Application of genomic arrays to the diagnosis and management of hematological neoplasms

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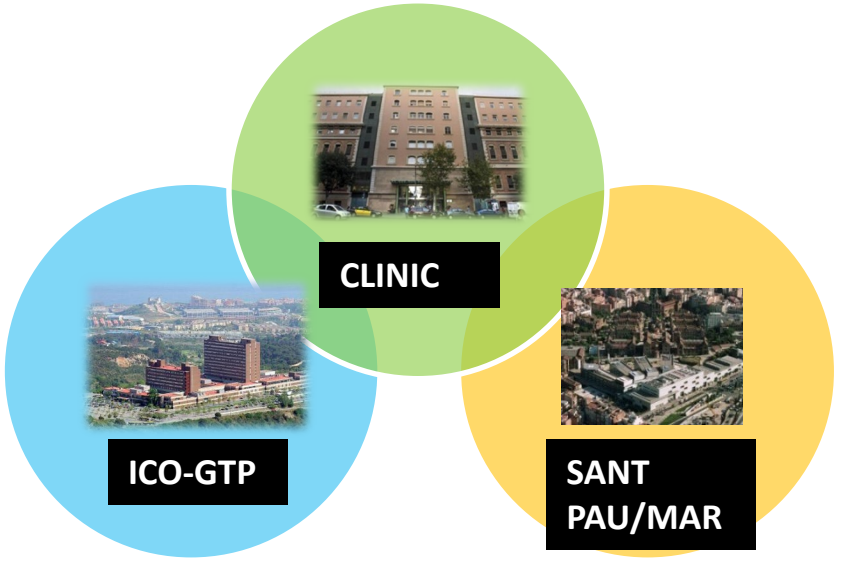
- > 25 research groups
- 250 researchers
- 200 papers/year
- 73 active papers
- 7 Plataforms
- 2 Spin off



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NEXT HOSPITALS: Hospital Trueta-Girona and Hospital Sant Joan de Deu-Barcelona

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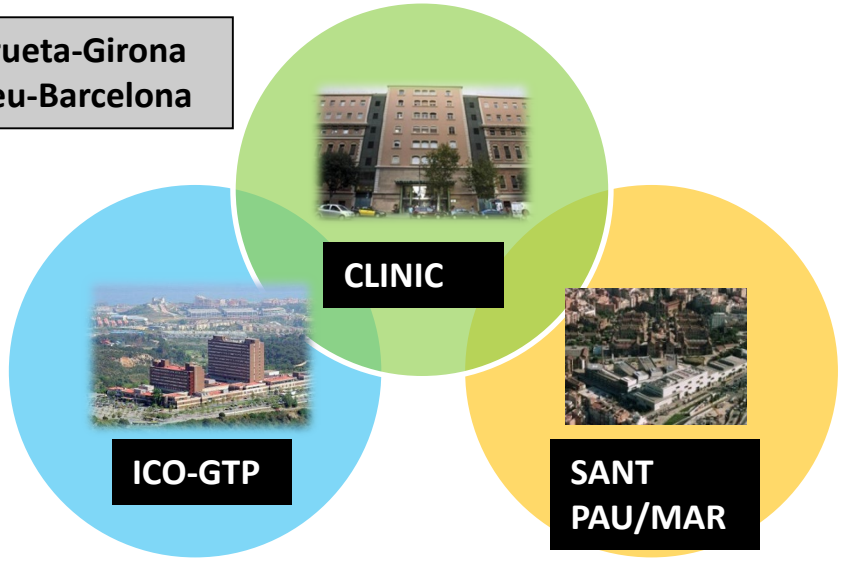


Unit of Microarrays in IJC

Jessica Tijero

Mar Mallo

Aida Silverio

Nuri de Haro

Institut de Recerca contra la Leucèmia Josep Carreras (IJC):

- **Mar Mallo**
- Jessica Tijero
- Nuri de Aro
- Aida Silverio

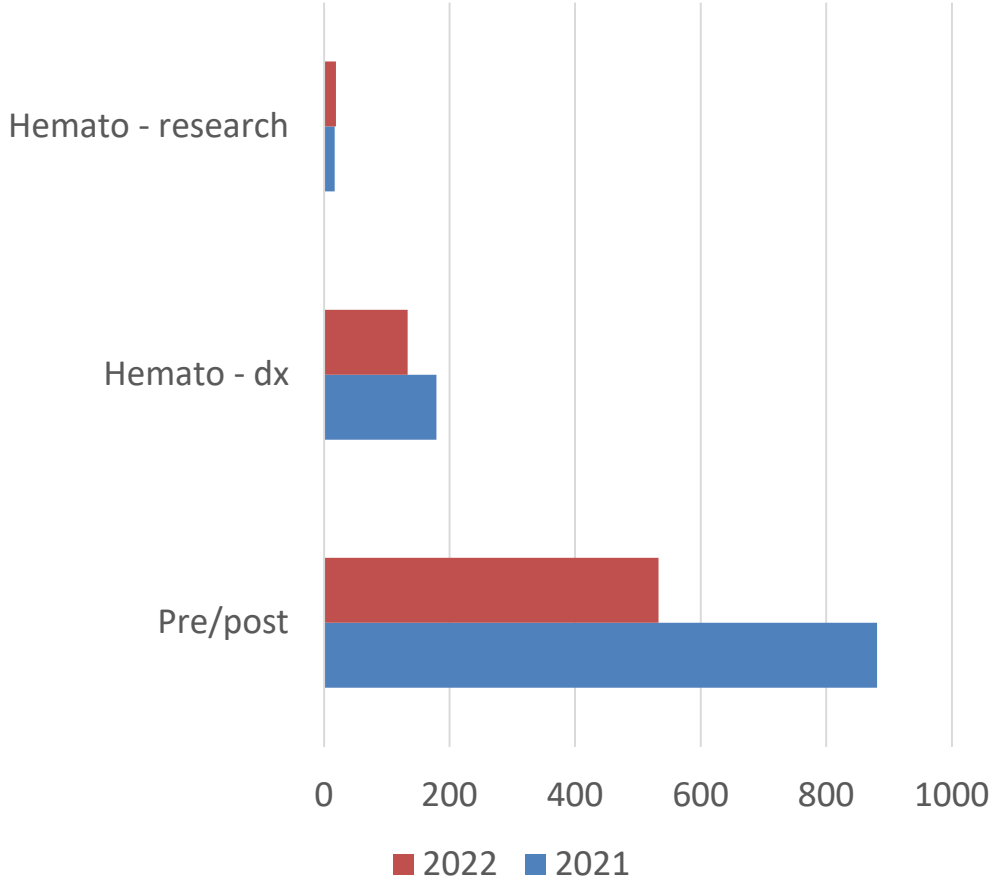
- Jordi Ribera
- Pamela Acha

Institut Català d'Oncologia (ICO):

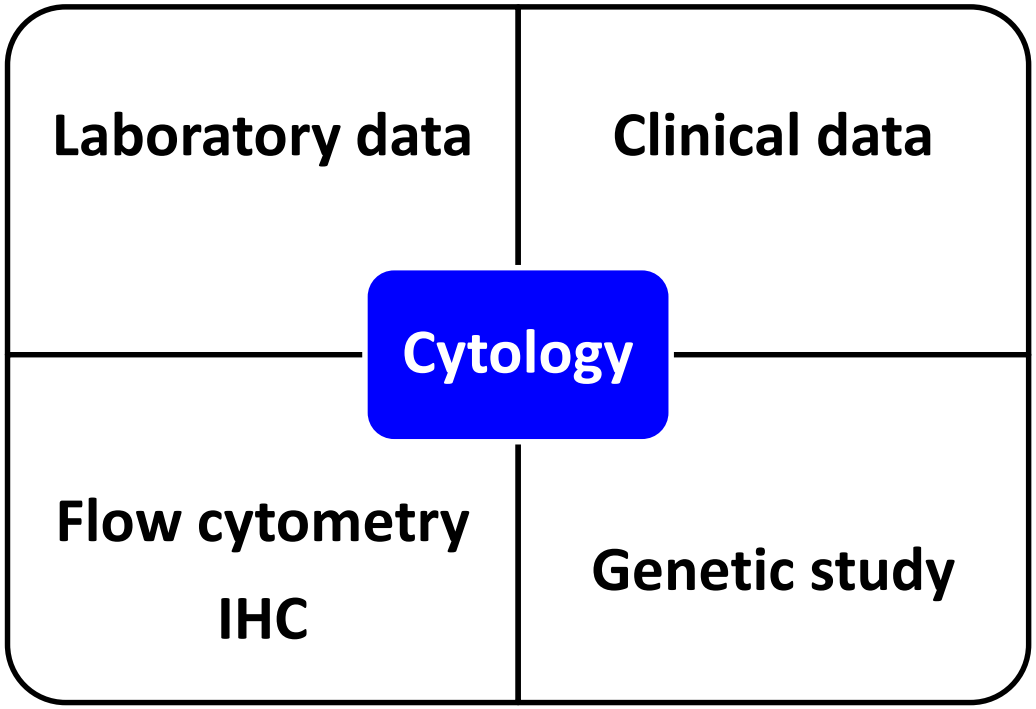
- Isabel Granada
- Adela Cisneros
- Neus Ruiz

Unit of Microarrays in IJC

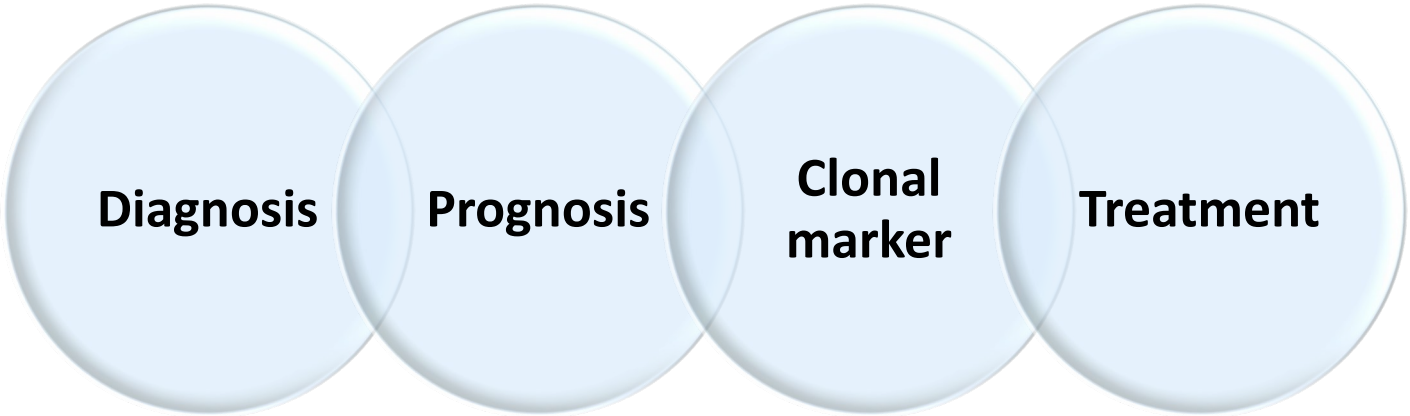
	2021	2022 Until July
Pre/post	881	533
Hemato - dx	179	133
Hemato - research	17	19
	1077	685



Diagnosis of haematological neoplasms



Conventional cytogenetics
Molecular biology/Sequencing
Fluorescence *In Situ* Hybridisation
Genomic arrays



Revlimid® (lenalidomide) capsules

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Institut de Recerca CONTRA LA LEUCÈMIA Josep Carreras

Cytogenomic techniques

	G-banding	FISH	CMA	WGS	Targeted sequencing panels		RT-PCR	MPSeq	WTS	OGM
Analyte	Chromosome in dividing cells	DNA in interphase nuclei and metaphase	DNA	DNA	DNA	RNA	RNA	DNA	RNA	DNA
Coverage	Whole	Targeted	Whole	Whole	Targeted	Targeted	Targeted	Whole	Whole	Whole
Distinction of individual cell clones	Yes	Yes	No	No	No	No	No	No	No	No
Analysis bias	Yes	Yes (if cultured)	No	No	Yes	Yes	No	No	No	No
Turnaround time (d)	3-7	4 h to 2 d	3-7	7-14	7-14	7-14	4 h to 5 d	7-14	14-21	7-10
Unmapped region detection	Yes	No	No	No	No	No	No	No	No	Some Alu and LINE elements
Ability to multiplex	Low	Low	High	High	High	High	High	High	High	Low to medium
Analytical sensitivity (%)	1*-3 out of 20 metaphases	1-10	10-20	20-30	1-10	5-10	~0.01	10	1-10	5-20
SVs	Yes	Yes	No	Yes (long-read or short-read deep sequencing)	No	Gene fusion	Limited	Yes	Yes	Yes
CNVs	Yes	Yes	Yes	Yes	Limited	Limited	Limited	Yes	Limited	Yes
SNVs	No	No	No	Yes	Yes	Yes	No	Limited	Yes	No
Disease status	Diagnosis, disease monitoring, relapse	Diagnosis, disease monitoring, relapse	Diagnosis, relapse	Diagnosis, relapse	Diagnosis, disease monitoring, relapse, MRD (if deep coverage)	Diagnosis, disease monitoring, relapse, MRD (if deep coverage)	Diagnosis, disease monitoring, MRD, relapse	Diagnosis, relapse	Diagnosis, relapse	Diagnosis, relapse
Well-established	High	High	High	Low	High	High	High	Low	Low	Low
Cost	++	++	+++	+++++	+++	+++	++	++++	++++	+++

*Depending on the clinical situation, 1 metaphase with a recurring abnormality may be considered evidence for an abnormal clone.

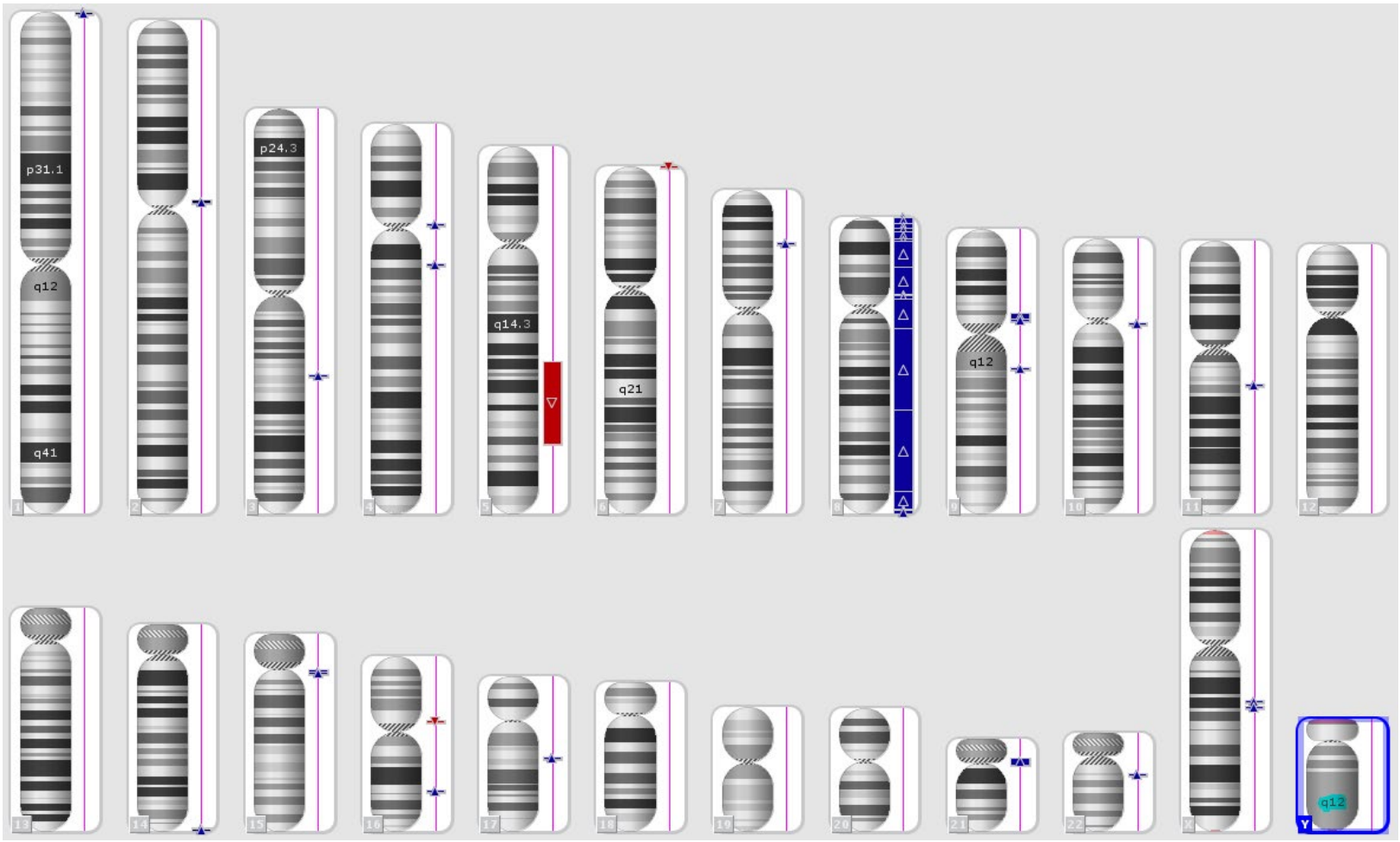
Akkari *et al.*, Blood, 2022

What can we detect using Genomic arrays

- **Gain/loss:** Type of copy-number change observed. It is recommended that the term “gain” be used rather than “duplication.”
- **CNAs (copy number alterations)**
- **Copy-neutral loss of heterozygosity (CN-LOH):** Allelic imbalance without an associated copy-number change. Uniparental disomy (UPD) should be used when the change is germline.
- **Amplification:** High copy-number gain of sequences, typically containing oncogene(s). Standard thresholds used to represent amplification typically range from 3–5 fold increases over >100 copies
- **Chromothripsis**
- **Intrachromosomal complexity**
- **Genomic complexity**

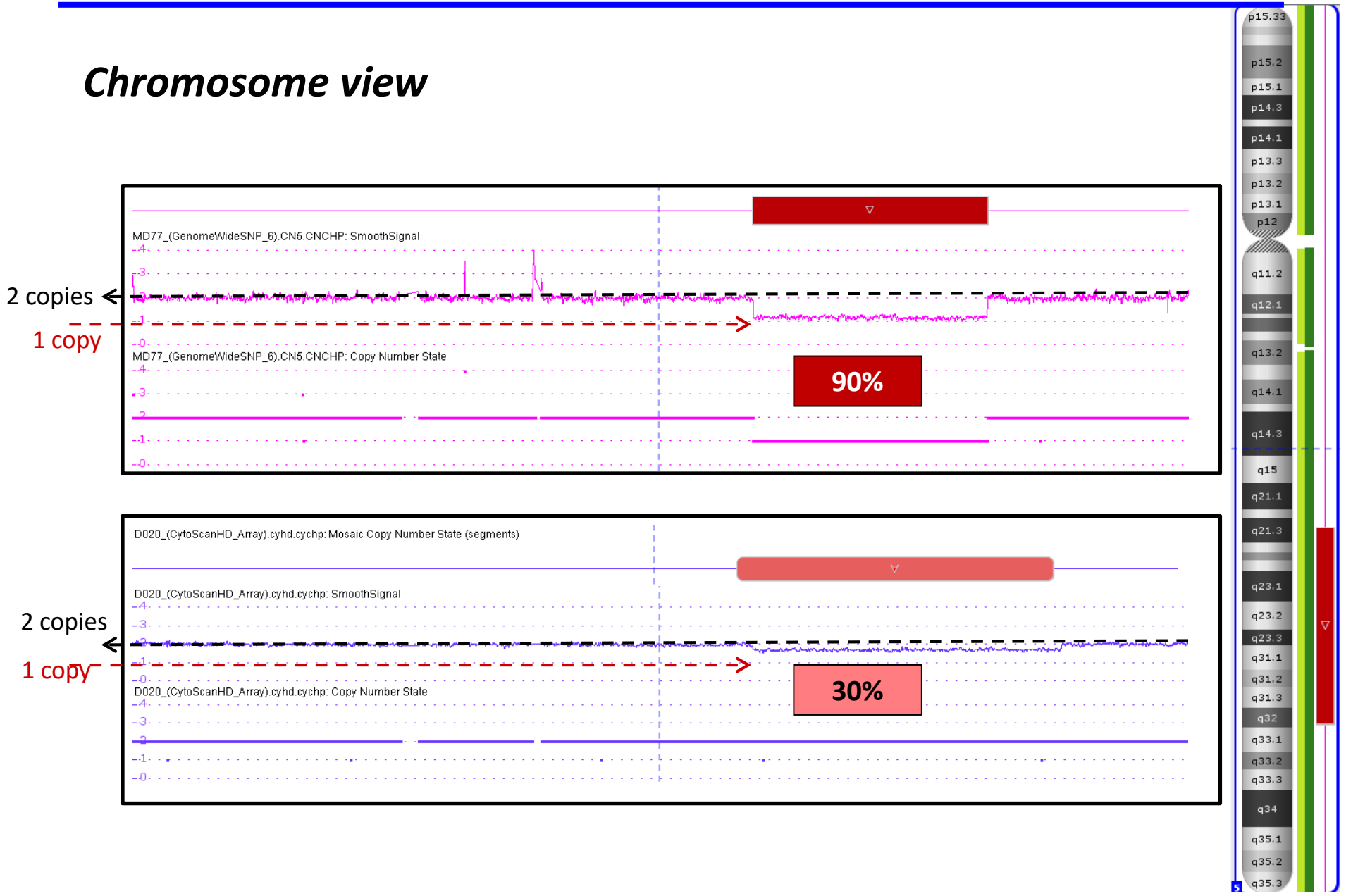
Data analysis: copy number

Genome view



Data analysis: Proportion of tumoral cells

Chromosome view



Application of arrays to hematological neoplasms

European recommendations and quality assurance for cytogenomic analysis of haematological neoplasms

Table 1 Recommended testing for different haematological neoplasms

Disease	Test	Requirement	Suggested methodology	Guidelines
CML	Karyotype	Mandatory	Chromosome banding	Baccarani et al. 2013 [24], 2015 [25]
	BCR-ABL1 gene fusion	Mandatory	FISH or molecular methods	
	ABL1 mutation when resistance to therapy	Mandatory	Molecular methods	
MPN	*JAK2, CALR, MPL* mutations depending on referral reason	Indicated	Molecular methods	Gong et al. 2013 [32] Xia and Hassejian 2016 [33]
	Karyotype	Optional	Chromosome banding	WHO 2017 [1]
Myeloid/lymphoid neoplasms with eosinophilia	Recurrent gene fusions involving *PDGFRA, PDGFRB, FGFR1, PCM1-JAK2*	Strongly recommended for most patients	FISH or molecular methods	Butt et al. 2017 [40]
	Karyotype	Recommended in absence of recurrent gene fusion	Chromosome banding	
MDS	Karyotype	Mandatory	Chromosome banding	Malcovati et al. 2013 [41]
	Targeted chromosome abnormalities -5/5q-,-7/7q-, *MECOM* (extended panel + 8,20q-del*TP53*)	Recommended[b]	FISH/ SNP array/ Molecular methods	
	High resolution chromosome analysis and aCN-LOH[c]	Recommended	SNP array	
	Mutation analysis of candidate genes	Recommended	Molecular methods	
AML	Karyotype	Mandatory	Chromosome banding	Döhner et al. 2017 [47]
	Gene mutations:*NMP1,CEBPA, RUNX1, FLT3,TP53, ASXL1*	Mandatory	Molecular methods	
	Recurrent gene fusions: *PML-RARA*, *CBFB-MYH11, RUNX1-RUNX1T*. Gene rearrangements of *KMT2A* and *MECOM*.	Recommended[a]	FISH or molecular methods	
ALL	Recurrent gene fusions (Age-related priority see Table 3)	Mandatory	FISH or molecular methods	Harrison et al. 2010 [57]
	Hyperdiploidy	Recommended	Chromosome banding or SNP-Array/ FISH	Moorman et al. 2010 [59]
	Recurrent microdeletions	Recommended in paediatric	MLPA, Array, molecular methods	Harrison et al. 2010 [57]
	Karyotype[d]	Mandatory		Hoelzer et al. 2016 [60]
CLL	Deletion 13q14, *ATM, TP53*, trisomy12	Mandatory	FISH, SNP-array or molecular methods	Hallek et al. 2018 [71]
	TP53 mutation/IGHV mutational status	Mandatory	Molecular methods	Malcikova et al. 2018 [75], Rosenquist et al. 2017 [76]
	Karyotype	Desirable for clinical trials		Hallek et al. 2018 [71]
Multiple myeloma	t(4;14)[e], t(14;16), deletion *TP53* [e] gain 1q/del(1p)	Recommended	FISH for gene rearrangements	Sonneveld et al. 2016 [82]
	t(11;14), t(14;20), ploidy status (extended panel)		FISH or Array, MLPA for copy number gains and losses	Caers et al. 2018 [83]
Other mature B-cell neoplasms	Recurrent gene rearrangements depending on differential diagnosis		FISH	WHO 2017 [1]
	MYC rearrangements for prognostic testing[f]			

Rack *et al.*, Leukemia, 2020

Application of arrays to hematological neoplasms

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	High resolution chromosome analysis and aCN-LOH[c]	Recommended	SNP array	
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Other mature B-cell neoplasms	Recurrent gene rearrangements depending on differential diagnosis		FISH	WHO 2017 [1]
	MYC rearrangements for prognostic testing[f]			

Rack *et al.*, Leukemia, 2020

When apply SNP arrays in hematologic cancers

- **Hematologic cancers with copy number alterations:**

MDS: gains and losses and LOH (IPSS-R and IPSS-M)

CLL: FISH of 11q, 12, 13q and 17p

Myeloma: copy number alterations

ALL: copy number alterations such as:

AMP21, hyperdiploid, Near haploid or tetraploid cases originated from haploid

Non useful: cases with balanced rearrangements:

- AML, NHL, MM… cases with translocations….

Application of arrays in CLL

Table IV. Detection of known recurrent CLL abnormalities by FISH and Cyto-array in 70 patients with CLL.

	FISH		Cyto-array				
Abnormality	*n* (%)	Median % altered nuclei (range)	*n* (%)	Median size (range)	MDR size	Concordance	*p*-Value*
Deletion 13q14	36 (51.4)	60% (14–92)	34 (48.6)	1.40 Mbp (0.14–31.13)	0.13 Mbp	95.7%	0.50
Trisomy 12	11 (15.7)	58% (12–74)	11 (15.7)	–	–	100%	–
Deletion 11q22	4 (5.7)	59.5% (12–93)	3 (4.3)	37.68 Mbp (20.12–46.61)	20.12 Mbp	98.6%	1.00
Deletion 17p13	5 (7.1)	19% (19–68)	2 (2.9)	21.41 Mbp (18.76–24.05)	18.76 Mbp	94.3%	0.63

CLL, chronic lymphocytic leukemia; FISH, fluorescence *in situ* hybridization; MDR, minimal deleted region.
*p-Value obtained in McNemar test to assess significance of discordance found.

- Complementary technique to Cytogenetics and FISH
- Could not replace Cytogenetics and FISH:
  - no detection of del(11q) and del(17p) in cases with low tumoral burden

Puiggros *et al.*, Leukemia and Lymphoma, 2012

Application of arrays in MDS

Disease	Overall CMA detection rate	Key and unique CMA aberrations	Altered gene(s)	Impact	References
MDS	28–83% (Normal karyotype only: 11–39%)	Total genomic alteration		Prognostic poor survival	[26,31,35,44,48]
		1p CN-LOH		Prognostic for progression to AML	[14,25,36,60,104]
		1q gain		Recurrent	[14,21,30,104]
		4q loss	*TET2*	Prognostic for poor survival	[14,21,23,24,46]
		4q CN-LOH	*TET2*	Prognostic for poor survival	[12,14,16,21,30,35-37,45,63,109]
		5q loss		5q loss "size" prognostic for progression to AML	[14,15,21,26,33,62,104,110]
		7q loss	*CUX1, EZH2*	Prognostic for poor survival	[14,15,18,30,32,38,45,60, 63,78,102,104,107,110]
		7q CN-LOH		Recurrent	[12,14,21,25,30,36,48,91,109]
		11q CN-LOH	*CBL*	Prognostic/ recurrent	[12,14,15,25,35,36,63,104]
		12p loss	*ETV6*	Recurrent	[14,16,30,32,46]
		13q loss	*?RB1*	Recurrent	[14,21,32,35,104]
		17p loss	*TP53*	Recurrent	[14,30,34,46,102]
		17p CN-LOH	*TP53*	Diagnostic for advanced MDS/sAML	[21,30,35,36,38]
		20q loss		Recurrent	[14,60,61,64,102,107,110]
		21q CN-LOH or deletion	*RUNX1*	Prognostic for progression to AML	[14,18,32,46,60,91]

- Most aberrations in MDS are gains (+8) and losses (5q-, -7, 7q-, 11q-, 12p-, i17q, 20q-…
- Translocations are very rare (less than 1%)

Application of arrays in MDS

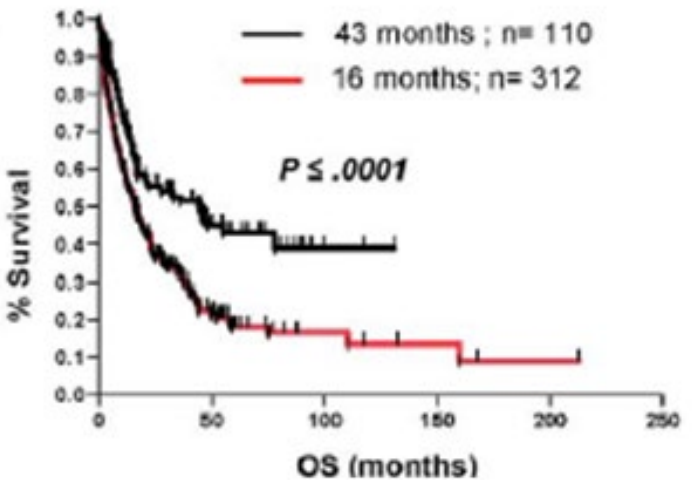
Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies

Ramon V. Tiu,[1,2] Lukasz P. Gondek,[1] Christine L. O'Keefe,[1] Paul Elson,[3] Jungwon Huh,[1,4] Azim Mohamedali,[5] Austin Kulasekararaj,[5] Anjali S. Advani,[2] Ronald Paquette,[6] Alan F. List,[7] Mikkael A. Sekeres,[2] Michael A. McDevitt,[8] *Ghulam J. Mufti,[5] and *Jaroslaw P. Maciejewski[1,2]

Blood, 2011

Table 2. Comparison of cytogenetic detection rate between MC and MC cytogenetics combined with SNA-A karyotyping

Disease group/MC	n (%)	MC + SNP-A	n (%)	P*
MDS (n = 250)				
NI†	17 (7)	Normal	65 (26)	
Normal	118 (47)	Abnormal	70 (28)	< .0001
Abnormal	115 (46)	No additional	47 (19)	
Abnormal		Additional	68 (27)	
MDS/MPN (n = 95)				
NI†	4 (4)	Normal	24 (25)	
Normal	55 (58)	Abnormal	35 (37)	< .0001
Abnormal	36 (38)	No additional	10 (11)	
Abnormal		Additional	26 (27)	
AML (n = 85)‡				
NI†	7 (8)	Normal	22 (26)	
Normal	40 (47)	Abnormal	25 (29)	.0002
Abnormal	38 (45)	No additional	15 (18)	
Abnormal		Additional	23 (27)	



Normal by cytogenetics and *microarrays*

Altered by cytogenetics and *microarrays*

Application of arrays in MDS

GENES, CHROMOSOMES & CANCER 00:00–00 (2013)

RESEARCH ARTICLE

Single Nucleotide Polymorphism Array Karyotyping: A Diagnostic and Prognostic Tool in Myelodysplastic Syndromes with Unsuccessful Conventional Cytogenetic Testing

Leonor Arenillas,[1] Mar Mallo,[2] Fernando Ramos,[3] Kathryn Guinta,[4] Eva Barragán,[5] Eva Lumbreras,[6] María-José Larrayoz,[7] Raquel De Paz,[8] Mar Tormo,[9] María Abáigar,[6] Carme Pedro,[10] José Cervera,[5] Esperanza Such,[5] María José Calasanz,[7] María Díez-Campelo,[6] Guillermo F. Sanz,[5] Jesús María Hernández,[6] Elisa Luño,[11] Silvia Saumell,[1] Jaroslaw Maciejewski,[4] Lourdes Florensa,[1] Francesc Solé[2*]

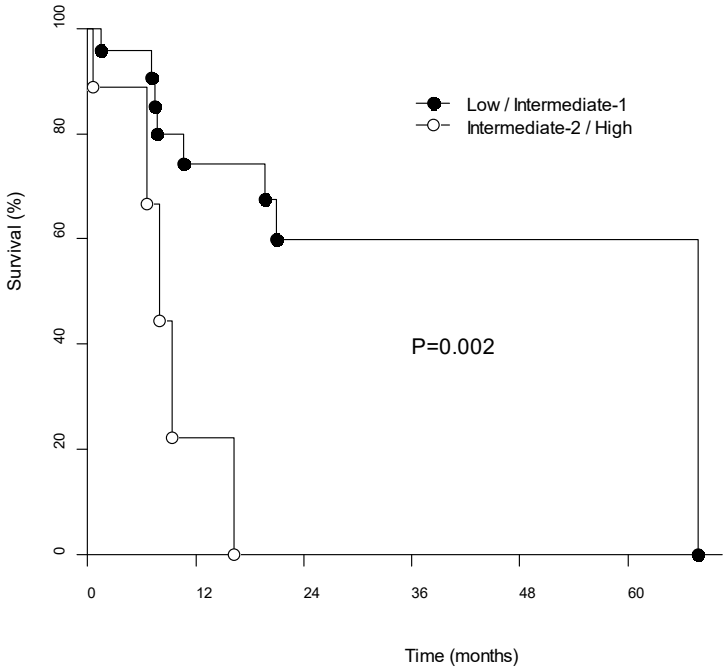
Without cytogenetic result
we could not apply
IPSS, IPSS-R nor IPSS-M

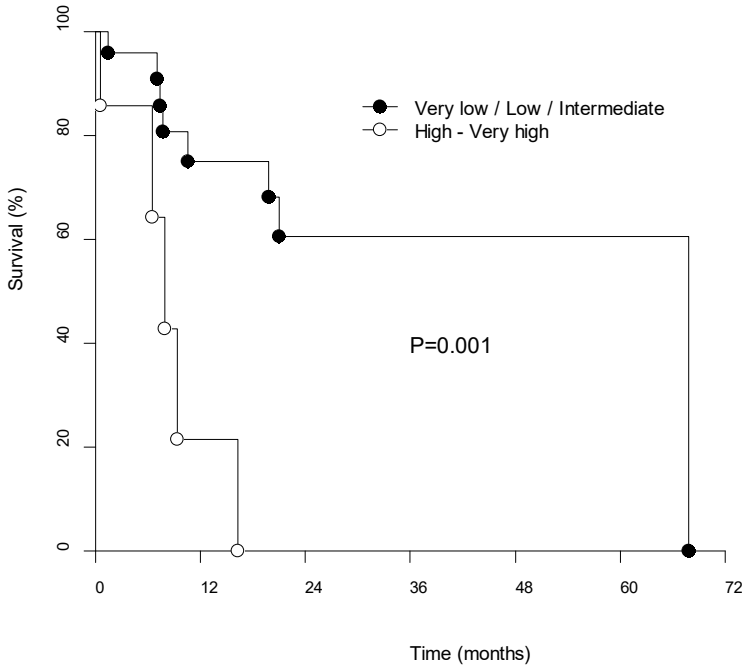
n=62 (56 MDS, 4 CMML, 2 RAEB-t)

↓

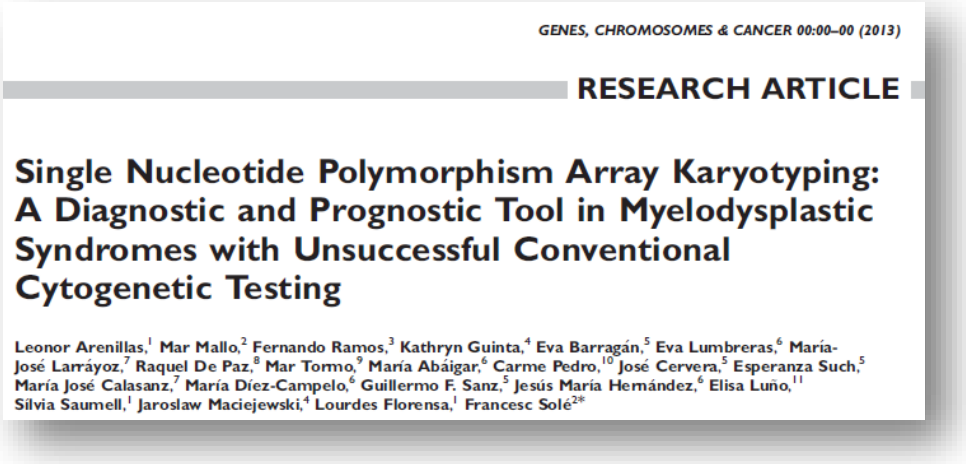
50% altered by SNP arrays

IPSS and **IPSS-R** application





Application of arrays in MDS



GENES, CHROMOSOMES & CANCER 00:00–00 (2013)

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Without cytogenetic result we could not apply IPSS, IPSS-R nor IPSS-M

In MDS cases without cytogenetic result it is recommended to apply SNP arrays. This could be also useful for patients with normal karyotype

Exemple 2 MDS

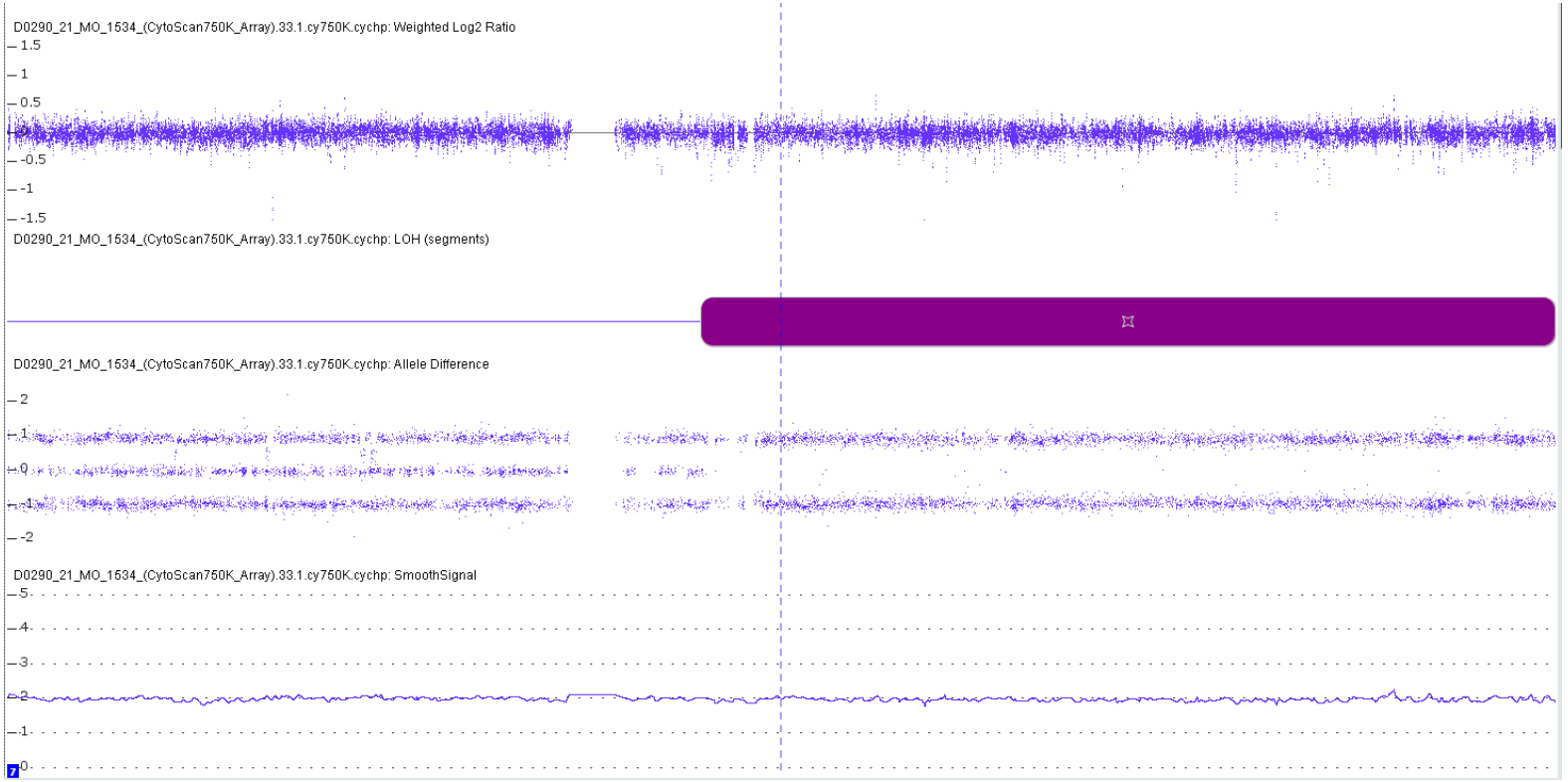
Gen	Chr	Tipo de variante	Cambio de secuencia	Cambio de aminoácido	VAF (%)			
					DX	S1	S2	P
SF3B1	chr2	Cambio de aminoácido	c.A2098G	p.K700E	46	34	35	42
TET2	chr4	Ganancia de stop	c.C2746T	p.Q916X	39	37	43	49
TET2	chr4	Ganancia de stop	c.G5620T	p.E1874X	38	27	40	46
EZH2	chr7	Cambio de aminoácido	c.G2051A	p.R684H	79	56	73	90
CUX1	chr7	Deleción tipo *frameshift*	c.2390delA	p.Q797Rfs*11		58	73	92

Suspected ROH in 7q → SNP-A

Exemple 2 MDS

Chr. 7 No copy number alterations but….

Exemple 2 MDS



Chr. 7q with CN-LOH at 7q (copy neutral LOH)

Application of arrays in ALL

Report the followng genetic alterations:

- Hypodiploidy
- IKZF1 and CDKN2A/B
- TP53 (17p-)

Other alterations:

- Insterstitial deletions that reveal fusions: P2RY8-CRLF2; EBF1-PDGFRB (Ph-like), or fusions at PAX5, PAX5 AMP, iAMP21, etc.
- t(9;22) o t(1;19)
- Hyperdiploidies

High risk genetic changes (to be transplanted):

- Rearrangement of MLL (KMT2A)

- **ALTERATIONS DETECTED BY SNP ARRAYS:**
  - Deletions of IKZF1 or CDKN2A/B
  - TP53 biallelic (deletion + mutation or LOH)
  - Low hypodiploidy in patients >35 YO

Application of arrays to ALL

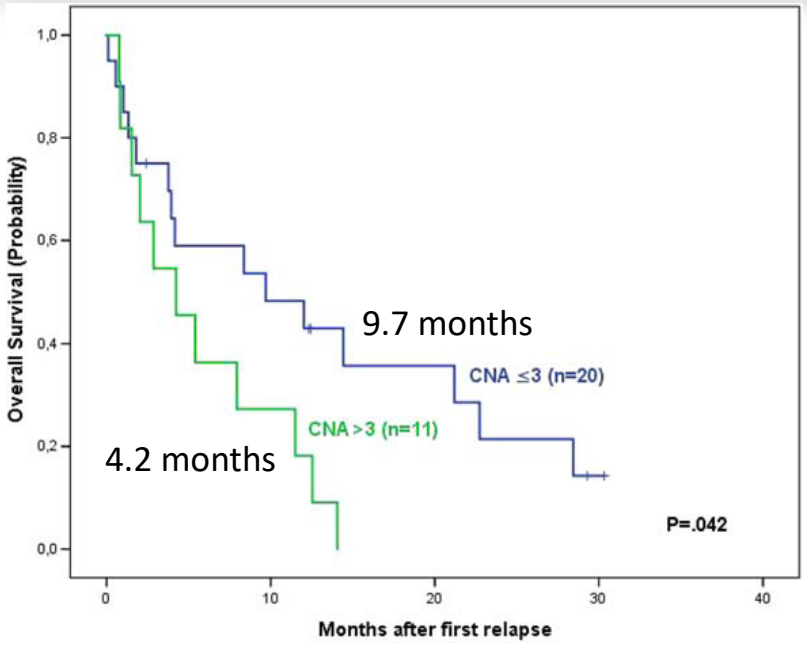
Received: 28 April 2017 | Revised: 22 July 2017 | Accepted: 22 July 2017
DOI: 10.1002/gcc.22486

RESEARCH ARTICLE

WILEY

Copy number profiling of adult relapsed B-cell precursor acute lymphoblastic leukemia reveals potential leukemia progression mechanisms

Jordi Ribera[1] | Lurdes Zamora[1,2] | Mireia Morgades[1,2] | Mar Mallo[1] | Neus Solanes[1] | Montserrat Batlle[1,2] | Susana Vives[1,2] | Isabel Granada[1,2] | Jordi Juncà[1,2] | Roberto Malinverni[1] | Eulàlia Genescà[1] | Ramon Guàrdia[3] | Santiago Mercadal[4] | Lourdes Escoda[5] | Joaquín Martinez-Lopez[6] | Mar Tormo[7] | Jordi Esteve[1,8] | Marta Pratcorona[1,9] | Carmen Martinez-Losada[10] | Francesc Solé[1] | Evarist Feliu[1,2] | Josep-Maria Ribera[1,2]
for the Spanish PETHEMA Group and the Spanish Society of Hematology



n=31 B- ALL patients at first relapse, and 21 paired diagnostic samples analyzed by MLPA and SNP-A

Relevance of **poor prognosis CNA**:

- Patients harboring biallelic losses of *CDKN2A/B* at 1st relapse are more prone to presenting a 2nd relapse
- Patients with *TP53* deletion showed higher deletion burden at relapse

The relapsed clone is already present at diagnosis as a minor subpopulation usually not detected by conventional methods

These subclones, selected by therapeutic pressure, have survival advantages

Ribera *et al.*, GCC, 2017

Guidelines: report recommendations

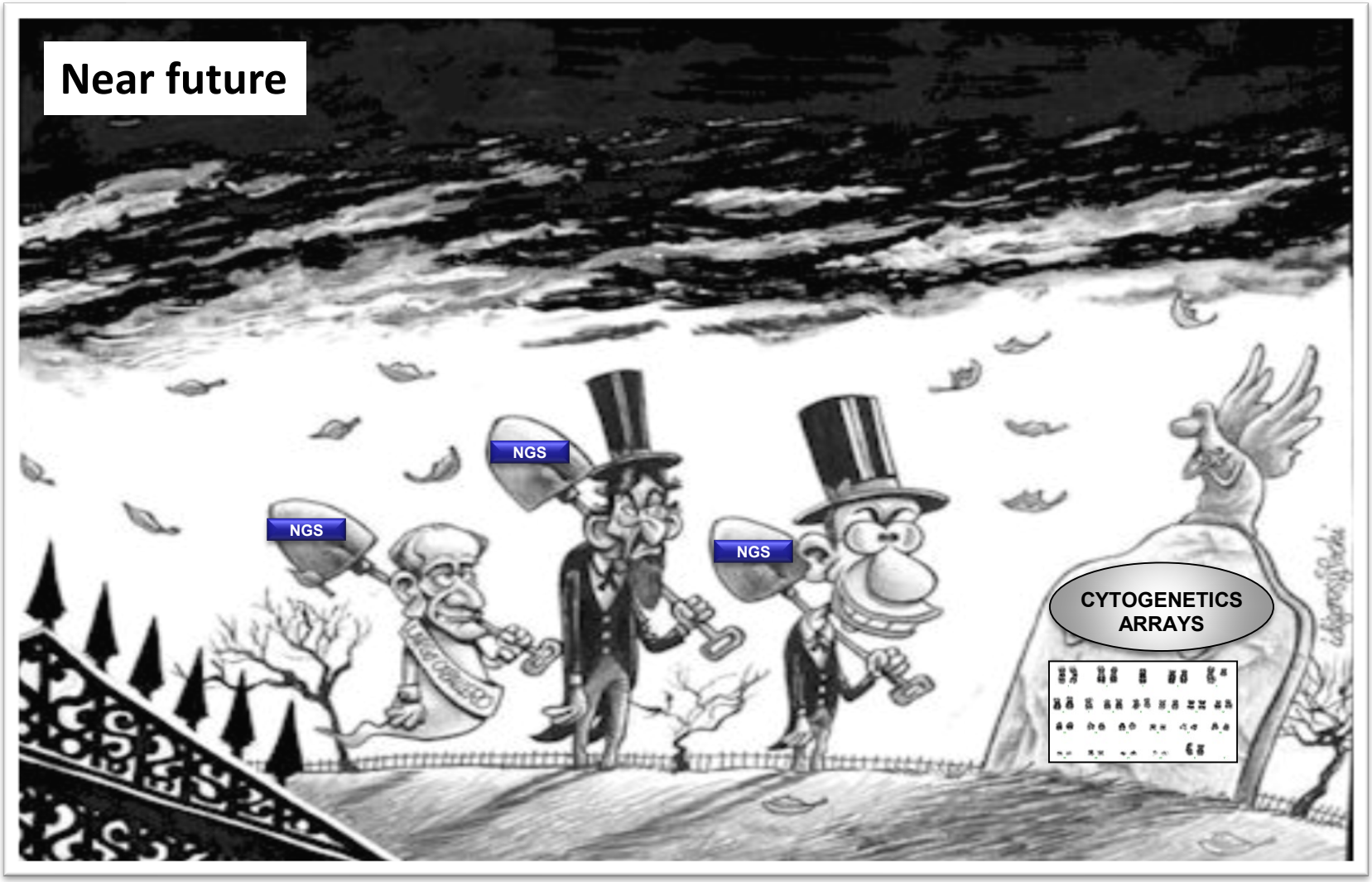
- ✓ Only CNAs >5Mb interpreted as abnormal, in order to reduce the detection of benign constitutional variants
- ✓ CNAs <5Mb when they encompass known tumoral related genes
- ✓ Focal CNA in T-cell receptor or immunoglobulin genes should be excluded
- ✓ Interpretation of CN-LOH needs to take into account: the size, level of mosaicism, the location (interstitial *vs.* terminal) and/or consanguinity. Studies showed that only large streches of CN-LOH (>10Mb) extending to the telomeres and/or in mosaic state could be considered as acquired. Otherwise, should be stated as "CN-LOH of uncertain origin"
- ✓ Plots from arrays containing SNPs may provide information regarding subclonal populations and ploidy level. **Visual inspection and manual review is mandatory**
  - ✓ **Guidelines for referring clinicians**: application of copy number arrays does not detect methylation anomalies or mRNA and microRNA expression

To sum up

- ✓ **Application SNP arrays in cases where the main genetic changes are copy number alterations:**
  - ✓ **CLL**: four FISH probes or just a single array? Cost effective technique
  - ✓ **ALL**: to detect CNA and to detect hyperdiploid, hypo haploid, … and alterations of known prognostic paper
  - ✓ **MM**: hyperdiploid or hypodiploid cases, and also detect loss of 17p or LOH of 17p (TP53)
  - ✓ **MDS**:
    - ✓ cases with normal karyotype
    - ✓ Cases without mitosis and then we could apply IPSS-R and IPSS-M
    - ✓ Detection of del(17p) or LOH at 17p. Multi hit status of TP53 (Bernard *et al.,* 2021)
- ✓ To complement NGS studies in cases with suspected LOH

What is the Genetic Technique of the Future?

What is the Genetic Technique of the Future?



What is the Genetic Technique of the Future?

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Genome Sequencing as an Alternative to Cytogenetic Analysis in Myeloid Cancers

Eric J. Duncavage, M.D., Molly C. Schroeder, Ph.D., Michele O'Laughlin, B.S., Roxanne Wilson, B.S., Sandra MacMillan, B.S., Andrew Bohannon, B.S., Scott Kruchowski, B.S., John Garza, B.S., Feiyu Du, M.S., Andrew E.O. Hughes, M.D., Ph.D., Josh Robinson, B.A., Emma Hughes, B.S., Sharon E. Heath, Jack D. Baty, B.A., Julie Neidich, M.D., Matthew J. Christopher, M.D., Ph.D., Meagan A. Jacoby, M.D., Ph.D., Geoffrey L. Uy, M.D., Robert S. Fulton, M.S., Christopher A. Miller, Ph.D., Jacqueline E. Payton, M.D., Ph.D., Daniel C. Link, M.D., Matthew J. Walter, M.D., Peter Westervelt, M.D., Ph.D., John F. DiPersio, M.D., Ph.D., Timothy J. Ley, M.D., and David H. Spencer, M.D., Ph.D.

11 March, 2021

What is the Genetic Technique of the Future?

blood®
Special Report

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, 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]

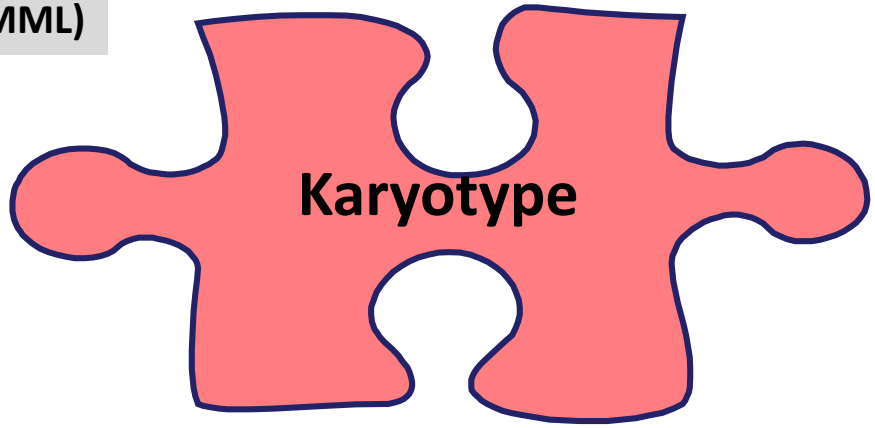
blood® 14 APRIL 2022 | VOLUME 139, NUMBER 15 **2273**

To conclude: What should we do?

Institut de Recerca CONTRA LA LEUCÈMIA Josep Carreras

Apply in all cases

IPSS-R (MDS)
CPSS (CMML)



To conclude: What should we do?

Institut de Recerca CONTRA LA LEUCÈMIA Josep Carreras

Apply in all cases

IPSS-R (MDS)
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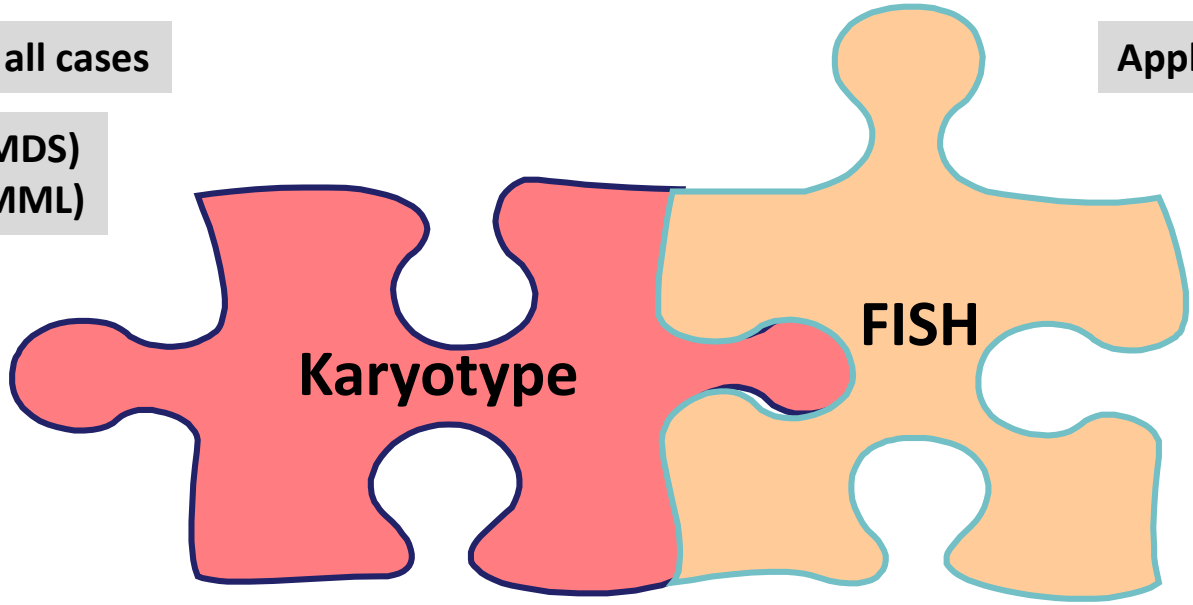


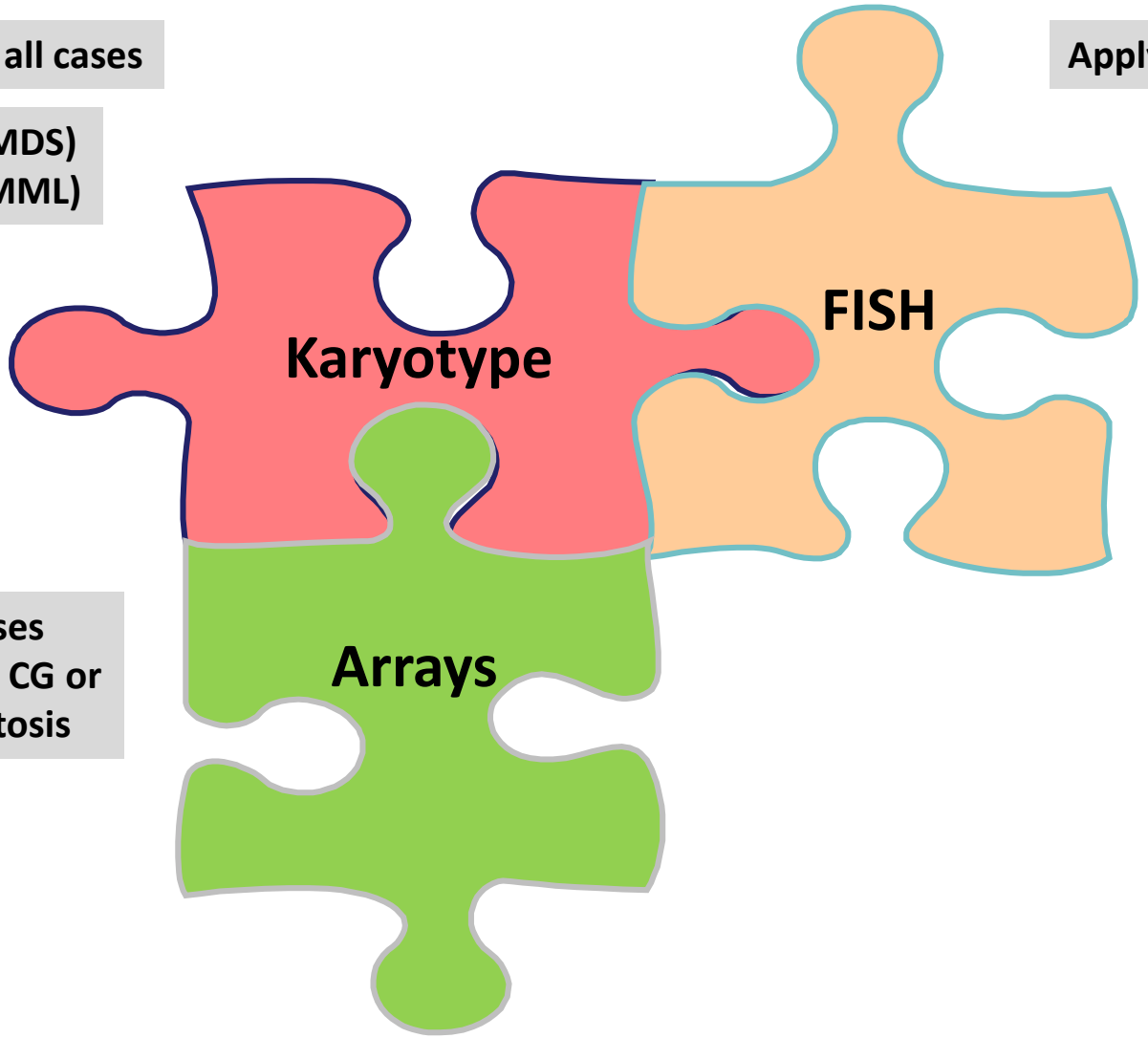
Apply in selected cases

To conclude: What should we do?

Institut de Recerca CONTRA LA LEUCÈMIA Josep Carreras

Apply in all cases

IPSS-R (MDS)
CPSS (CMML)



Apply in selected cases

Apply in cases wih normal CG or without mitosis

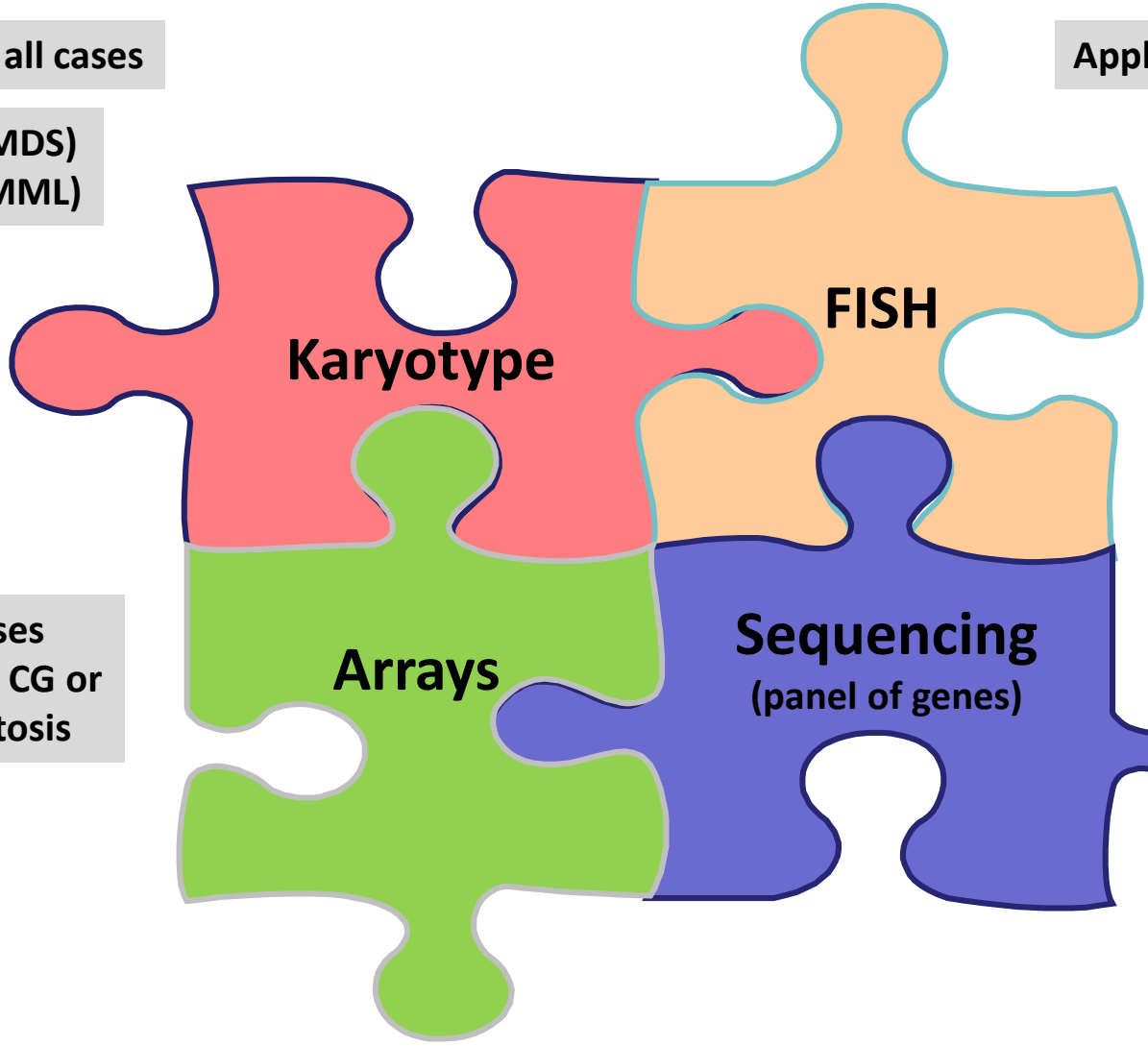
To conclude: What should we do?

Institut de Recerca CONTRA LA LEUCÈMIA Josep Carreras

Apply in all cases

IPSS-R (MDS)
CPSS (CMML)

Apply in selected cases

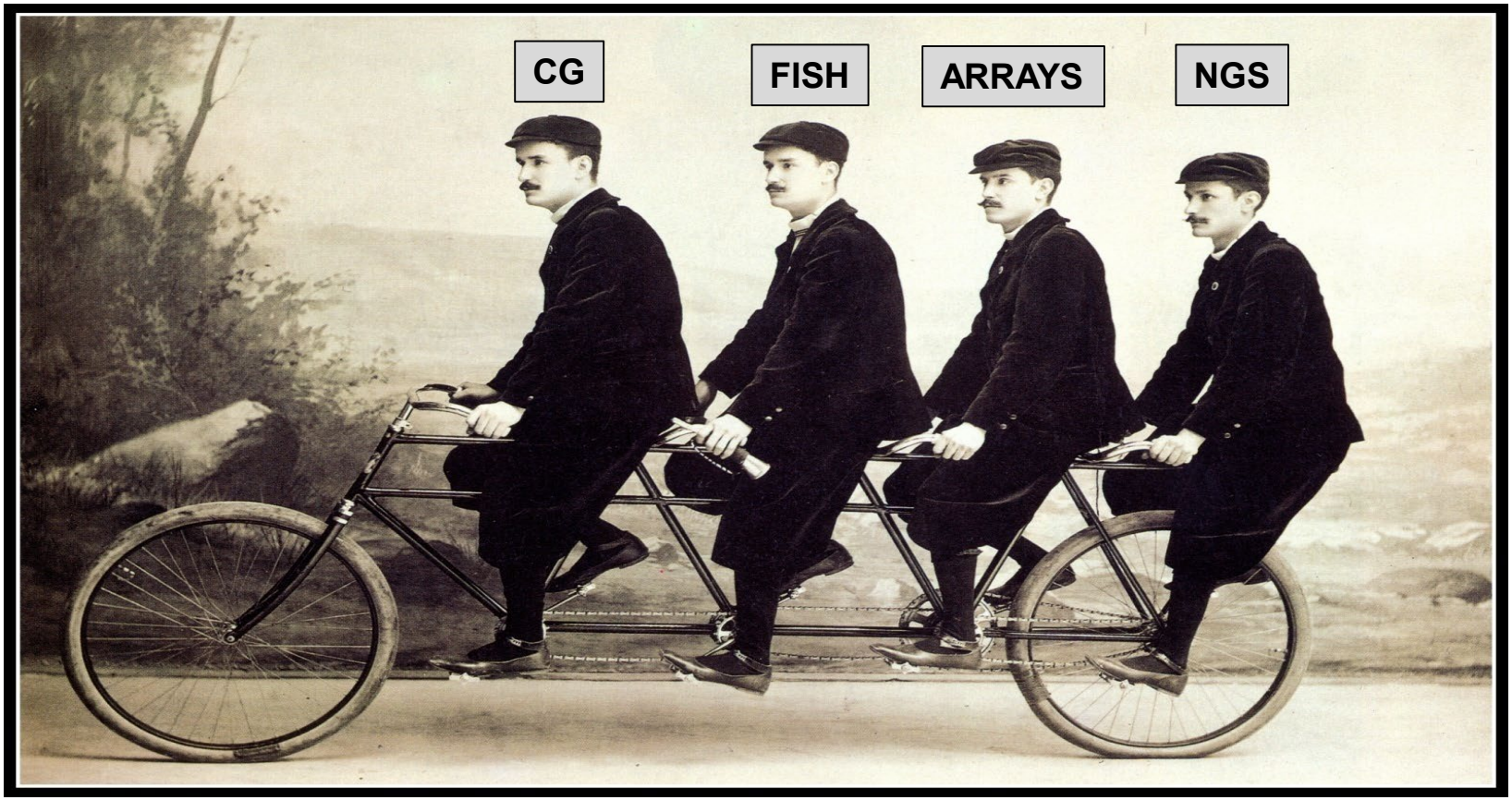


Apply in cases wih normal CG or without mitosis

Start to introduce

IPSS-M (MDS)

CONSIDERATIONS: What should we do?



All techniques have their part to play...

Thank you!

Institut de Recerca
CONTRA LA LEUCÈMIA
Josep Carreras

ICO
Institut Català d'Oncologia

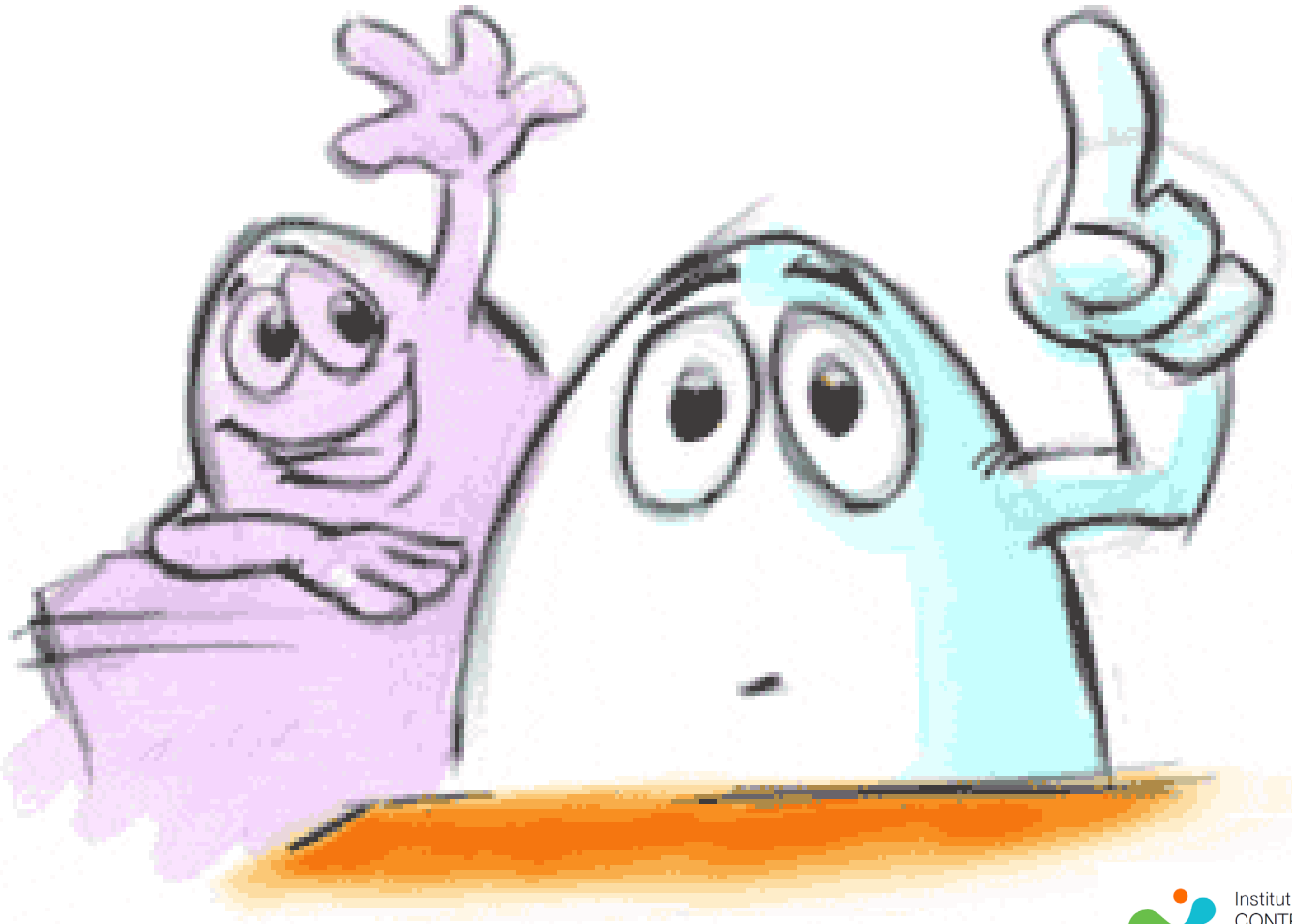
Institut Català d'Oncologia:

- Isabel Granada
- Adela Cisneros
- Neus Ruiz

Institut de Recerca contra la Leucèmia Josep Carreras:

- Jordi Ribera
- Pamela Acha

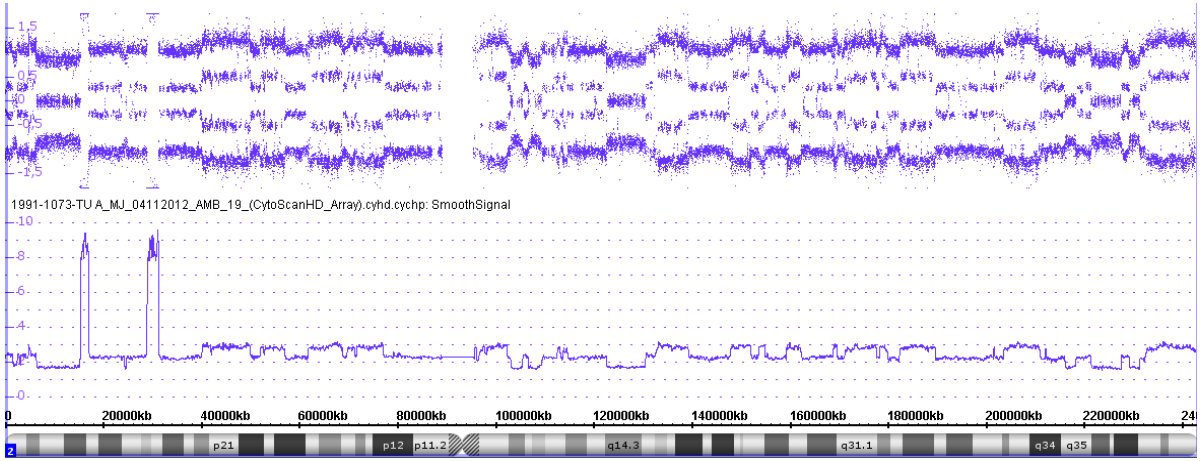
Thank you!



Institut de Recerca
CONTRA LA LEUCÈMIA
Josep Carreras

Specific terms

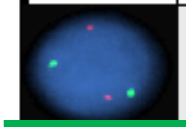
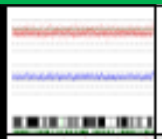
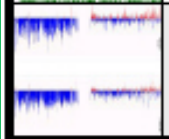
- **Chromothripsis:** A copy-number profile that has alternating copy states in a single region—typically a single chromosome or chromosome arm—that contains at least ten distinct alternating copy-number segments



- **Intrachromosomal complexity:** Summary of chromosomal regions that include more than two copy-number states, and contain at least five distinct copy-number segments

- **Genomic complexity:** Pattern of chromosome instability predominantly due to structural alterations resulting in widespread gains and losses of chromosomes or chromosomal regions in the majority of chromosomes

Comparative cytogenetic techniques

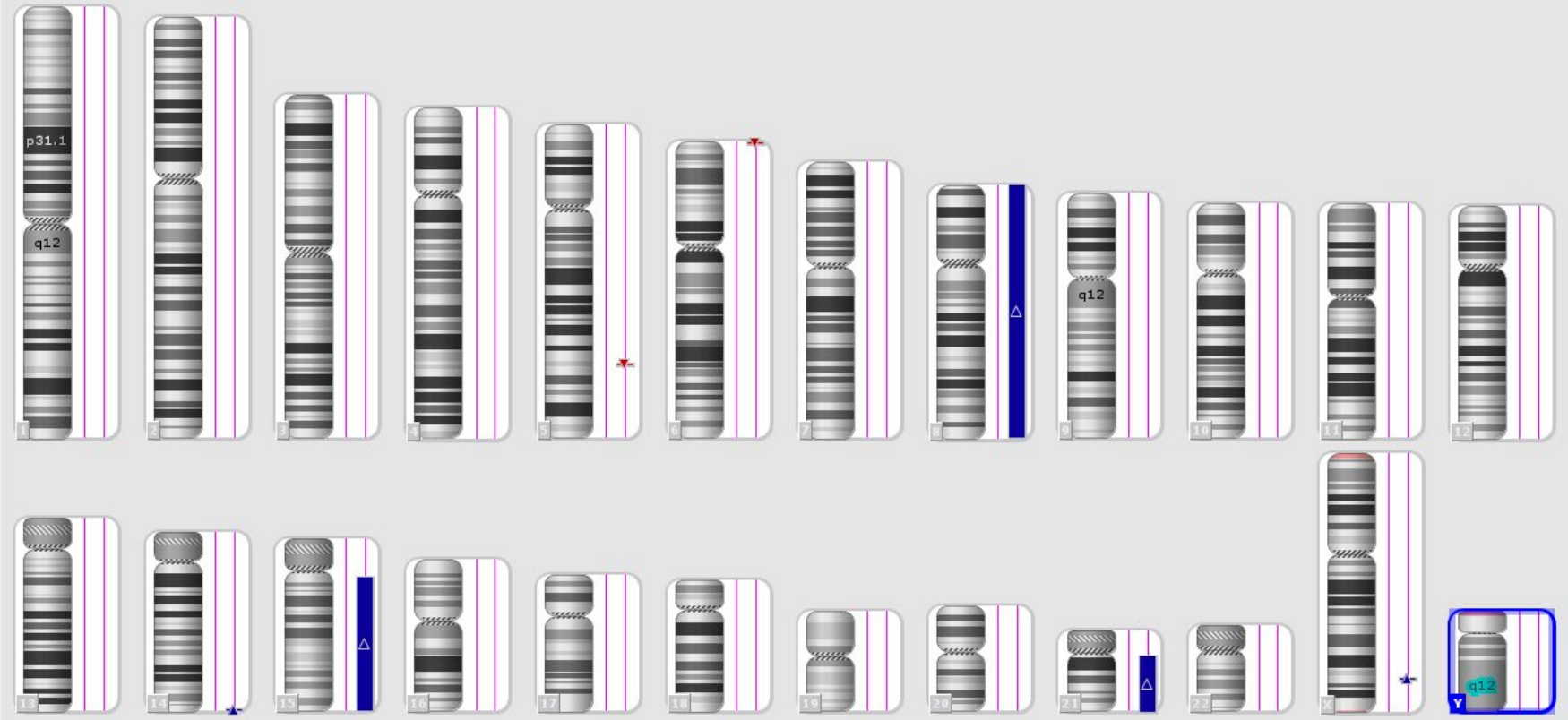
Output of Method	Method	Resolution	Sensitivity	UPD Detection	Dividing Cells Needed	Distinction of Individual clones	Screening for New Lesions	Balanced lesions
	Metaphase Cytogenetics	Low	10%	No	Yes	Yes	Yes	Yes
	FISH	Low	High	No	No	Yes	No	No
	SNP	High	2 - 30%	Yes	No	No	Yes	No
	CGH	High	2 -30%	No	No	No	Yes	No

Maciejewski J *et al*. Application of Array-based Whole Genome Scanning Technologies as a Cytogenetic Tool in Hematologic Malignancies. Br J Haematol 2009;146(5):479-88

Exemple 1 MDS

49,XX,+8,+15,+21

Exemple 1 MDS



49,XX,+8,+15,+21

Genomic arrays – ROH detection

Genotype detection: A-B

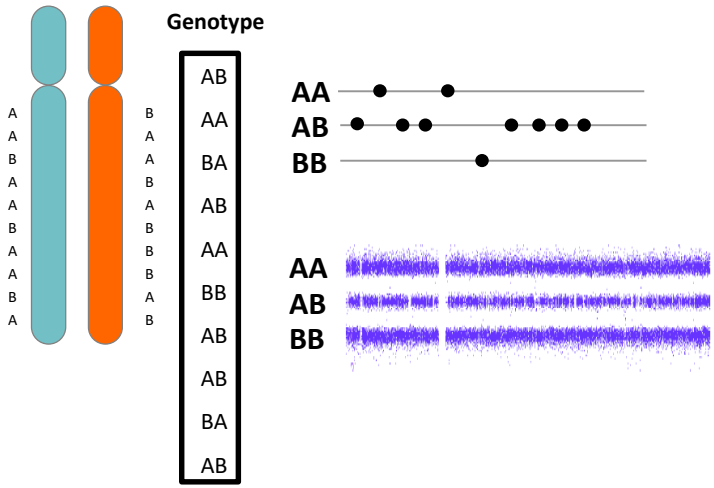
A	0,5
B	-0,5

Genotype “AA” = 0,5+0,5 = 1

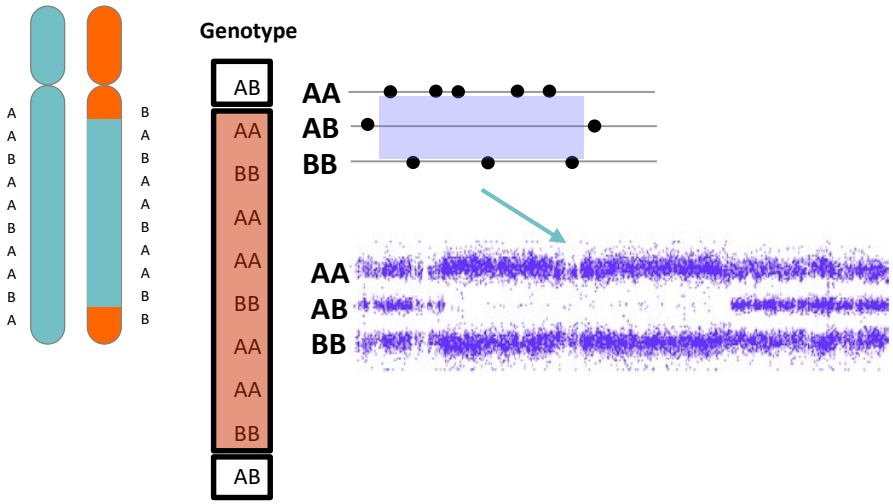
Genotype “AB” = 0,5 - 0,5 = 0

Genotype “BB” = -0,5-0,5 = -1

Region of heterozygosis



Region of homozygosis



What is the Genetic Technique of the Future?

	G-banding	FISH	CMA	WGS	Targeted sequencing panels		RT-PCR	MPSeq	WTS	OGM
Analyte	Chromosome in dividing cells	DNA in interphase nuclei and metaphase	DNA	DNA	DNA	RNA	RNA	DNA	RNA	DNA
Coverage	Whole	Targeted	Whole	Whole	Targeted	Targeted	Targeted	Whole	Whole	Whole
Distinction of individual cell clones	Yes	Yes	No	No	No	No	No	No	No	No
Analysis bias	Yes	Yes (if cultured)	No	No	Yes	Yes	No	No	No	No
Turnaround time (d)	3-7	4 h to 2 d	3-7	7-14	7-14	7-14	4 h to 5 d	7-14	14-21	7-10
Unmapped region detection	Yes	No	No	No	No	No	No	No	No	Some Alu and LINE elements
Ability to multiplex	Low	Low	High	High	High	High	High	High	High	Low to medium
Analytical sensitivity (%)	1*-3 out of 20 metaphases	1-10	10-20	20-30	1-10	5-10	~0.01	10	1-10	5-20
SVs	Yes	Yes	No	Yes (long-read or short-read deep sequencing)	No	Gene fusion	Limited	Yes	Yes	Yes
CNVs	Yes	Yes	Yes	Yes	Limited	Limited	Limited	Yes	Limited	Yes
SNVs	No	No	No	Yes	Yes	Yes	No	Limited	Yes	No
Disease status	Diagnosis, disease monitoring, relapse	Diagnosis, disease monitoring, relapse	Diagnosis, relapse	Diagnosis, relapse	Diagnosis, disease monitoring, relapse, MRD (if deep coverage)	Diagnosis, disease monitoring, relapse, MRD (if deep coverage)	Diagnosis, disease monitoring, MRD, relapse	Diagnosis, relapse	Diagnosis, relapse	Diagnosis, relapse
Well-established	High	High	High	Low	High	High	High	Low	Low	Low
Cost	++	++	+++	+++++	+++	+++	++	++++	++++	+++

*Depending on the clinical situation, 1 metaphase with a recurring abnormality may be considered evidence for an abnormal clone.

Akkari *et al.*, Blood, 2022

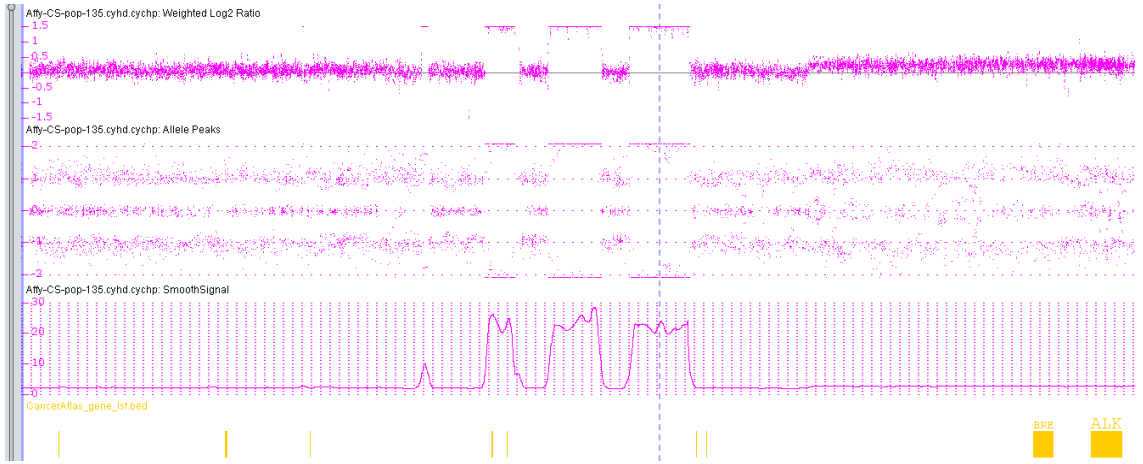
Specific terms

- **Gain/loss:** Type of copy-number change observed. It is recommended that the term “gain” be used rather than “duplication.”

- **CNAs (copy number alterations)**

- **Copy-neutral loss of heterozygosity (CN-LOH):** Allelic imbalance without an associated copy-number change. Uniparental disomy (UPD) should be used when the change is germline.

- **Amplification:** High copy-number gain of sequences, typically containing oncogene(s). Standard thresholds used to represent amplification typically range from 3–5 fold increases over >100 copies



Genomic arrays – regions of homozygosity

- **LOH: *loss of heterozygosity***

  Describes the fact that heterozygosity (previously present in that region) has been lost. It happens in deletions and can also happen without a change in the copy number

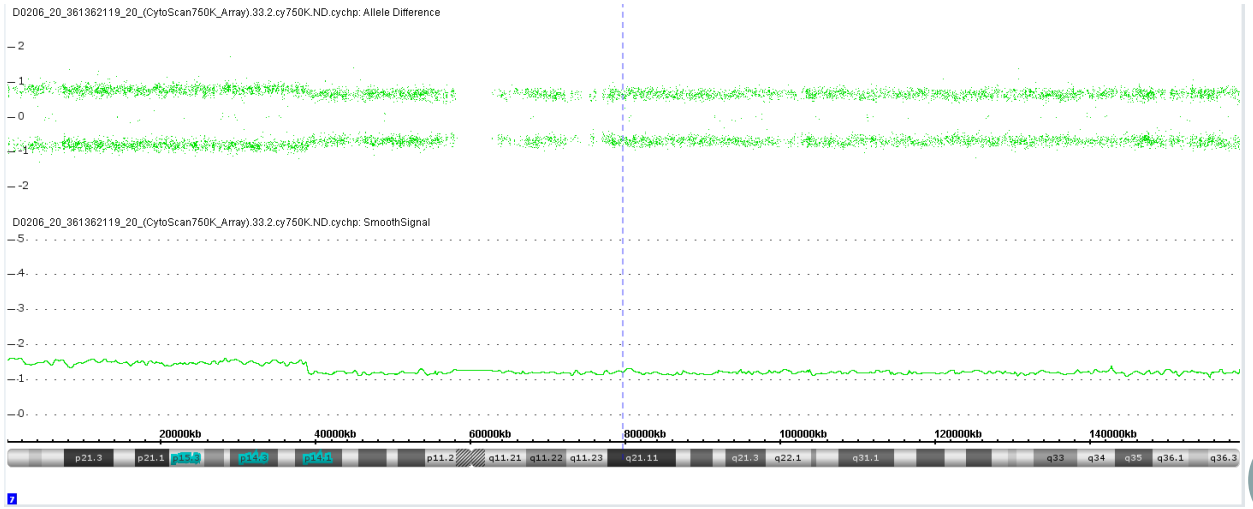
- **AOH: *absence of heterozygosity***

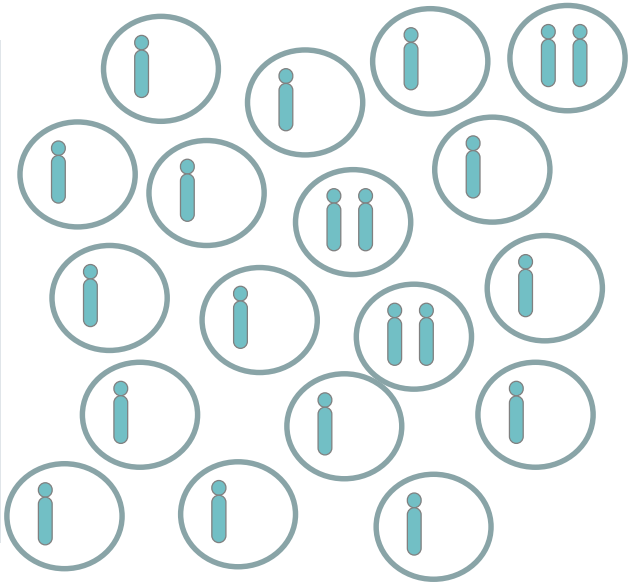
  Describes the observation, at a specific time, that there is no heterozygosity. It happens in deletions and can also happen without a change in the copy number

- **ROH: *runs of homozygosity* / LCSH: *long contiguous stretch of homozygosity / CN-LOH: copy neutral loss of heterozygosity***

  Specific term for homozygosity without copy number alteration. It does not apply for hemizygous deletions

Exemple 3: ALL-B





Compatible with hypodiploid karyotype with endorreduplication
False hyperdiploid case

Hyperdiploid: Good prognosis
Hypodiploid: Poor prognosis

Our reports

Recomendations:

Single page (if it is possible)

Specify lmitations

If QC is not correct, analyse with caution

Lab reference number: 50303

MICROARRAYS REPORT

Surname	Dukakis	**First name**	Elena
Date of birth	11/11/1939	**Medical record number**	-
External ID	738AA18	**Internal ID**	D410/18
Sample type	DNA from PB	**Sample reception date**	24/05/2018
Reason for request/Clinical indication	Newly diagnosed CLL. Prognostic indicators?		
Sex	Female		
Referring clinician	Dr EQA		

Technique and methods

Genetic imbalances analysis have been performed with the **CytoScan 750K** microarray (Affymetrix®) with a coverage from the whole genome (750.000 probes). Samples have been processed with GeneChip® System (GCS) 3000 Affymetrix® platform, according to the manufacturer manual (*CytoScan Assay* P/N 703038 Rev.3). For the analysis, Chromosome Analysis Suite (Affymetrix®) v. 3.2, with NetAffx na 33.2 (UCSC hg19) version of annotations was used.

According to the detection analysis parameters (a minimum of 25 altered markers), an average resolution of 110 Kb is reached. For all altered regions, those with an overlap above 50% with any polymorphic region were excluded (copy number variants extracted from an internal database from Affymetrix® and the *Database of Genomic Variants*), as well as centromeric regions.

Results

arr[GRCh37] 4p16.2p15.1(4614863_31159849)x1,11q22.3(107930039_108447899)x1,13q14.2q14.3 (50523537_51694092)x1

Clinical interpretation

Female chromosomal sex. There is a loss of 26Mb in 4p16.2p15.1, from 4614863bp to 31159849bp; a loss of 517Kb in 11q22.3, from 107930039bp to 108447899bp; and a loss of 1.1Mb in 13q14.2q14.3 from 50523537bp to 51694092bp.

Deletions of 11q and 13q are recurrent alterations in CLL. Deletion of 11q involves ATM gene; and deletion of 13q involves *MIR15, MIR16-1* and *DLEU* (Type I or *RB1* not included). The presence of 11q deletion is associated with adverse prognosis.

Limitations

This result is subject to the limitations on the type of study, mainly the non-detection of chromosomal rearrangements smaller than the resolution of the microarray, low mosaicism percentages and balanced rearrangements. A normal result does not exclude the possibility that the clinical phenotype may be due to genetic causes not tested in this genetic test. The relevance and significance of chromosomal abnormalities detected, and possible polymorphic variants are interpreted according to the criteria and information sources available that can change after the date of the report.

Result validated by the service chief	**Result validated by the technical assistant**
Signature	Signature
Date	21/06/2018

Page **1** of **1**

Guidelines: report recommendations

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)

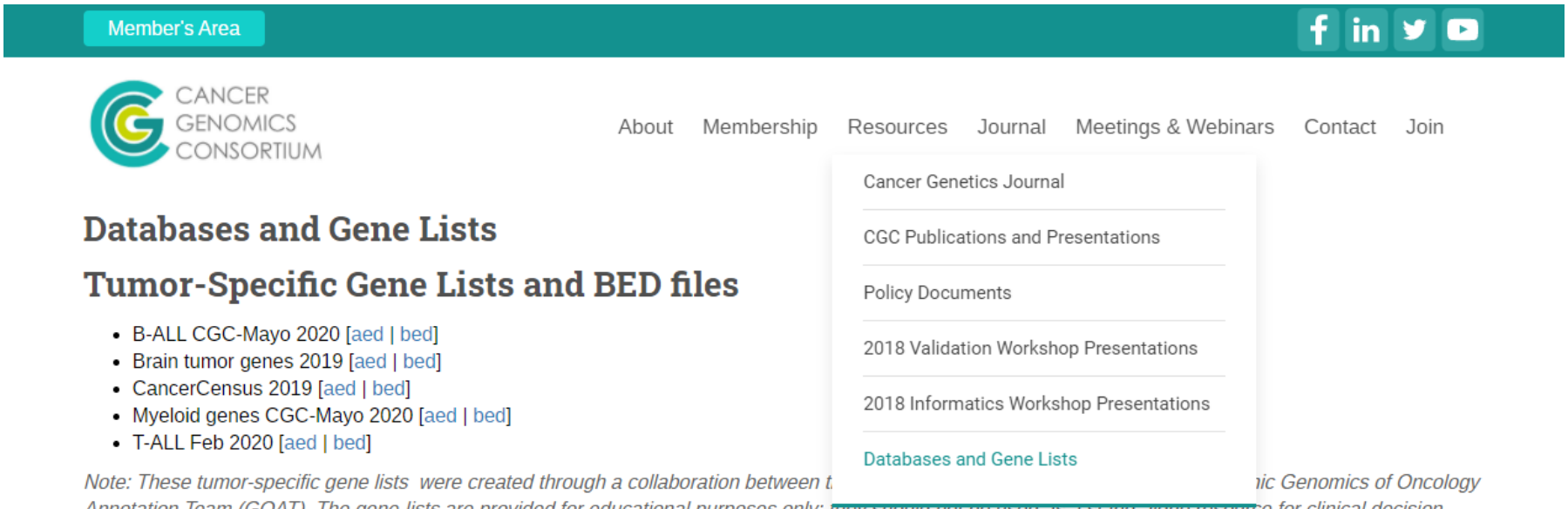
Fady M. Mikhail, MD, PhD[1], Jaclyn A. Biegel, PhD[2], Linda D. Cooley, MD, MBA[3], Adrian M. Dubuc, PhD[4], Betsy Hirsch, PhD[5], Vanessa L. Horner, PhD[6], Scott Newman, PhD[7], Lina Shao, MD, PhD[8], Daynna J. Wolff, PhD[9] and Gordana Raca, MD, PhD[2]

Tier 1: Variants with strong clinical significance *(Diagnostic, prognostic, and/or therapeutic)*	**Tier 1A** • Acquired variants that define a specific entity in the WHO classification, are included in professional guidelines (e.g., NCCN, COG, IPSS), and/or can be treated with an FDA-approved drug • Germline pathogenic variants associated with cancer predisposition
	Tier 1B Acquired variants associated with a specific neoplasm, prognosis, or treatment response, as shown by high or good quality evidence (Levels 1, 2, and 3 CEBM evidence) with expert consensus and/or confirmed and reproduced by independent groups
Tier 2: Variants with some clinical significance *(Diagnostic, prognostic, and/or therapeutic)*	• Recurrent acquired variants observed in different neoplasms but not specific to a particular tumor type ----------OR---------- • Acquired variants associated with a specific neoplasm, prognosis, or treatment response, as shown by average quality evidence (Levels 4 and 5 CEBM evidence)
Tier 3: Clonal variants with no documented neoplastic disorder association	• Acquired variants with no documented neoplastic disorder association • All variants that do not meet the criteria for Tiers 1 and 2, and cannot be classified as constitutional benign or likely benign
Tier 4: Benign or likely benign Variants	• Constitutional benign or likely benign variants that are listed in the ClinGen curated benign variants and/or in the Database of Genomic Variants (DGV) with ≥1% population frequency • They usually do not encompass COSMIC cancer genes

MDS	del(5q)	Tier 1A
	CN-LOH 7q	Tier 1B
	+21	Tier 2

Mikhail *et al.*, Genetics in Medicine, 2019

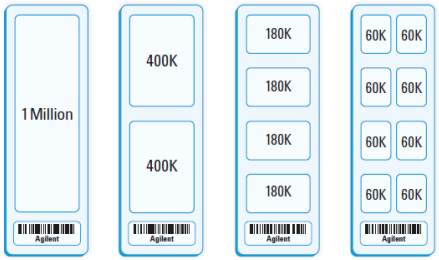
Resources


Member's Area
CANCER GENOMICS CONSORTIUM
About
Membership
Resources
Journal
Meetings & Webinars
Contact
Join
Cancer Genetics Journal
CGC Publications and Presentations
Policy Documents
2018 Validation Workshop Presentations
2018 Informatics Workshop Presentations
Databases and Gene Lists
Databases and Gene Lists
Tumor-Specific Gene Lists and BED files
B-ALL CGC-Mayo 2020 [aed | bed]
Brain tumor genes 2019 [aed | bed]
CancerCensus 2019 [aed | bed]
Myeloid genes CGC-Mayo 2020 [aed | bed]
T-ALL Feb 2020 [aed | bed]
Note: These tumor-specific gene lists were created through a collaboration between t ic Genomics of Oncology Annotation Team (GOAT). The gene-lists are provided for educational purposes only; they should not be used as a stand-alone resource for clinical decision making or reporting, as they may contain errors or omissions. Review of the data by an appropriately trained medical professional is required for clinical reporting. All BED and AED files are GRCh37/hg19 based. AED files are only viewable in Affymetrix ChAS software, but BED files should allow viewing in other software or genome browsers.
Compendium of Cancer Genome Aberrations
The CGC is developing the Compendium of Cancer Genome Aberrations (CCGA), a collaborative multi-institutional project to document and describe genomic aberrations in cancer as resource for day-to-day use in clinical reporting. The CCGA is a wiki database designed to host up-to-date and integrative molecular genetics and cytogenetic features of specific cancers, highlighting actionable and diagnostically-important features. The first page on Acute Myeloid Leukemia (AML) and Related Precursor Neoplasms is nearly complete.

Genomic arrays - types

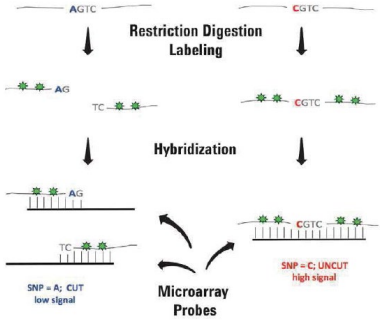
aCGH

- CN probes: oligonucleotides
- Competitive hybridization: tumoral DNA vs. control DNA



aCGH + SNP

- SNP probes (2xSNP)
- CN probes: oligonucleotides
- Detection through a restriction enzyme specific for each SNP



- Competitive hybridization: tumoral DNA vs. control DNA

SNP

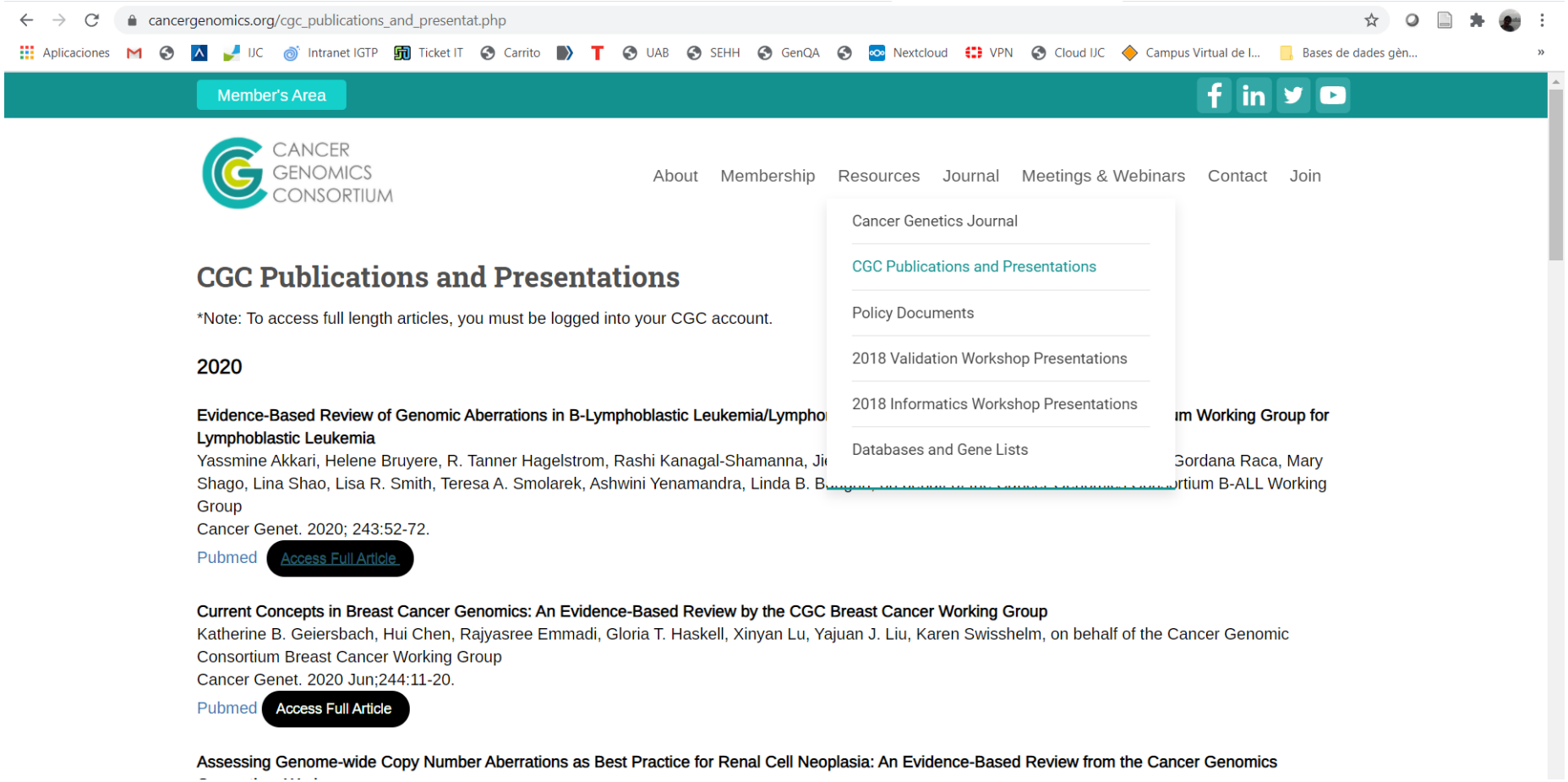
- SNP probes (2xSNP)
- CN probes: oligonucleotides
- Detection by fluorescence intensity

Genomic arrays. Advantages and Disadvantages

	aCGH	aSNP
Advantages	Robust platform: good coverage and resolution	
	"Custom" design	
	Two-colour hybridisation (1 array) BACs: “home-made” design	ROH detection
Disadvantages	BACs: low resolution and specificity (co-hybridisation)	One-colour hybridisation (2 arrays) Sealed platform
	Interpretation becomes more tedious as array resolution increases	

Choice of array depends on the study to be performed

Application of arrays to hematological neoplasms

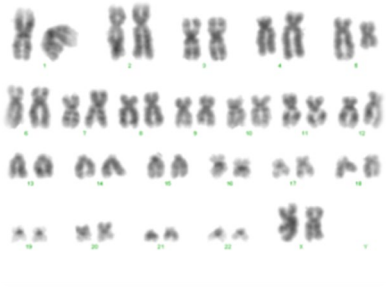


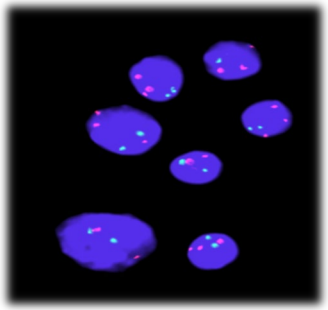
Strategy for cytogenetic study: Past / Present

Conventional Cytogenetics

- **Altered**
- **Normal**
- **No metaphases**

FISH





Information on
1 *loci*

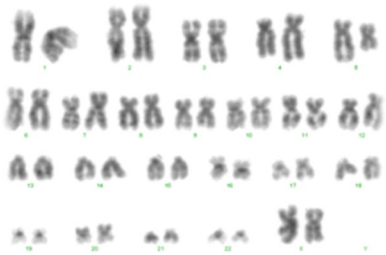
Strategy for cytogenetic study : Present / Future

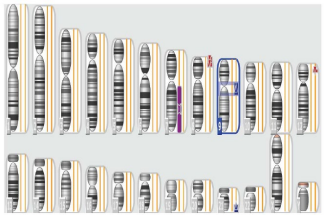
Conventional Cytogenetics

Altered | **Normal** | **No metaphases**

ALL

Arrays
FISH translocations (ALL)





Information on the **whole** genome

Application of arrays to ALL

ARTICLES

nature genetics

The genomic landscape of hypodiploid acute lymphoblastic leukemia

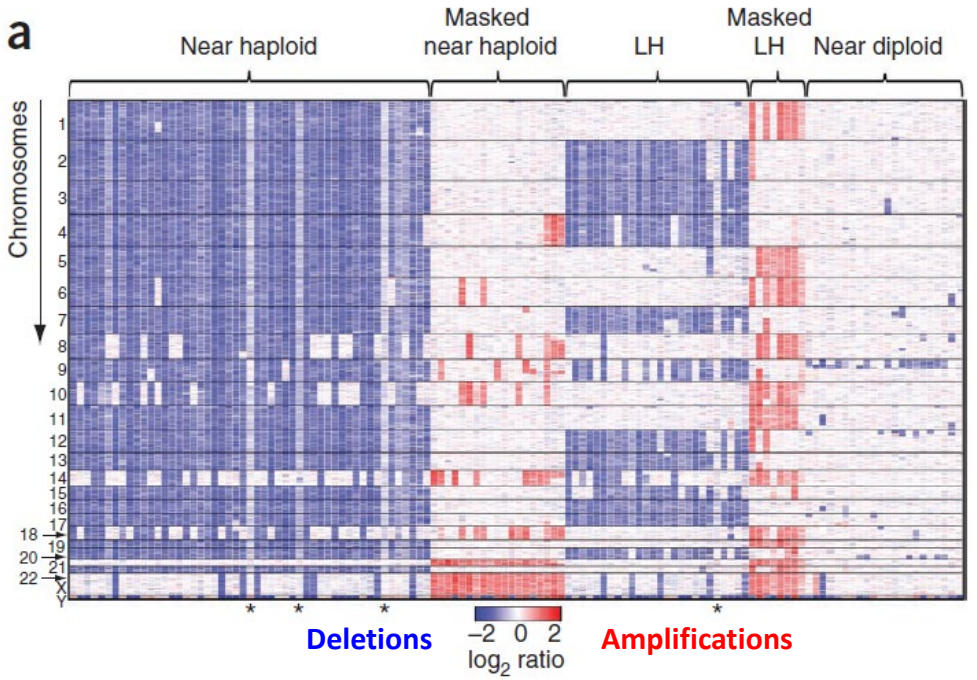
Panel: **Definition of cytogenetic risk groups**

Good risk*
- High hyperdiploidy (51–65 chromosomes)
- *ETV6–RUNX1*

Intermediate risk
- t(1;19)(q23;p13)
- *IGH–CEBP*
- *IGH–ID4*
- del(6q)
- Abnormal 9p
- Abnormal 11q
- dup(1q)
- -7
- dic(9;20)(p13;q11)
- dic(9;12)(p11–21;p11–13)
- Any other abnormality
- Normal karyotype

Poor risk†
- t(9;22)(q34;q11.2)
- iAMP21
- *MLL* translocations
- Near haploidy (<30 chromosomes)
- Low hypodiploidy (30–39 chromosomes)
- t(17;19)(q23;p13)
- Abnormal 17p
- Loss of 13q

Doubling of either a low-hypodiploid or a near-haploid clone results in an apparently high-hyperdiploid karyotype, which is often misclassified for risk



CN-LOH observed for diploid chromosomes in masked hypodiploid cases, consistent with duplication of the hypodiploid clone

Holmfeldt *et al.*, Nature Genetics, 2013

Application of arrays to ALL

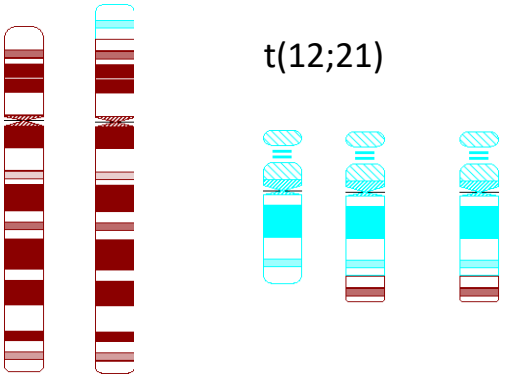
Received: 27 February 2017 | Revised: 31 May 2017 | Accepted: 5 June 2017
DOI: 10.1002/gcc.22477

RESEARCH ARTICLE

WILEY

Copy number alterations determined by single nucleotide polymorphism array testing in the clinical laboratory are indicative of gene fusions in pediatric cancer patients

Tracy M. Busse[1] | Jacquelyn J. Roth[2] | Donna Wilmoth[3] | Luanne Wainwright[3] | Laura Tooke[3] | Jaclyn A. Biegel[1,4]



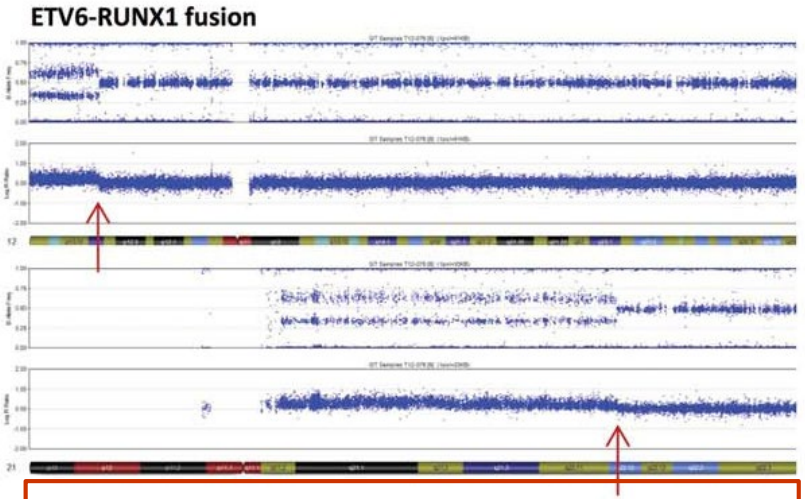
n=1,211 pediatric oncology patients

↓

132 CNA with SNP-A that demonstrated a structural rearrangement and indicated an associated gene fusion event

↓

1/3 of hematologic cases and in <10% of the solid tumor cases, the observed CNA stemmed from a gain or loss of the derivative chr associated with a translocation

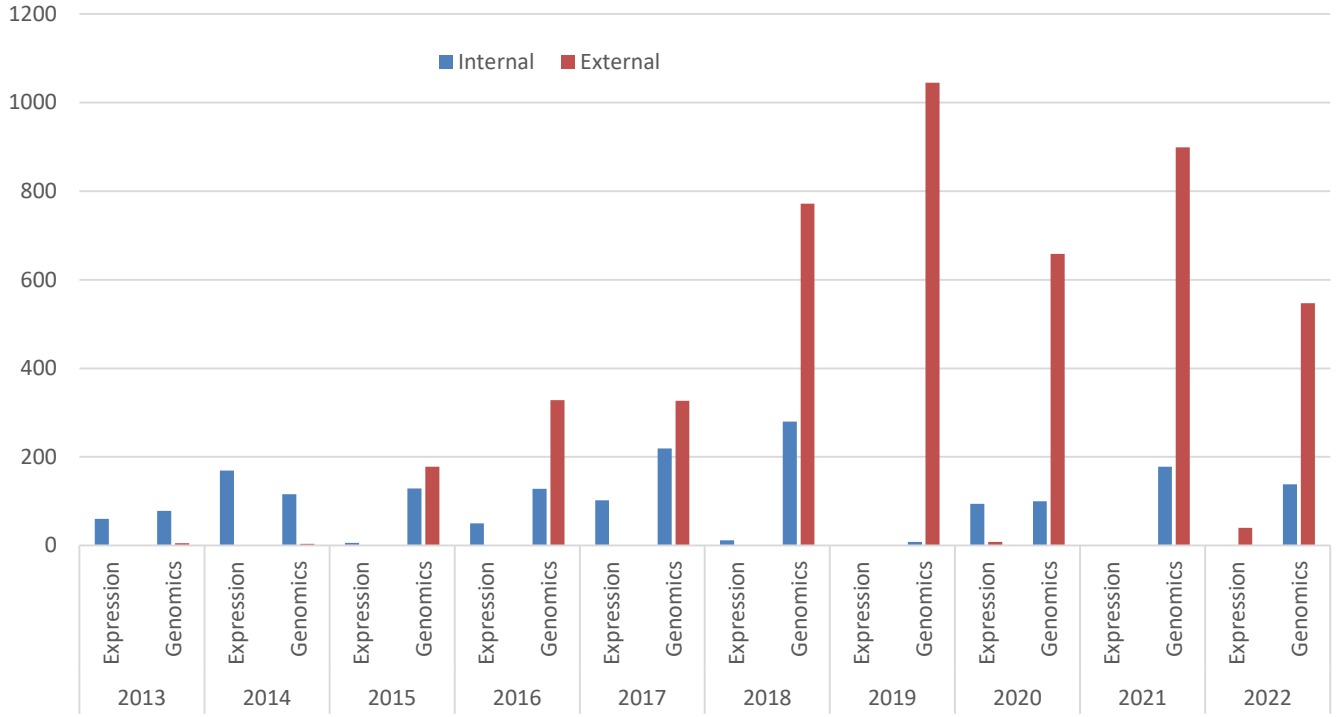


CNA gains in 12p and 21q with breakpoints in ETV6 and RUNX1 were consistent with a duplication of the der(21) in an ETV6-RUNX1 fusion

Busse *et al.*, GCC, 2017

Unit of Microarrays in IJC

	2013		2014		2015		2016		2017		2018		2019		2020		2021		2022	
	Expression	Genomics	Expression	Genomics	Expression	Genomics	Expression	Genomics	Expression	Genomics	Expression	Genomics	Expression	Genomics	Expression	Genomics	Expression	Genomics	Expression	Genomics
Internal	60	78	169	116	6	129	50	128	102	219	12	280	0	8	94	100	0	178	0	138
External	0	5	0	4	0	178	0	328	0	327	0	772	0	1045	8	658	0	899	40	547
Total	***60***	***83***	***169***	***120***	***6***	***307***	***50***	***456***	***102***	***546***	***12***	***1052***	***0***	***1053***	***102***	***758***	***0***	***1077***	***40***	***685***
Total	***143***		***289***		***313***		***506***		***648***		***1064***		***1053***		***860***		***1077***		***725***	



Unit of Microarrays in IJC

- Samples per year:
- SNP arrays:
  - Diagnosis:
    - MDS
    - ALL
    - Prenatal and post natal
  - Research:
- Expression arrays

Application of arrays in ALL

Received: 30 April 2018 | Revised: 14 June 2018 | Accepted: 18 June 2018
DOI: 10.1002/gcc.22664

WILEY

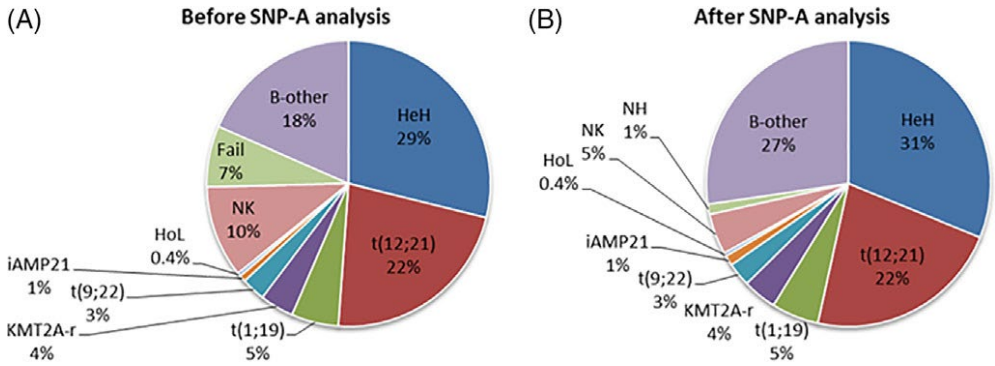
BRIEF REPORT

Improved cytogenetic characterization and risk stratification of pediatric acute lymphoblastic leukemia using single nucleotide polymorphism array analysis: A single center experience of 296 cases

Linda Olsson[1] | Kristina B. Lundin-Ström[2] | Anders Castor[3] | Mikael Behrendtz[4] | Andrea Biloglav[2] | Ulrika Norén-Nyström[5] | Kajsa Paulsson[2] | Bertil Johansson[1,2]

n= 296 ALL cases:

67% of T-ALL alteraciones >5Mb o
91% of B-ALL mejor caracterización



Mejor caracterización citogenética de LAL-T y LAL-B: 29% de los casos →
información importante para la estratificación del riesgo

Olsson *et al.*, GCC, 2018

What is the Genetic Technique of the Future?

	G-banding	FISH	CMA	WGS	Targeted sequencing panels		RT-PCR	MPSeq	WTS	OGM
Analyte	Chromosome in dividing cells	DNA in interphase nuclei and metaphase	DNA	DNA	DNA	RNA	RNA	DNA	RNA	DNA
Coverage	Whole	Targeted	Whole	Whole	Targeted	Targeted	Targeted	Whole	Whole	Whole
Distinction of individual cell clones	Yes	Yes	No	No	No	No	No	No	No	No
Analysis bias	Yes	Yes (if cultured)	No	No	Yes	Yes	No	No	No	No
Turnaround time (d)	3-7	4 h to 2 d	3-7	7-14	7-14	7-14	4 h to 5 d	7-14	14-21	7-10
Unmapped region detection	Yes	No	No	No	No	No	No	No	No	Some Alu and LINE elements
Ability to multiplex	Low	Low	High	High	High	High	High	High	High	Low to medium
Analytical sensitivity (%)	1*-3 out of 20 metaphases	1-10	10-20	20-30	1-10	5-10	~0.01	10	1-10	5-20
SVs	Yes	Yes	No	Yes (long-read or short-read deep sequencing)	No	Gene fusion	Limited	Yes	Yes	Yes
CNVs	Yes	Yes	Yes	Yes	Limited	Limited	Limited	Yes	Limited	Yes
SNVs	No	No	No	Yes	Yes	Yes	No	Limited	Yes	No
Disease status	Diagnosis, disease monitoring, relapse	Diagnosis, disease monitoring, relapse	Diagnosis, relapse	Diagnosis, relapse	Diagnosis, disease monitoring, relapse, MRD (if deep coverage)	Diagnosis, disease monitoring, relapse, MRD (if deep coverage)	Diagnosis, disease monitoring, MRD, relapse	Diagnosis, relapse	Diagnosis, relapse	Diagnosis, relapse
Well-established	High	High	High	Low	High	High	High	Low	Low	Low
Cost	++	++	+++	+++++	+++	+++	++	++++	++++	+++

*Depending on the clinical situation, 1 metaphase with a recurring abnormality may be considered evidence for an abnormal clone.

Akkari *et al.*, Blood, 2022

Application of arrays in CLL

ELSEVIER

Cancer Genetics 228–229 (2018) 236–250

Cancer Genetics

REVIEW ARTICLE

Assessing copy number aberrations and copy-neutral loss-of-heterozygosity across the genome as best practice: An evidence-based review from the Cancer Genomics Consortium (CGC) working group for chronic lymphocytic leukemia

Kathy Chun[a,1,3], Gail D. Wenger[b,3], Alka Chaubey[c], D.P. Dash[d], Rashmi Kanagal-Shamanna[e], Sibel Kantarci[f], Ravindra Kolhe[g], Daniel L. Van Dyke[h], Lu Wang[i,2], Daynna J. Wolff[j], Patricia M. Miron[k,l,*]

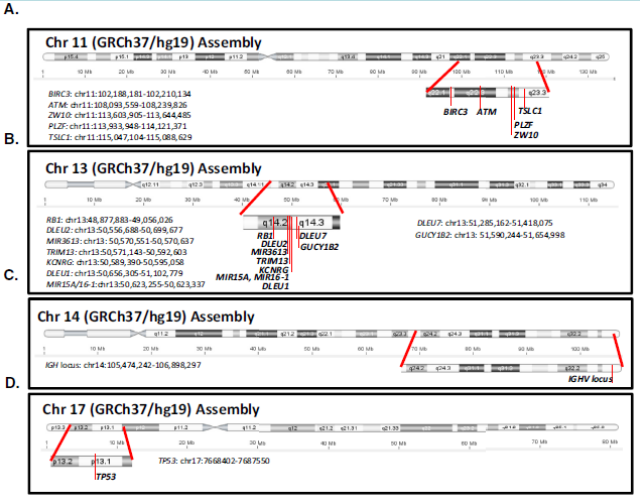
ROH with prognostic value

Table 2 Recurring regions of CN-LOH in CLL.

CN-LOH	Candidate gene	Association	Strength of evidence for prognosis (Level*)	References
13q	*miR15a/16–1*	Biallelic deletion of 13q	Established (1)	[34–36,46,48,49,91,99]
17p13	*TP53*	Homozygous *TP53* mutations	Established (1)	[34,36,43,49,94]
11q13-qter	Includes *ATM*	Monoallelic *ATM* deletion	Suspected (2)	[36,49]
20q11	Unknown	None	N/A (3)	[43,93]
1p36	Unknown	None	N/A (3)	[36,97]

* Level 1: present in WHO classification or professional practice guidelines; Level 2: recurrent in well-powered studies with suspected clinical significance; Level 3: recurrent, but uncertain prognostic significance

CNA with prognostic value



Application of arrays in MM

ELSEVIER

Cancer Genetics 228–229 (2018) 184–196

Cancer Genetics

REVIEW ARTICLE

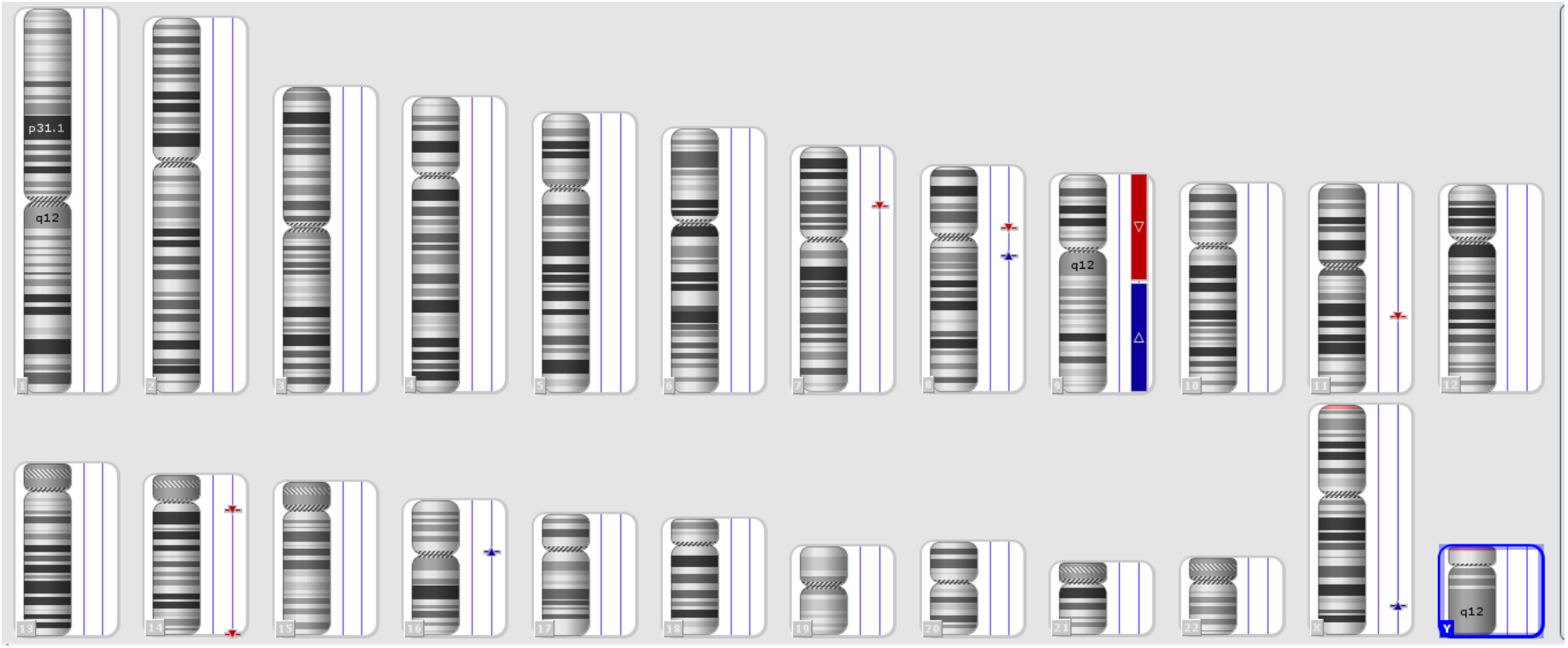
Assessing genome-wide copy number aberrations and copy-neutral loss-of-heterozygosity as best practice: An evidence-based review from the Cancer Genomics Consortium working group for plasma cell disorders

Trevor J. Pugh[a,*], James M. Fink[b], Xinyan Lu[c], Susan Mathew[d], Joyce Murata-Collins[e], Pascale Willem[f], Min Fang[g,*], on behalf of the Cancer Genomics Consortium Plasma Cell Disorders Working Group

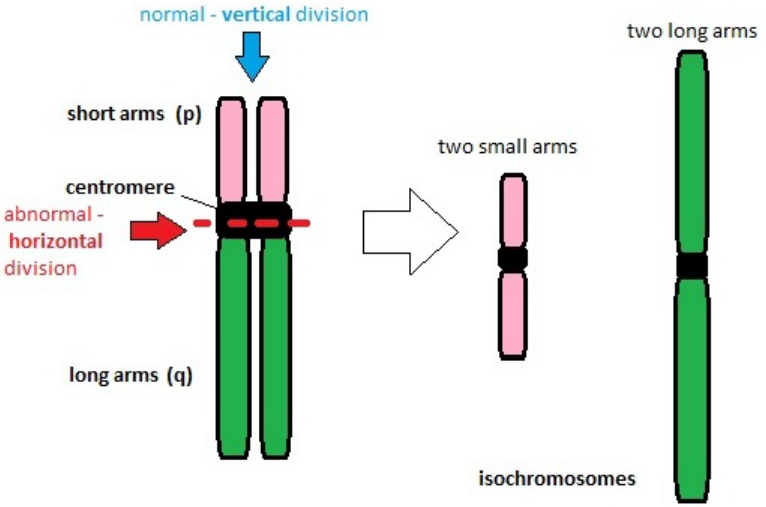
Evidence Level	Chromosomal Abnormality	Significance	Genes
Level 1	Hyperdiploidy (+3,+5,+7,+9,+11,+15,+21)	Good prognosis	
Well established evidence in NCCN guideline, WHO criteria, FDA-approved, COG recommendation, or based on large body of publications.	t(4;14)	Poor prognosis, predicts bortezomib response	*IGH*
	t(6;14)	Good prognosis	*IGH*
	t(14;16)	Poor prognosis	*IGH*
	t(11;14)	Good prognosis	*IGH*
	t(14;20)	Poor prognosis	
	del(1p)	Poor prognosis	
	1q+	Poor prognosis	
	del(13q)	Poor prognosis	
	16q	Poor prognosis	
	del(17p)	Poor prognosis (Level 1), predicts response (Level 2)	
Level 2 Emerging evidence by one large study or multiple case reports	1p CN-LOH	Recurrent	
	+2	Recurrent	
	del(4q)	Recurrent	
	del(5p), 5q+, del(5q)	Recurrent	
	6p+	Recurrent	
	del(6q)	Recurrent	
	7q+	Recurrent	
	del(8p)	Recurrent	
	8q24.2+	Recurrent	*MYC*
	9p+	Recurrent	
	del(10q23.31)	Recurrent	*PTEN*
	11q+	Recurrent	
	del(12p) or 12p CN-LOH	Recurrent	
	del(13q32.2)	Recurrent	*TGDS*
	del(14q)	Good prognosis	
	14q CN-LOH	Recurrent	
	16 CN-LOH	Recurrent	
	17 CN-LOH	Recurrent	
	17q25+	Recurrent	
	+18	Recurrent	
	+19, 19q+	Recurrent	
	del(20p)	Recurrent	
	+20, 20q+	Recurrent	
	del(22)	Recurrent	
	22q21+	Associated with relapse	*PRAME*
	del(X), X+, X CN-LOH	Recurrent	
	Xq+ in males	Poor prognosis	

*See supplemental Table 1 for references and Level 3 alterations.

Exemple 2: ALL-B



Exemple 2: ALL-B



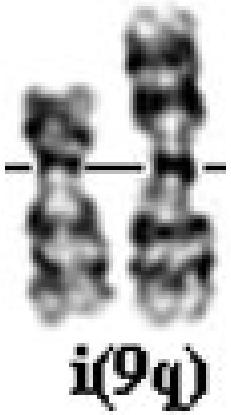


Imagen extraída del Atlas de genética y citogenética en Oncología y Hematología

Exemple 3: ALL-B

LOSS

GAIN