Disclosures

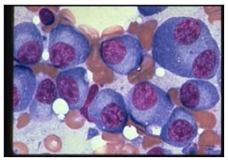
In compliance with ACCME policy, ASH requires the following disclosures to the activity audience:

Research support/P.I.	**N/A**
Employee	**N/A**
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

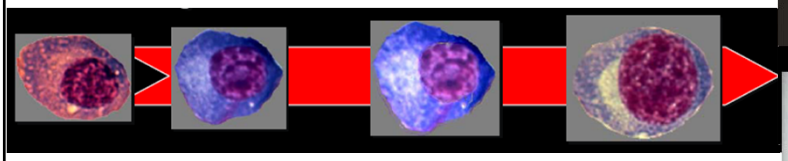


SNP-based mapping arrays reveal high genomic complexity in monoclonal gammopathies: from MGUS to myeloma status

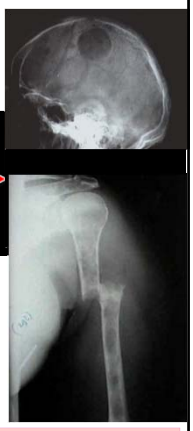
Lucía López Corral, María Eugenia Sarasquete, Sílvia Beà, Ramón García Sanz, María Victoria Mateos, Luis Antonio Corchete, Joan Bladé, Albert Oriol, Miguel T. Hernández García, Pilar Giraldo, José Hernández, Marcos González, Jesús María Hernández Rivas, Jesús F. San Miguel, Norma C. Gutiérrez.

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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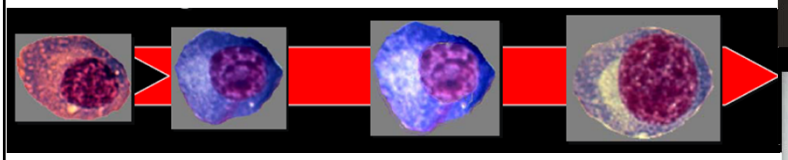

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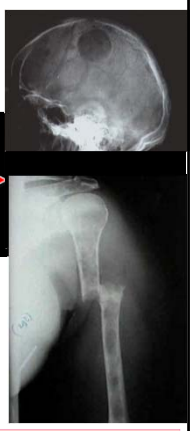

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[1]Landgren *et al*, Leukemia 2009. [2]Weiss *et al*, Expert. Rev. Hematol 2010

Monoclonal gammopathies: IMWG criteria[1]

Introduction

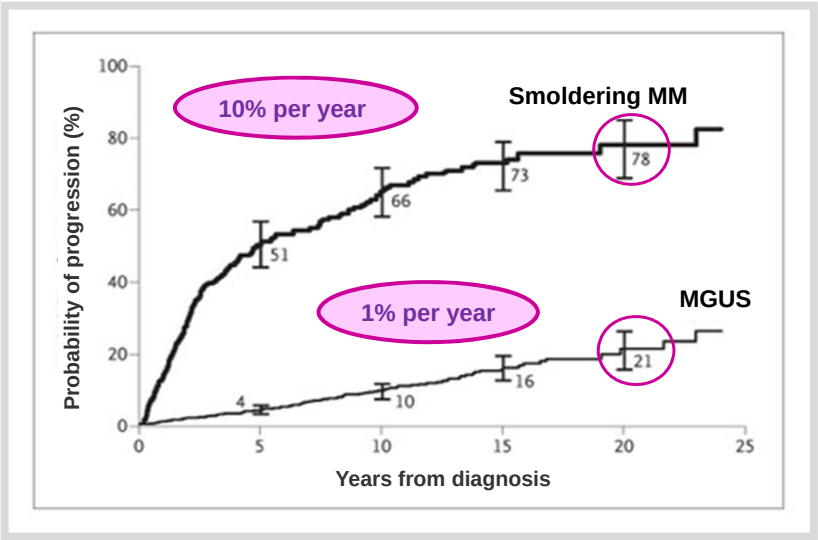
	MGUS	SMM	MM
• Monoclonal protein	< 30 g/L	≥30 g/L	YES
	and	and/or	and
• BMPC (%)	< 10%	≥ 10%	> 10%[b]
	and	and	and
• CRAB symptoms[a]	NO	NO	YES

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 insuficiency (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

[1]International Working Group (BJH 2003; 121:749)

MGUS and SMM: risk of progression to symptomatic MM

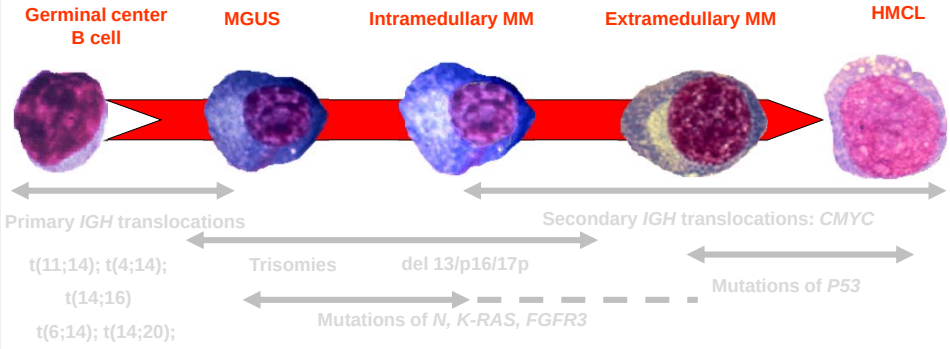
Introduction



Kyle et al, NEJM (2007)

Multistep transformation model

Introduction



This suggests

- The chromosomal regions explored are not involved in the malignant transformation
- The progression to MM is associated with subtle genetic lesions beyond the resolving power of classical techniques

Hallek (modified), Blood 1998

Multistep transformation model

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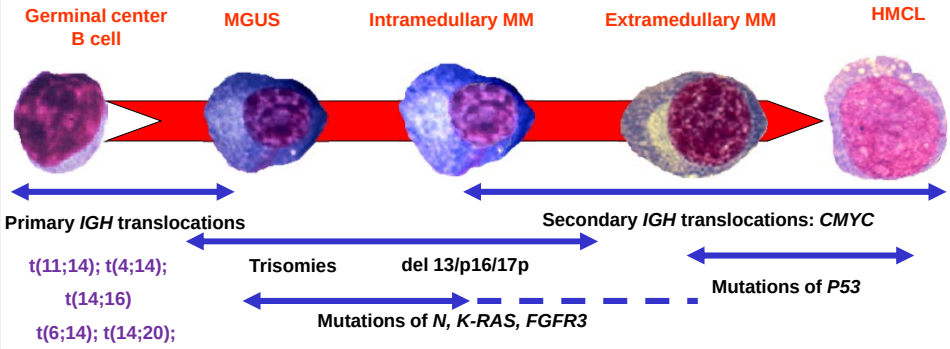

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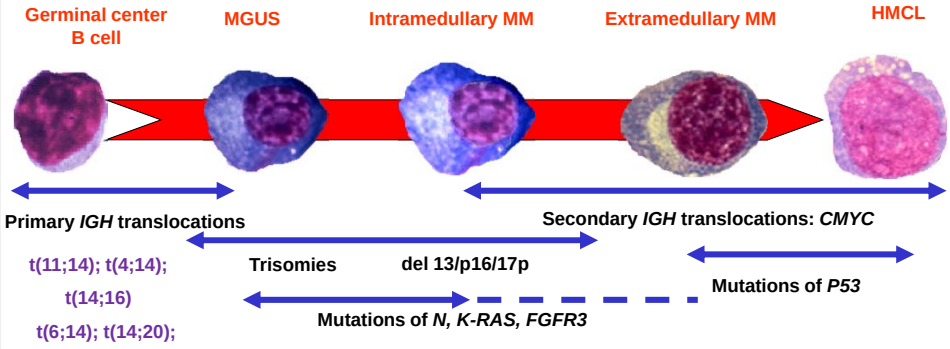

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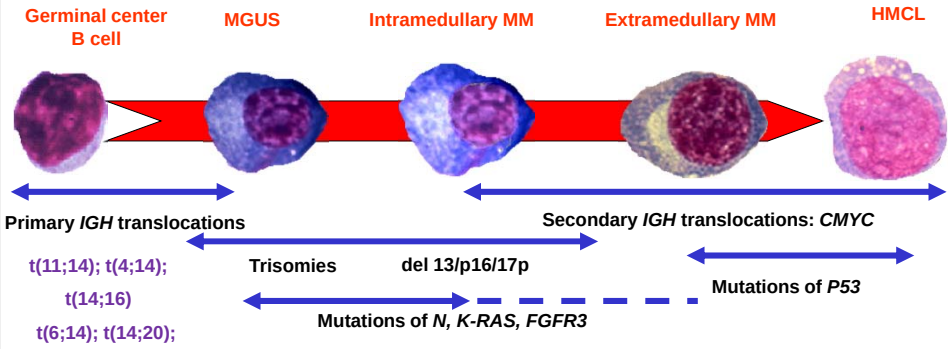

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Hallek (modified), Blood 1998

Aims

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 gammopathies[1]

- BM aspiration

- MGUS[2] → (n=20)
- HR-SMM[3] → (n=20)
- MM[4] → (n=34)

Paired peripheral blood → (n=10)

- Cytogenetic Lab

AutoMACs separation system

- Positive selection (CD138+)

Purity >90%

→ DNA[5]: Genome-wide Human SNP array 6.0

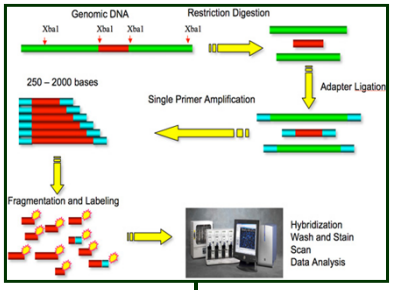
[1] IMWG 2003
[2] >24 months of stable disease
[3]Kyle etl al, NEJM 2007; Perez-persona et al, Blood 2007
[4]Newly diagnosed untreated patients
[5]Only high quality DNA was used (ND-1000 spectrophotometer)

The study was approved by the research ethics committees and written informed consent was obtained (Helsinky 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)*

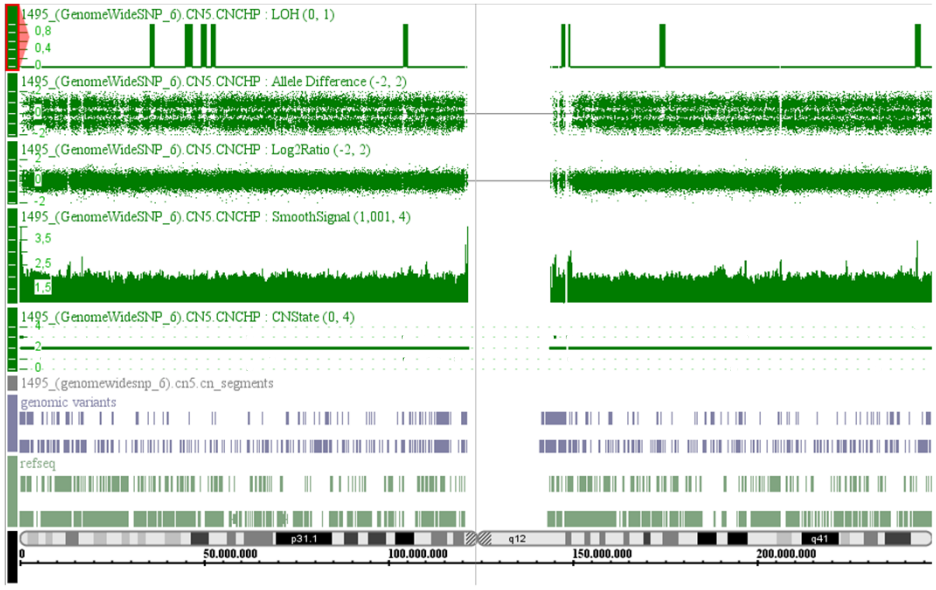
ANALYSIS *(Genotyping Console 4.0 –Affymetrix-, dCHIP y ChAS –Affymetrix-, SPSS 15)*

Criteria

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

Example of a normal chromosome

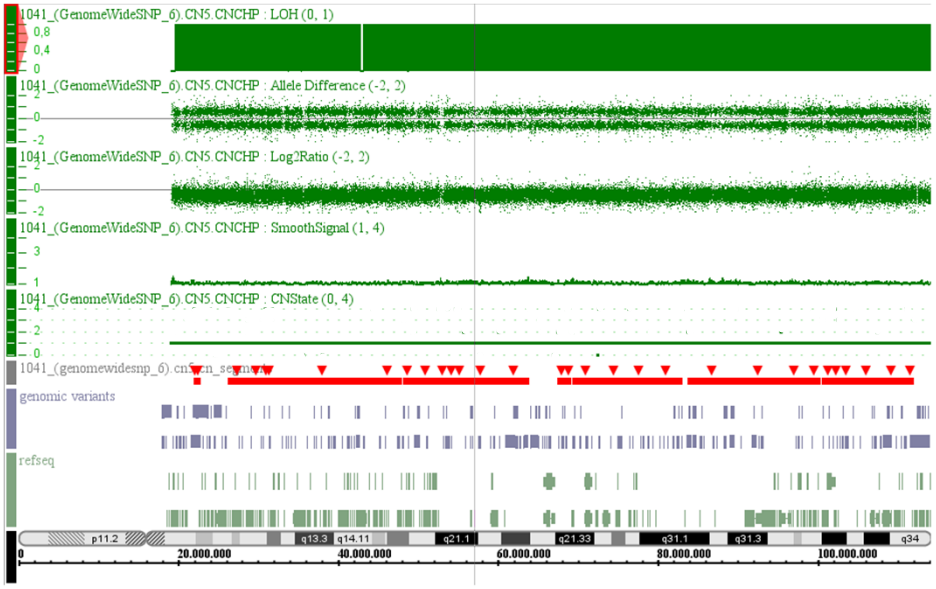
Material and Methods



Chromosome 1

Example of a monosomy

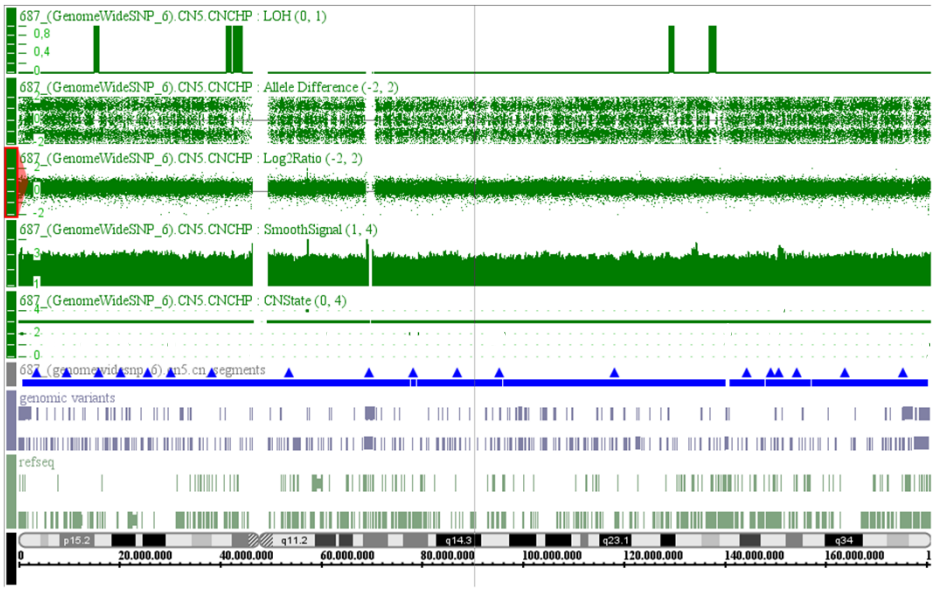
Material and Methods



Chromosome 13

Example of a trisomy

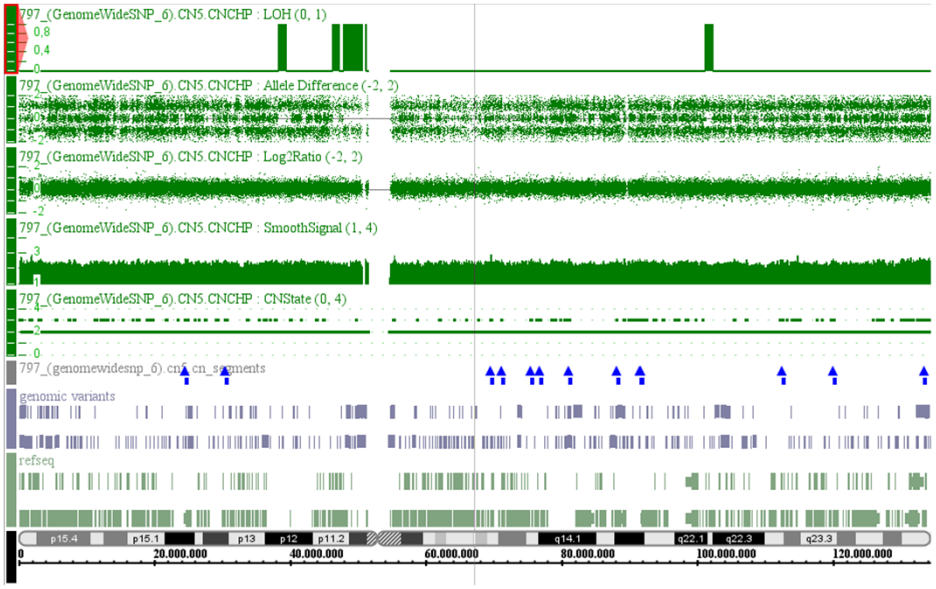
Material and Methods



Chromosome 5

Example of a trisomy in a minor subclone

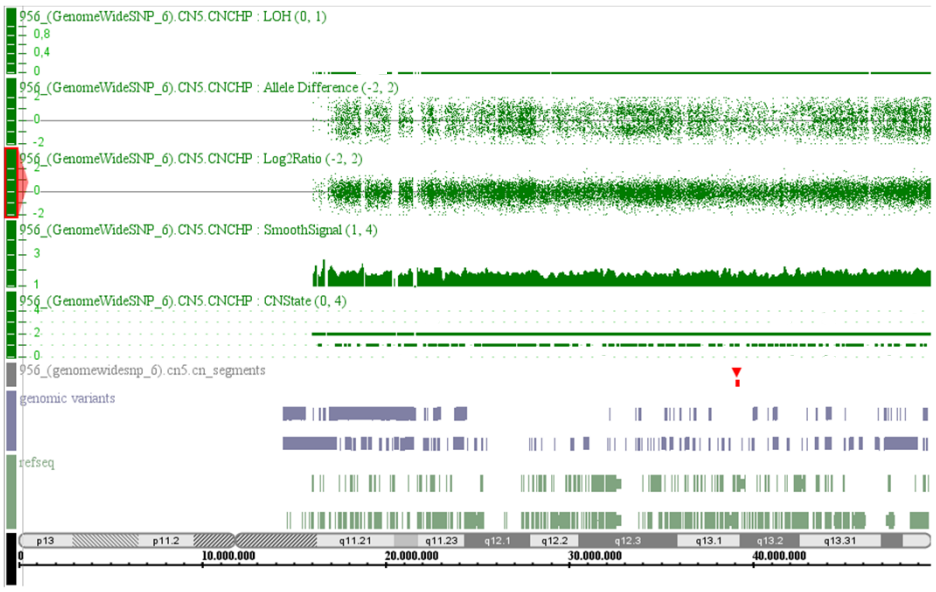
Material and Methods



Chromosome 11

Example of a monosomy in a minor subclone

Material and Methods



Chromosome 22

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)

Copy number abnormalities

Results

- **CNA[1] 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 (0-8)	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 CNA[1] from MGUS to HR-SMM and to MM

[1]CNA= copy number alterations

Copy number abnormalities

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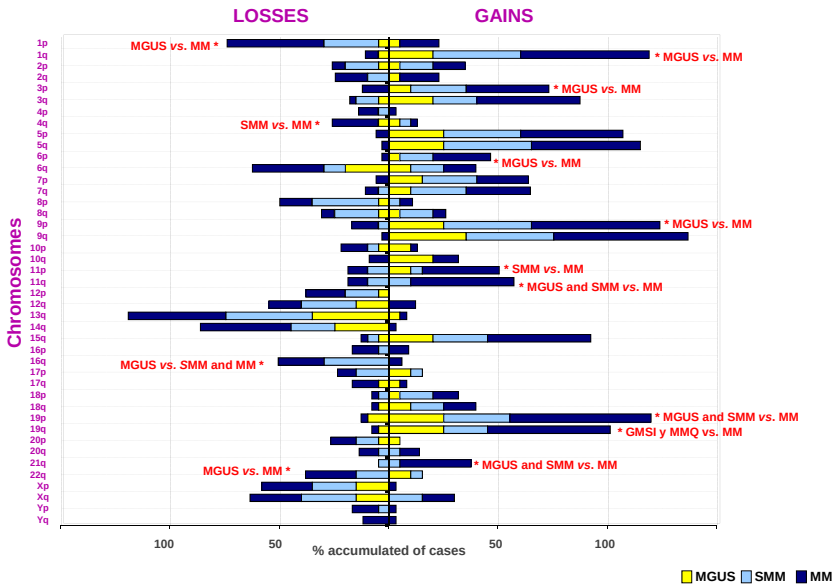
Progressive increase in the incidence of CNA[1] from MGUS to HR-SMM and to MM

[1]CNA= copy number alterations

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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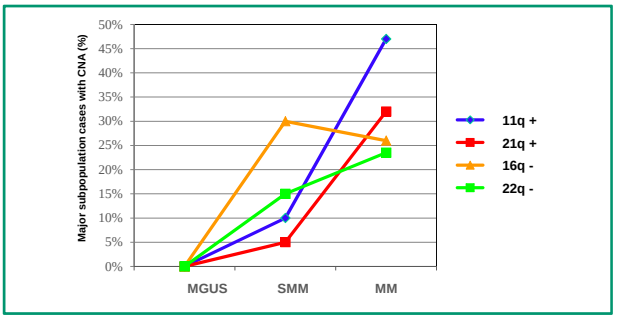
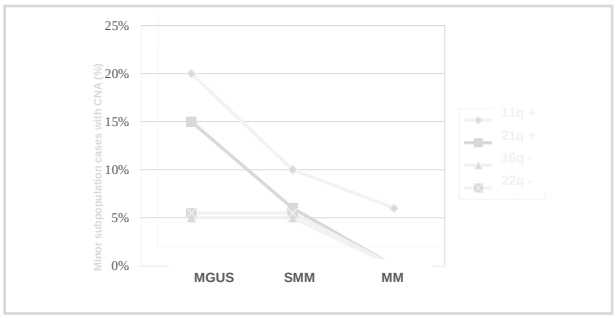
Percentages of cases carrying specific CNA as major subpopulations

Results





Percentages of cases carrying specific CNA as minor subpopulations

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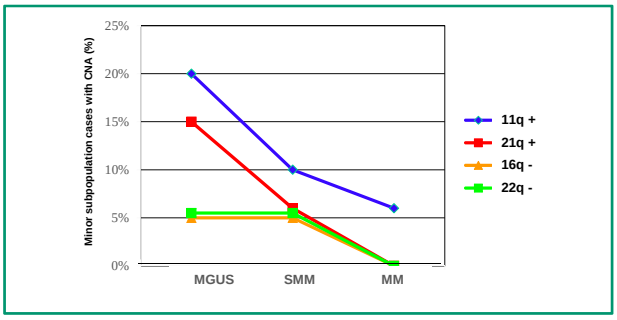

Percentages of cases carrying specific CNA as minor and major subpopulations

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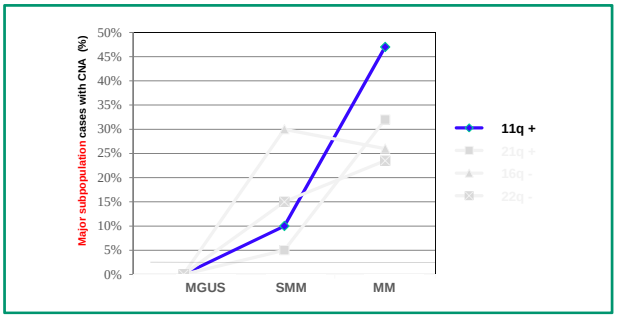
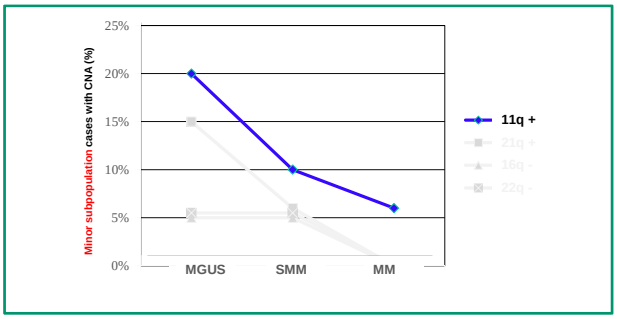




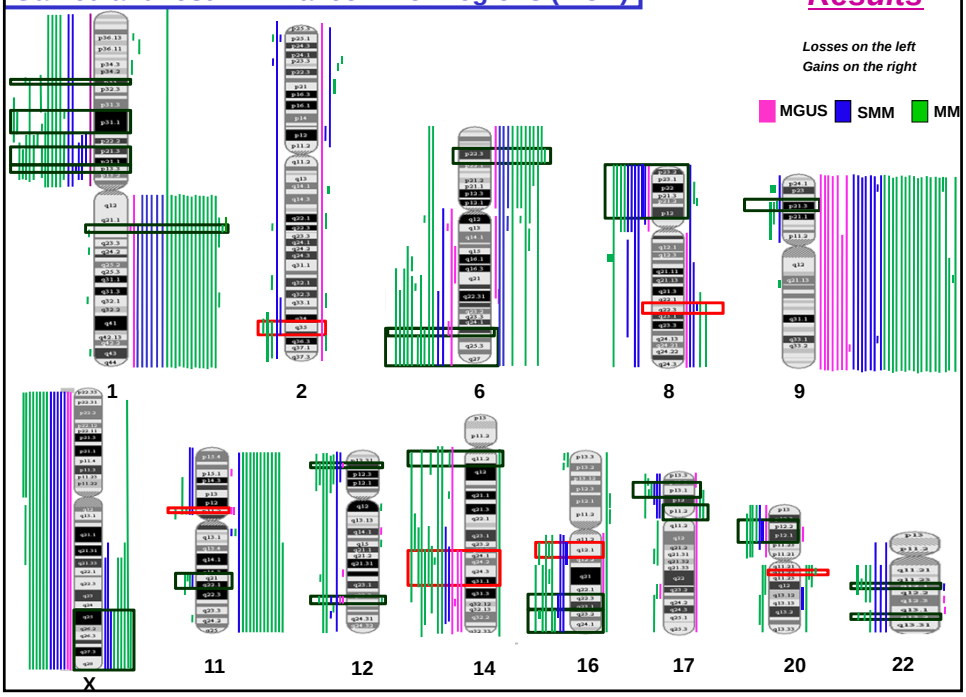
Gained and lost minimal common regions (MCR)

Results

Cytogenetic band	Start (Kb)	End (Kb)	Size (Kb)	Known genes	MGUS (n=20)	SMM (n=20)	MM (n=34)	% of CNA
				Gains				
1q21.3	152964.81	153134.74	169.93	*KCNN3*	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	*HS3ST2*	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	66%
18q12.2	31747.57	31927.42	179.84	*p15rs, c18orf21*	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	*CD53, C1ORF103*	5%	25%	0	86%
1p31.1	71647.0	71918.0	271.0	*NEGR1*	5%	10%	21%	48%
1p33	46494.0	51297.4	4803.4	*FAF1, CDKN1C*	5%	10%	18%	43%
2q35	218972.7	219106.7	134.0	*CTDSP1, VIL1, USP37*	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	*LATS1, NUP43, PCMT1*	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	*CDKN2A, CDKN2B*	0	5%	6%	60%
11p11.2	45957.2	46103.5	146.3	*PHF21A*	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	82603.4	15605.3	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	*MYH10*	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%

Gained and lost minimal common regions (MCR)

Results



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.

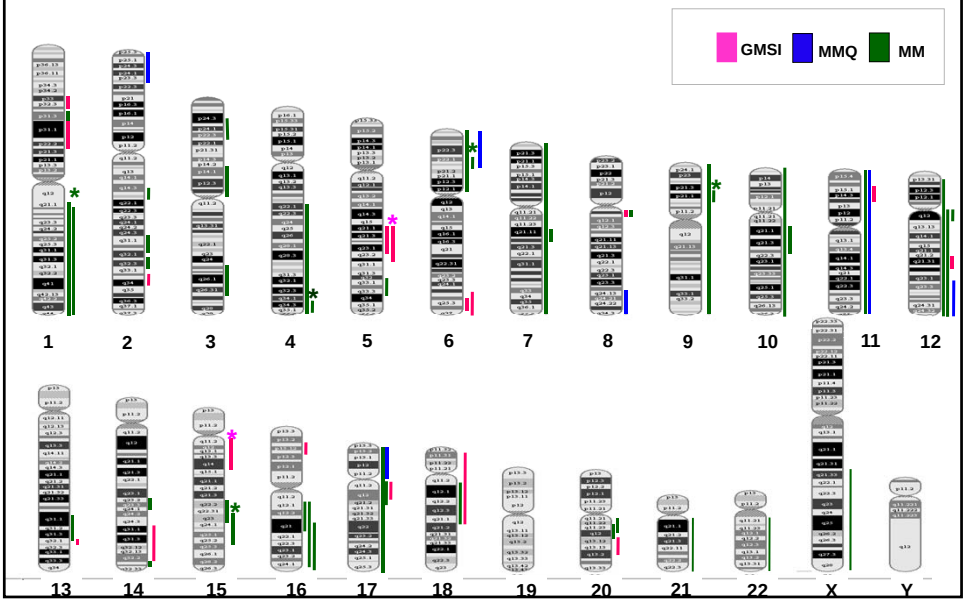
Patients	Band	Start (Kb)	End (Kb)	Size (Kb)	Genes
15_MGUS	1q25.1	173068.5	173063.2	5.3	*RABGAP1L*
13_MGUS	1q31,1	195077.6	194981.6	96.0	*CFHR1, CFHR3*
15_MGUS	2p22.3	34590.3	34546.8	43.5	
19_MGUS	2p22.3	34590.6	34549.7	40.9	
66_MM	3q26.1	163626.5	163612.0	14.5	
11_MGUS	6q14.1	79092.9	79020.7	72.3	
9_MGUS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

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)

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



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

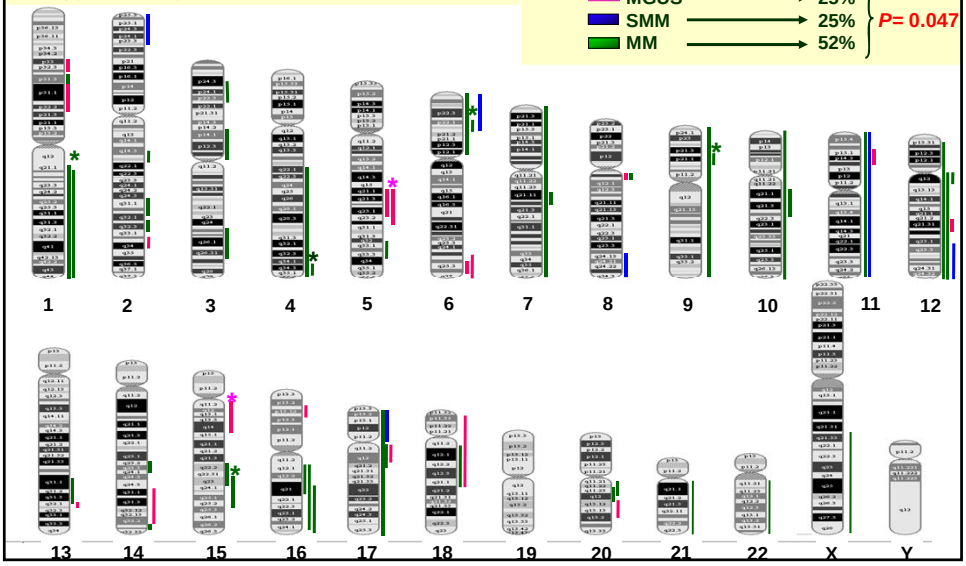
Results

- 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



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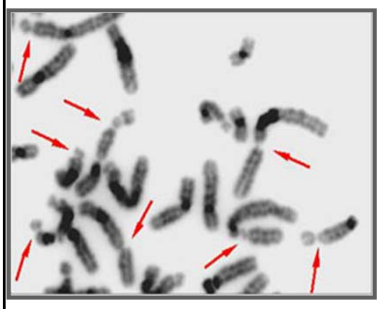
Correlation with fragile sites (FRA)

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)

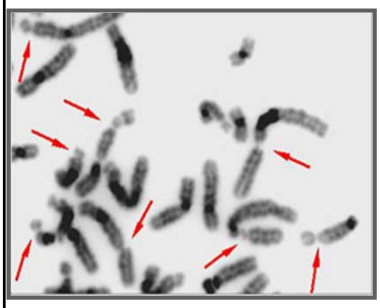
- 55% of MCR
- 65% of CNN-LOH and 58% of CNG-LOH
- 40% of HZD

FRA

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

Correlation with fragile sites (FRA)

Results



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Summary

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- **The transition from MGUS to MM was not associated with a particular chromosomal imbalance, but rather with an expansion of altered clones that were already present in MGUS.**
- **More than a half of the genetic lesions were located at fragile sites.**

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