, Kathryn Foucar, Naseema Gangat, Umberto Gianelli, Lucy A. Godley, Nicola Goekbuget, Jason R. Gotlib, Eva Hellström-Lindberg, Gabriela Hobbs, Ronald Hoffman, Elias J. Jabbour, Jean-Jacques Kiladjian, Richard A. Larson, Michelle M. Le Beau, Mignon L. Loh, Bob Löwenberg, Elizabeth A. Macintyre, Luca Malcovati, Charles G. Mullighan, Charlotte M Niemeyer, Olatoyosi Odenike, Seishi Ogawa, Alberto Orfao, Elli Papaemmanuil, Francesco Passamonti, Kimmo Porkka, Ching-Hong Pui, Jerald P Radich, Andreas Reiter, María Rozman, Martina Rudelius, Michael R Savona, Charles Schiffer, Annette Schmitt-Graeff, Akiko Shimamura, Jorge Sierra, Wendy Stock, Richard M. Stone, Martin S. Tallman, Juergen Thiele, Hwei-Fang Tien, Alexandar Tzankov, Alessandro M. Vannucchi, Paresh Vyas, Andrew H. Wei, Olga K. Weinberg, Agnieszka Wierzbowska, Mario Cazzola, Hartmut Döhner, Ayalew Tefferi

- While the primary prognostic classification may not have changed much, many new recurrent rearrangements are now recognized.
- Some of these rearrangements are cryptic.
- Cryptic translocations and variants of these rearrangements can be difficult to confirm/detect without specific FISH probes.

Supplemental Table 6. Acute myeloid leukemia (AML) with other rare recurring translocations

AML with t(1;3)(p36.3;q21.3)/*PRDM16::RPN1*	
AML with t(3;5)(q25.3;q35.1)/*NPM1::MLF1*	
AML with t(8;16)(p11.2;p13.3)/*KAT6A::CREBBP*	
AML (megakaryoblastic) with t(1;22)(p13.3;q13.1)/*RBM15::MRTF1**	
AML with t(5;11)(q35.2;p15.4/ *NUP98::NSD1**	CRYPTIC
AML with t(11;12)(p15.4;p13.3)/*NUP98::KMD5A**	CRYPTIC
AML with *NUP98* and other partners*	?CRYPTIC
AML with t(7;12)(q36.3;p13.2)/*ETV6::MNX1**	CRYPTIC
AML with t(10;11)(p12.3;q14.2)/*PICALM::MLLT10*	
AML with t(16;21)(p11.2;q22.2)/*FUS::ERG*	
AML with t(16;21)(q24.3;q22.1)/*RUNX1::CBFA2T3*	
AML with inv(16)(p13.3q24.3)/*CBFA2T3::GLIS2**	CRYPTIC

And don't forget the RARA variants in APL…

t(1;17)(q42.3;q21.2)/IRF2BP2::RARA;
t(5;17)(q35.1;q21.2)/NPM1::RARA;
t(11;17)(q23.2;q21.2)/ZBTB16::RARA;
cryptic inv(17q) or del(17)(q21.2q21.2)/STAT5B::RARA, STAT3::RARA;
Other genes rarely rearranged…
RARA:TBL1XR1 (3q26.3), FIP1L1 (4q12), BCOR (Xp11.4)

AML Workflow – Karyotype and FISH (Reflex)

The Standard Reflex Strategy

3-5 days (Prelim)
5-14 days (Final)

Based on morphology

Acute Myeloid Leukemia → KARYOTYPE → FISH

CBF – Based on Morphology

- t(8;21)
- t(15;17)
- Inv(16)

The Upfront FISH/PCR with Reflex Strategy

3-5 days (Prelim)
5-14 days (Final)

Based on morphology

Acute Myeloid Leukemia → KARYOTYPE → FISH (reflex as needed)

Acute Myeloid Leukemia → FISH (upfront)

Standard Reflex (Fail + Inc)

- KMT2A Rearrangement
- Deletion 5q/Monosomy 5
- Deletion 7q/Monosomy 7

"Optional" Reflex – to confirm karyotype finding, clinical suspicion or always…

- RARA Break Apart
- DEK::NUP214
- MECOM Rearrangement
- Trisomy 8
- Deletion 20q
- Deletion 17p
- ETV6 Rearrangement

Why conventional analysis won't scale for hematologic malignancies…→

cost

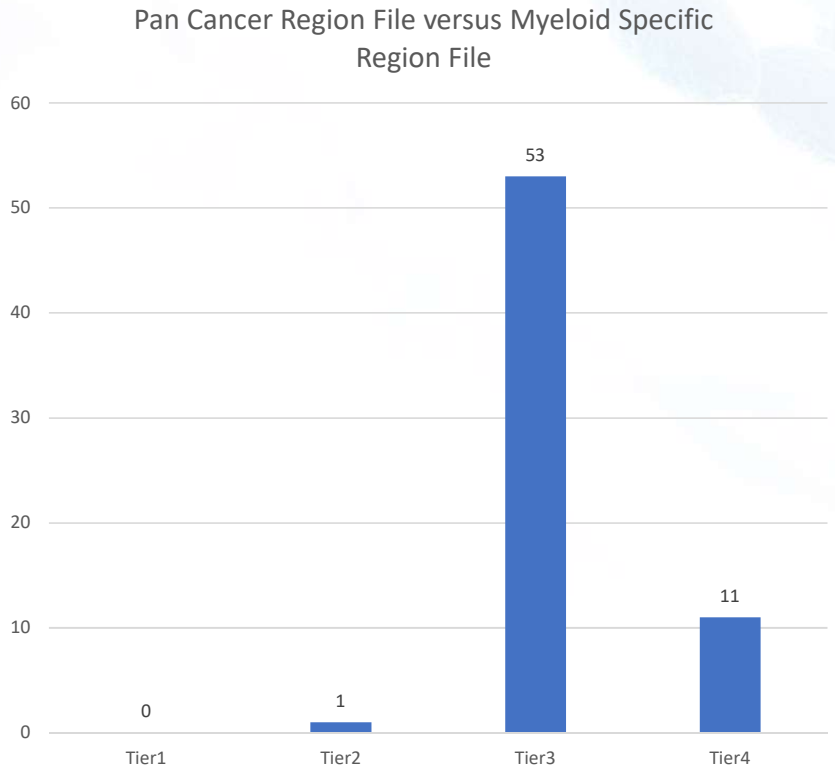
workload

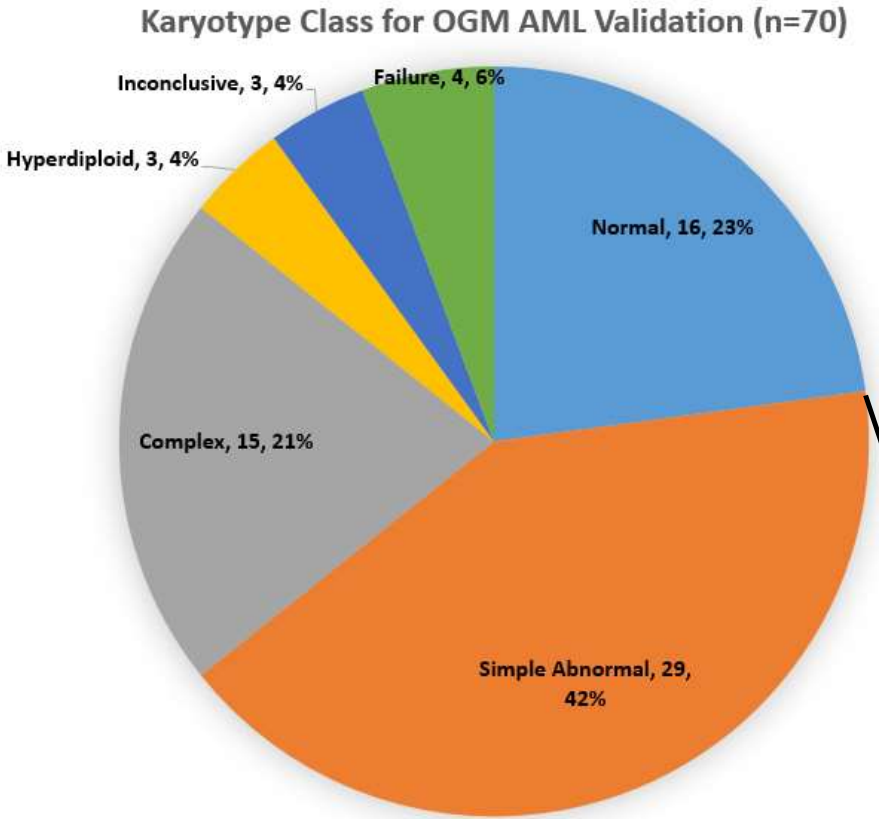
Clinical Validation

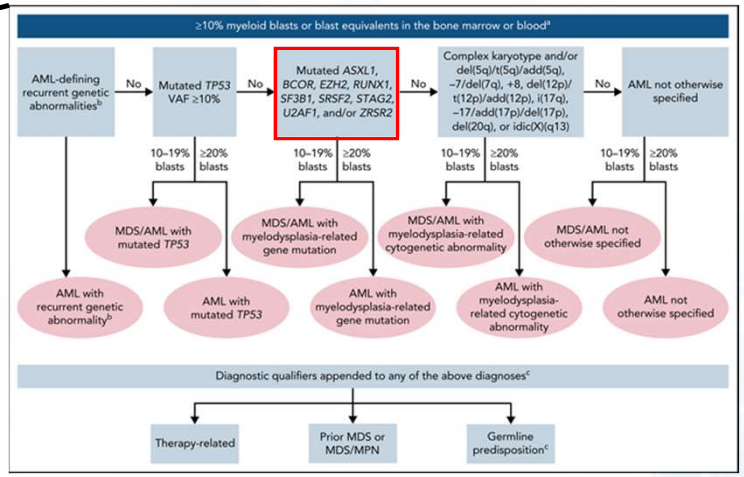
- Clinical Validation on patients with Acute Myeloid Leukemia
- 70 patients (mostly prospectively collected, although a few samples from the Princess Margaret Leukemia Tissue Bank were used to represent rare and challenging samples).
- Abnormalities Detected in 70 Patient Cohort:
  - Standard of Care (largely karyotype and FISH) detected 150 abnormalities
  - OGM detected 186 abnormalities that met reporting criteria.
- Therefore, the overall Diagnostic Utility of OGM compared to Standard of Care is an increase of 10.29%
- Cases where OGM detected a Tier 1 or Tier 2 biomarker that was missed by Standard of Care: 36% of cases.

Filtering with a Region (BED) File

- After evaluating large SVs and copy number abnormalities a region specific filtering approach can be used to target SVs for reporting.
- Can identify important Tier 1/Tier 2 biomarkers in a complex genome.
- Can reduce interpretation of non-relevant SVs.
  - In a cohort of 70 AML patients, use of a myeloid specific region file (~150 targets) reduced the number of SVs that were interpreted by approx. 1 SV per sample.
  - The overwhelming majority were Tier 3.
  - One Tier 2, a deletion in BRCA2 was eliminated as it was not part of the myeloid region file.
  - No Tier 1 abnormalities were removed.

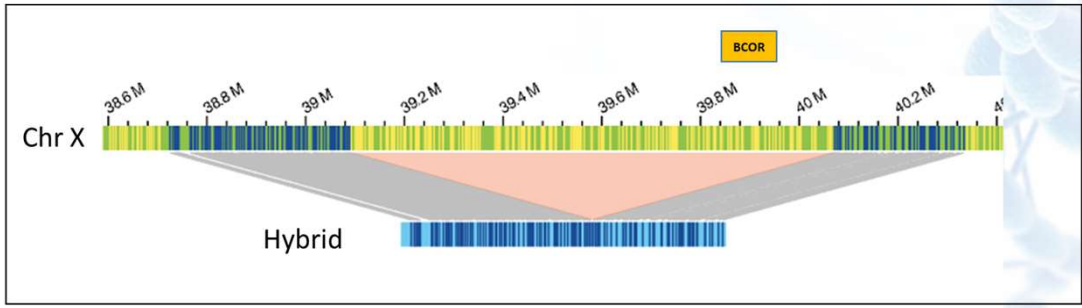






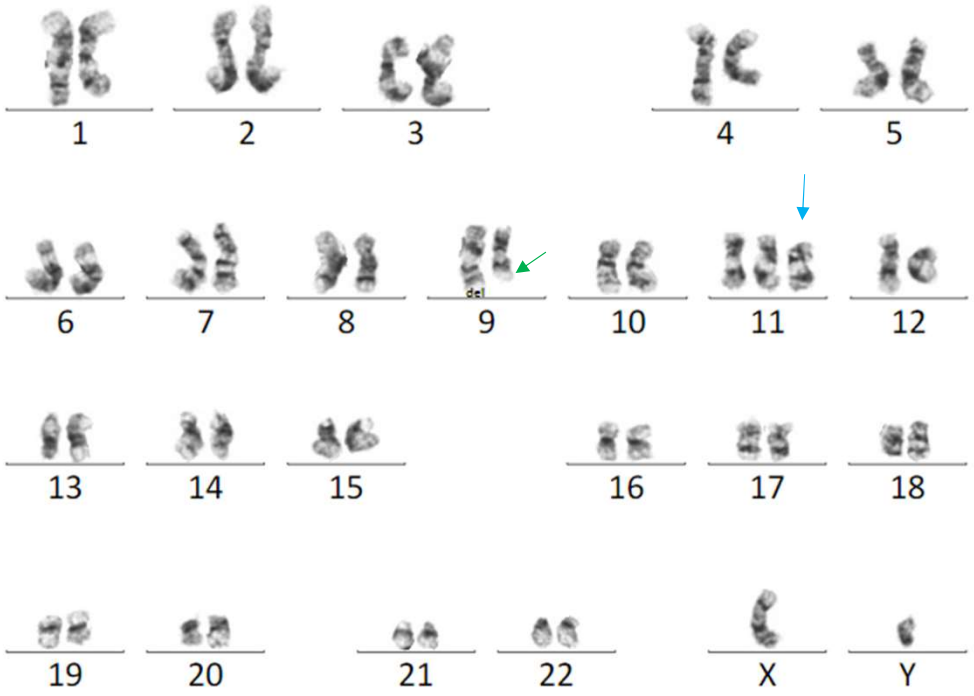
Example 1: Karyotype: 46,XY[20]

BCOR deletion (chr X) in XY patient with 90% loss of BCOR region.

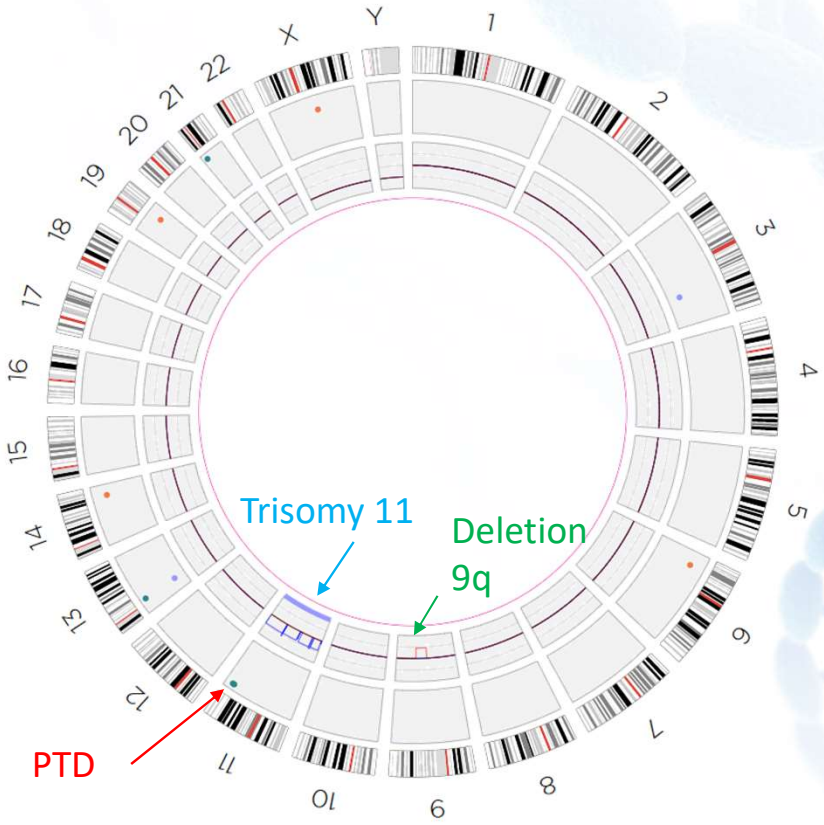


Δ in DIAGNOSIS, Δ in PROGNOSIS

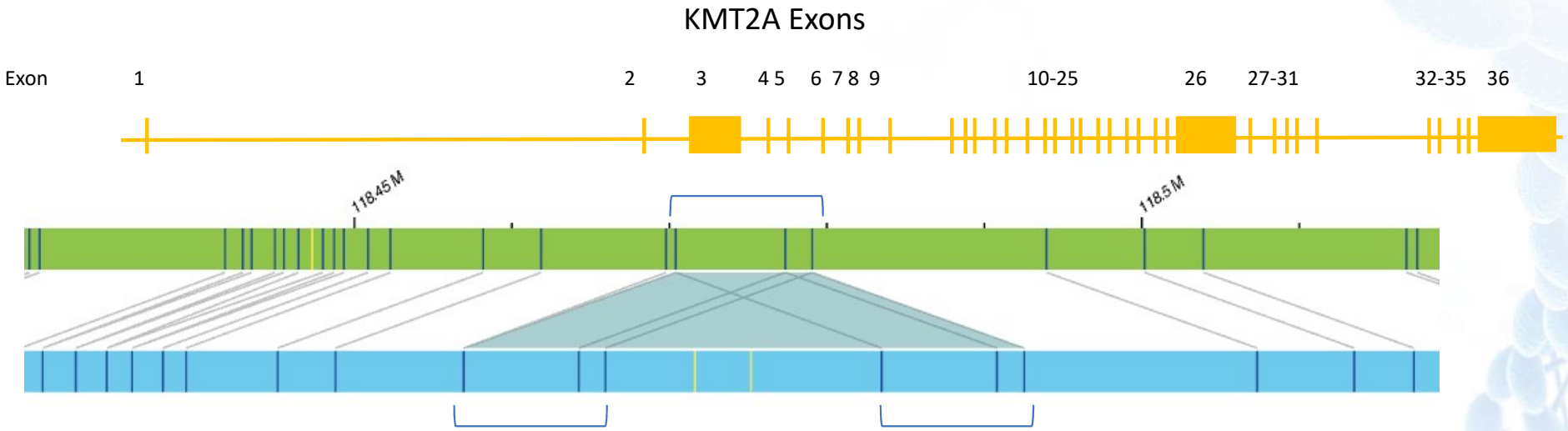
What does a KMT2A-PTD Look Like on OGM?



47,XY,del(9)(q13q22),+11[10]



Genome View – 11q23



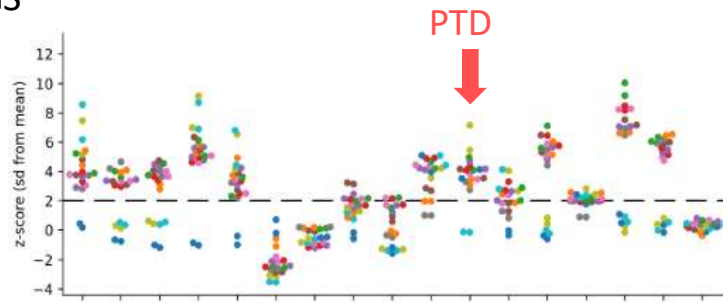
ogm ins(11;?)(q23.3;?)

KMT2A-PTDs range in size from approximately 20kb to 50kb (in our experience). They are detected by the SV pipeline in OGM, not the copy number pipeline. SV pipeline 5kb or greater (unbal SV), CNV >500kb

Classified by Karyotype Studies as a "Simple Abnormal" Karyotype

Karyotype: 48,XY,+8,+19[20]

NGS



OGT Software:

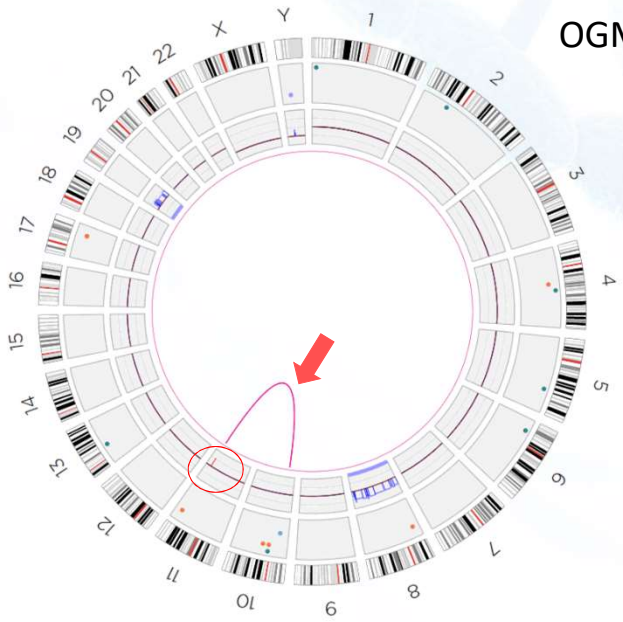
- PTD positive exons 2-11
- High confidence

MLPA

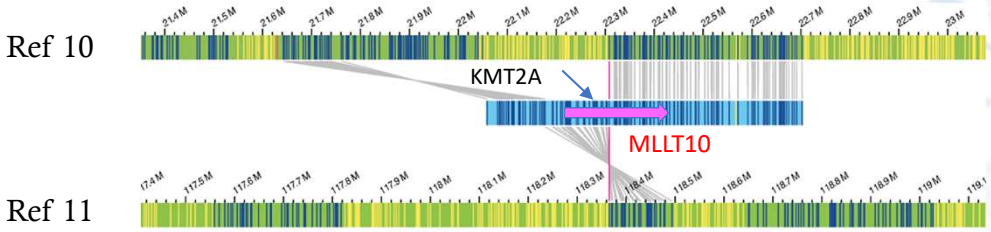
	Probe Name	Bin Size	Height Ratio
21	ETS1_11q24.3	181.6	0.963
31	KMT2A_11q23.3	185.9	0.558
32	KMT2A_11q23.3_2	196.7	0.998
53	TIRAP_11q24.2	331.2	1.013

- No PTD
  - 1 copy exon 36
  - 2 copies exon 4

OGM



ogm (8)x3,t(10;11)(KMT2A::MLLT10),ins(11;?)(q23.3;?),(19)x3

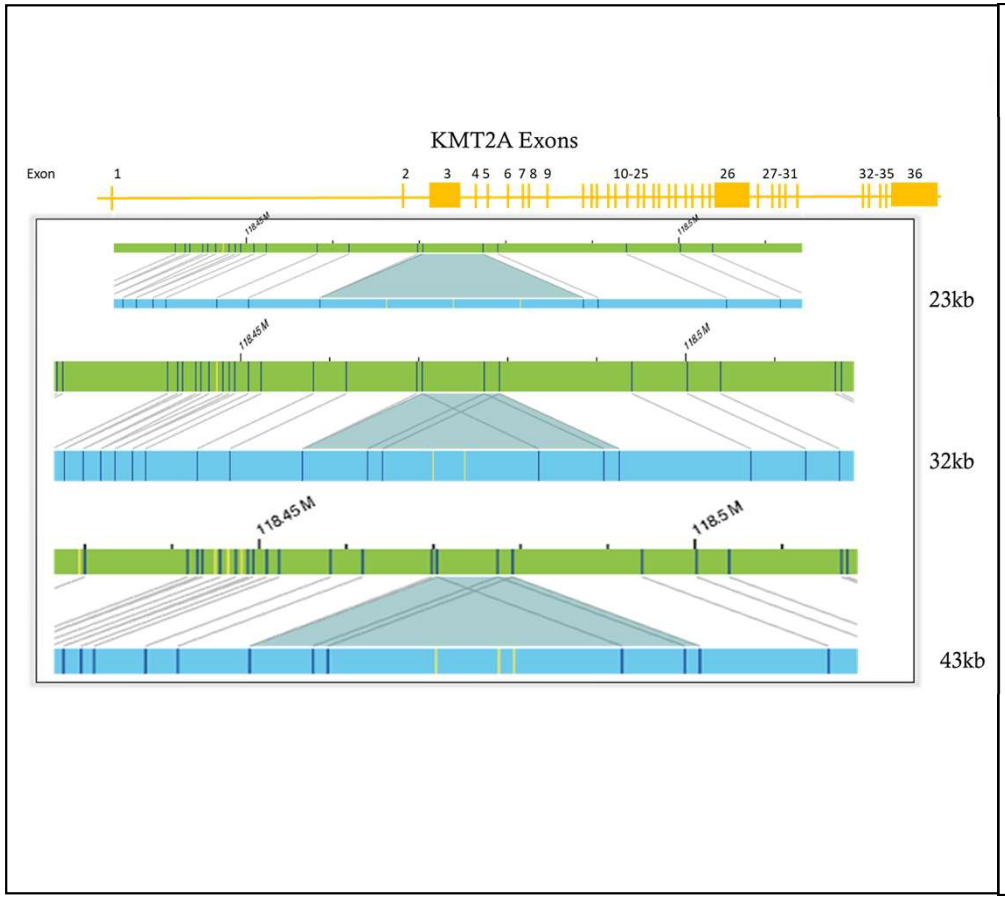


Deletion 5'-KMT2A and cryptic insertion of MLLT10

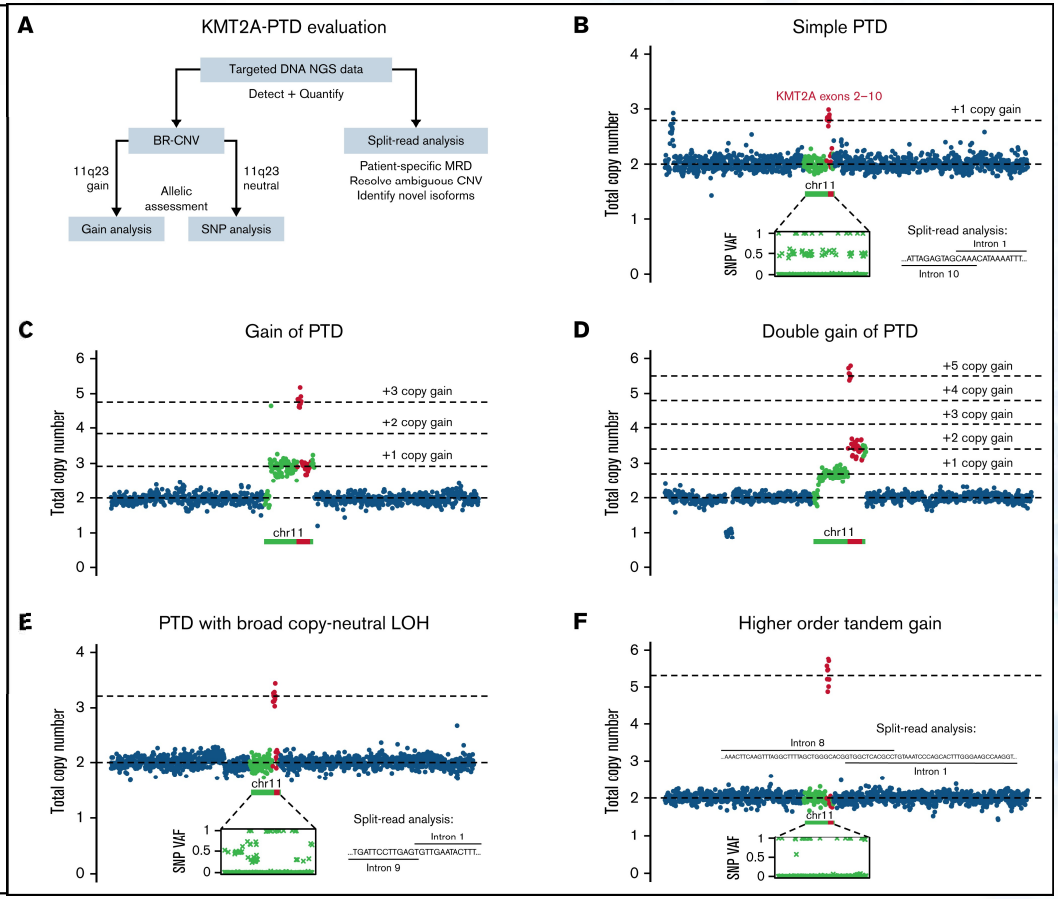
OGM Resolves Discordant NGS/MLPA Results for PTDs

	Classical Cytogenetics		NGS			MLPA				OGM
Case	G-banding	KMT2A FISH	PTD	Length	Avg Z-score	PTD	exon4/exon 36	exon 4	exon 36	KMT2A-PTD
1	47,XY,+11[19]/46,XY[1]	N/T	Yes	exons 2-8	3.56	Yes	1.3	1.8	1.3	N/T
2	47,XY,del(11)(p11.2p15),+del(11)[13]/48,XY,+11,+13[6]/46,XY[2]	Negative	Yes	exons 2-8	2.87	Yes	1.5	2.0	1.4	N/T
3	46,XY[24]	Negative	Yes	exons 3-9	3.14	Yes	1.3	1.3	1.0	N/T
4	46,XX,del(12)(p12p13)[22]	Negative	Yes	exons 2-8	5.53	Yes	2.1	2.2	1.0	N/T
5	46,XY[20]	Negative	Yes	exons 2-8	3.98	Yes	1.6	1.6	1.0	N/T
6	46,XY[11]	N/T	Yes	exons 3-10	2.54	Yes	1.3	1.4	1.0	N/T
7	46,XX[21]	Negative	Yes	exons 3-8	2.57	Yes	1.4	1.5	1.0	N/T
8	46,XY,inv(7)(q11.2q22)?c[22]	Negative	Yes	exons 1-7	5.11	Yes	1.5	1.6	1.0	N/T
9	Inconclusive	Negative	Yes	exons 2-10	2.65	Yes	2.1	2.3	1.1	N/T
10	46,XY[20]	N/T	Yes	exons 2-8	3.36	Yes	1.6	1.6	1.0	N/T
11	46,XY[20]	N/T	Yes	exons 3-11	3.64	Yes	1.9	1.9	1.0	N/T
12	46,XX[21]	N/T	Yes	exons 1-8	5.15	Yes	1.8	1.9	1.0	N/T
13	46,XY,del(7)(q22q32)[17]/46,XY[3]	N/T	Yes	exons 2-8	2.75	Yes	1.7	1.9	1.1	N/T
14	46,XY[22]	Negative	Yes	exons 3-8	3.45	Yes	1.5	1.5	1.0	N/T
15	Inconclusive	Negative	Yes	exons 2-8	4.44	Yes	1.9	1.9	1.0	N/T
16	46,XY,+1,der(1;14)(q10;q10)[15]/46,XY[5]	N/T	Yes	exons 4-8	2.78	Yes	1.3	1.3	1.0	N/T
17	46,XY[20]	Negative	Yes	exons 3-7	2.74	Yes	1.6	1.5	1.0	N/T
18	46,XY[20]	Negative	Yes	exons 3-6	2.81	Yes	1.4	1.4	1.0	N/T
19	47,XY,del(9)(q13q22),+11[10]	N/T	Yes	exons 1-10	8.07	Yes	1.6	2.1	1.3	Yes
20	46,XX[20]	N/T	Yes	exons 2-8	4.14	Yes	2.7	2.0	0.7	Yes
21	45,XX,-7[5]/49,XX,+8,+13,+22[1]/46,XX[17]	N/T	Yes	exons 2-10	4.84	Yes	1.9	1.9	1.0	Yes
22	N/T	Negative	Yes	exons 3-10	4.24	Yes	1.1	1.0	0.9	Yes
23	46,XY[20]	N/T	Yes	exons 2-4	2.56	Yes	1.6	1.6	1.0	Yes
24	46,XY,del(11)(p11.2p15)[19]/46,XY[1]	Negative	Yes	exons 2-10	5.72	Yes	1.8	1.8	1.0	No
25	Inconclusive	Positive	Yes	exons 3-11	2.95	Inconclusive	1.7	1.1	0.6	No
26	46,XY,20,+21[8]/46,idem,der(3)inv(3)(p23q27)inv(3)(q?21q26.2)[12]	N/T	Yes	exons 3-10	2.85	Inconclusive	2.2	1.1	0.5	N/T
27	48,XY,+8,+19[20]	N/T	Yes	exons 1-9	2.91	Inconclusive	1.8	1.0	0.6	No
28	45,XX,-7[10]/46,XX[11]	N/T	Yes	exon 3	3.26	No	1.0	1.0	1.0	N/T
29	46,XY,i(7)(p10),der(16)t(11;16)(q13;q24)[2]/48,sl,+4,+10[7]/49,sdl1,+8[6]/46,XY[5]	Negative	Yes	exon 1	3.55	No	1.0	1.4	1.4	N/T
30	39~41,X,-Y,add(3)(p12),add(3)(q11.2),-5,der(7;22)(q10;q10),-11,-12,add(12)(q21),-17,add(19)(q13.3),add(21)(p11.2),-22,+mar1,+mar2,1dmin[cp6]/46,XY[14]	N/T	Yes	exons 5-11	2.86	No	0.9	0.9	1.0	N/T
31	46,XX[24]	N/T	Yes	exon 8	5.78	No	0.9	1.0	1.0	N/T
32	47,XY,+11[5]/46,XY[21]	N/T	Yes	exons 5-11	3.16	No	1.1	1.1	1.0	N/T

Allelic complexity of KMT2A partial tandem duplications

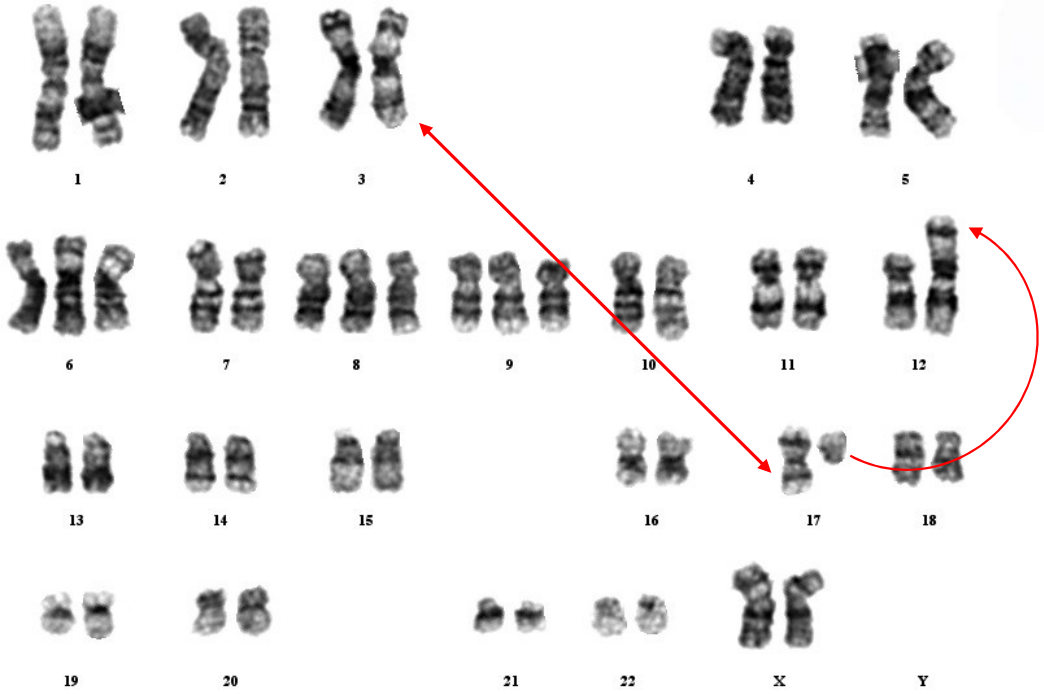


Some examples of different sized insertions in KMT2A detected by OGM

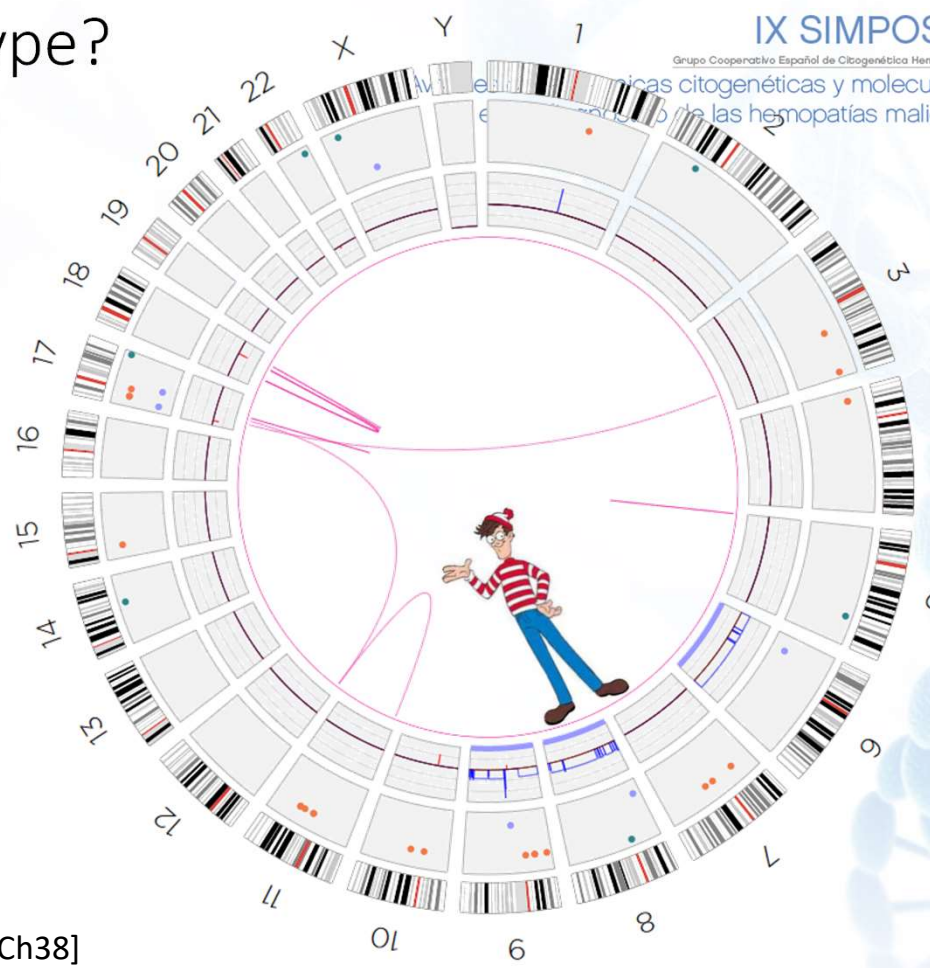


B) Single copy PTD, C) dup(11q) with 2 extra PTD copies, D) complex karyotype with extra PTD copies, E) copy neutral LOH, F) LOH plus higher order gain of PTD.

What's hiding in your complex karoytype?

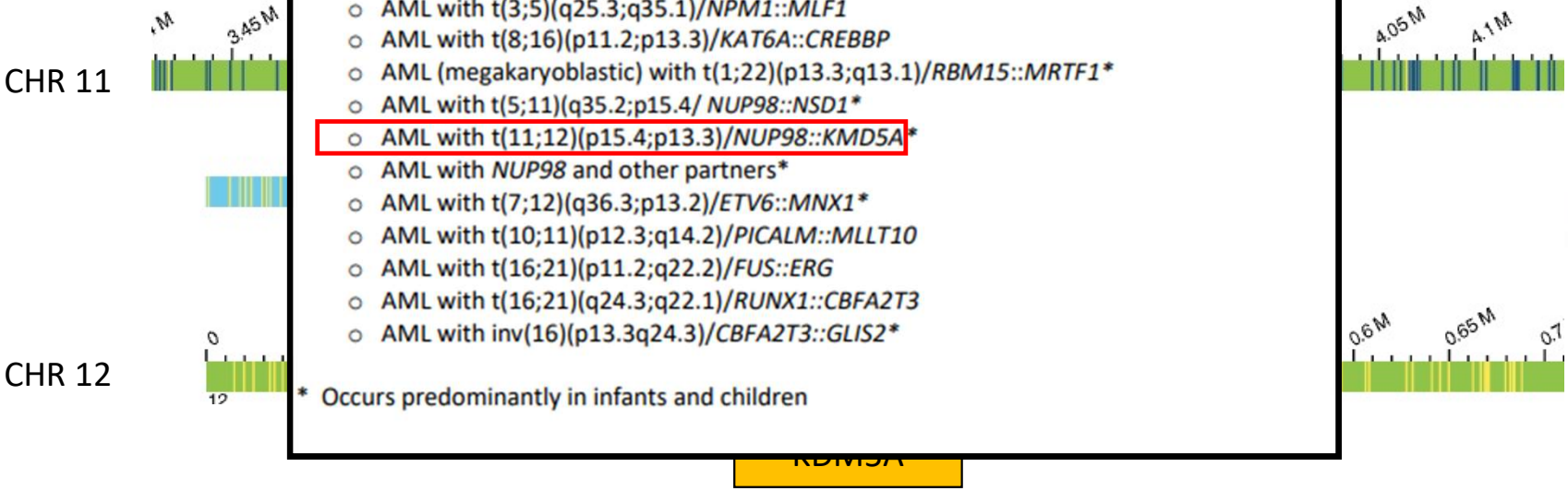


Karyotype:
48~49,XX,+6,+8,+9,t(12;17)(p13;q11.2),
i(17)(q10),inv(18)(q11.2q21)[cp20]



ogm[GRCh38]
t(3;17)(q25.2;p11.2),+6,+8,+9,t(11;12)(p15.4;p13.33),t(12;17)(p13.33;q11.2),
fus(17;17)(q12;q12),fus(18;18)(q11.2;q21.2-q21.33)

NUP98 Rearrangements are often cryptic



Supplemental Table 6. Acute myeloid leukemia (AML) with other rare recurring translocations

- AML with t(1;3)(p36.3;q21.3)/*PRDM16*::*RPN1*
- AML with t(3;5)(q25.3;q35.1)/*NPM1*::*MLF1*
- AML with t(8;16)(p11.2;p13.3)/*KAT6A*::*CREBBP*
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.1)/*RBM15*::*MRTF1**
- AML with t(5;11)(q35.2;p15.4/ *NUP98*::*NSD1**
- AML with t(11;12)(p15.4;p13.3)/*NUP98*::*KMD5A**
- AML with *NUP98* and other partners*
- AML with t(7;12)(q36.3;p13.2)/*ETV6*::*MNX1**
- AML with t(10;11)(p12.3;q14.2)/*PICALM*::*MLLT10*
- AML with t(16;21)(p11.2;q22.2)/*FUS*::*ERG*
- AML with t(16;21)(q24.3;q22.1)/*RUNX1*::*CBFA2T3*
- AML with inv(16)(p13.3q24.3)/*CBFA2T3*::*GLIS2**

* Occurs predominantly in infants and children

Dx = AML – MRC

Cytogenetics:

46,XY,del(5)(q31q35)[19]/46,XY[1]

FISH:

EGR1 (5q31): Negative

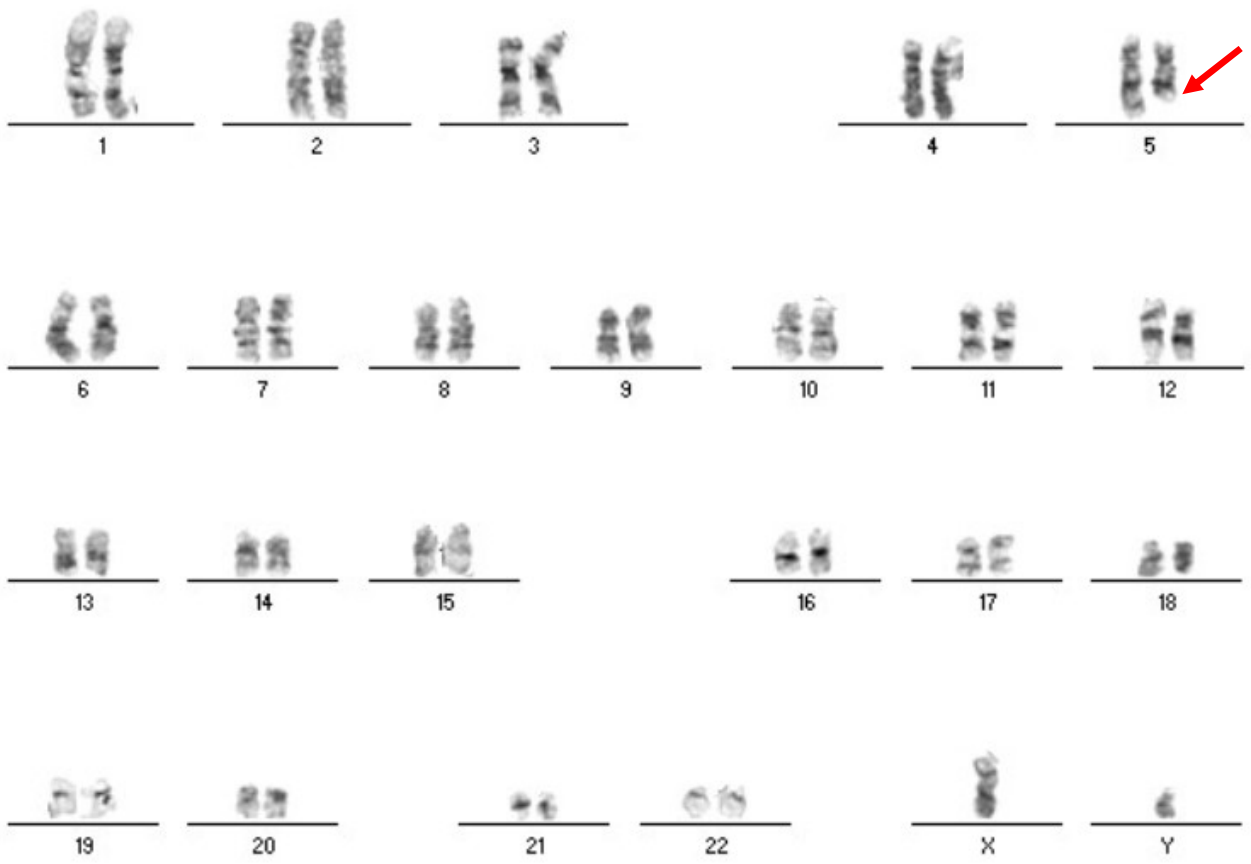
PDGFRB (5q32): Positive for 5’ deletion

Molecular:

FLT3-ITD +ve (1.92%)

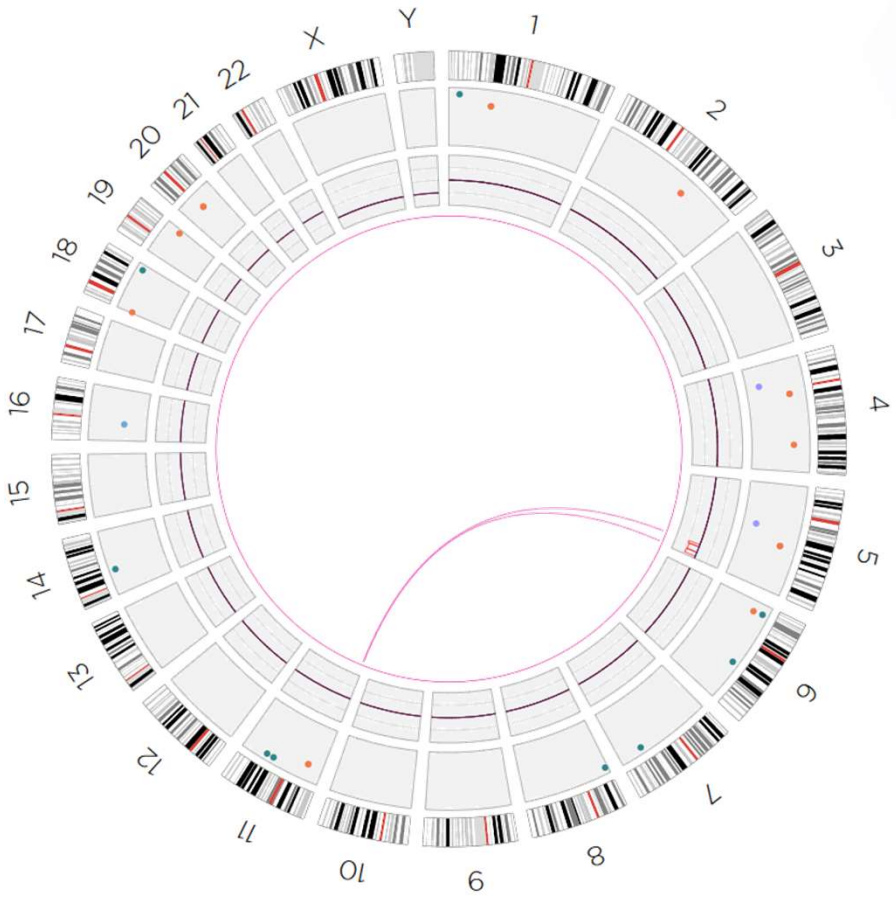
NGS:

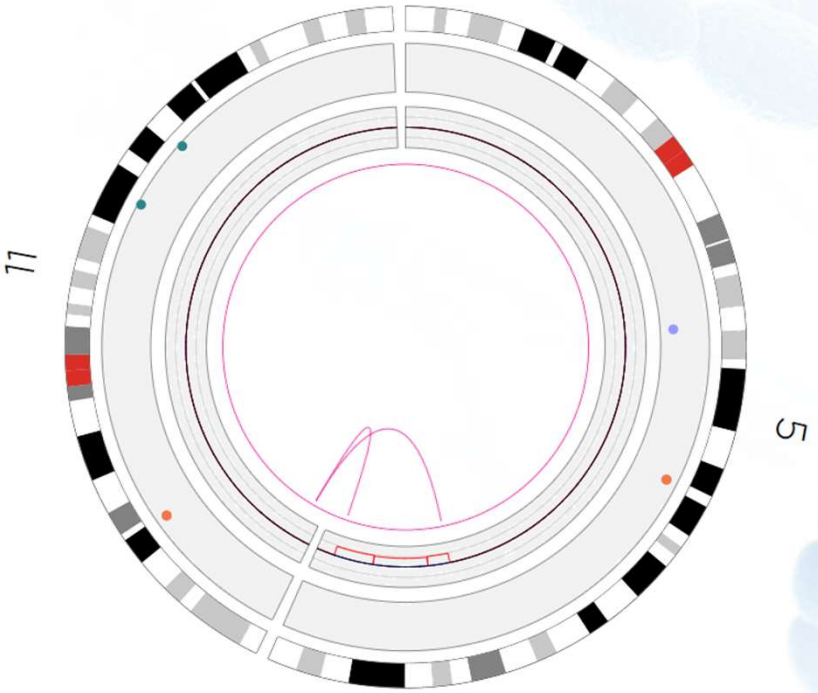
WT1 c.1156_1159dupTCGG (39%)



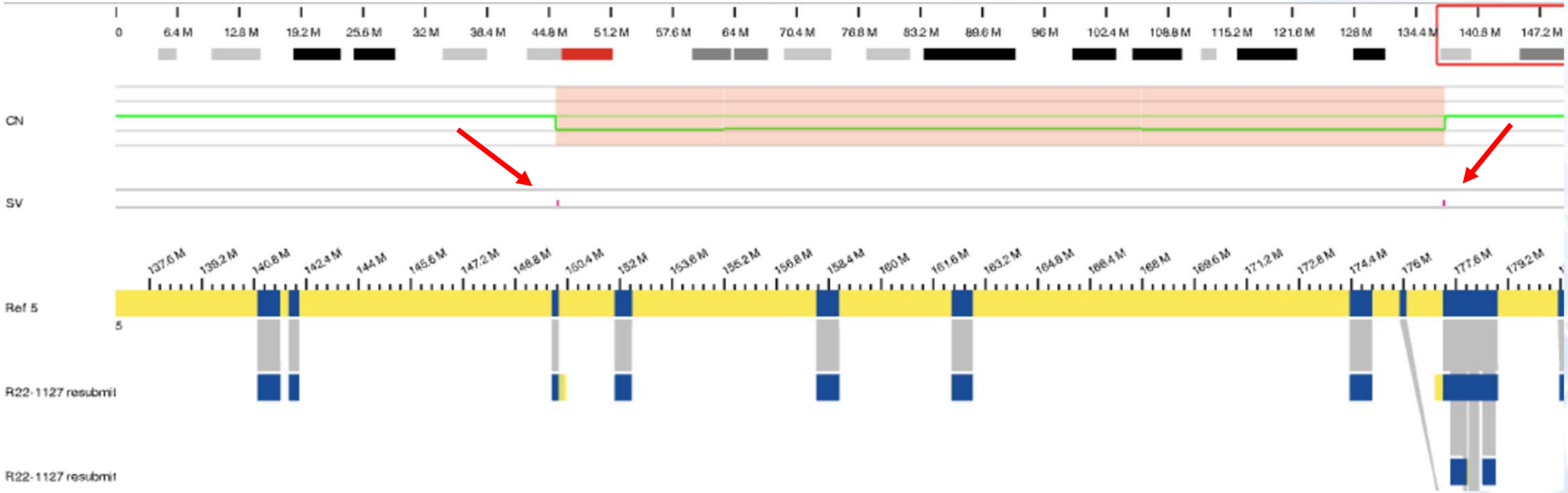
Conventional work-up required 1 karyotype and 2 FISH….and was still inaccurate

Del(5q) with Cryptic Translocation

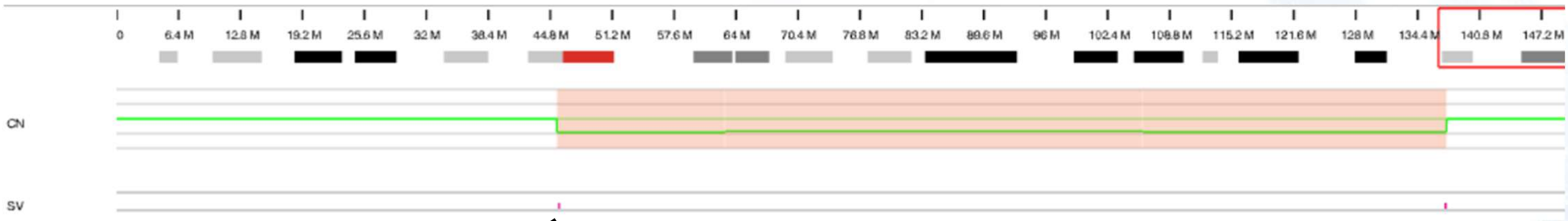




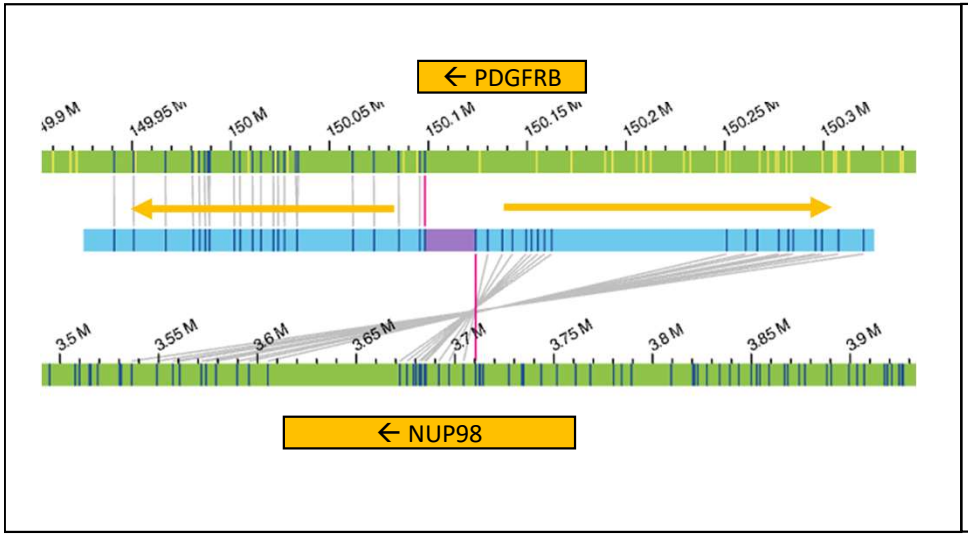
OGM reveals “deletion bridges” between rearrangement breakpoints…



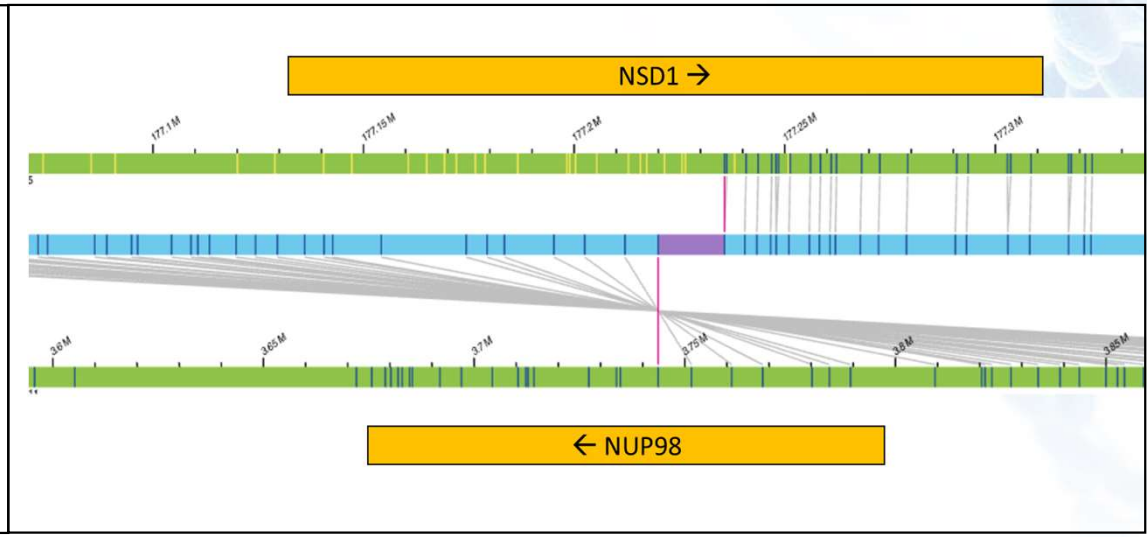
Gene Orientation is Critical to Determining Clinical Significance



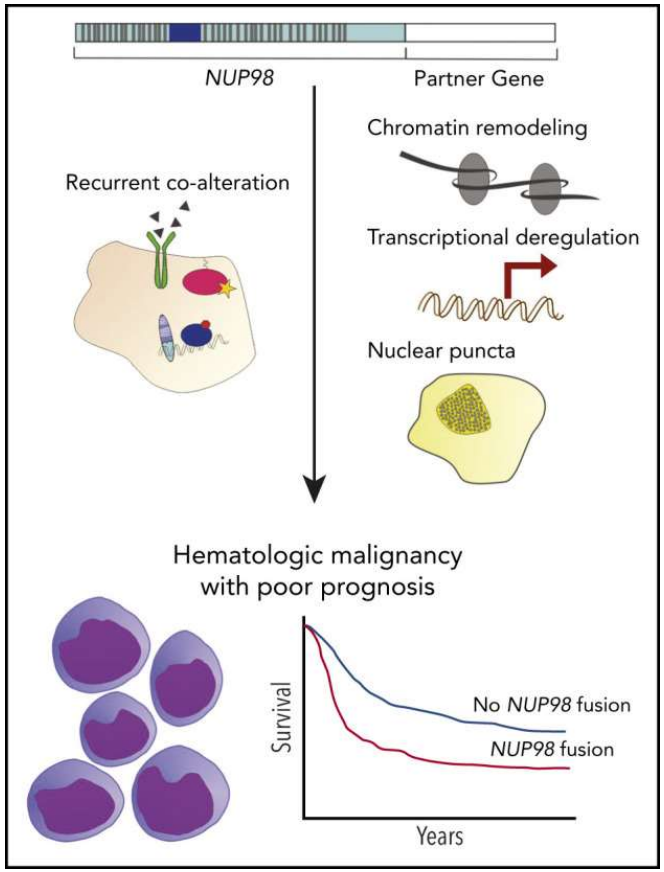
Centromeric Breakpoint

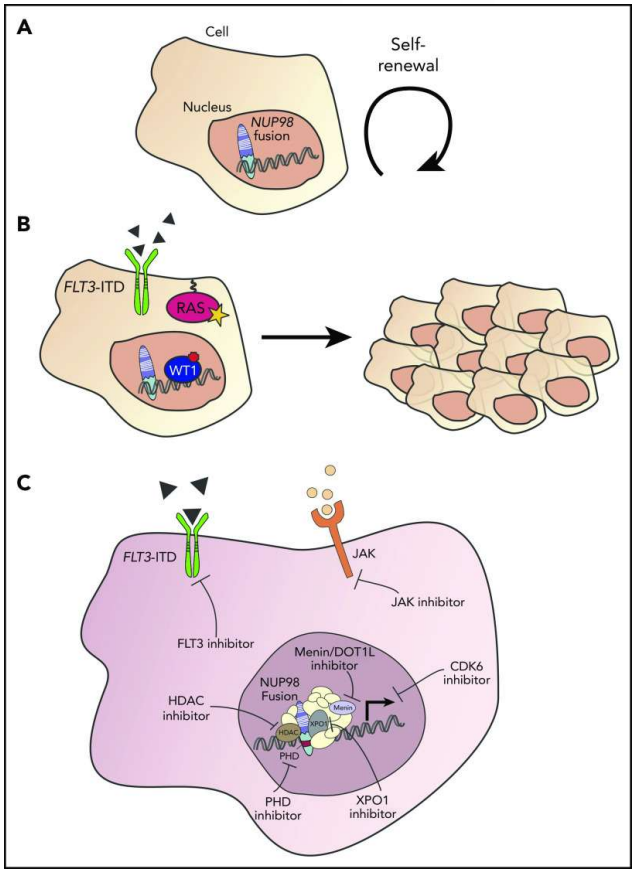


Telomeric Breakpoint



Improved Profiling will enable better targeted therapy





Michmerhuizen NL, Klco JM, Mullighan CG. Mechanistic insights and potential therapeutic approaches for NUP98-rearranged hematologic malignancies. Blood. 2020 Nov 12;136(20):2275-2289. PMID: 32766874

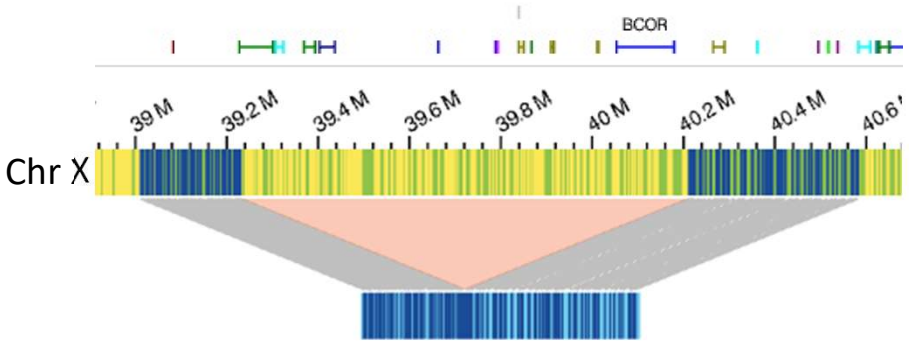
Table 4.

Recurrent coalterations with *NUP98* gene fusions

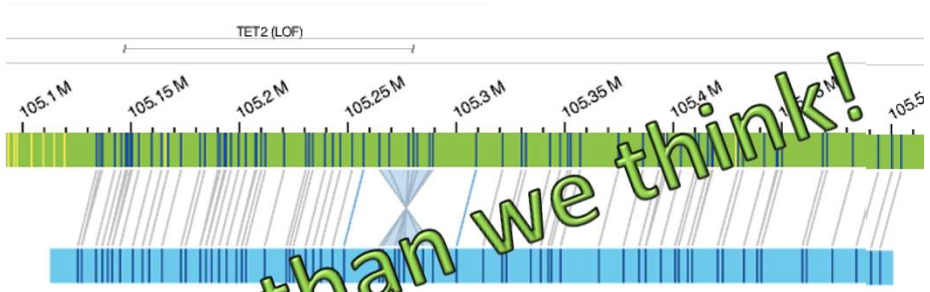
Coalteration	Estimated frequency
FLT3-ITD mutation	48%-92% *NUP98-NSD1*[31,34,59,60,70] 7%-27% *NUP98-HOXA9*[65,66]
WT1 mutation	33%-55% *NUP98-NSD1*[59,60,131] 44% *NUP98-HOXA9*[66]
NRAS mutation	11%-29% *NUP98-NSD1*[59,60,131] 22% *NUP98-HOXA9*[66]
KRAS mutation	11%-17% *NUP98-NSD1*[59,60] 22% *NUP98-HOXA9*[66]
RB1 loss	80%-100% *NUP98-KDM5A*[3,62]
BCR-ABL fusion	
CEPBA mutation	
NOTCH1 mutation	
MYC mutation	
KIT mutation	
ASXL1 mutation	
Trisomy 8	

For the most common coalterations, estimated frequencies wer

The “SV Gap” Between Cytogenetics and Molecular

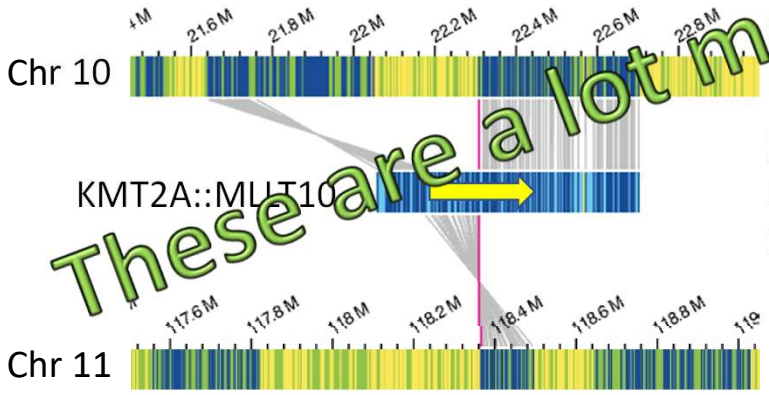


Xp11.4(39230013_40212446)x1[VAF 0.880] (BCOR deletion) ~ 1 MB



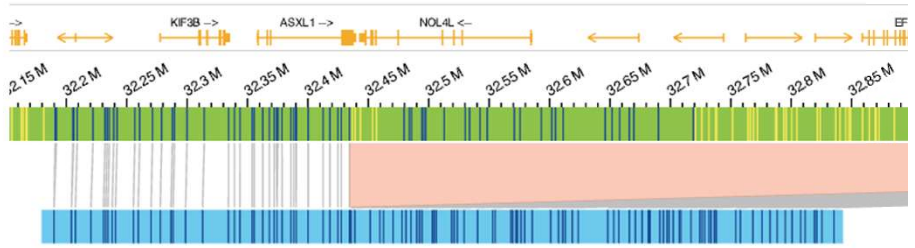
n(4)(q24q24)(TET2, exons 9-11) 38,262 bp

These are a lot more common than we think!



Not seen by karyotype or FISH

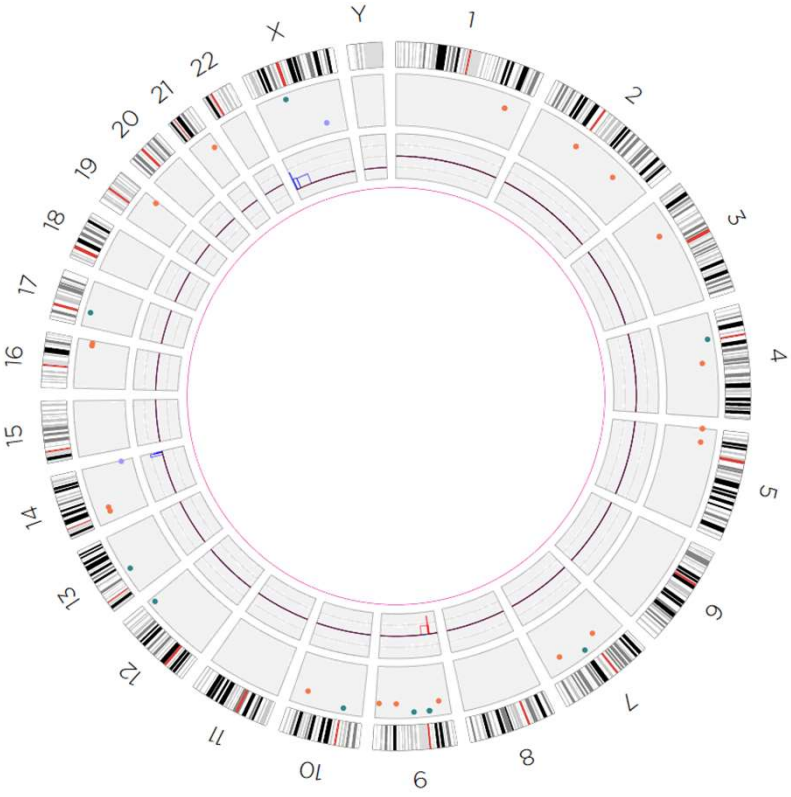
ins(11;10)(q23.3;p12.31p12.31)

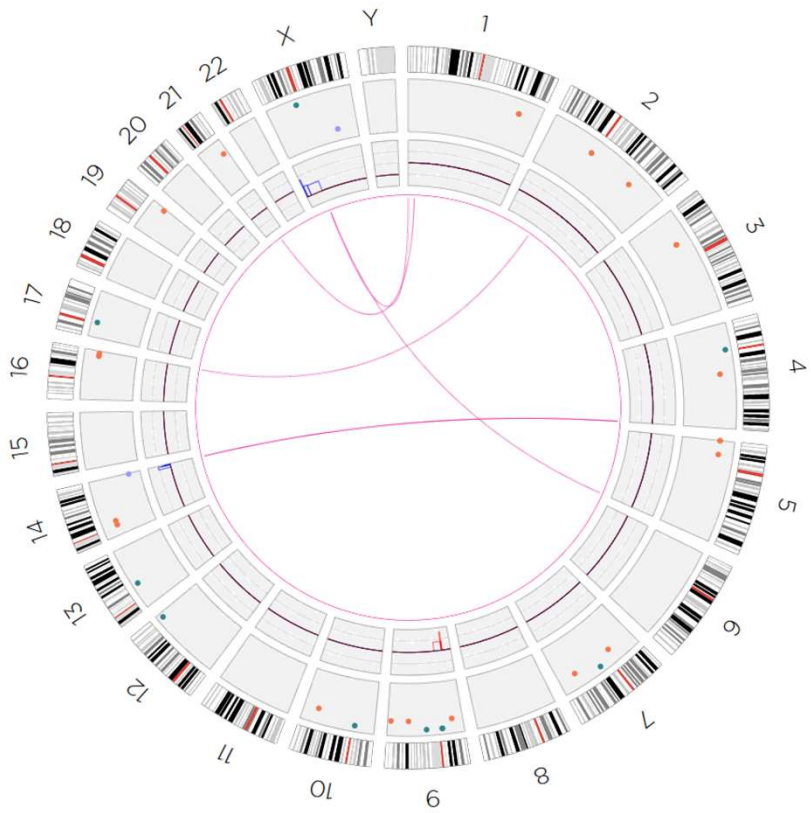


20q11.21q11.22(32374390_34350337)x1~2 (ASXL1 deletion)

Genomic Blindspots: Very telomeric translocations!

CBA: 46,XY,add(5)(q35),add(9)(p12),del(14)(q32)[19]/46,XY[2]





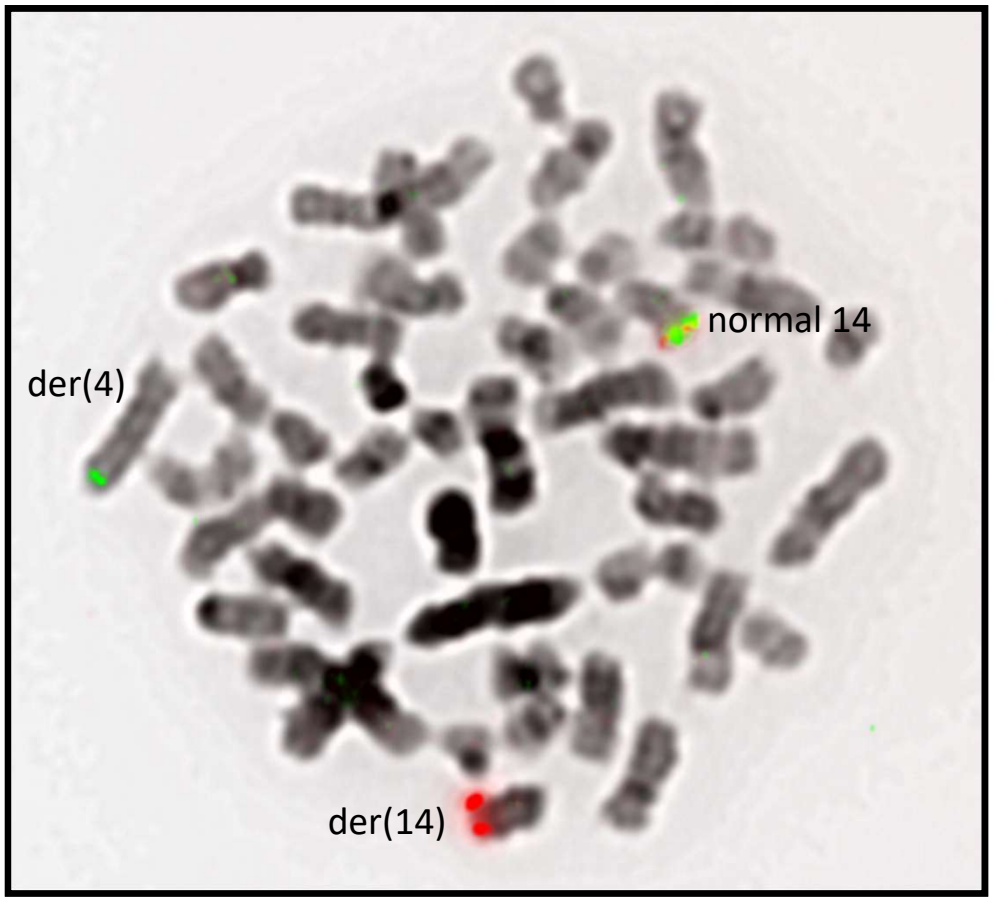
add(5)(q35) =
der(5)t(X;5)
Self molecules 114
VAF 0.33
Confidence 0

del(14) =
t(4:14)(DUX4::IGH)
Self molecules 58
VAF 0.54
Confidence 0.02

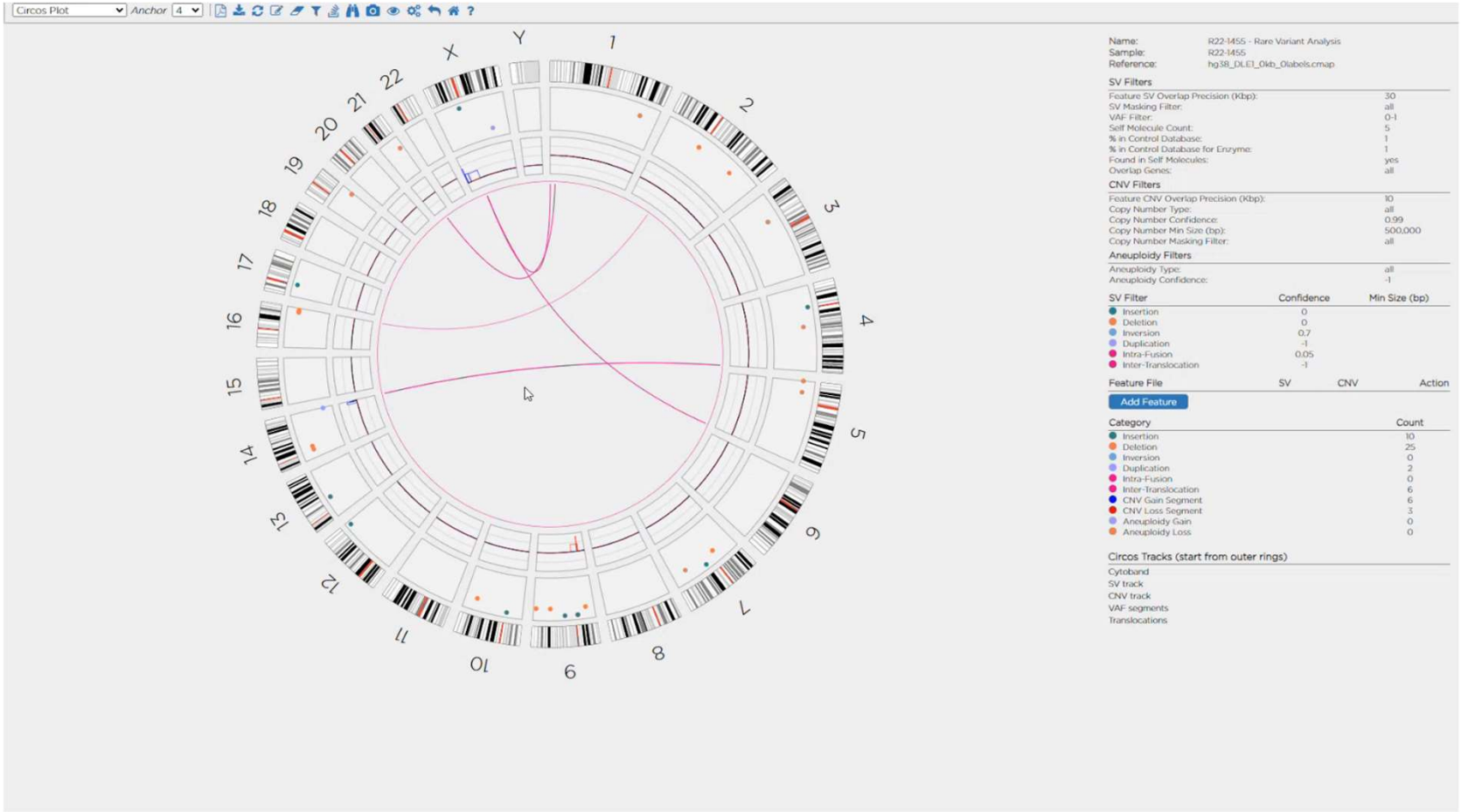
Confidence Setting: Recommended

Confidence Setting: All (Inter Chromosomal Fusions)

IGH Break-Apart FISH



DUX4::IGH – Relaxing filter settings to find clinically significant translocations with low confidence…



Enumerating "Cytogenetically Visible" Events: Rethinking the complex genome…

- Calls made only by the copy number algorithm: >5MB
- Intra- and Inter-Chromosomal rearrangements:
  - any cytogenetically visible balanced or unbalanced, intra- or inter-chromosomal rearrangement should be counted.
  - Including both recurrent disease-specific translocations, but also other rearrangements that would be detected by CBA that are somatic. ***Note, that while cryptic translocations would not be counted by CBA (as they are not detected by the technique), they should be counted by OGM.***
- Catastrophic Genome Events:
  - chromoanagenesis (chromothripsis, chromoanasynthesis and chromoplexy)
- Recurrent Clinically Significant Copy Number Changes:
  - hyperdiploidy, hypodiploidy, iAMP21

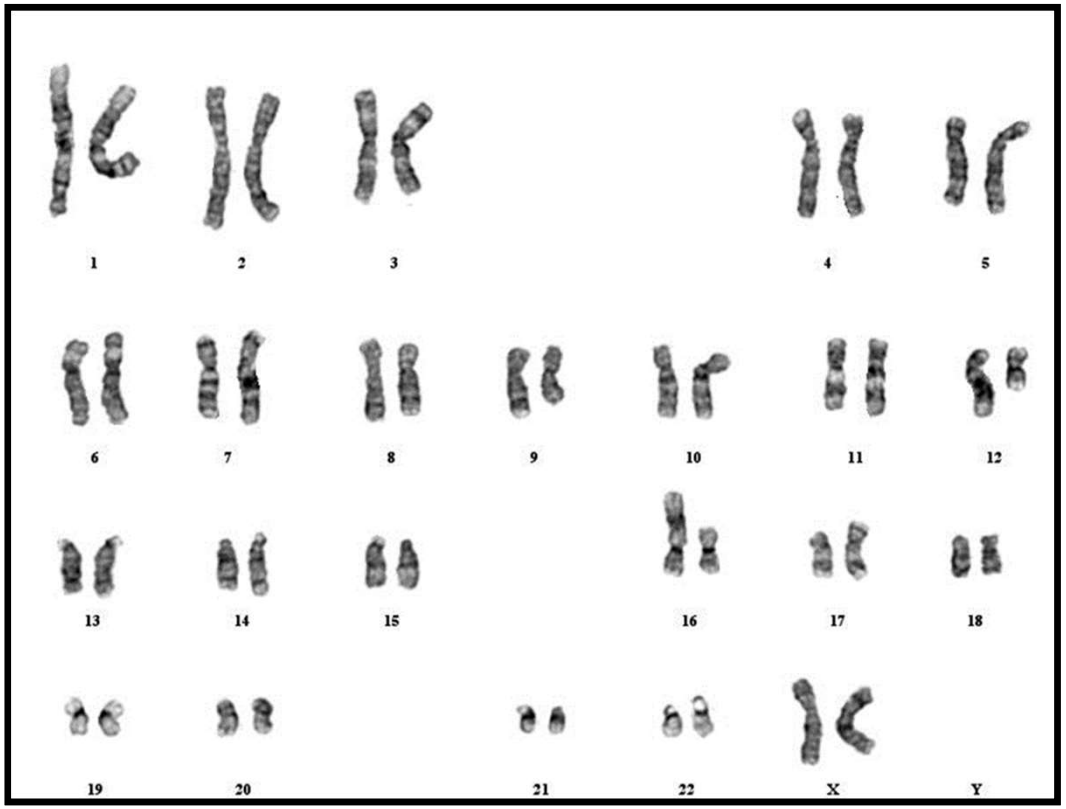
Chromoanagenesis Defined?

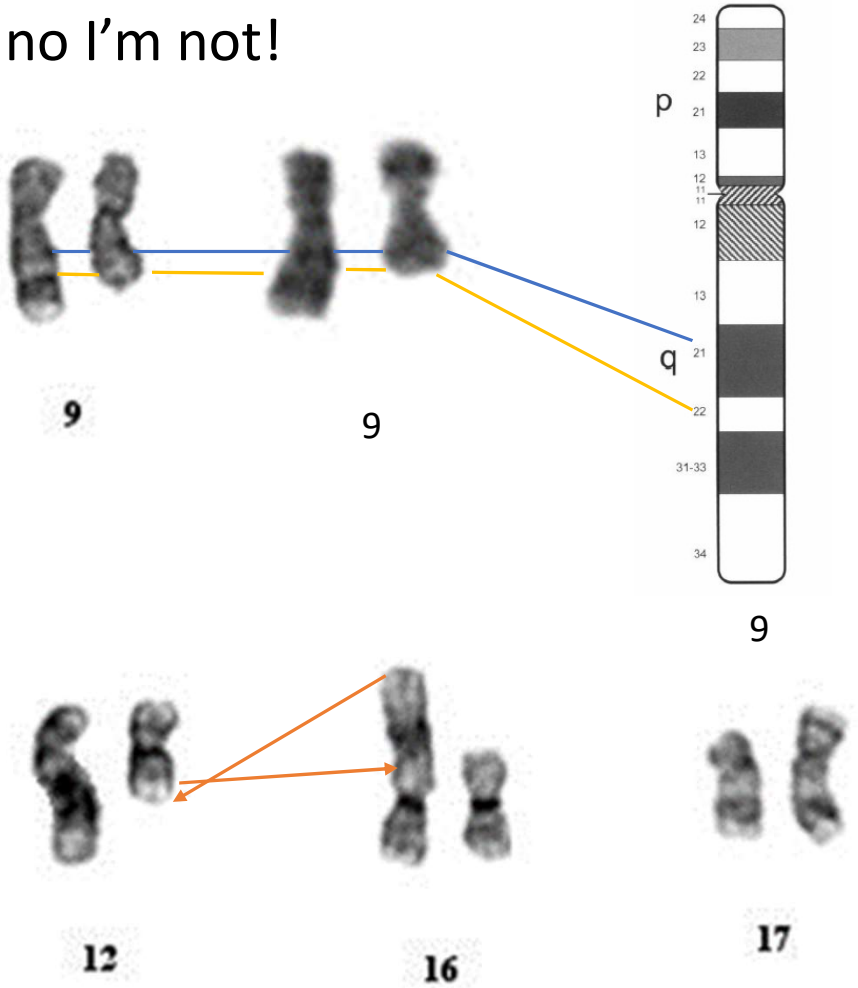
- **Chromothripsis:** The phenomenon is currently defined as a mutational event driven by multiple double-strand breaks (DSBs) occurring in a single catastrophic event between a limited numbers of chromosomal segments, and followed by the reassembly of the DNA fragments in random order and orientation to form complex derivative chromosomes.

- **Chromoanasynthesis:** Like chromothripsis, chromoanasynthesis events involve a combination of structural rearrangements. However, the occurrence of localized multiple copy-number changes, particularly region-focused duplication and triplication and short stretches of micro-homologies at the breakpoint junctions, are both the hallmarks of replication-based mechanism with iterative template switches and define the chromoanasynthesis phenomenon.

- **Chromoplexy:** this phenomenon is characterized by the interdependent occurrence of multiple inter-and intra-chromosomal translocations and deletions.

Cytogenetic Split Personality: I'm complex, no I'm not!

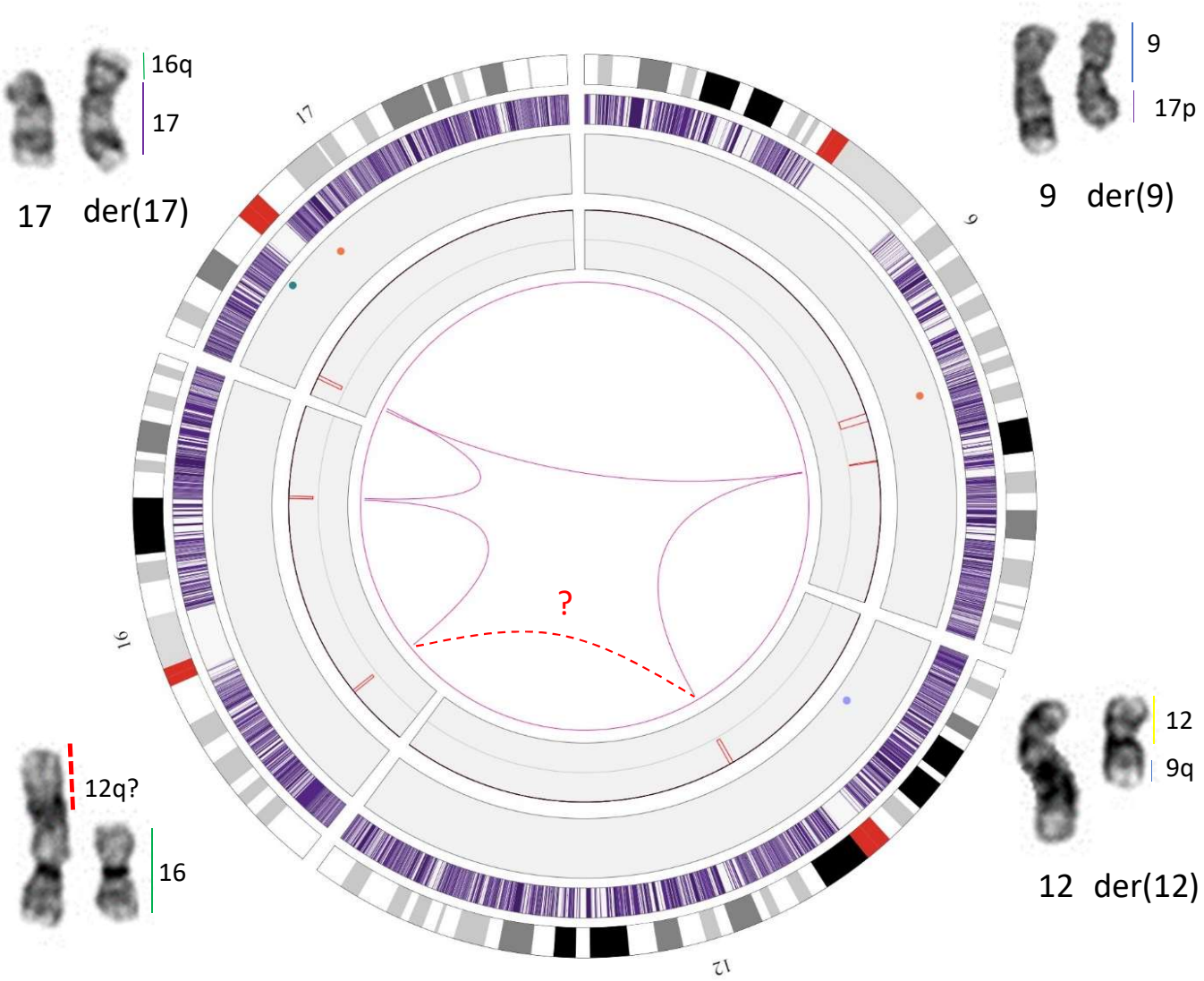




46,XX,del(9)(q22),t(12;16)(q13;p11.2),
del(16)(q22q23),add(17)(p11.2)

Chromoplexy – multiple rearrangements often with deletion bridges

A complex karyotype in AML carries a poor prognosis. But an inv(16), even with other abnormalities, is still good. ***Understanding the underlying molecular pathology of the structural variation is important for diagnosis and patient management!***



Complex Genomes: Chromothripsis and Chromoanasynthesis

- Both chromothripsis and chromoanasynthesis result in the focal rearrangement of a region or regions of the genome.
- However, we should be careful with "operational definitions" of these events. E.g. ">10 copy number changes on chromosome with a copy number between 1-2". While these definitions might encompass a percentage of chromothripsis or chromoanasynthesis they likely don't capture them all.
- Also, these events do not happen "in isolation". It can be difficult to tell if certain events have happened in a stepwise fashion versus an "all at once" mechanism.
- Cth and Cha are also not mutually exclusive of other rearrangements or each other.
- We recommend the use of complex genome "cx" in the nomenclature.

With more accurate classification of complex genomes we may be able to better define prognosis.

Conclusions

- OGM is an emerging clinical tool with unique advantages over conventional approaches and also compared to srGS or lrGS approaches.
- OGM will improve detection of many additional clinically relevant biomarkers. Likely, multi-factor risk adjusted models will need to be developed from combined OGM and NGS-panel data sets (e.g. IPSS-M)
- Data will help to drive new therapeutic approaches as we take a more nuanced approach to treatment selection.
- OGM still provides a whole genome structural analysis in reasonable TAT and at reasonable cost.
- Ultimately, clinical management (Tx) will drive genomic biomarker testing requirements. Currently, nearly all heme malignancy classification/prognostic systems require a full genome structural analysis.

Acknowledgements

IX SIMPOSIO
Grupo Cooperativo Español de Citogenética Hematológica
Avances de las técnicas citogenéticas y moleculares en el diagnóstico de las hemopatías malignas

UHN Toronto General Toronto Western Princess Margaret Toronto Rehab

Ana Baptista, MLT
Shabnam Salehi-Rad, MLT
Olivia King, MLT
Kate Harris, MLT
Dr. Jose-Mario Capo-Chichi
Dr. Barb Morash
Dr. Tracy Stockley

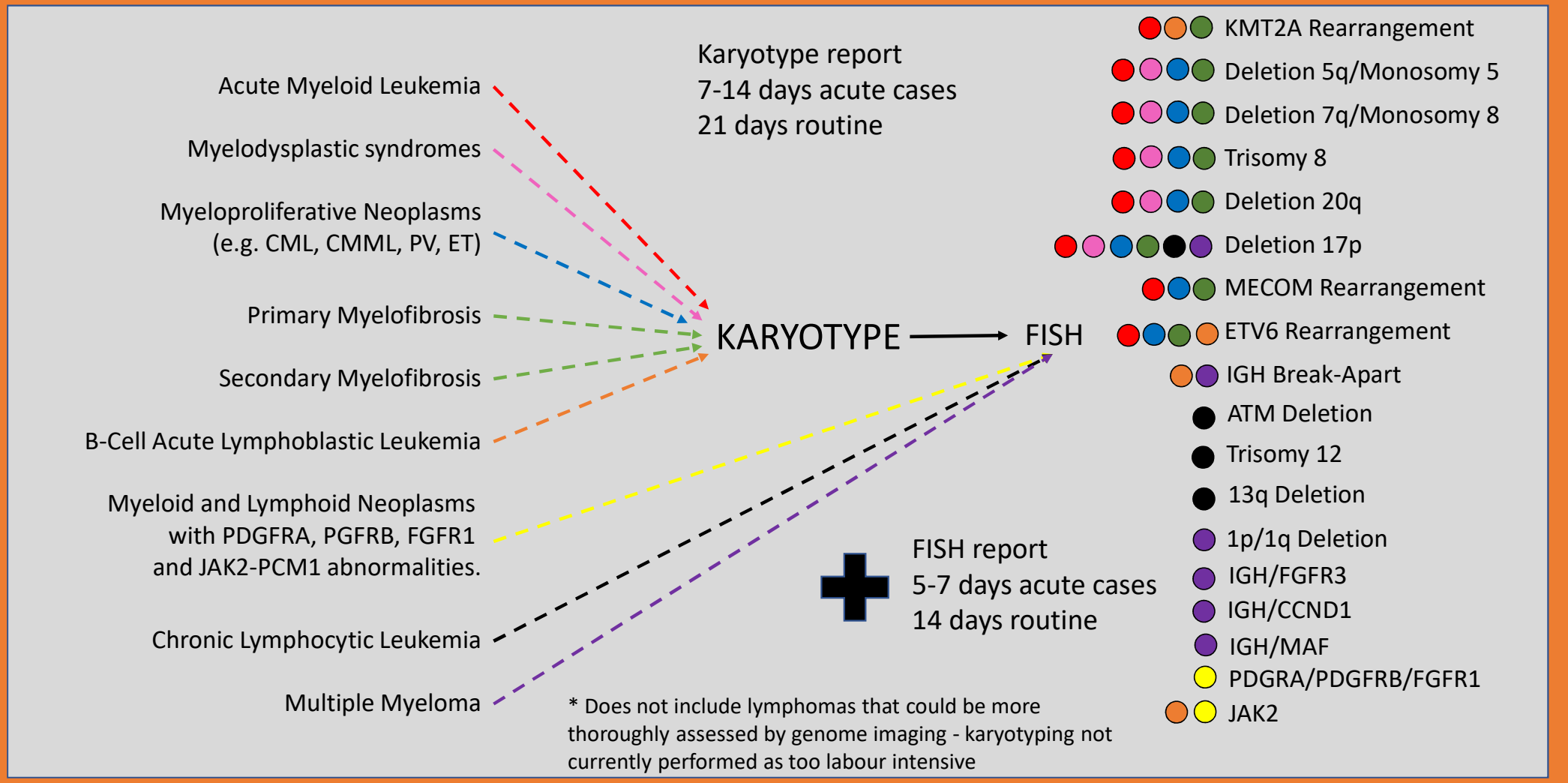
bionano GENOMICS

Dr. Alka Chaubey
Dr. Alex Hastie
Dr. Andy Pang
Alvin Yee
Chad Collier
Dr. Ben Clifford
Neil Miller
Mike Diaz
and many others!!

INTERNATIONAL WORKING GROUP

Adam C. Smith
Rashmi Kanagal-Shamanna
Barbara Dewaele
Katrina Rack
Alex Hoischen
Kornelia Neveling
Marian Stevens-Kroef
Gordana Raca
Brynn Levy
Ravindra Kolhe
Nikhil Sahajpal
Blanca Espinet
Anna Puiggros
Francesc Sole
Mar Mallo
Tuomo Mantere

Cytogenetics and Hematologic Malignancies



Lack of SV Database Resources

nature

Explore content | About the journal | Publish with us

nature > articles > article

Article | Open Access | Published: 27 May 2020

A structural variation reference for medical and population genetics

Ryan L. Collins, Harrison Brand, Konrad J. Karczewski, Xuefang Zhao, Jessica Alföldi, Laurent C. Francioli, Amit V. Khera, Chelsea Lowther, Laura D. Gauthier, Harold Wang, Nicholas A. Watts, Matthew Solomonson, Anne O'Donnell-Luria, Alexander Baumann, Ruchi Munshi, Mark Walker, Christopher W. Whelan, Yongqing Huang, Ted Brookings, Ted Sharpe, Matthew R. Stone, Elise Valkanas, Jack Fu, Grace Tiao, Genome Aggregation Database Production Team, Genome Aggregation Database Consortium, ... Michael E. Talkowski + Show authors

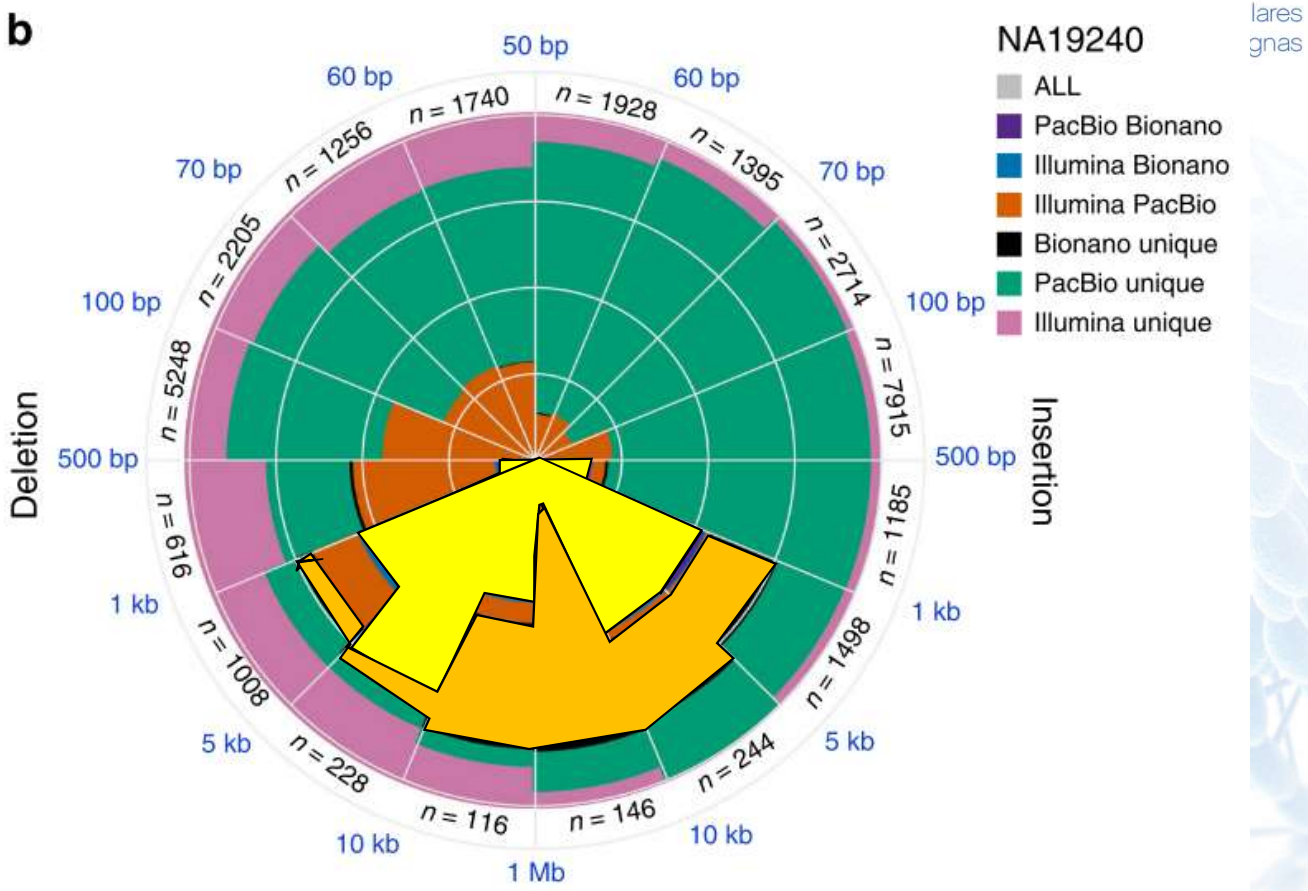
Nature **581**, 444–451 (2020) | Cite this article

55k Accesses | **235** Citations | **187** Altmetric | Metrics

"…we estimate that SVs are responsible for 25–29% of all rare protein-truncating events per genome."

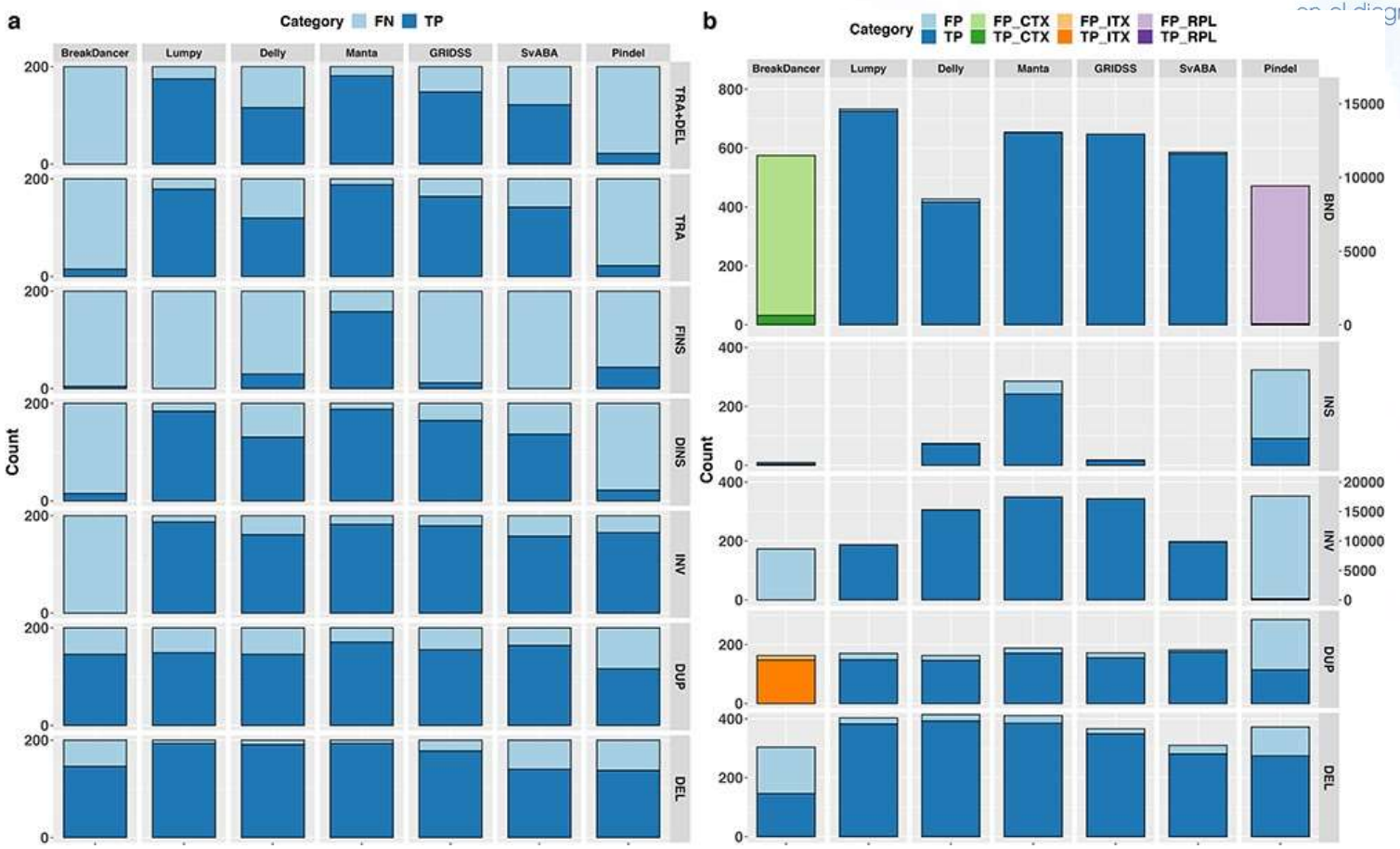
"We found strong correlations between natural selection against damaging SNVs and rare SVs that disrupt or duplicate protein-coding sequence, which suggests that genes that are highly intolerant to loss-of-function are also sensitive to increased dosage".

SV Detection Radar Plot



Chaisson, et al. (2019). Multi-platform discovery of haplotype-resolved structural variation in human genomes. Nature Communications, 10(1), 1784. https://doi.org/10.1038/s41467-018-08148-z

Figure 5 Performance of SV callers in detecting different SV types.



Brief Bioinform, Volume 22, Issue 3, May 2021, bbaa056, https://doi.org/10.1093/bib/bbaa056

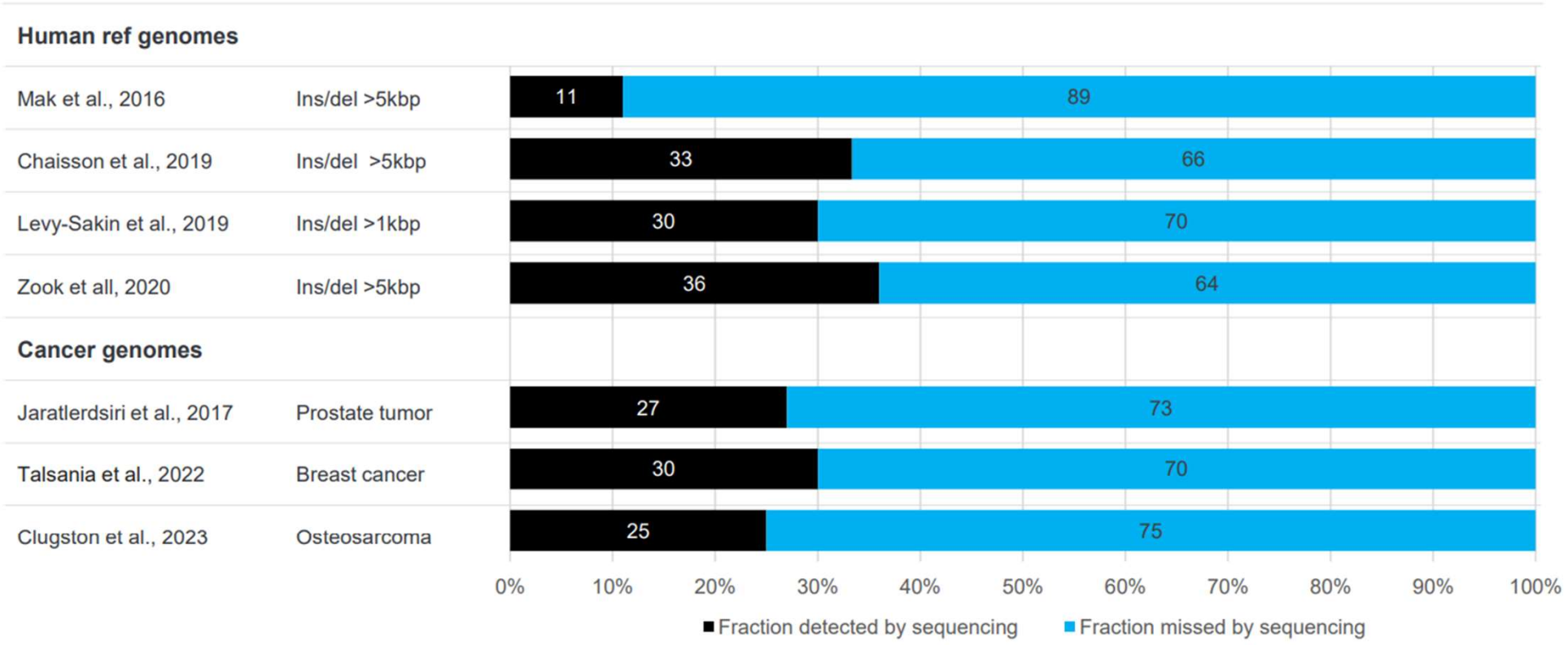
Short-read GS callers for SVs have variable performance. Some callers were designed for specific types of SVs and have high rates of FP and FN calls on other types of SVs.

In order to use these clinically they must have very high sensitivity and specificity for SVs of all types.

Using “meta-methods” often doesn’t improve call accuracy.

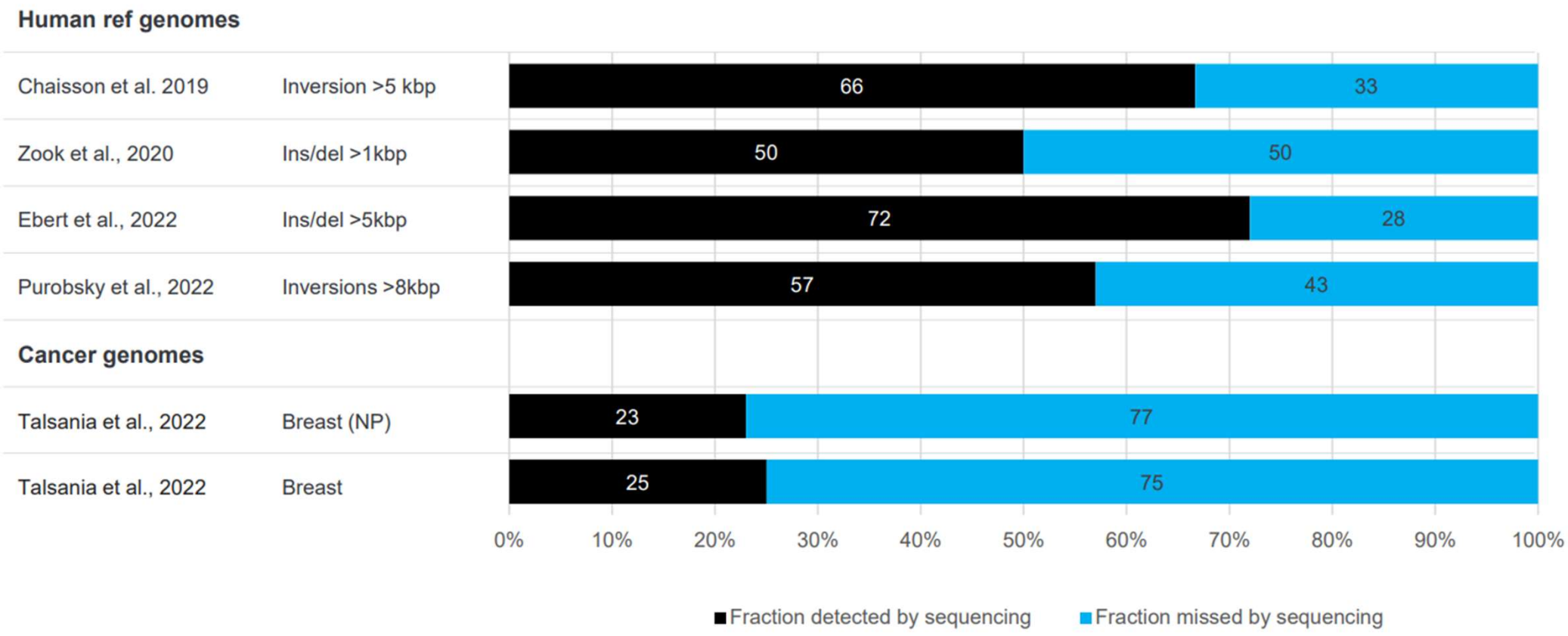
At the end of the day, False Negative and False Positive rates make a big difference to Clinical Utility..

Benchmark Studies Show How Much SVs Short-Read Misses



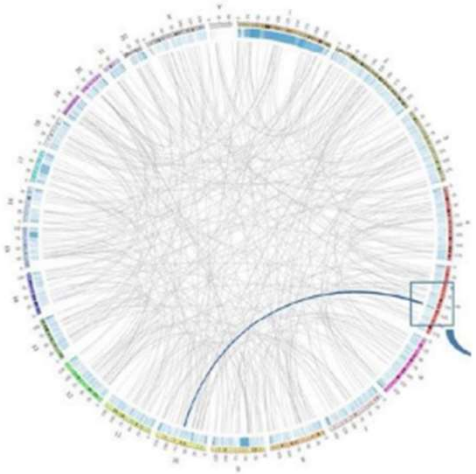
Mak, et al. *Genetics*. 2016;202(1):351-362., Chaisson, et al. *Nat Commun*. 2019;10(1):1784., Levy-Sakin, et al. *Nat Commun*. 2019;10(1):1025., Zook, et al. *Nat Biotechnol*. 2020;38(11):1347-1355. Jaratlerdsiri, et al. Oncotarget. 2017;8(14):23588-23602. Talsania, et al. Genome Biol. 2022;23(1):255. Clugston, et al., unpublished poster presentation

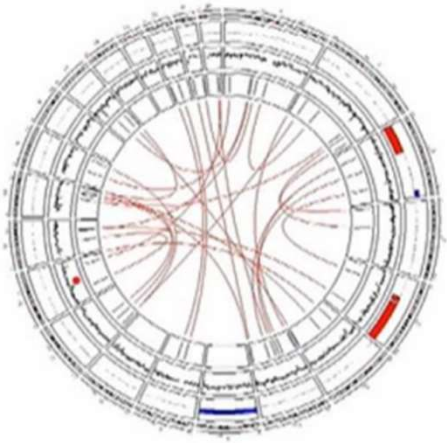
Long-Read Sequencing Performs Better but Still Misses Many SVs

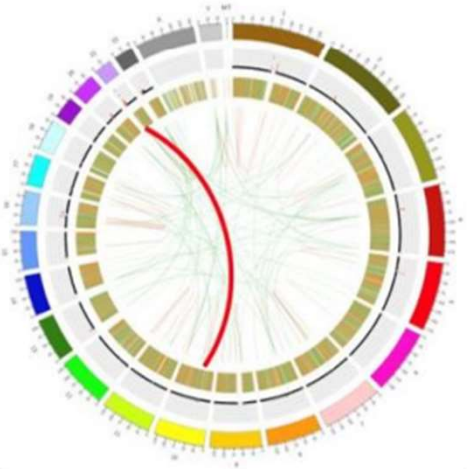


Chaisson, et al. *Nat Commun*. 2019;10(1):1784. Zook, et al. *Nat Biotechnol*. 2020;38(11):1347-1355. Ebert, et al. Science. 2021;372(6537). Porubsky, et al. Cell. 2022;185(11):1986-2005.e26. Talsania, et al. Genome Biol. 2022;23(1):255.

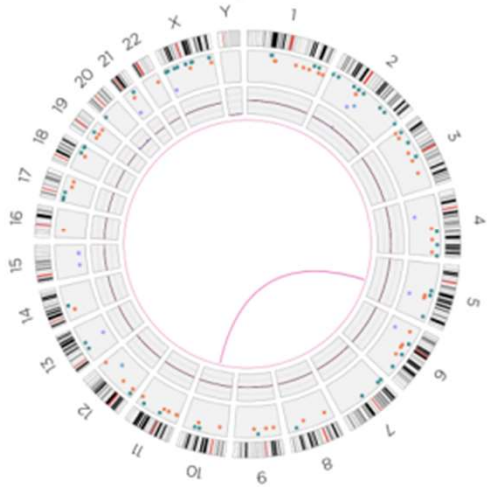
Nanopore

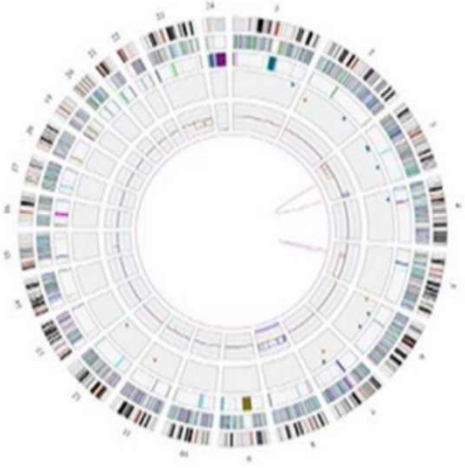


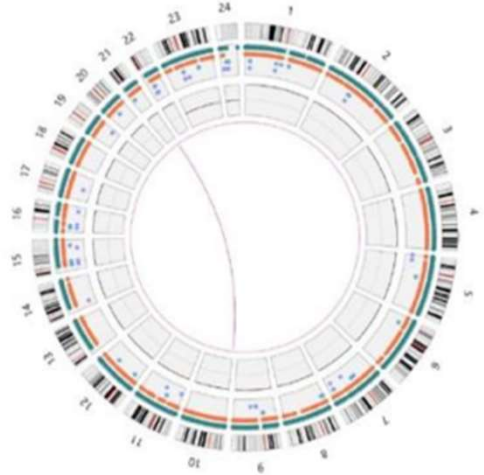




OGM







Scharf et al. J Med Genet. 2022;59(10):976-983. doi:10.1136/jmedgenet-2021-108147

Rodney Scott - Human Genetics Society of Australasia ASM2022 Perth Australia

Reproductive disorder case - Personal communication - Unpublished

Recent comments in publications from leading US sequencing consortiums

Utility of long-read sequencing for All of Us

M. Mahmoud[1,2], Y. Huang[3], K. Garimella[3], P. A. Audano[4], W. Wan [3], N. Prasad[5], R. E. Handsaker[6], S. Hall[5], A. Pionzio[5], M. C. Schatz[7], M. E. Talkowski[8,9], E. E. Eichler[10,11], S. E. Levy[12], F. J. Sedlazeck[1,2,13]

- "The percentage of SVs agreed upon by all three technologies is approximately 22.00%; ONT and HiFi agreed on 53.86% of all SVs"
- "For long reads to advance, several major considerations must be addressed including costs, throughput, robustness of software cycles, and predictable/variable yields from sequence components or DNA quality fluctuations."

Beyond the exome: what's next in diagnostic testing for Mendelian conditions

Monica H. Wojcik[1,2,3], Chloe M. Reuter[4], Shruti Marwaha[4], Medhat Mahmoud[5], Michael H. Duyzend[1,2,6], Hayk Barseghyan[7,8], Bo Yuan[9], Philip M. Boone[1,2,6], Emily E. Groopman[1,2,6], Emmanuèle C. Délot[8,10,11], Deepti Jain[12], Alba Sanchis-Juan[1,6], Genomics Research to Elucidate the Genetics of Rare Diseases (GREGoR) Consortium, Lea M. Starita[13,14], Michael Talkowski[1,6,15,16], Stephen B. Montgomery[17,18,19], Michael J. Bamshad[13,14,20], Jessica X. Chong[13,20], Matthew T. Wheeler[4], Seth I. Berger[21], Anne O'Donnell-Luria[1,2,22], Fritz J. Sedlazeck[5,23], Danny E. Miller[13,20,24,*]

- "A direct comparison of lrGS, srGS, and OGM on the same sample showed that 1 in 3 deletions and 3 in 4 insertions larger than 10 kb were detectable only by OGM."