

Disclosures

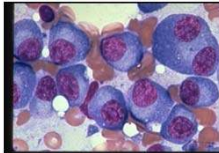
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Consultant	N/A
Major stockholder	N/A
Speakers' bureau	N/A
Scientific advisory board	N/A

53rd ASH Annual Meeting ♦ San Diego, California

NA = not applicable (no conflicts listed)

Presentation includes discussion of the following off-label use of a drug or medical device: N/A



**SNP-based mapping arrays reveal high genomic complexity in monoclonal gammopathies:
from MGUS to myeloma status**

Lucía López Corral, María Eugenia Sarasquete, Sílvia Beà, Ramón García Sanz, María Victoria Mateos, Luis Antonio Corchete, Joan Bladé, Albert Oriol, Miguel T. Hernández García, Pilar Giraldo, José Hernández, Marcos González, Jesús María Hernández Rivas, Jesús F. San Miguel, Norma C. Gutiérrez.

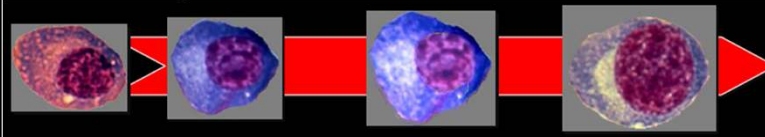


ASH, San Diego. 12/12/2011



Monoclonal Gammopathies: from early to late stages

Introduction



Normal PC

MGUS

SMM

MM

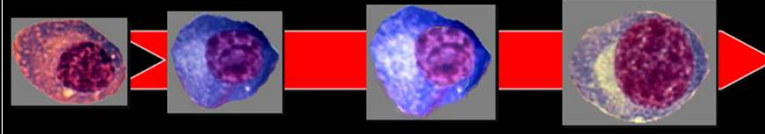


• MM is a malignant and incurable disorder characterized by the accumulation of clonal plasma cells in the bone marrow.

•MM evolves from a previous premalignant condition in most patients^{1,2}.

Monoclonal Gammopathies: from early to late stages

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MGUS

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¹Landgren *et al*, Leukemia 2009. ²Weiss *et al*, Expert. Rev. Hematol 2010

Monoclonal gammopathies: IMWG criteria¹

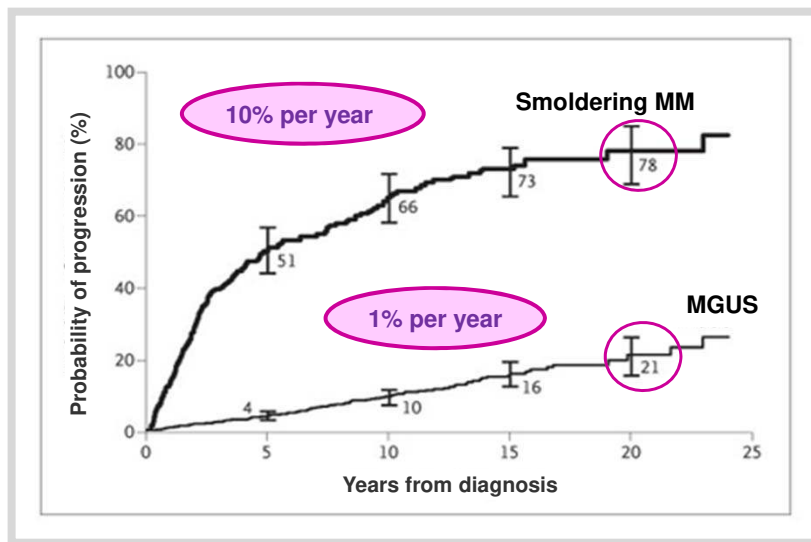
Introduction

	MGUS	SMM	MM
• Monoclonal protein	< 30 g/L	≥30 g/L	YES
	and	and/or	and
• BMPC (%)	< 10%	≥ 10%	> 10% ^b
	and	and	and
• CRAB symptoms^a	NO	NO	YES

a) Myeloma Related Organ or Tissue Impairment (end organ damage) related to Plasma cell proliferative process: anemia with 2 g/dL below the normal level or <10 g/dL, or serum calcium level >10 mg/L (0.25 mmol/L) above normal or >110 mg/dL (2.75 mmol/L), or lytic bone lesions or osteoporosis with compressive fractures, or renal insufficiency (creatinine >2 mg/dL or 173 mmol/L), [CRAB: Calcium increase, Renal impairment, Anemia and Bone lesion] or symptomatic hyperviscosity, amyloidosis or recurrent bacterial infections (>2 episodes in 12 m).
 b) For symptomatic multiple myeloma, a minimum level of M-component or BM plasma cell infiltration (although usually it is >10%, is not required, provided than this two features coexists with the presence of end organ damage
¹International Working Group (BJH 2003; 121:749)

MGUS and SMM: risk of progression to symptomatic MM

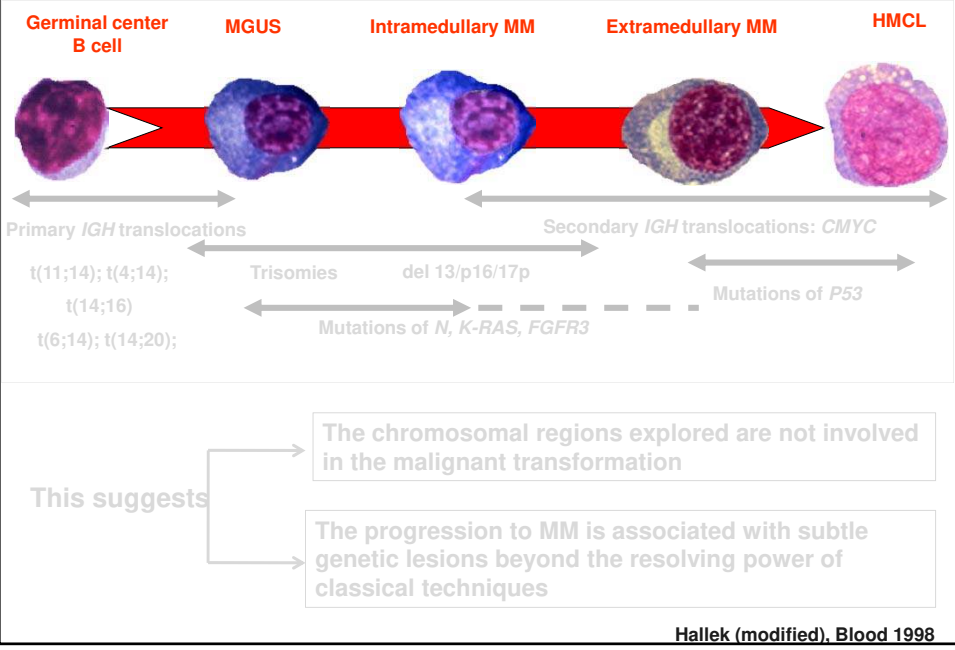
Introduction



Kyle et al, NEJM (2007)

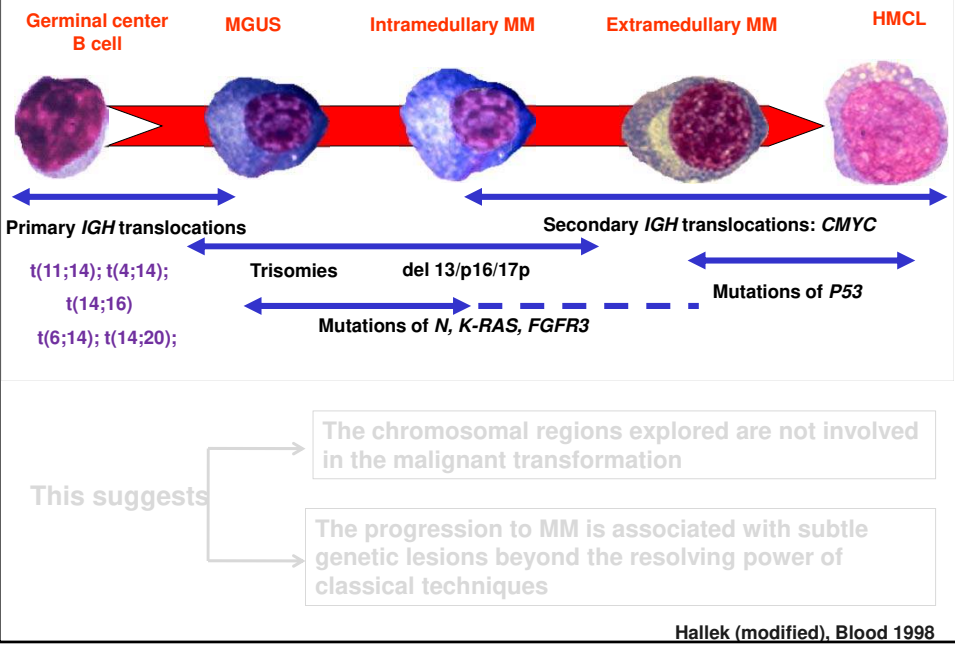
Multistep transformation model

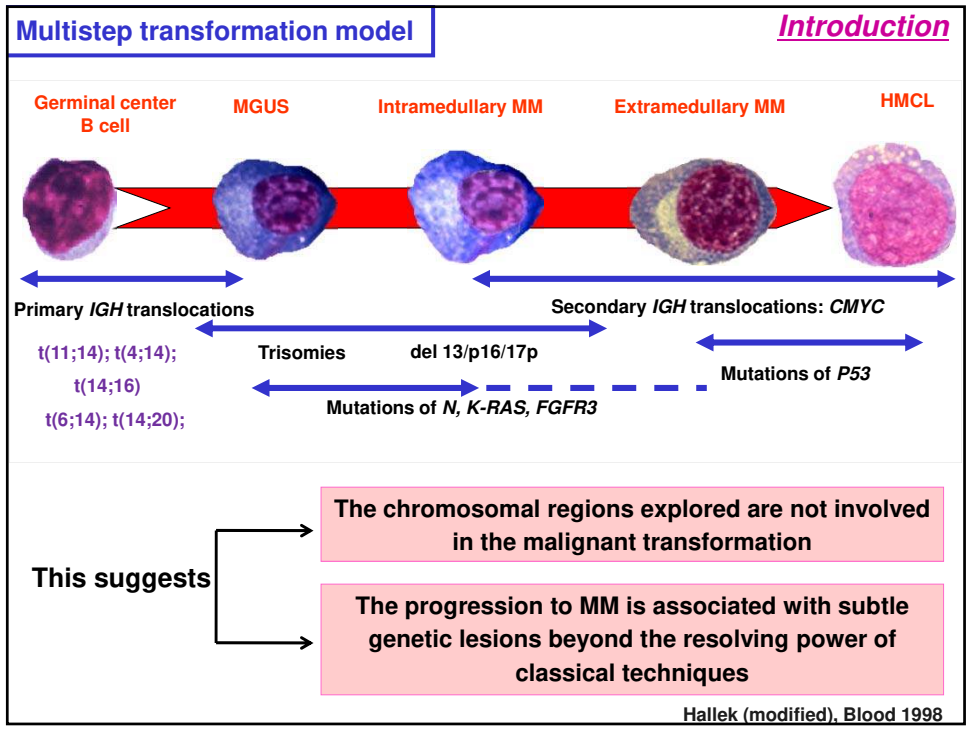
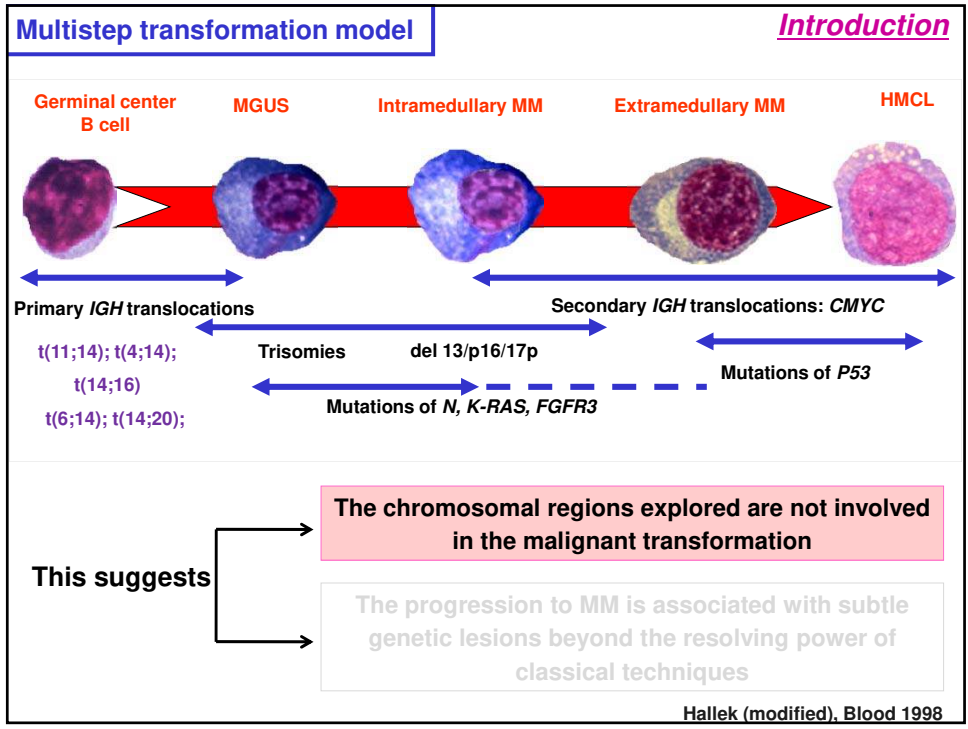
Introduction



Multistep transformation model

Introduction





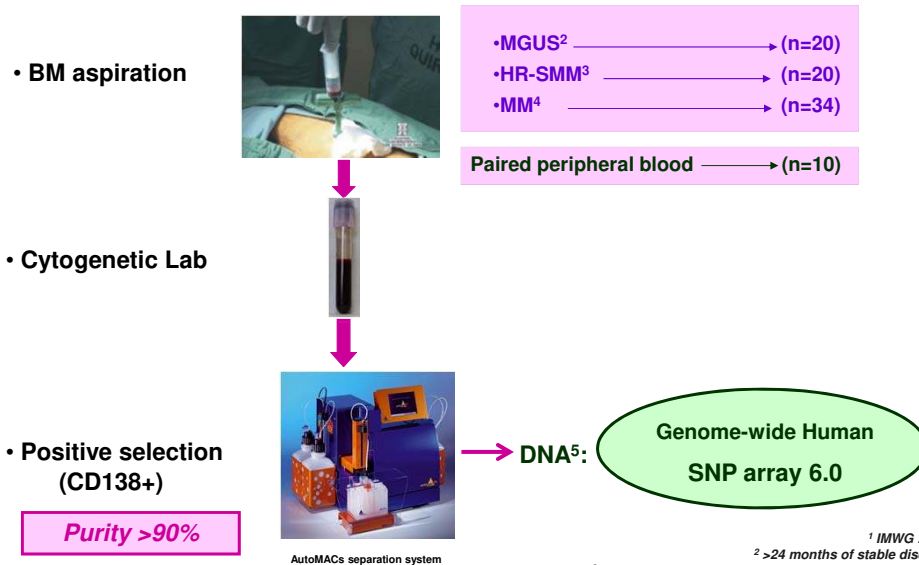
A comprehensive high-resolution analysis of genomic imbalances from the early to late stages of monoclonal gammopathies:

DNA: SNP-arrays

1. Copy number abnormalities (CNA)
2. Copy number neutral LOH (CNN-LOH)
3. Correlation with fragile sites (FRA)

Material and Methods

74 patients with monoclonal gammopathies¹

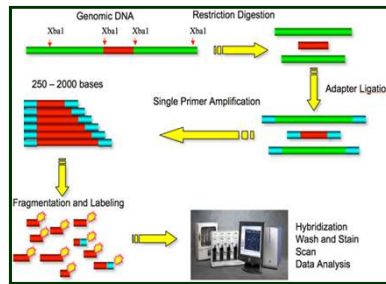


¹IMWG 2003
²>24 months of stable disease
³Kyle et al, NEJM 2007; Perez-persona et al, Blood 2007
⁴Newly diagnosed untreated patients
⁵Only high quality DNA was used (ND-1000 spectrophotometer)
The study was approved by the research ethics committees and written informed consent was obtained (Helsinki declaration).

SNP-arrays methodology

Material and Methods

Genome-Wide Human *SNP-Array 6.0* assay protocol (Affymetrix)



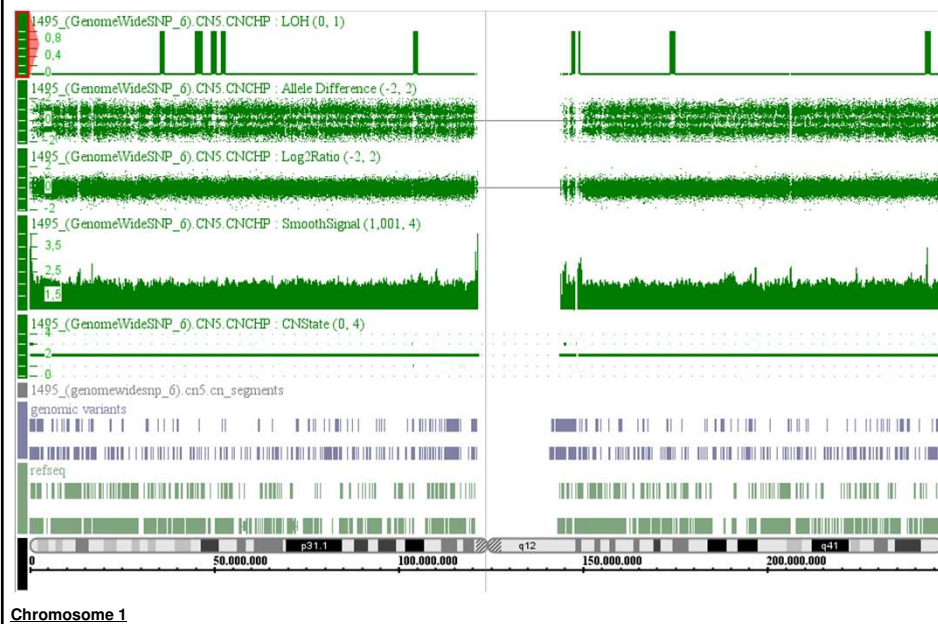
- **Processing** (*Fluidics Station 450, GeneChip Scanner 3000 7G and AGCC*)
- **Filter** (*Contrast quality control > 0.4 y MAPD < 0.35*)
- **Normalization** (*240 hapmap file*)

ANALYSIS (*Genotyping Console 4.0 –Affymetrix-, dCHIP y ChAS –Affymetrix-, SPSS 15*)

- Criteria
1. > 10 markers per segments
 2. > 100 Kb minimum genomic sizes
 3. <50% overlap with known CNV
 4. CNN-LOH >5 Mb

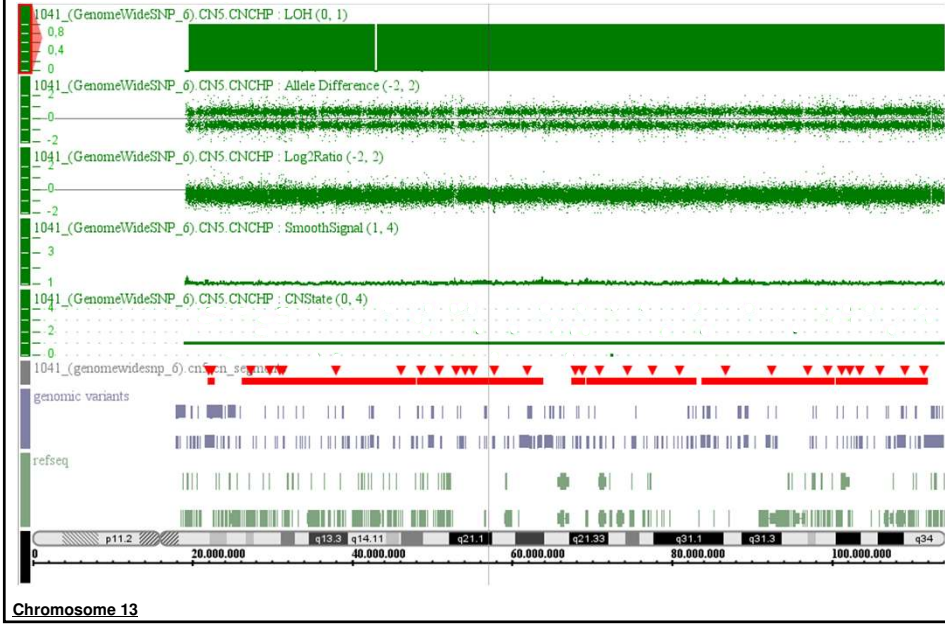
Example of a normal chromosome

Material and Methods



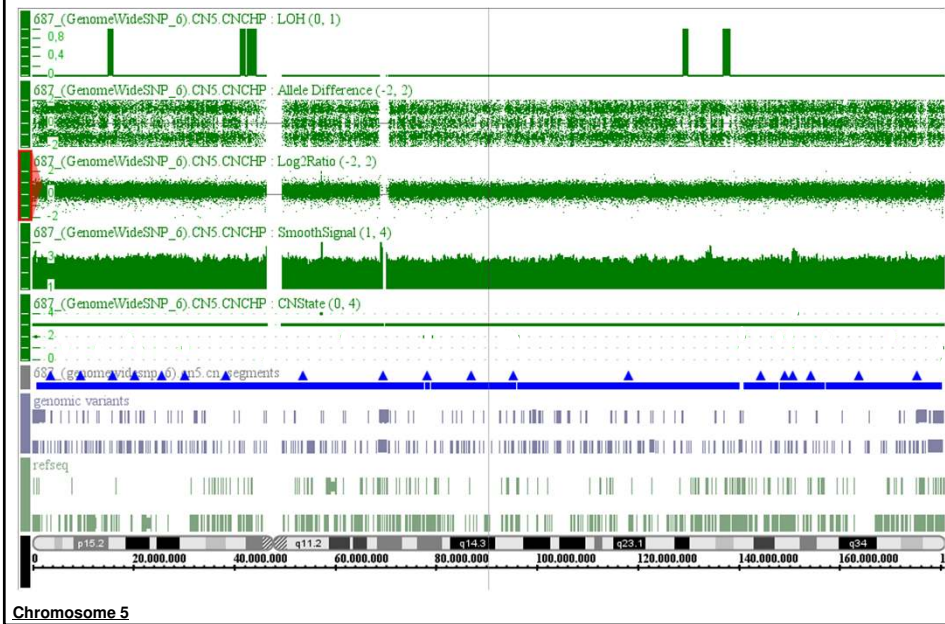
Example of a monosomy

Material and Methods



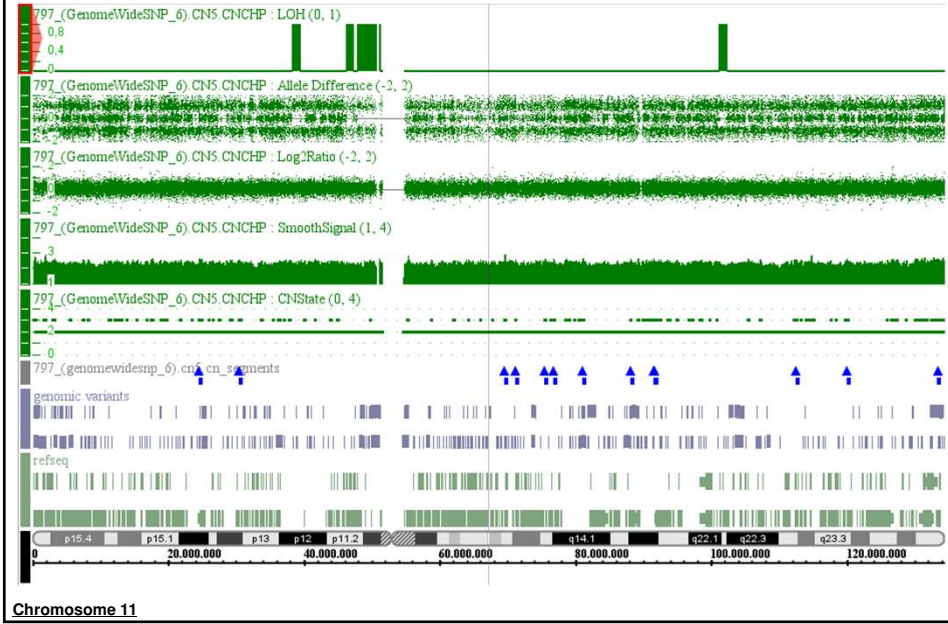
Example of a trisomy

Material and Methods



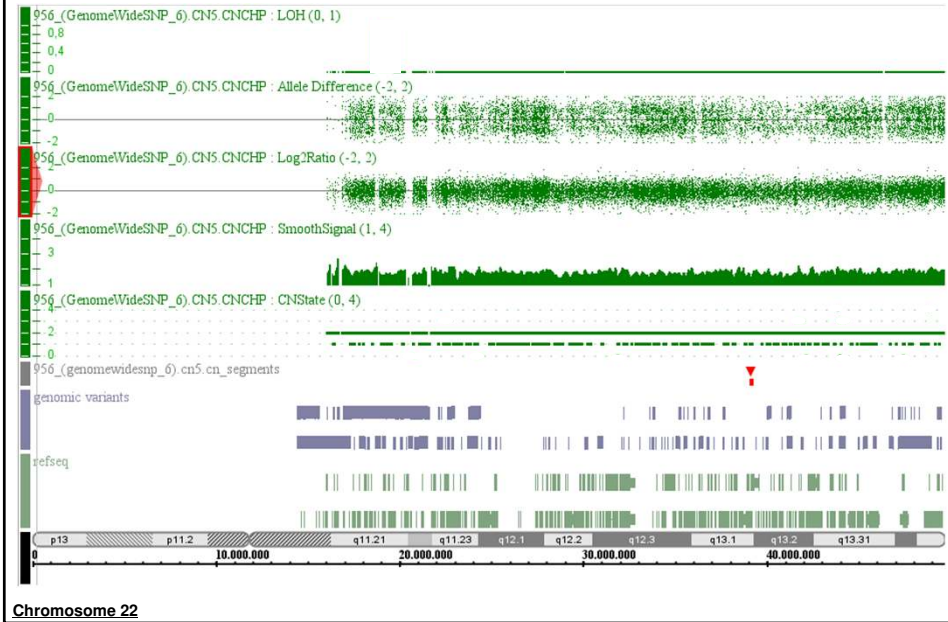
Example of a trisomy in a minor subclone

Material and Methods



Example of a monosomy in a minor subclone

Material and Methods



A comprehensive high-resolution analysis of genomic imbalances from early to late stages of monoclonal gammopathies:

DNA: SNP-arrays

- 1. Copy number abnormalities (CNA)
- 2. Copy number neutral LOH (CNN-LOH)
- 3. Correlation with fragile sites (FRA)

- CNA¹ were identified in 93% (69/74 patients)
- Two MGUS and three SMM patients with no CNA

	Global CNA Median (range)	GAINS Median (range)	LOSSES Median (range)
MGUS (n=20)	5 (0-12)	1,5 (0-9)	1,5 (0-9)
HR-SMM (N=20)	7,5 (0-23)	3 (0-12)	3,5 (0-14)
MM (N=34)	12 (1-32)	6,5 (1-20)	4 (0-29)
	MGUS vs MM P= 0,006	SMM vs MM p=0,025 MGUS vs MM P= 0,000	MGUS vs MM P= 0,033



Progressive increase in the incidence of CNA¹ from MGUS to HR-SMM and to MM

¹CNA= copy number alterations

Copy number abnormalities

Results

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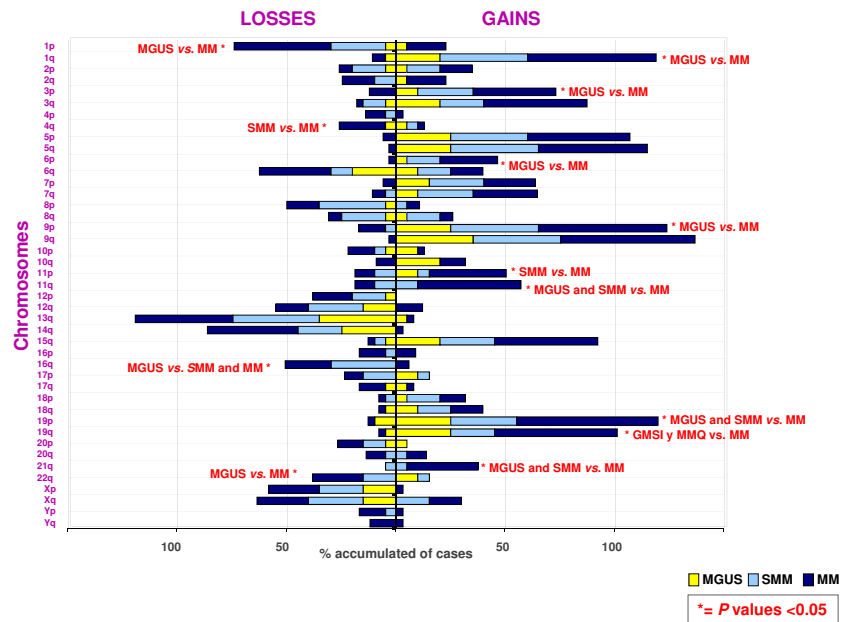
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Progressive increase in the incidence of CNA¹ from MGUS to HR-SMM and to MM

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Percentages of aberrations per chromosome

Results



Significantly different frequencies of gains and losses

Results

COPY NUMBER GAINS				
	MGUS (n=20) patients (%)	SMM (n=20) patients (%)	MM (n=34) patients (%)	P value
1q	20% (4/20)	40% (8/20)	59% (20/34)	P=0.013 (MGUS vs MM)
3p	10% (2/20)	25% (5/20)	38% (13/34)	P=0.05 (MGUS vs MM)
6p	5% (1/20)	15% (3/20)	26% (9/34)	P=0.05 (MGUS vs MM)
9p	25% (5/20)	40% (8/20)	59% (20/34)	P=0.034 (MGUS vs MM)
11p	10% (2/20)	5% (1/20)	35% (12/34)	P=0.019 (MGUS vs MM)
11q	0% (0/20)	10% (2/20)	47% (16/34)	P=0.001 (MGUS vs MM) P=0.013 (SMM vs MM)
19p	25% (5/20)	30% (6/20)	65% (22/34)	P=0.011 (MGUS vs MM) P=0.029 (SMM vs MM)
19q	25% (5/20)	20% (4/20)	56% (19/34)	P=0.05 (MGUS vs MM) P=0.022 (SMM vs MM)
21q	0% (0/20)	5% (1/20)	32% (11/34)	P=0.004 (MGUS vs MM) P=0.022 (SMM vs MM)
COPY NUMBER LOSSES				
1p	5% (1/20)	25% (5/20)	44% (15/34)	P=0.006 (MGUS vs MM)
4q	5% (1/20)	0% (0/20)	21% (7/34)	P=0.038 (SMM vs MM)
16q	0% (0/20)	30% (6/20)	21% (7/34)	P=0.02 (MGUS vs SMM) P=0.038 (MGUS vs MM)
22q	0% (0/20)	15% (3/20)	23% (8/34)	P=0.020 (MGUS vs MM)

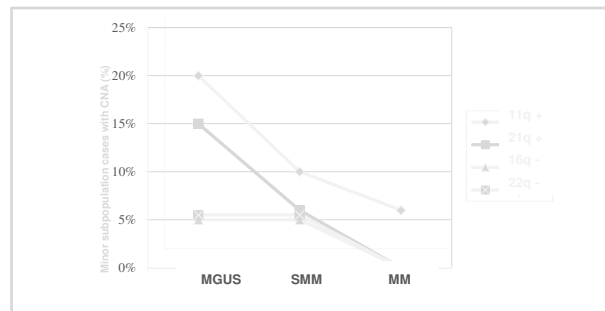
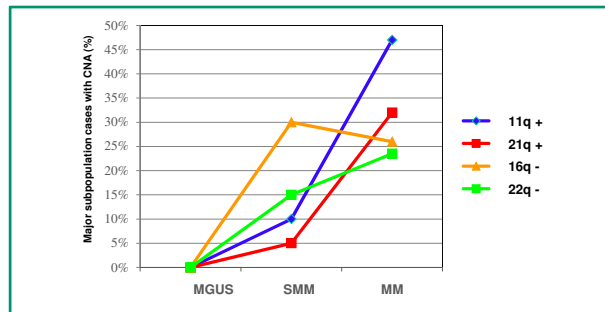
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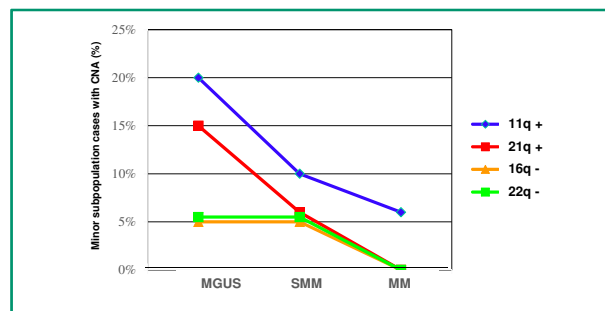
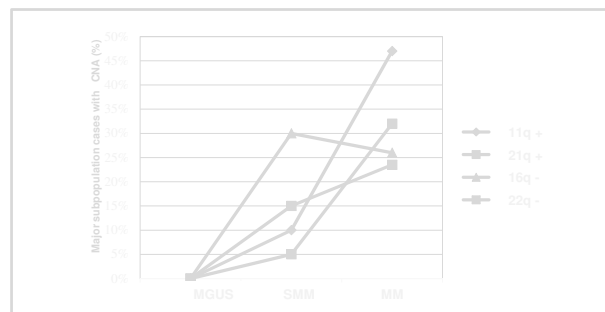
Percentages of cases carrying specific CNA as major subpopulations

Results



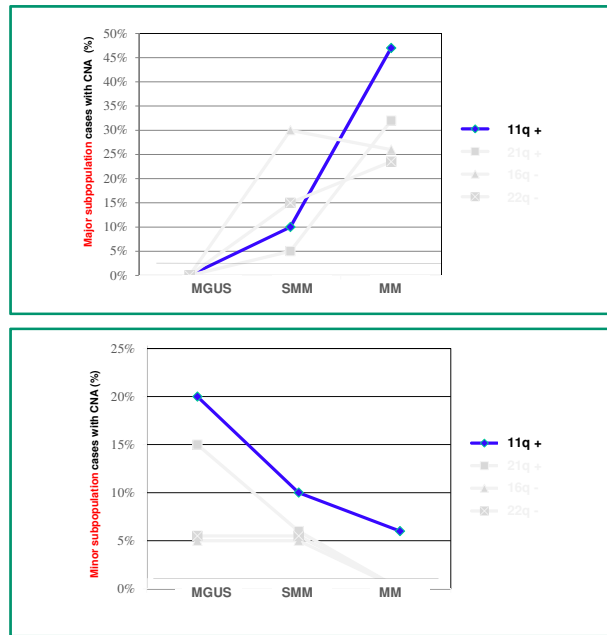
Percentages of cases carrying specific CNA as minor subpopulations

Results



Percentages of cases carrying specific CNA as minor and major subpopulations

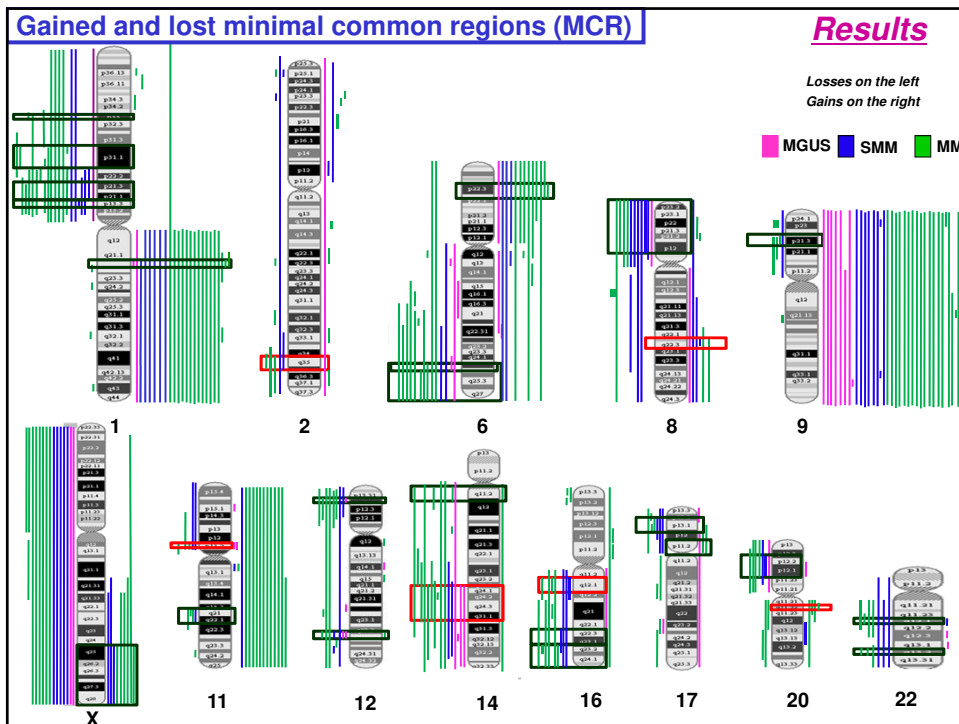
Results



Gained and lost minimal common regions (MCR)

Results

Cytogenetic band	Start (Kb)	End (Kb)	Size (Kb)	Known genes	Gains			% of CNA
					MGUS (n=20)	SMM (n=20)	MM (n=34)	
1q21.3	152964.81	153134.74	169.93	<i>KCNN3</i>	10%	30%	53%	81%
6p22.3-p25.3	0.00	19360.12	19360.12	64	5%	15%	23%	92%
8q22.2-q22.3	100858.16	102001.30	1143.14	11	5%	15%	3%	83%
16p12.1	22640.22	22820.24	180.02	<i>HS3ST2</i>	0	0	6%	66%
16p11.2	29041.61	31888.22	2846.61	87	0	0	6%	66%
17p11.2	13139.43	17043.83	3904.40	-	5%	5%	0	68%
18q12.2	31747.57	31927.42	179.84	<i>p15rs, c18orf21</i>	5%	15%	15%	90%
20q11.22	31333.73	32156.79	823.06	12	0	0	9%	75%
Xq25-q28	120740.55	154850.49	34109.94	201	0	15%	15%	100%
					Losses			
Cytogenetic band	Start (Kb)	End (Kb)	Size (Kb)	Known genes	GMSI (n=20)	SMM (n=20)	MM (n=34)	% of CNA
1p21.1-p21.3	94498.2	106994.8	12496.6	41	10%	25%	32%	86%
1p13.3	111176.8	111283.1	106.3	<i>CD53, C10RF103</i>	5%	25%	0	88%
1p31.1	71647.0	71918.0	271.0	<i>NEGR1</i>	5%	10%	21%	48%
1p33	46494.0	51297.4	4803.4	<i>FAF1, CDKN1C</i>	5%	10%	18%	43%
2q35	218972.7	219106.7	134.0	<i>CTDSP1, VIL1, USP37</i>	0	5%	9%	57%
6q25.1-tel	152509.3	170800.0	18290.7	79	5%	10%	26%	70%
6q25.1	150054.0	150181.9	127.9	<i>LATS1, NUP43, PCMT1</i>	5%	10%	26%	70%
8p12-p23.3	0.0	31757.6	31757.6	185	5%	30%	12%	92%
9p21.3	20951.4	24056.6	3105.2	<i>CDKN2A, CDKN2B</i>	0	5%	6%	60%
11p11.2	45957.2	46103.5	146.3	<i>PHF21A</i>	0	10%	6%	80%
11q21-q22.2	95833.4	102185.8	6352.4	20	0	5%	6%	60%
12q24.11	107500.0	110200.0	2700.0	38	10%	10%	12%	61%
12p13.2	10000.0	12600.0	2600.0	46	5%	15%	15%	90%
14q24.1-q31.1	66998.1	82803.4	15805.3	117	15%	10%	21%	52%
14q11.2	19515.7	22506.4	2990.7	66	5%	0	15%	26%
16q22.3-23.1	71827.4	73762.6	1935.1	15	0	20%	12%	61%
16q23.2-24.3	79145.6	88826.9	9681.3	96	0	15%	15%	61%
16q12.1	46498.5	49153.4	2655.0	15	0	20%	12%	61%
17p13.1	8340.931	8471.997	131.066	<i>MYH10</i>	0	15%	6%	83%
20p12.1-p12.2	11332.8	15284.9	3952.1	9	5%	10%	9%	86%
22q13.2	40365.4	49520.2	9154.8	107	0	15%	3%	36%
22q12.1	26888.5	28527.7	1639.2	62	0	15%	12%	64%



Homozygous deletions (HZD)

Results

- 12 HZD corresponding to 5 MGUS (25%), 1 SMM (5%) and 3 MM (9%).
- 10 different chromosomal regions involved.
- Median size: 210 Kb. Total of genes: 23.

Patients	Band	Start (Kb)	End (Kb)	Size (Kb)	Genes
15_MGUS	1q25.1	173068.5	173063.2	5.3	<i>RABGAP1L</i>
13_MGUS	1q31.1	195077.6	194981.6	96.0	<i>CFHR1, CFHR3</i>
15_MGUS	2p22.3	34590.3	34546.8	43.5	
19_MGUS	2p22.3	34590.6	34549.7	40.9	
66_MM	3q26.1	163626.5	163612.0	14.5	
11_MGUS	6q14.1	79092.9	79020.7	72.3	
9_MGUS	8p11.23-p11.22	39507.6	39350.8	156.8	<i>ADAM3A</i>
10_MGUS	8p11.23-p11.22	39506.4	39354.1	152.3	<i>ADAM3A</i>
56_MM	11q22.1-q22.2	102013.9	101523.2	490.7	<i>TRPC6, ANGPTL5, KIAA1377, C11orf70, YAP1, BIRC3, BIRC2, TMEM123, MMP7, MMP20</i>
40_SMM	13q32.1	94715.6	93912.0	803.6	<i>DCT, TGDS, GPR180, SOX21, ABCC4</i>
74_MM	19q13.31	48434.2	48239.1	195.0	<i>PSG2, PSG5, PSG4, PSG9</i>
40_SMM	22q11.22	21556.1	21110.4	445.7	<i>ZNF280B, ZNF280A, PRAME, GGTL4</i>

Results

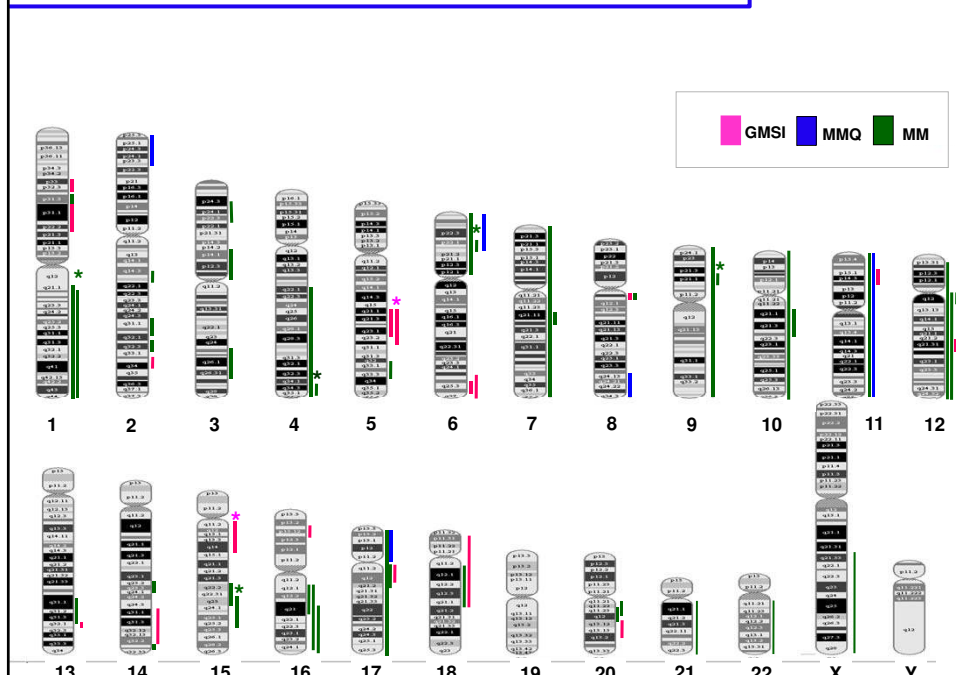
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Copy number neutral-LOH and Copy number gain-LOH*

Results



Copy number neutral-LOH and Copy number gain-LOH*

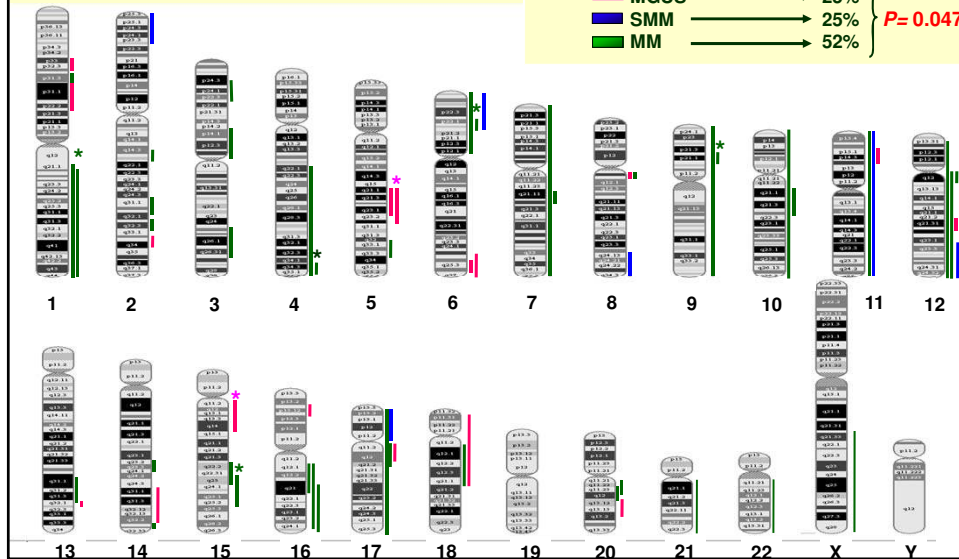
Results

- 38% of patients (28/74) showed CNN-LOH
- 58 CNN-LOH (52 partial, 6 complete). Median: 2 (1-5)
- 7 copy number gain LOH*

Frequency of CNN-LOH according to entity:

MGUS	→ 25%
SMM	→ 25%
MM	→ 52%

P = 0.047



Results

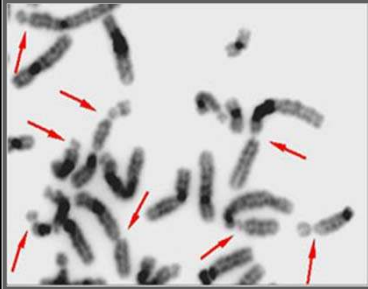
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Correlation with fragile sites (FRA)

Results



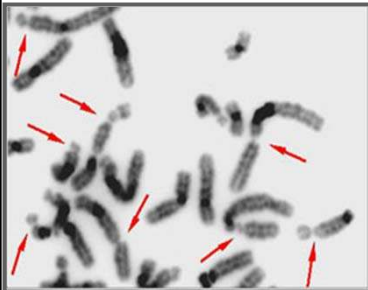
- ± 110 FRA described*
- Correlation between FRA and cancer breakpoints in solid tumors.
- For example: FRA16D (16q23.3): *WWOX*. Underexpressed in MM cases with 16q LOH or t(14;16)

- 55% of MCR
 - 65% of CNN-LOH and 58% of CNG-LOH
 - 40% of HZD
- FRA

*<http://www.ncbi.nlm.nih.gov/Locuslink/>

Correlation with fragile sites (FRA)

Results



- ± 110 FRA described*
- Correlation between FRA and cancer breakpoints in solid tumors.
- For example: FRA16D (16q23.3): *WWOX*. Underexpressed in MM cases with 16q LOH or t(14;16)

- 55% of MCR
 - 65% of CNN-LOH and 58% of CNG-LOH
 - 40% of HZD
- FRA

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Summary

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Acknowledgements



Hospital Universitario de Salamanca (*Hematology department*):

[Norma C. Gutierrez](#), [Maria Eugenia Sarasquete](#) and [Jesús F. San Miguel](#).

[Ramón García Sanz](#), [Marcos González](#), [María Victoria Mateos](#) and [Jesús M. Hernández Rivas](#).

[Luis Antonio Corchete](#), [Isabel Isidro](#), [Teresa Prieto](#) and [Sara González](#).



Hospital Clínic de Barcelona (*Institut d'Investigacions Biomediques*):

[Silvia M. Beà](#).



Centro de Investigación del Cáncer (*Genomic Unit*):

[Encarna Fermiñán](#).

[Eva García](#).



Grupo Español del Mieloma (*GEM*)