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 FEHH Sociedad Española de Hematología y Hemoterapia · Fundación Española de Hematología y Hemoterapia

Patrones variantes de FISH en mieloma múltiple

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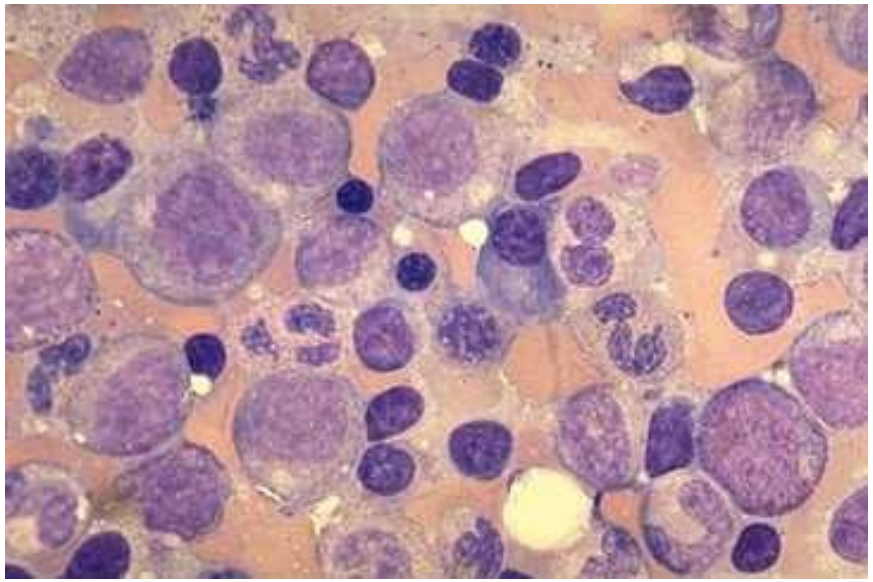
www.gcecgh.org

#gcecgh

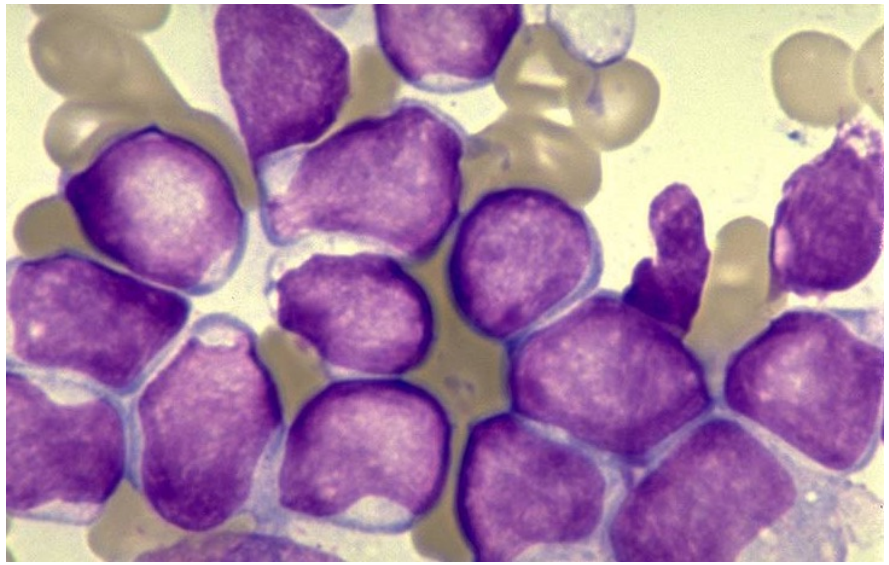
Conflictos de interés

Research Support/P.I.	N/A
Employee	N/A
Consultant	N/A
Major Stockholder	N/A
Speakers Bureau	N/A
Honoraria	Janssen, Amgen
Scientific Advisory Board	N/A

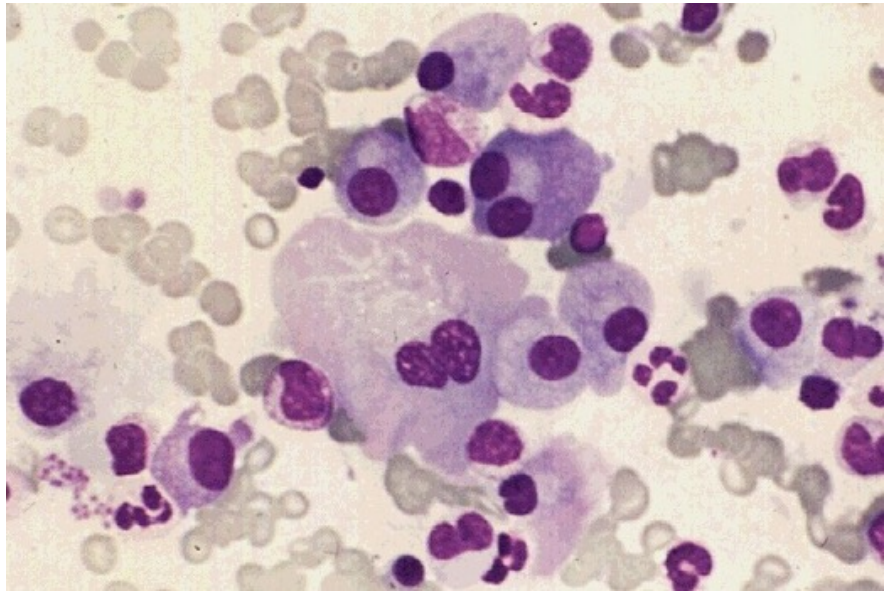
Normal BM



Acute leukemia



Myeloma

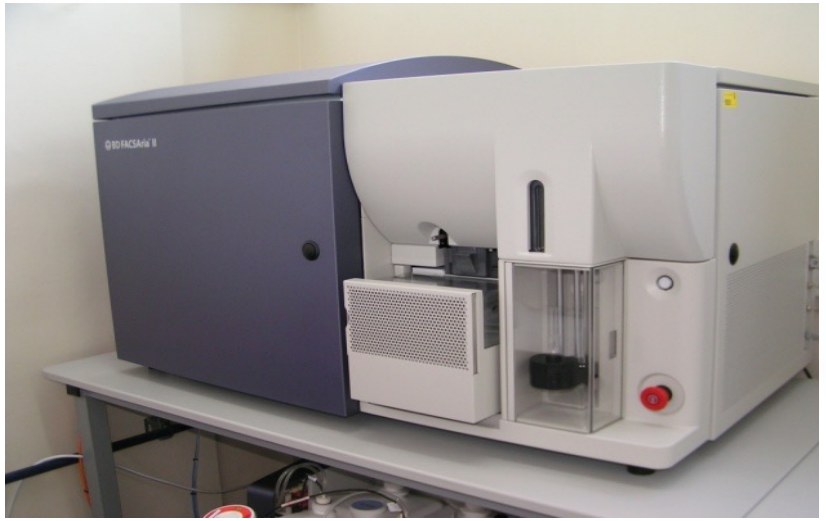


Low infiltration of tumoral cells in the bone marrow

The plasma cells need to be selected enabling an unambiguous identification

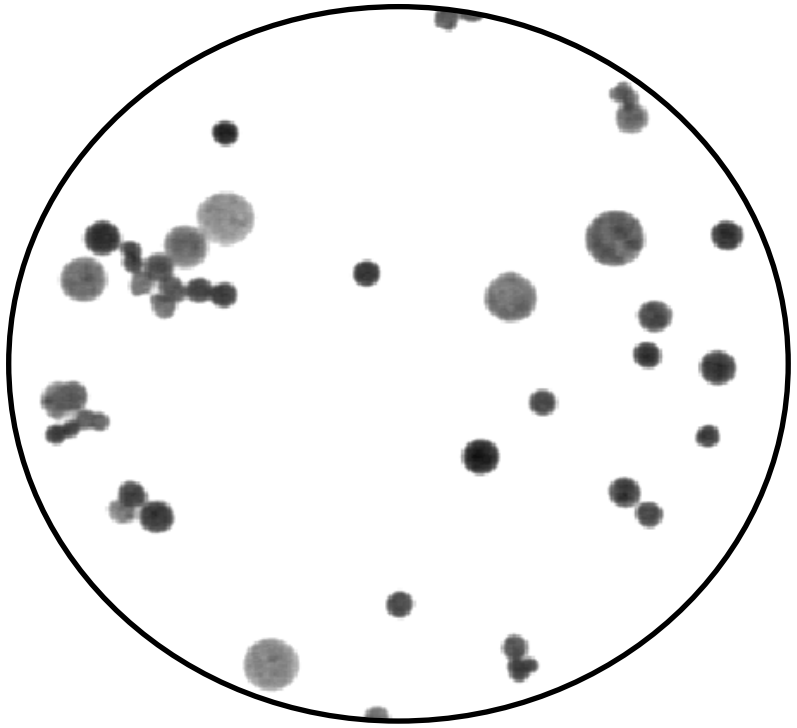
Immunomagnetic separation

Flow cytometry, FACSAria II cell sorter

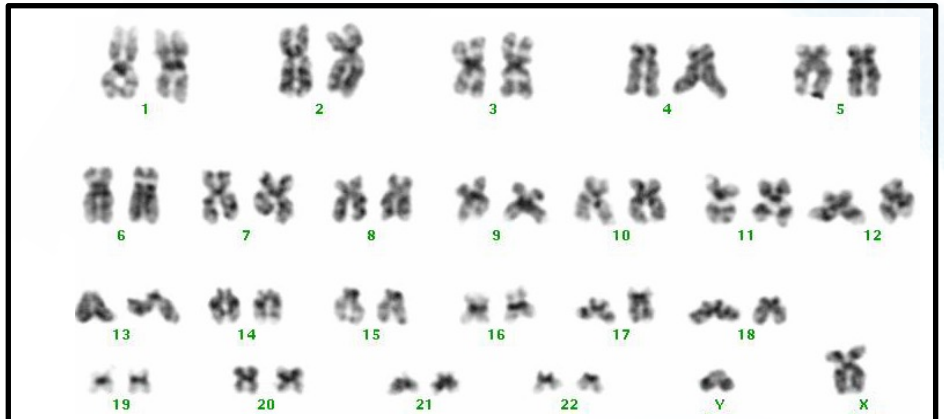


Cell sorting results in a pure PC population which enables further analyses to be performed

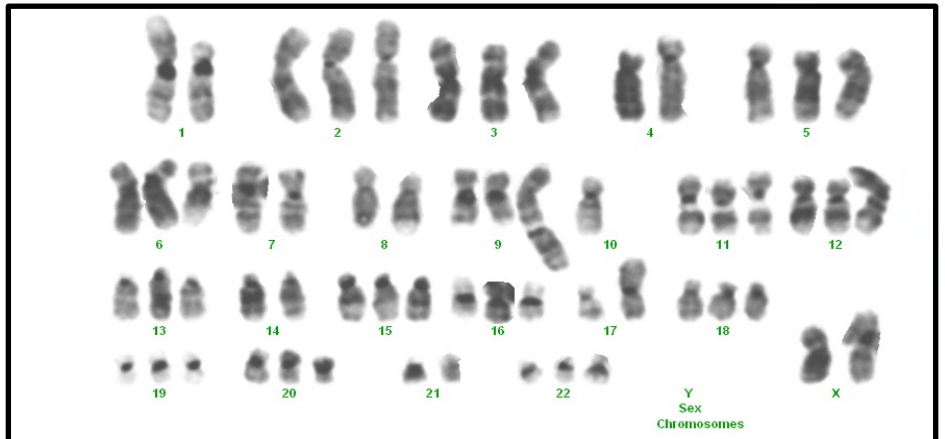
Low mitotic index



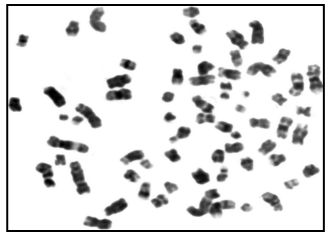
Failure ~ 10-25%



Normal Karyotype ~ 40-50%

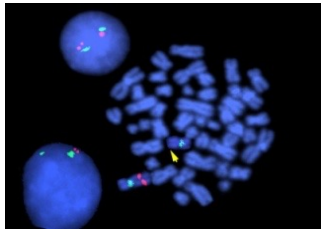


Abnormal Karyotype ~ 30-40%



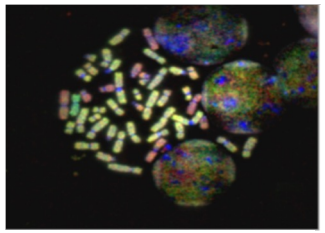
Conventional Cytogenetics

5-10 Mb



Fluorescence *in situ* hybridization

100 Kb-5 Mb



Comparative genomic hybridization (CGH)

50 Kb

SNP-arrays

NGS

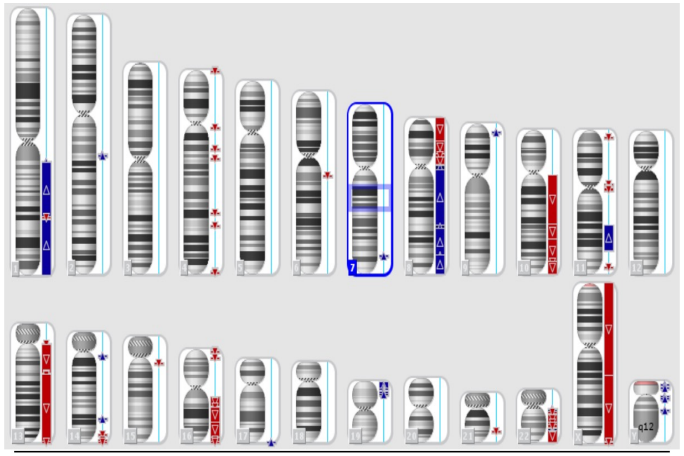
base-pair

DNA

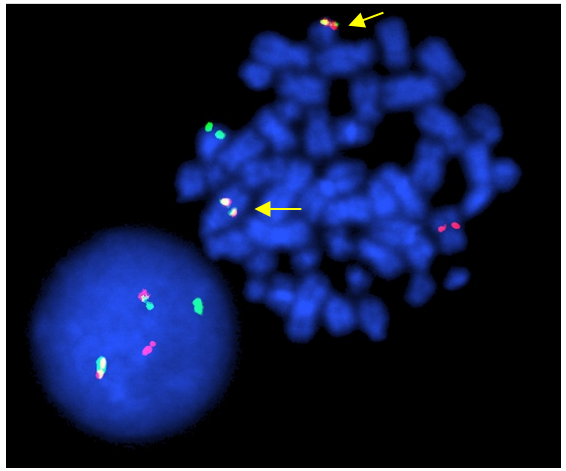
Copy number abnormalities

Translocations

Point mutations



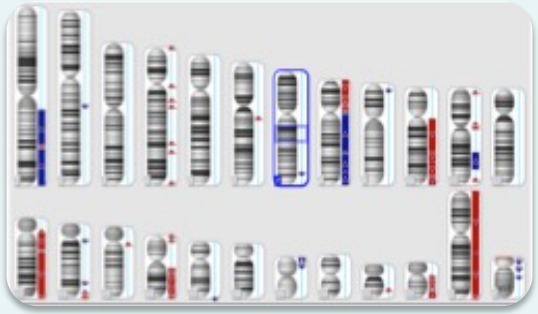
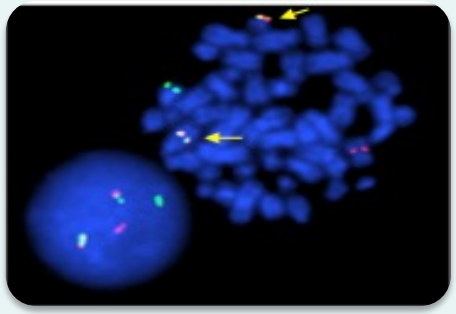
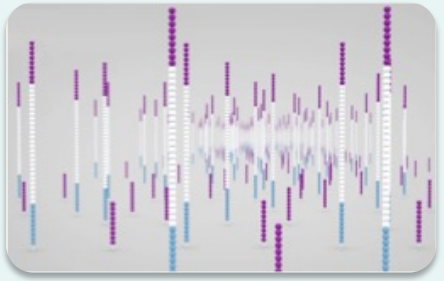
Array comparative genomic hybridization (aCGH) SNP-arrays



FISH

Next-generation sequencing

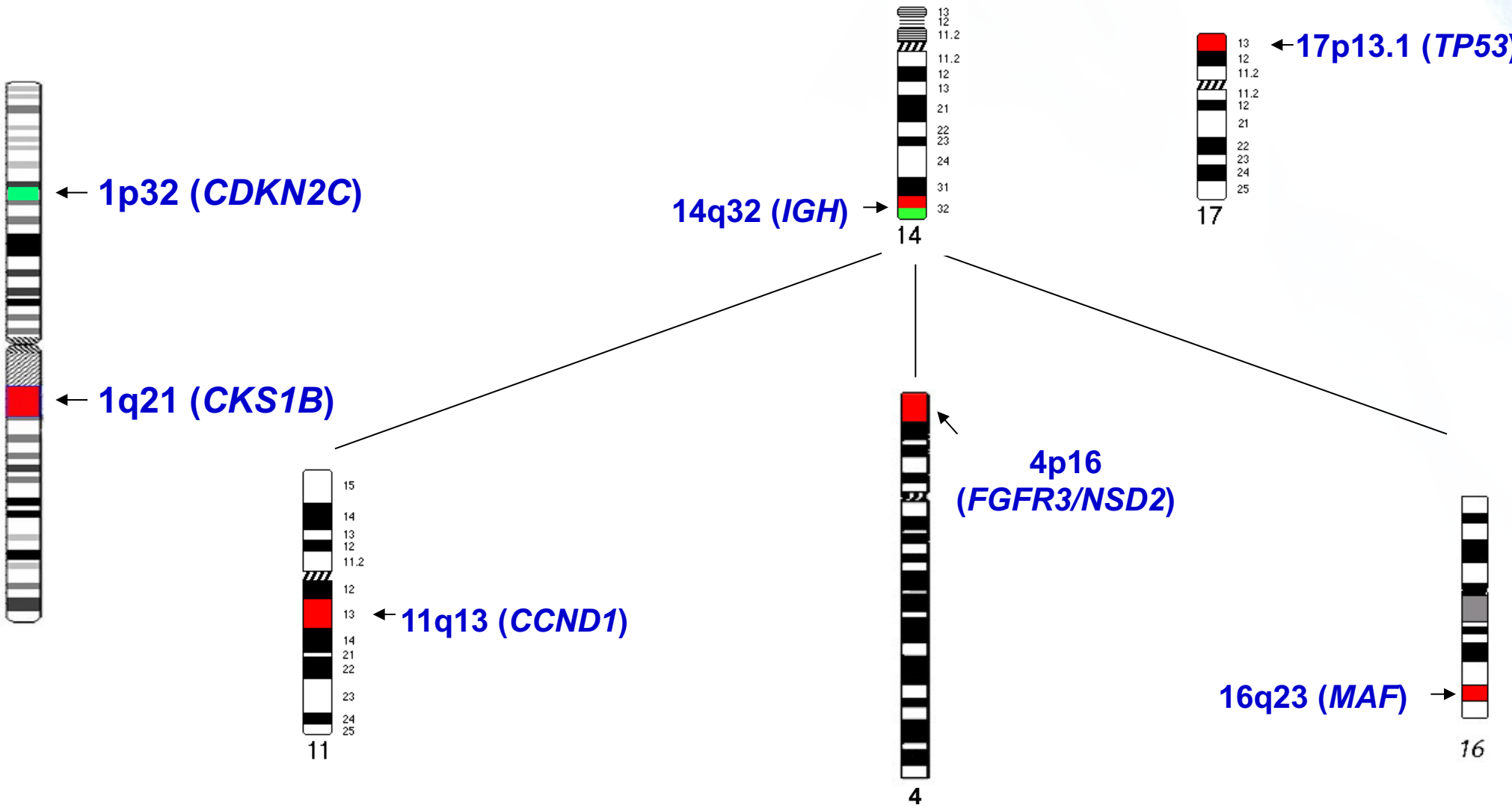
Genetic markers with prognostic significance

Genomic imbalances	Translocations	Point mutations
		
1q gain/amp 1p deletion 17p deletion Hyperdiploidy Monosomy 13	<u>IGH translocations</u> t(4;14) t(14;16) t(11;14) <u>MYC translocations</u> **?**	*TP53* *KRAS, NRAS, BRAF* *DIS3, FAM46C*

Prognostic value	adverse
	neutral/not well-defined

R2-ISS: t(4;14), del(17p), 1q+
D'Agostino M, JCO 2022

FISH-probe panel (recommendation)



GCECGH

GRUPO COOPERATIVO ESPAÑOL DE CITOGENÉTICA HEMATOLÓGICA

IX SIMPOSIO

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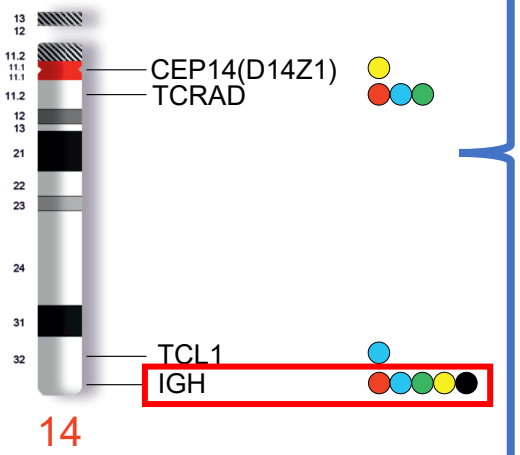
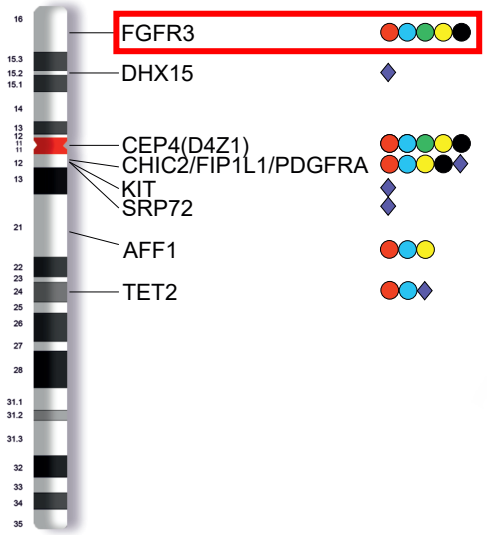
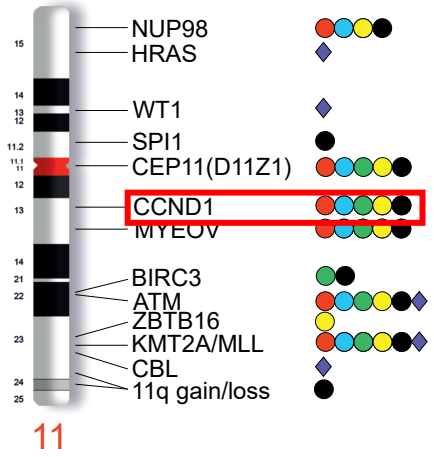
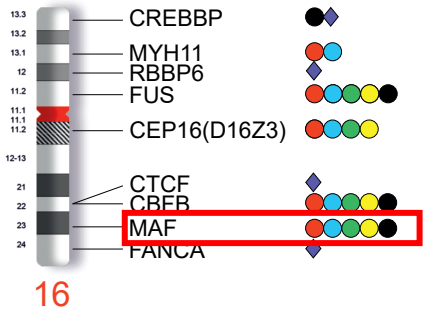
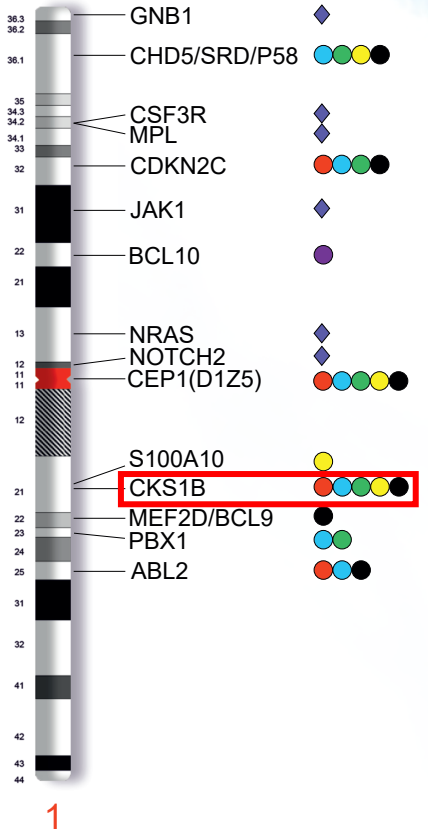
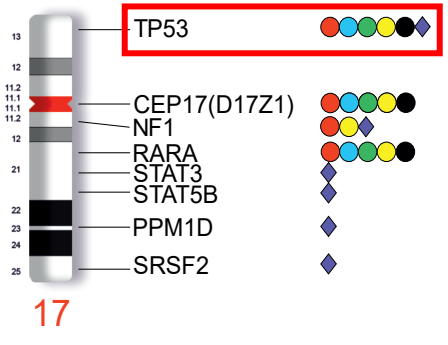






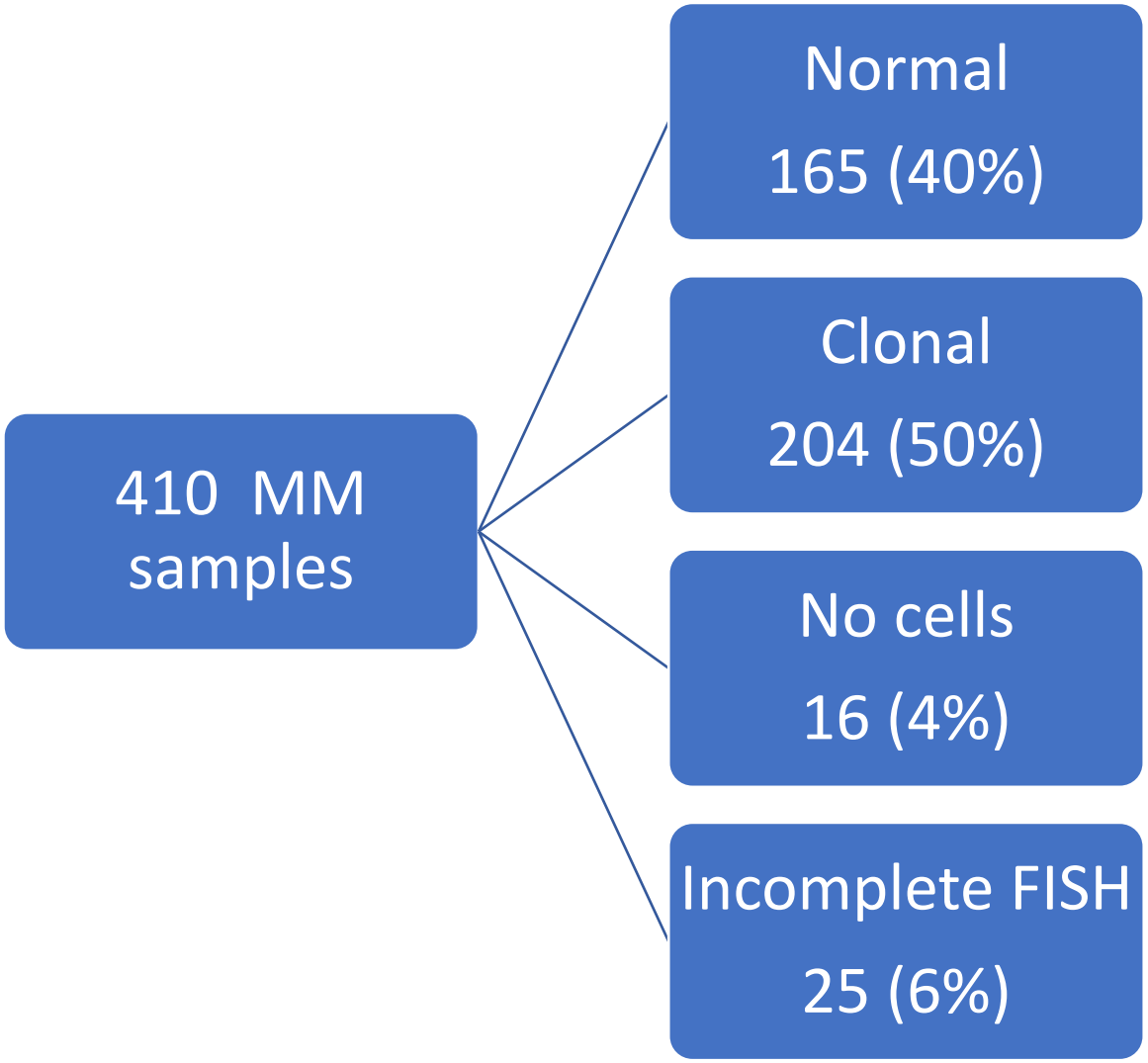






Metasystems
Cytocell
Abbott(Vysis)
Leica(Kreate)
Zytovision
NGS

MM samples received at the laboratory during 5 months, between November to April

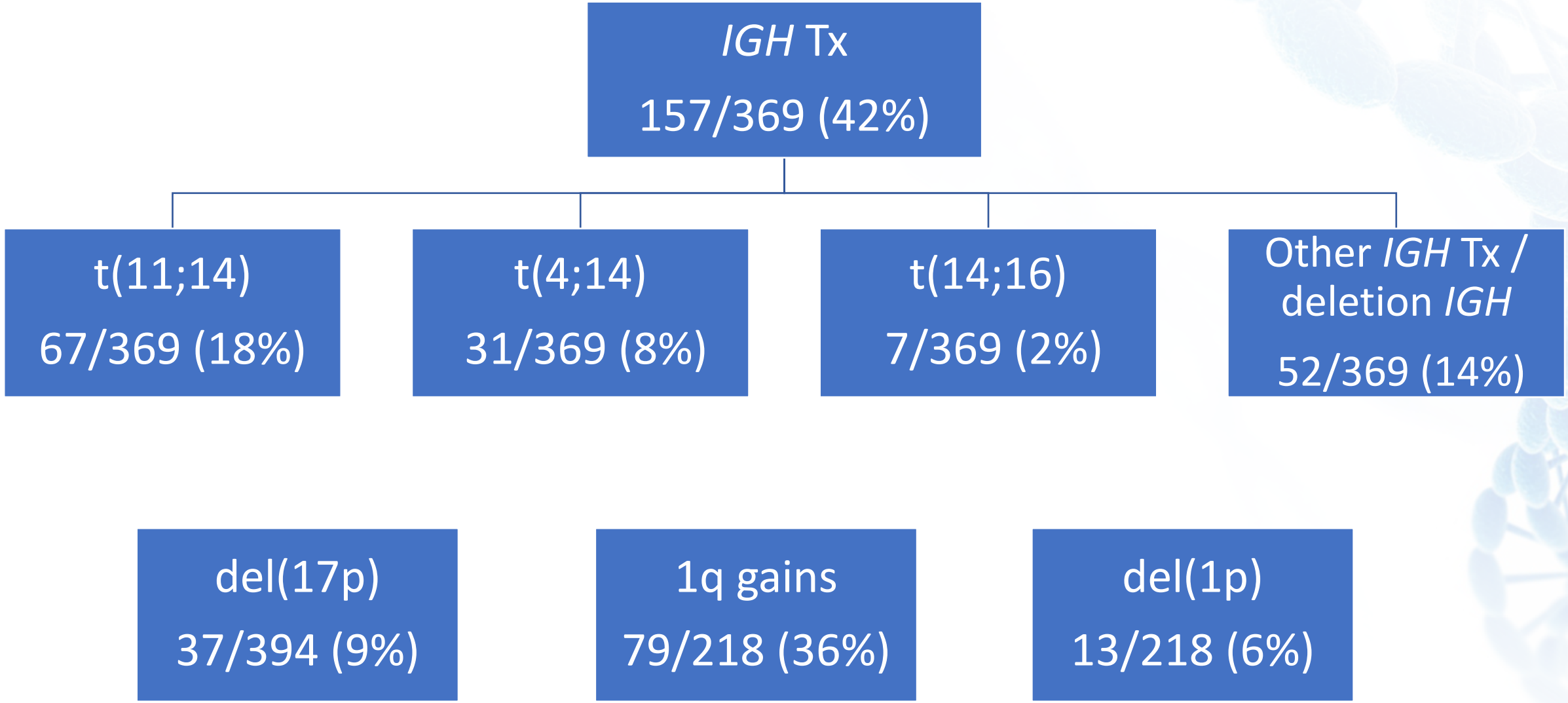


- Samples received as a diagnosis of MM.
- MGUS excluded.

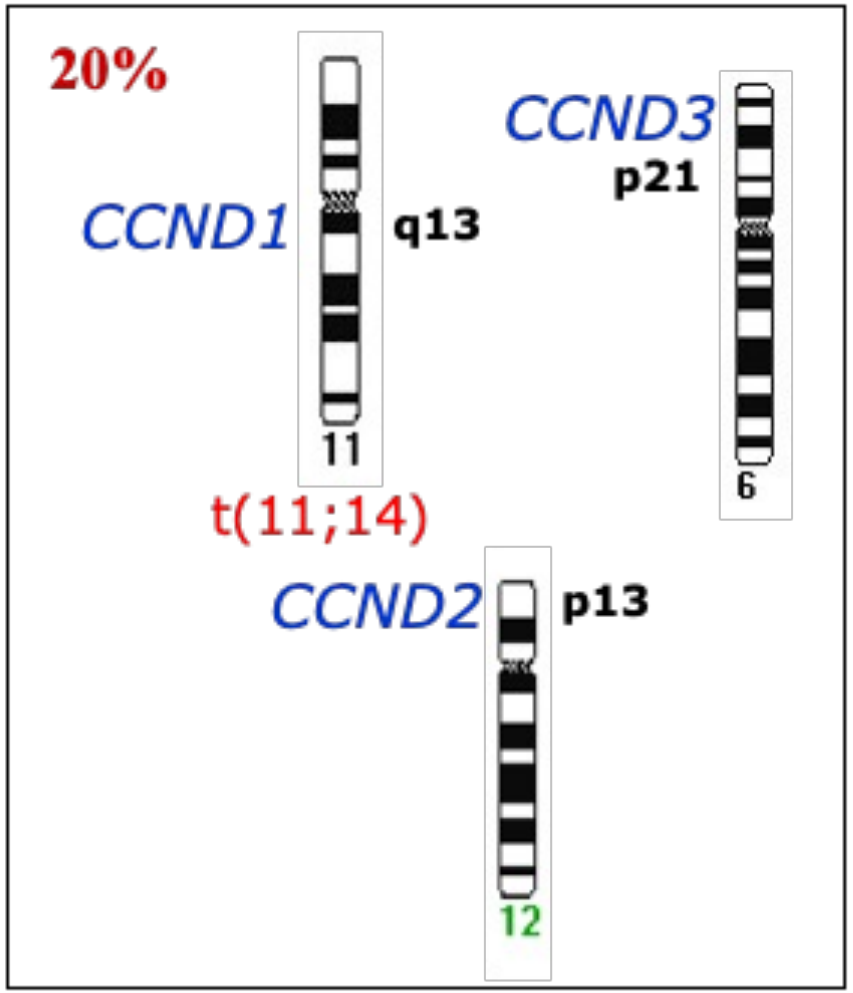
del(17p) → *IGH* → 1p/1q

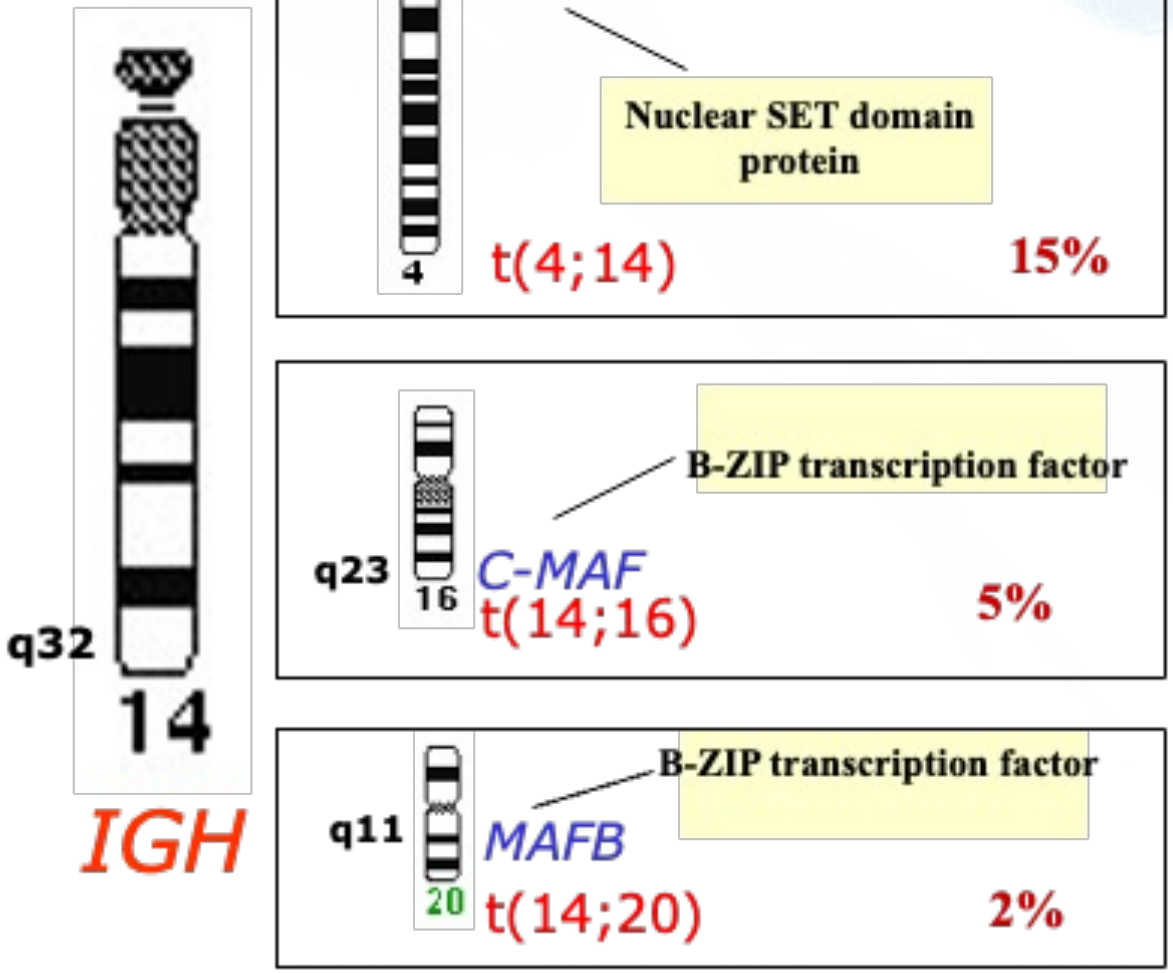
t(4;14)
t(14;16)
t(11;14)

MM samples received at the laboratory during 5 months, between November to April



IGH translocations

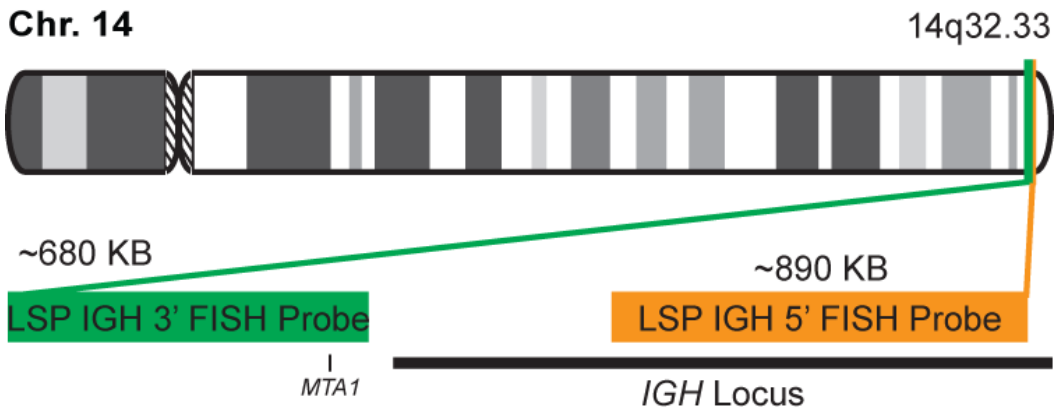




Chesi M, Blood 1998; Avet-Loiseau H, GCC 1999

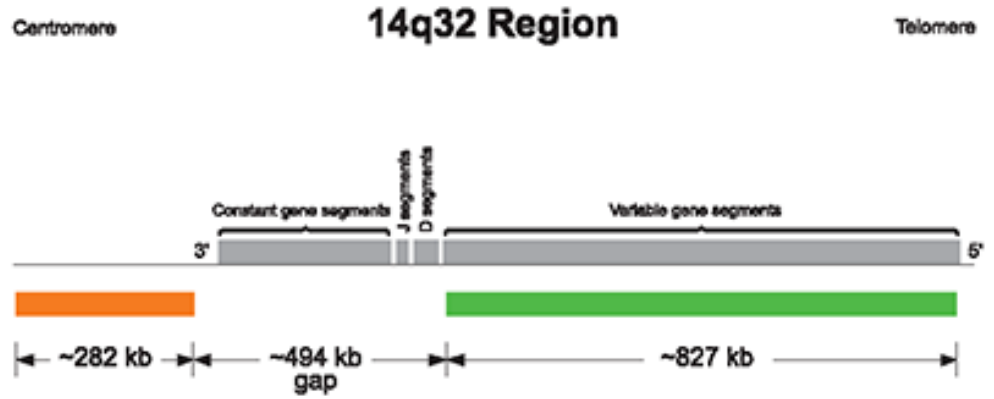
IGH translocations

IGH probes



IGH Break Apart FISH Probe Kit

CytoTest®



LSI IGH Dual Color, Break Apart Rearrangement Probe

Abbott

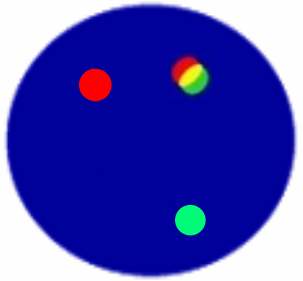
IGH probe

CytoTest

Abnormal

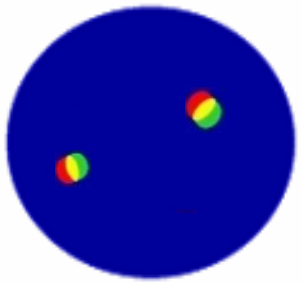
N = 157

1F1R1G



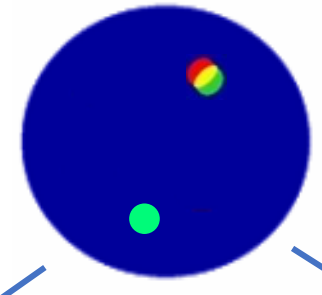
N = 63 (40%)

Normal

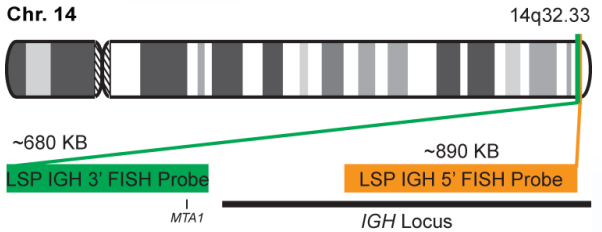


N = 212

1F1G



N = 47 (30%)



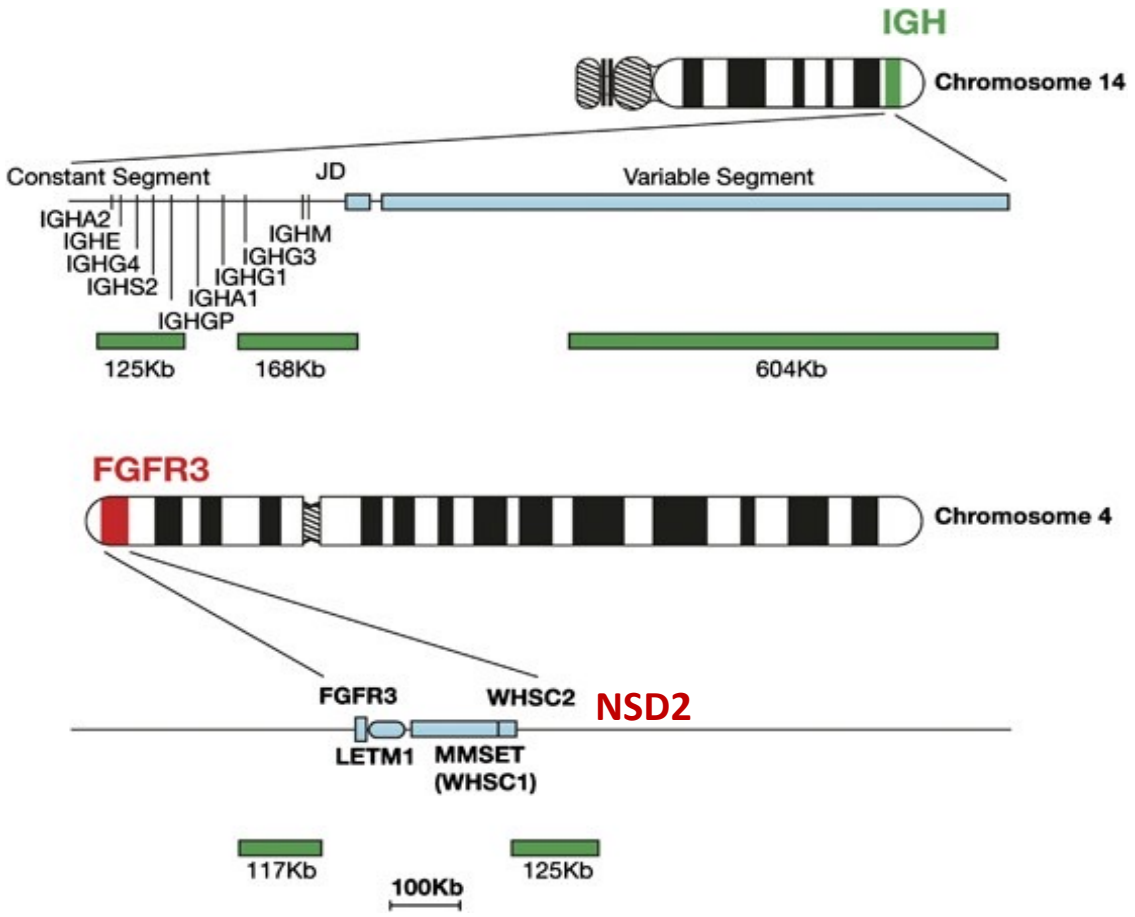
- *IGH* translocation
- Deletion of the *IGH* variable region

Various patterns

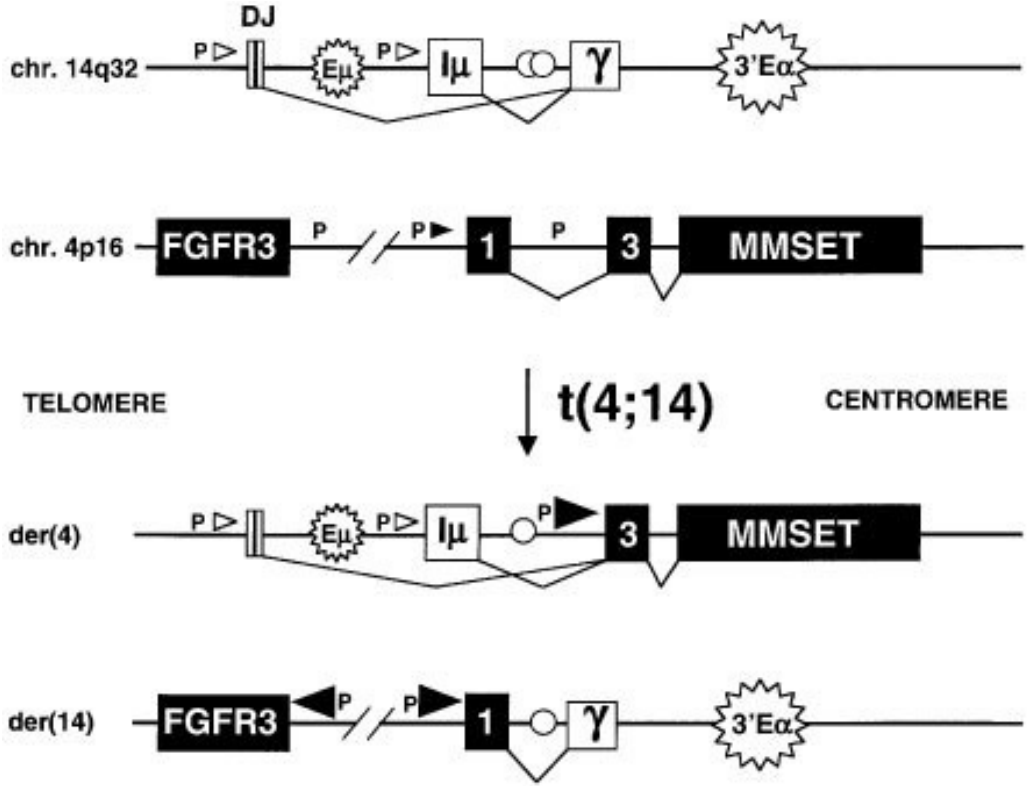
N = 47 (30%)

t(4;14) FGFR3/NSD2::IGH

Dysregulation of both *FGFR3* and *MMSET* (*NSD2*) contributes to neoplastic transformation in MM with t(4;14)



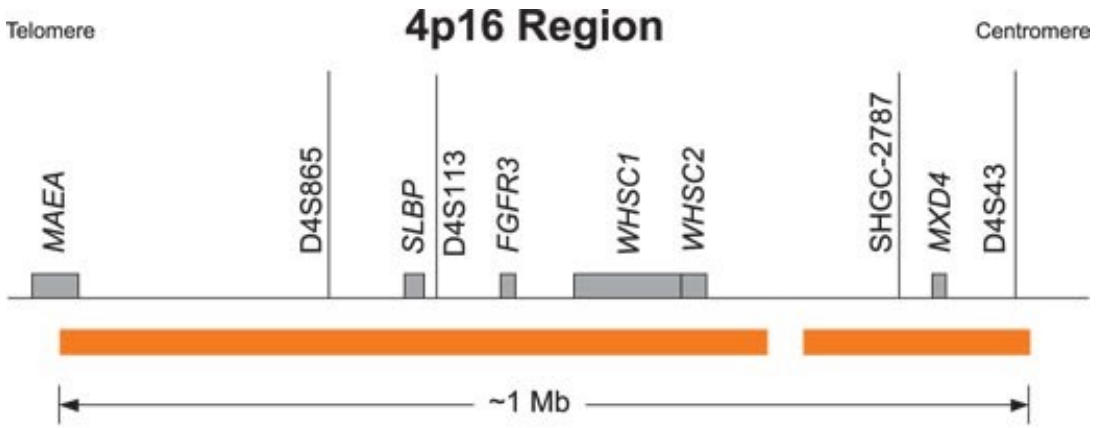
The t(4;14) translocation was the first example of an *IGH* translocation that simultaneously dysregulated two genes with oncogenic potential: *FGFR3* on der(14) and *MMSET* on der(4)



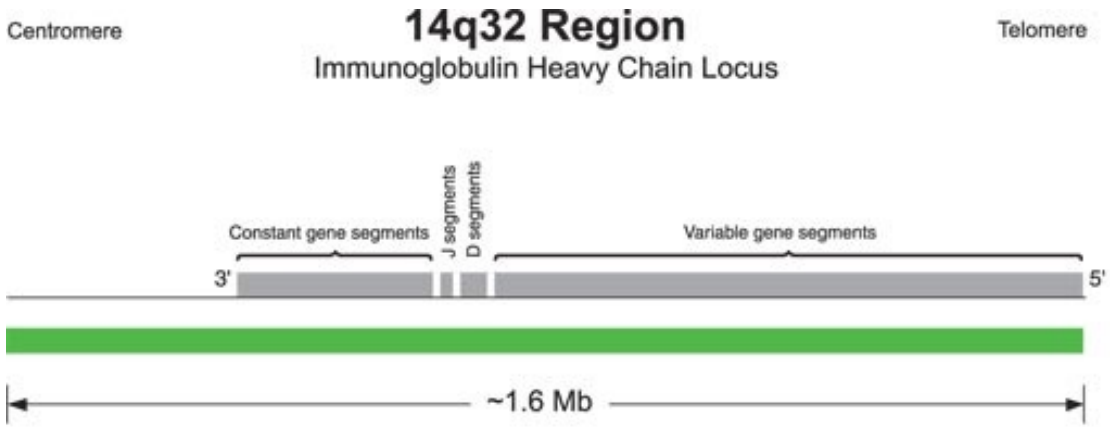
Chesi M, Nat Genet 1997; Blood 1998

t(4;14): FGFR3/NSD2::IGH

Vysis LSI *IGH/FGFR3* Dual Color Dual Fusion Probes



LSI FGFR3 SpectrumOrange Probe



LSI IGH SpectrumGreen Probe

Abbott

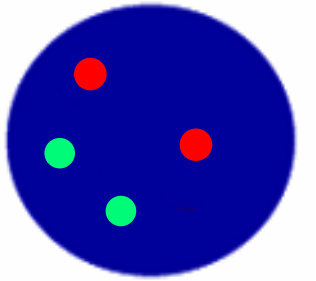
FGFR3::IGH probe

Abbott (Vysis)

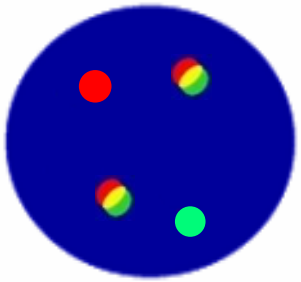
t(4;14)

N = 31

Normal

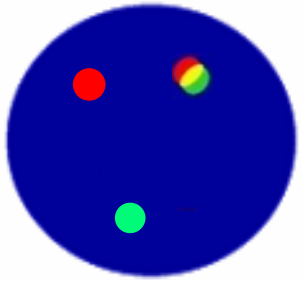


2F1R1G



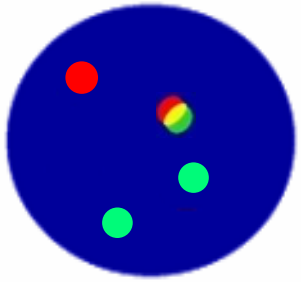
N = 13 (42%)

1F1R1G



N = 4 (13%) → Loss of the der(14) chromosome

1F1R2G



N = 1 (3%) → Loss of *FGFR3* with no evidence of CH deletion

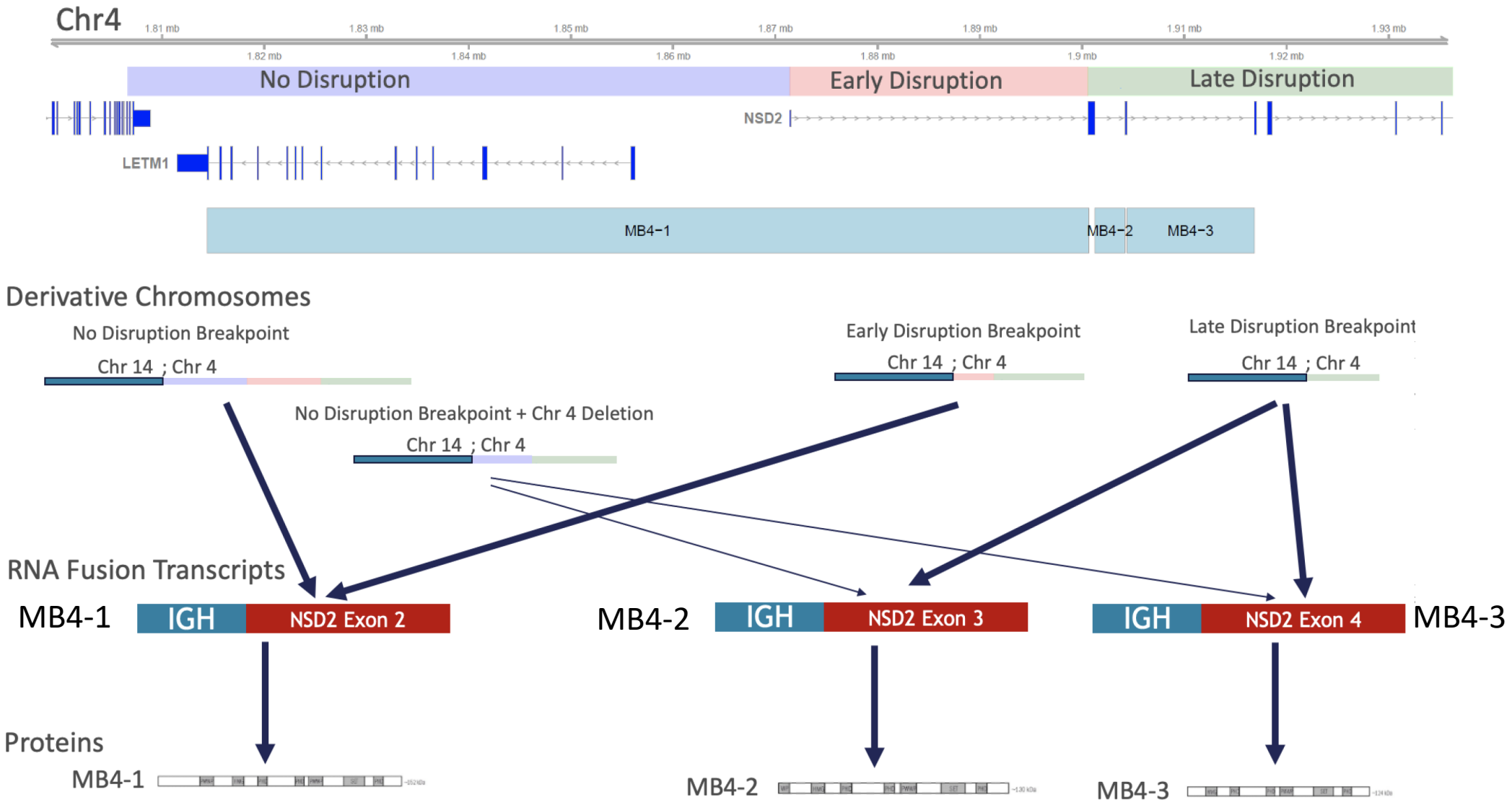
Loss of *FGFR3* expression

2F1R1G
3F1R1G
2F1R2G
FRG

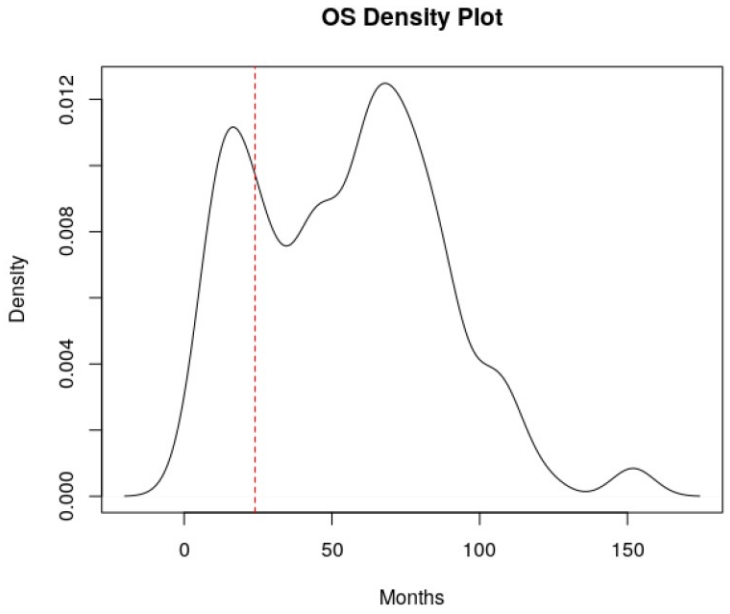
N = 13 (42%)

Santra M, Blood 2003

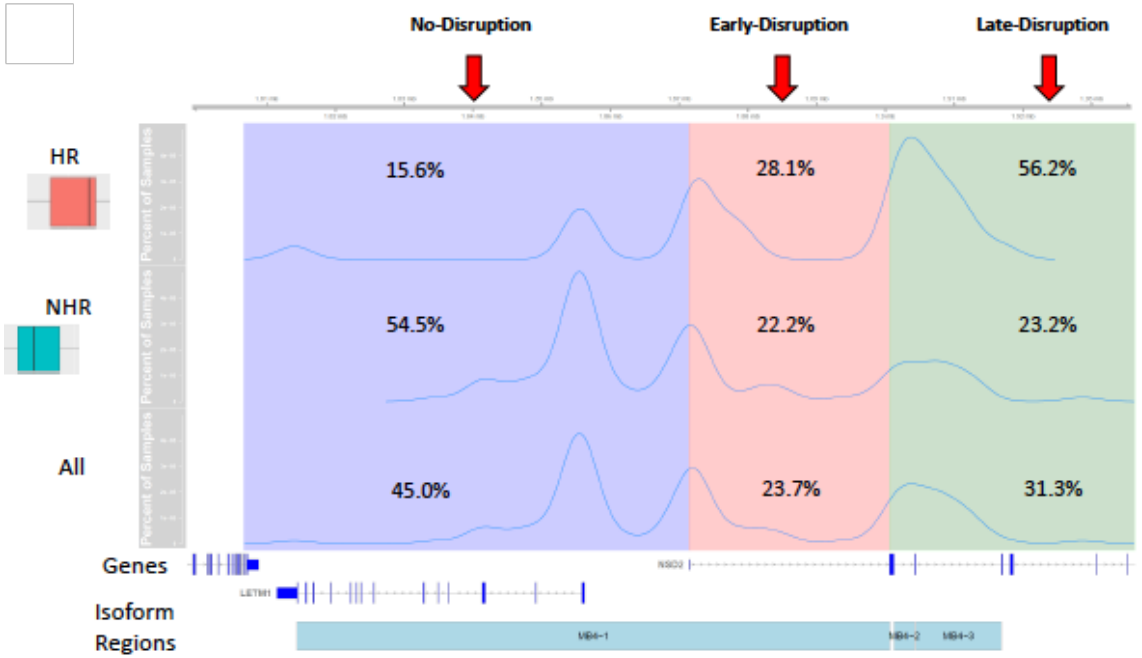
t(4;14) translocation breakpoint within the NSD2 gene



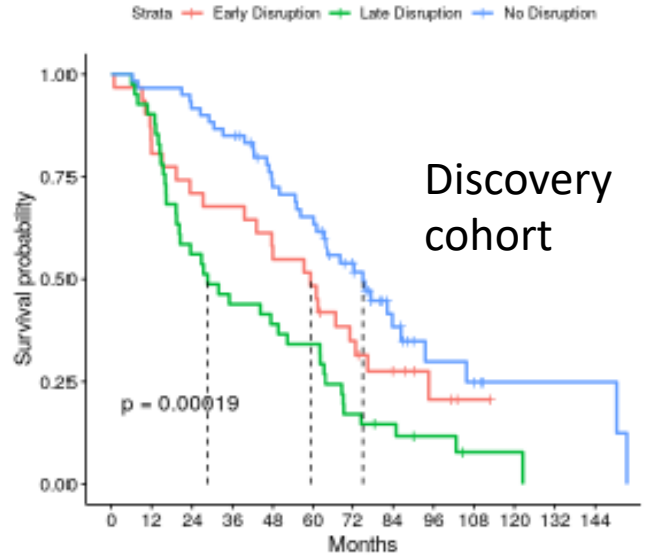
Stong N. Blood 2022



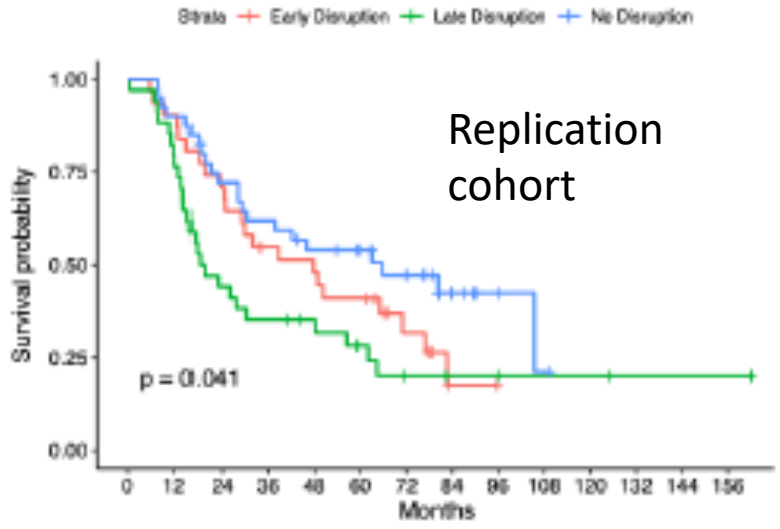
High Risk **(HR):** OS < 24 months
Non High Risk **(NHR):** OS > 24 months



HR t(4;14): 56% have a late-disruption translocation
NHR t(4;14): 54.5% have a breakpoint in the no-disruption category

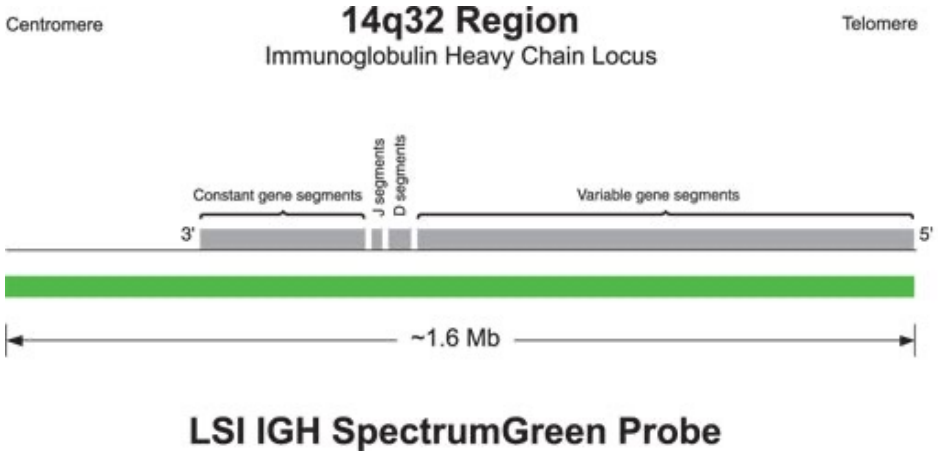


Discovery cohort

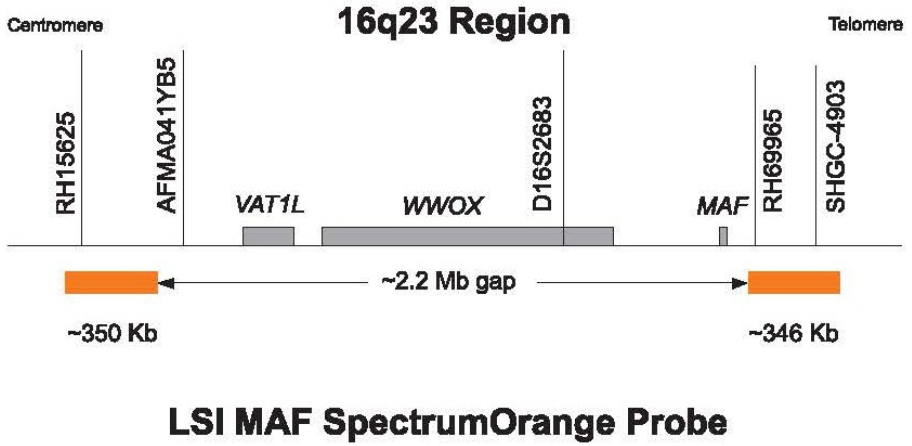


Replication cohort

t(14;16): IGH::MAF

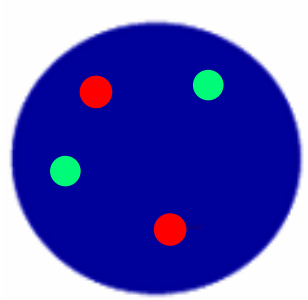


LSI IGH SpectrumGreen Probe



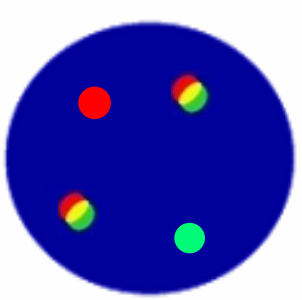
LSI MAF SpectrumOrange Probe

Normal



2F1R1G

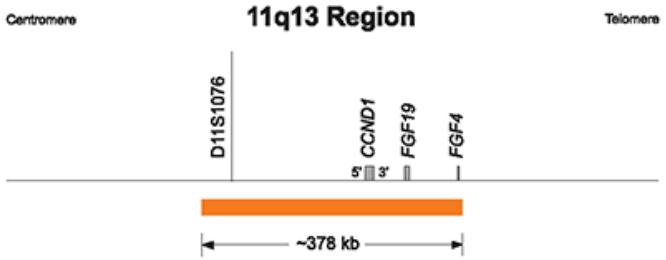
t(14;16)
N = 7



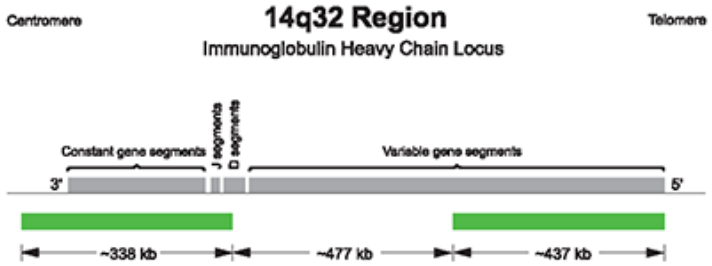
N= 3 (43%)

t(11;14): CCND1::IGH

Vysis LSI *IGH/CCND1* DF FISH Probe Kit

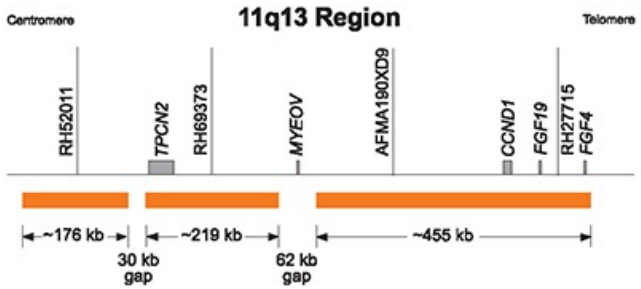


LSI CCND1 SpectrumOrange Probe

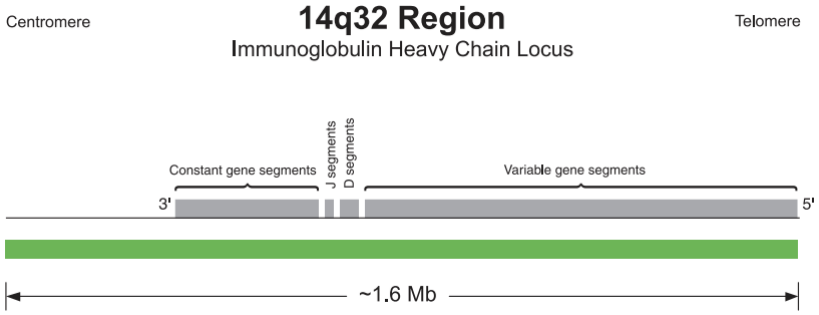


LSI IGH SpectrumGreen Probe

Vysis *IGH/CCND1* XT DF FISH Probe Kit



LSI CCND1 XT SpectrumOrange Probe



Abbott

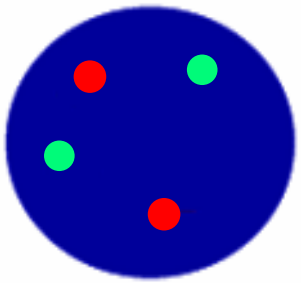
IGH::CCND1 probe

Abbott (Vysis)

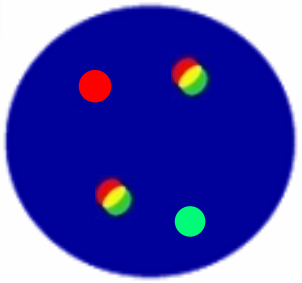
t(11;14)

N = 67

Normal

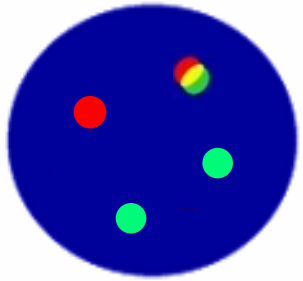


2F1R1G



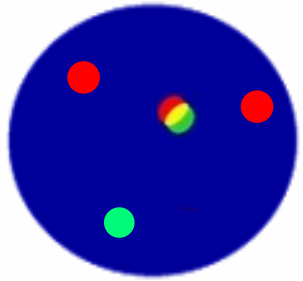
N = 10 (15%)

1F1R2G



N = 8 (12%)

1F2R1G



N = 11 (16%)

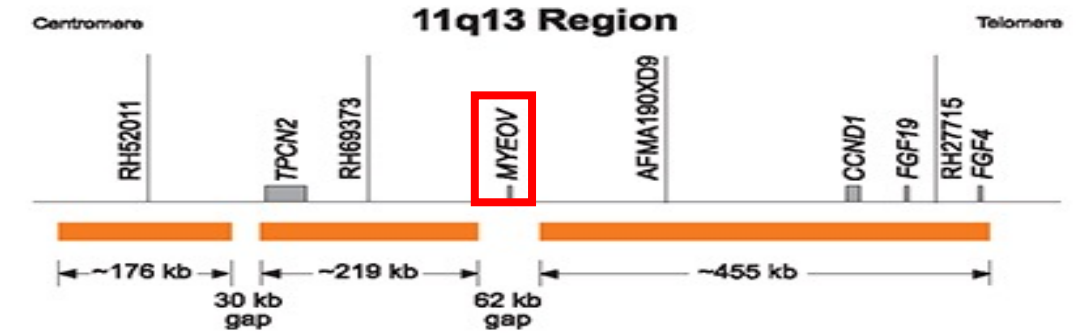
2F1R2G

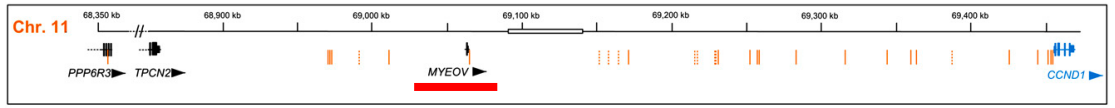
1F1R2G

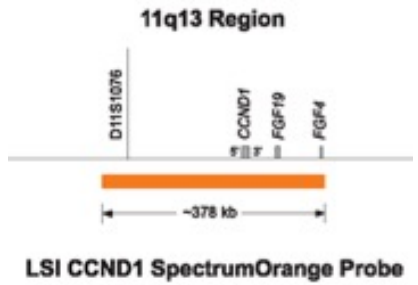
1F2R1G

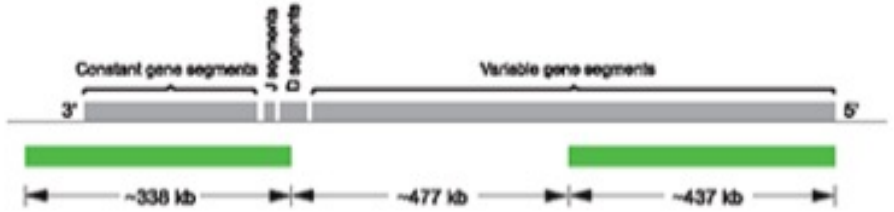
1F2R2G

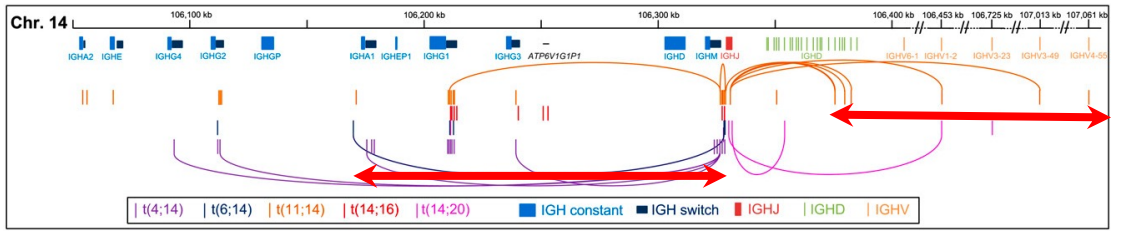
N = 38 (57%)





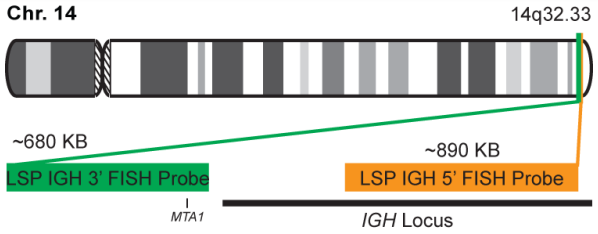




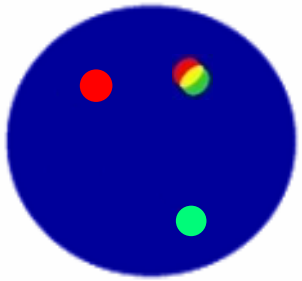


Walker BA, Blood 2013

Correspondence between *IGH* probe and fusion probes for specific *IGH* translocations



1F1R1G



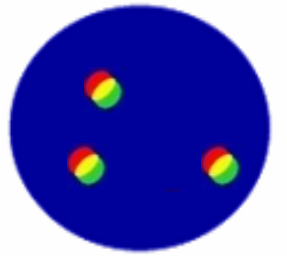
IGH Tx	N = 63
t(11;14)	24
t(4;14)	12
t(14;16)	1
Other *IGH* Tx	19
No cells	7

1F1G

IGH Tx	N = 47
t(11;14)	16
t(4;14)	1
Other *IGH* Tx/IGHv deletion	27
No cells	3

Gains and losses of genes involved in *IGH* translocations

IGH probe
CytoTest



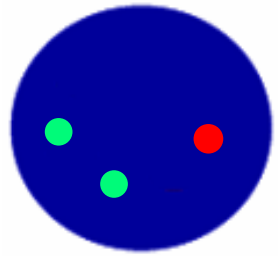
N= 11

Trisomy 14

N= 21

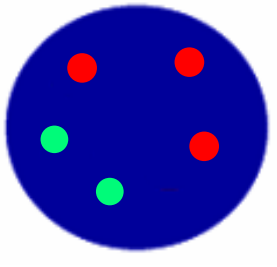
Monosomy 14/del(14q32)

FGFR3::IGH probe
Abbott (Vysis)



N= 5

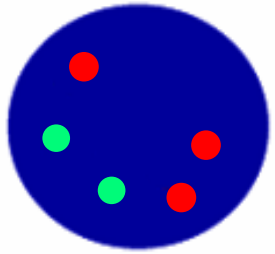
Monosomy 4/del(4p16)



N= 4

Trisomy 4

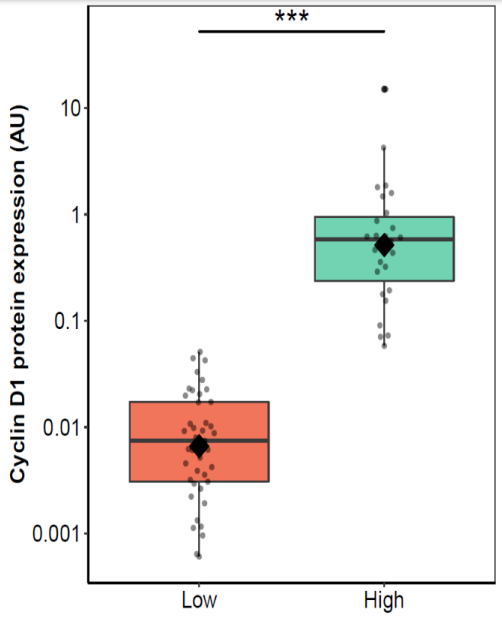
IGH::CCND1 probe
Abbott (Vysis)

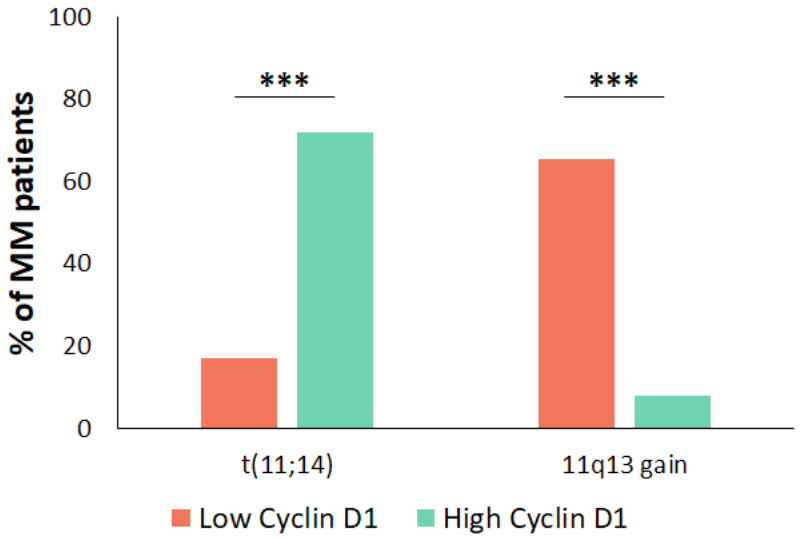


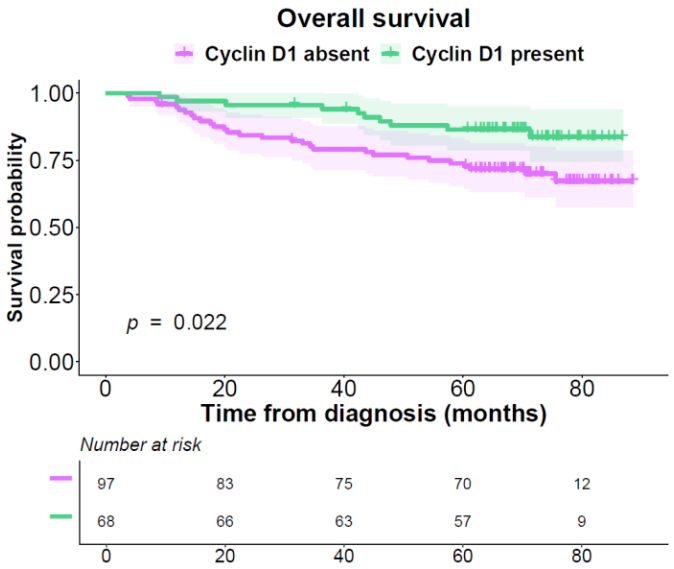
N= 25

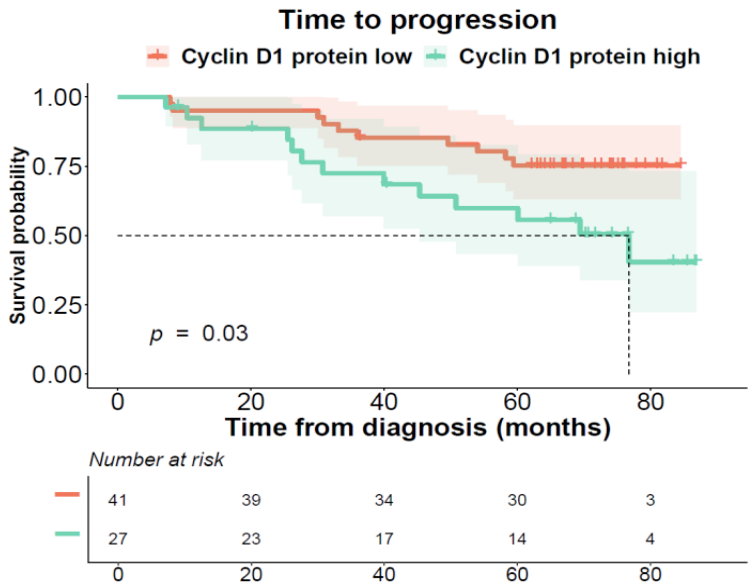
Trisomy 11

Cyclin D1 expression









Patients overexpressing cyclin D1 at lower levels corresponded mainly to cases with 11q13 gains

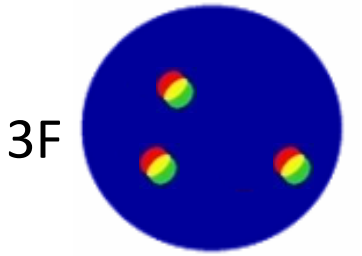
Patients with high levels of cyclin D1 protein had significantly shorter TTP than did those with low levels

More favorable outcome for MM patients with 11q13 than for those with t(11;14).

Preprint: Research Square 10.21203/rs.3.rs-2616507/v1

Gains and losses of genes involved in *IGH* translocations

IGH probe
CytoTest



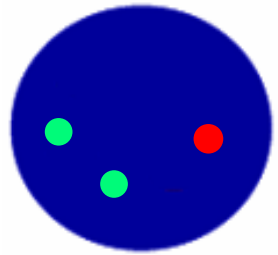
N = 11

Trisomy 14

N = 21

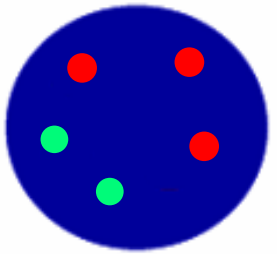
Monosomy 14/del(14q32)

FGFR3::IGH probe
Abbott (Vysis)



N = 5

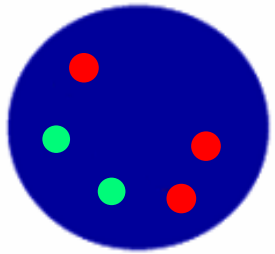
Monosomy 4/del(4p16)



N = 4

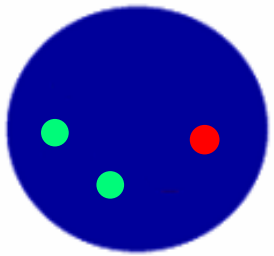
Trisomy 4

IGH::CCND1 probe
Abbott (Vysis)



N = 25 → Trisomy 11

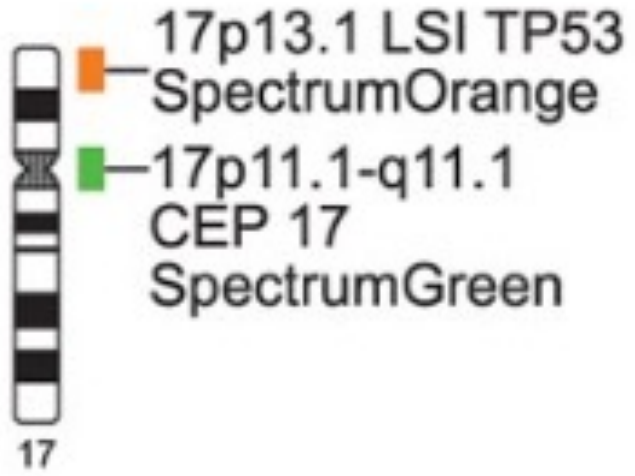
IGH::MAF probe
Abbott (Vysis)

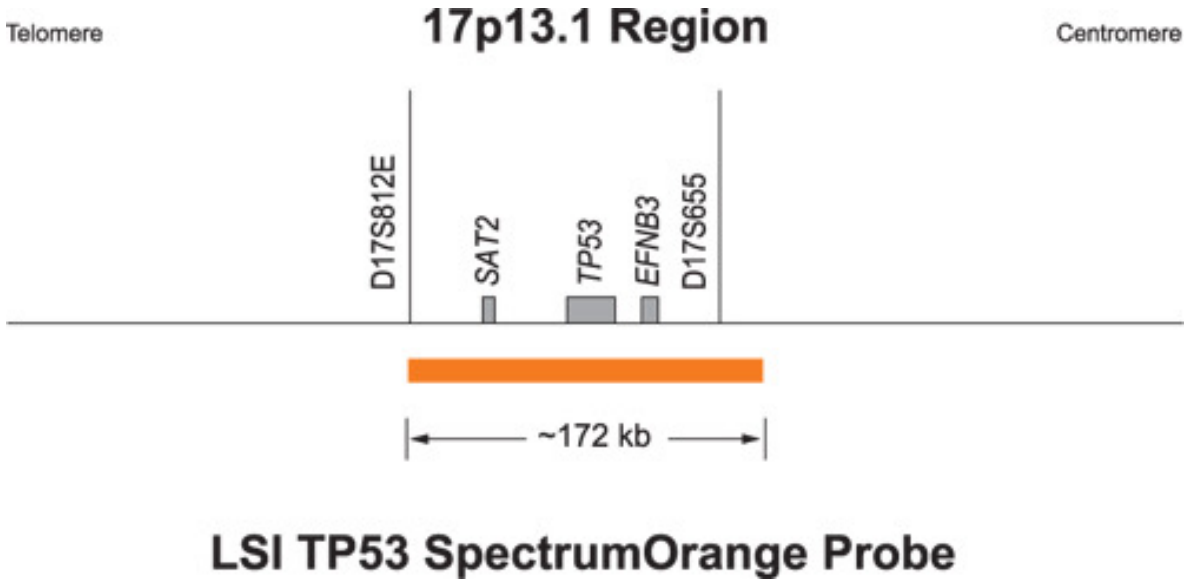


N = 10 → del(16q23) — *WWOX* (2.4-fold reduction in expresión)

Walker BA, Blood 2010

17p probe



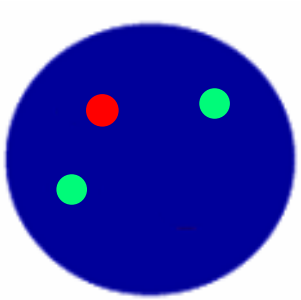


Abbott

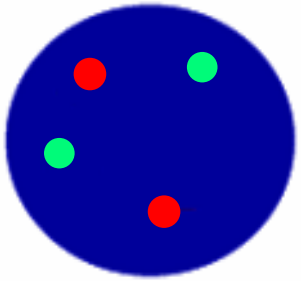
TP53 probe

Abbott (Vysis)

1R2G

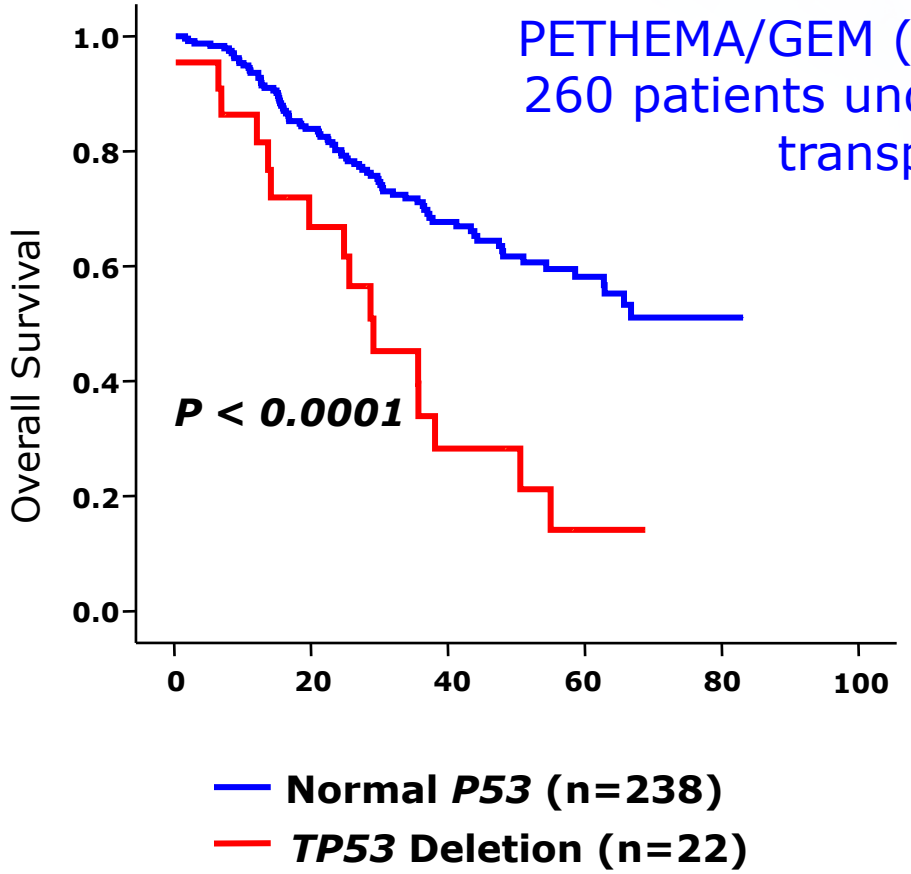


N= 37 (9%)



N = 394

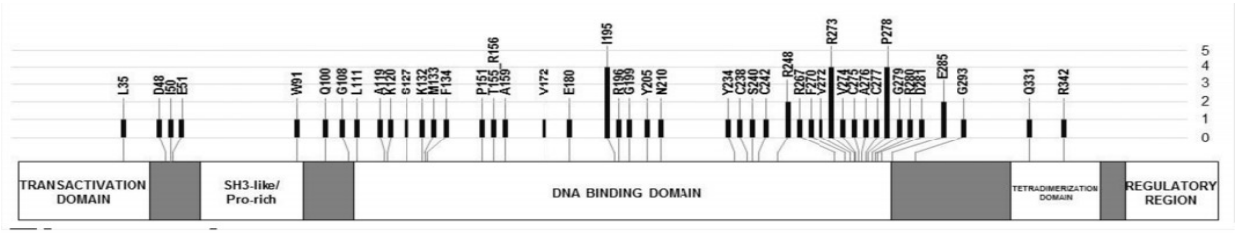
PETHEMA/GEM (Spanish MM group):
260 patients undergoing autologous transplantation



Bi-allelic TP53 inactivation is associated with poorer prognosis

Herrero AB, Int J Mol Sci 2016

Double hit MM



Lionetti M, Expert Rev Mol Diagn 2017

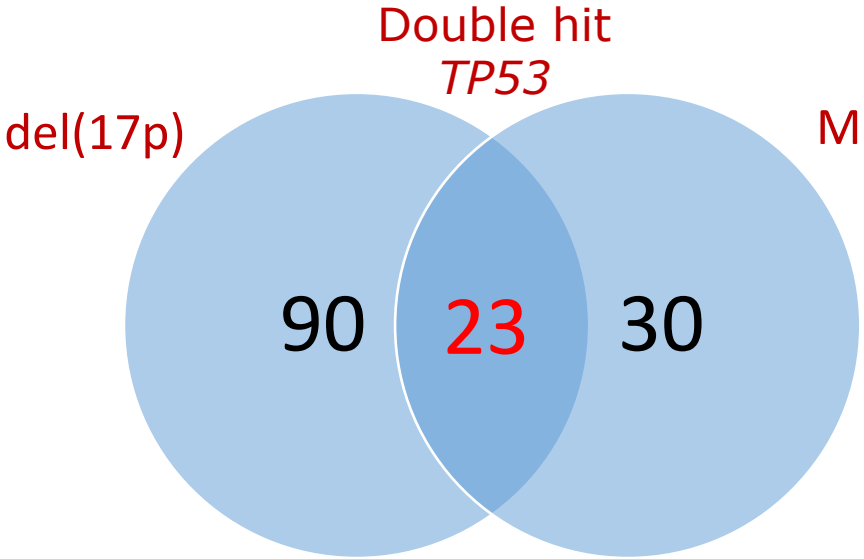
Del(17p)
8-10%

↓

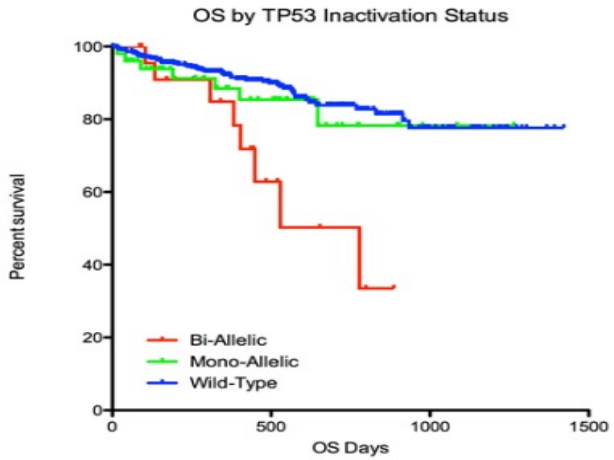
Inactivación bialélica
2-4%

↑

Mutación *TP53*
5-8%

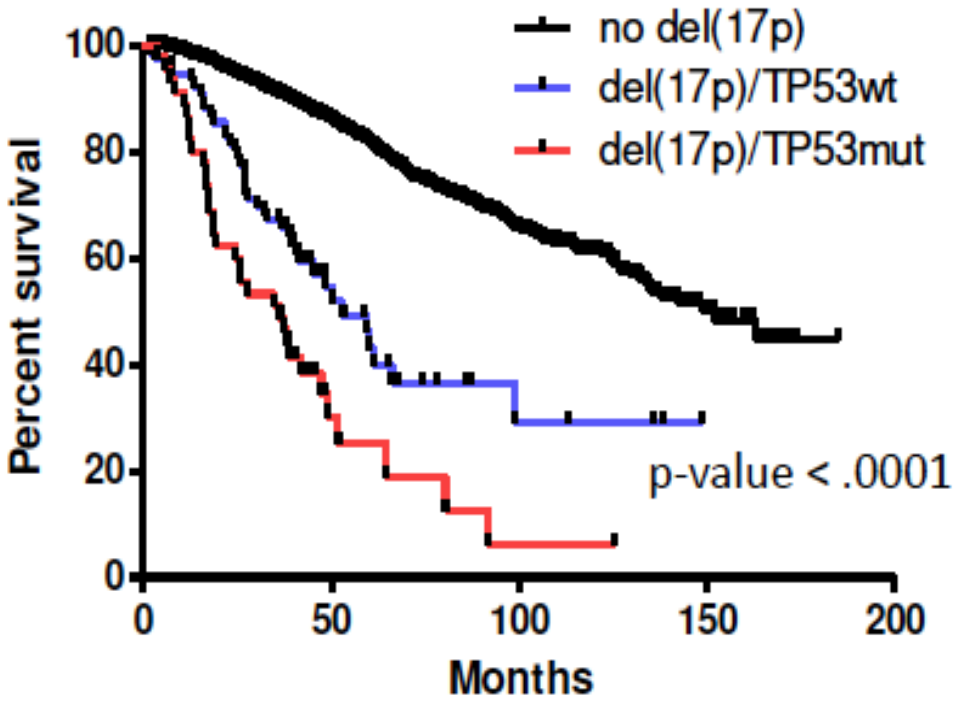


CoMMpass Trial



Bi-Allelic = Del + Del, Del + Mut, or Mut + Mut
Mono-Allelic = Deletion or Mutation alone
Wild-Type = No Deletion and No Mutation Detected

del(17p) without *TP53* mutation confers a poor prognosis in intensively treated newly diagnosed patients with MM



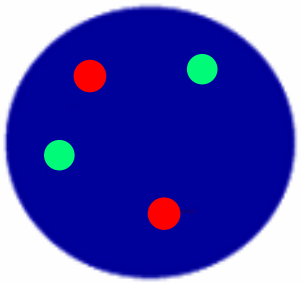
Event/N	Median survival (mo)
601/2505	152,2
39/76	52,8
33/45	36

- 121 newly diagnosed MM patients with a del(17p) in > 55% of plasma cells.
- One-third of these patients had an additional mutation in *TP53*.
- Uniformly treated with intensive therapy, including ASCT.
- Outcome was compared to a large control population (2,505 patients) lacking del(17p).

Corre J, Blood 2021

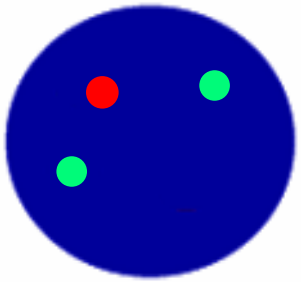
TP53 probe

Abbott (Vysis)



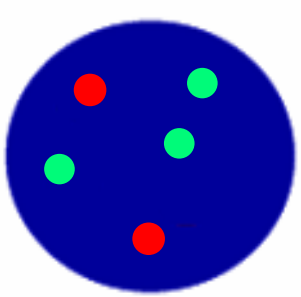
N = 357

1R2V

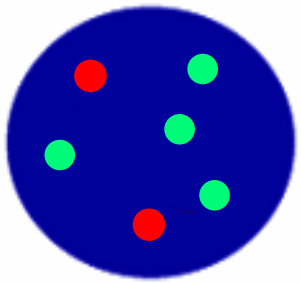


N = 37 (9%)

2R3V



2R4V



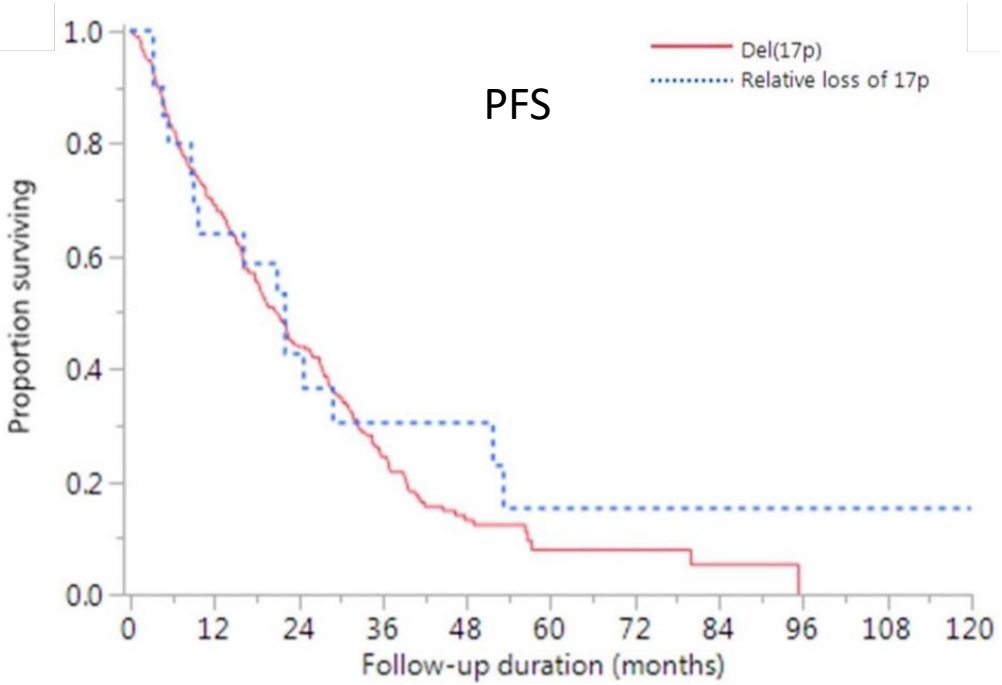
N = 4

del(17p) in presence of trisomy or tetrasomy involving chromosome 17

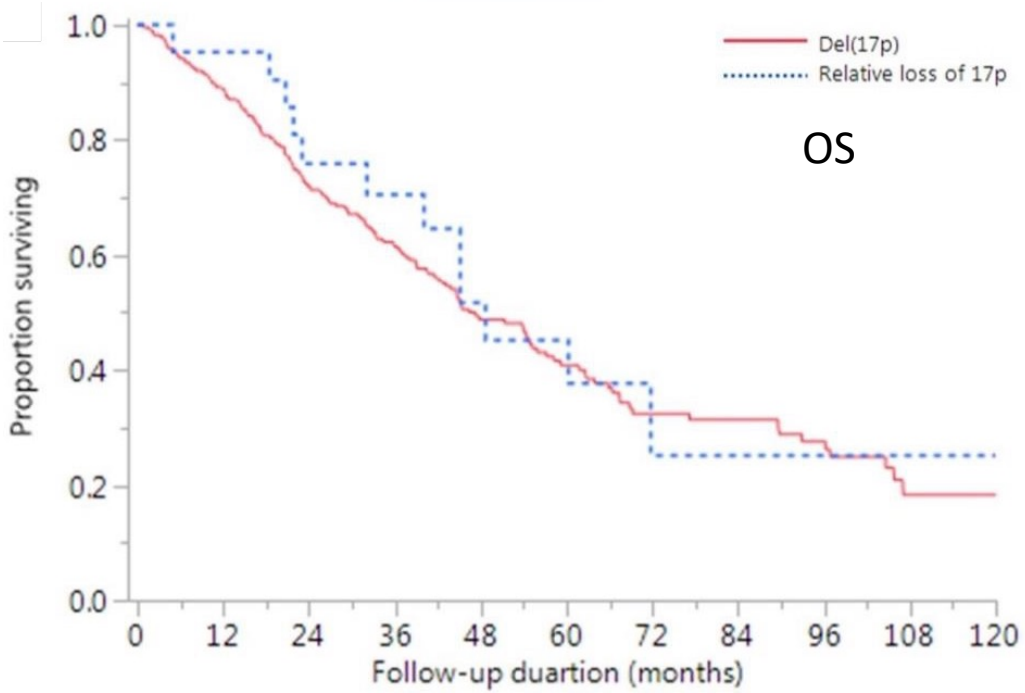
Mayo Clinic (Rochester): FISH testing. (between 2004 and August 2016) → 310 patients with newly diagnosed MM with del(17p)

Relative loss of 17p: del(17p) in presence of trisomy or tetrasomy involving chromosome 17 → **21/310 (6.8%) patients**

PFS

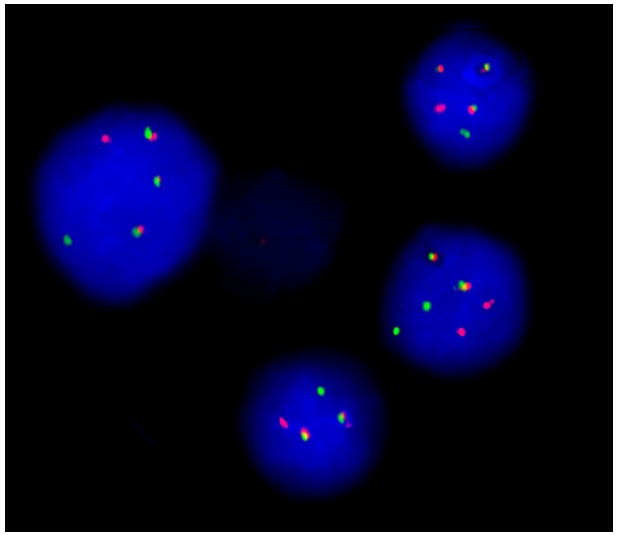


OS

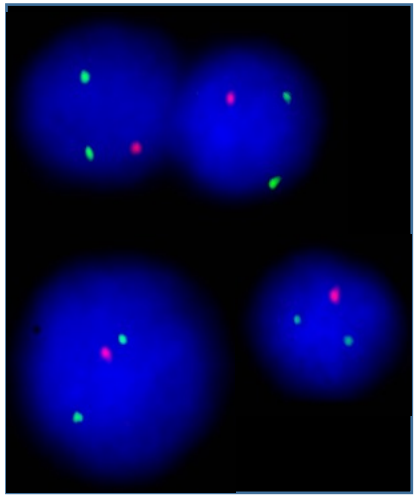


Lakshman A, Blood Cancer J 2019

Diagnosis



t(14;16)

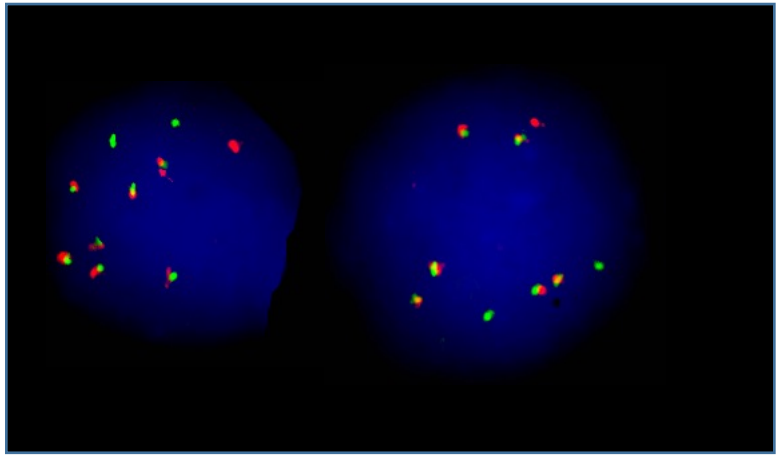


del(17p)

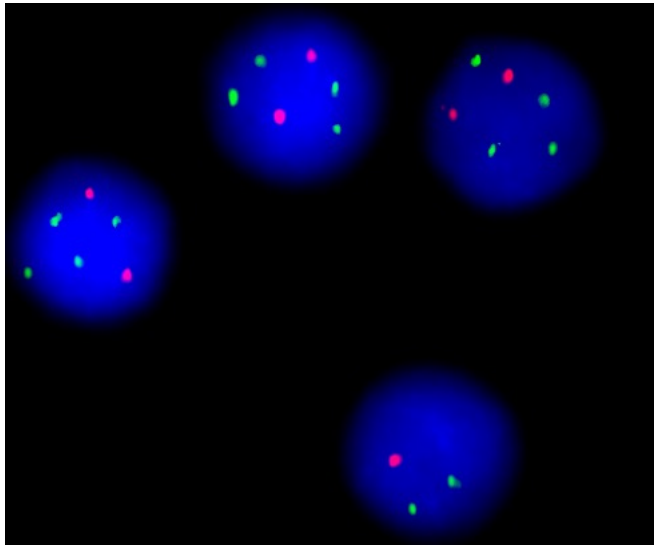
Endoreduplication

Polyploidization

Relapse

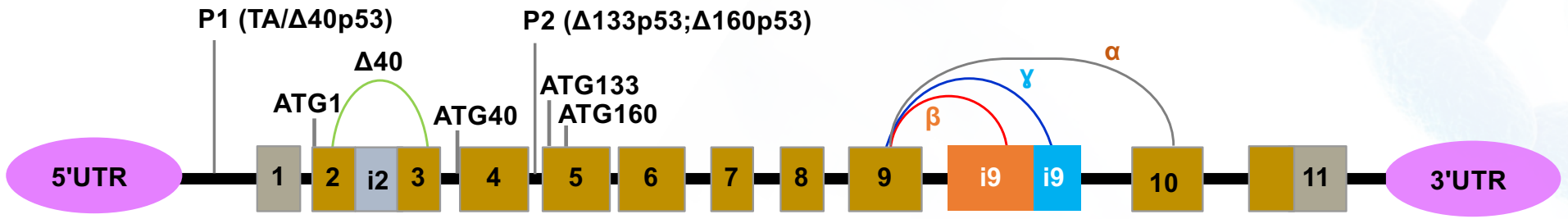


t(14;16)



del(17p)

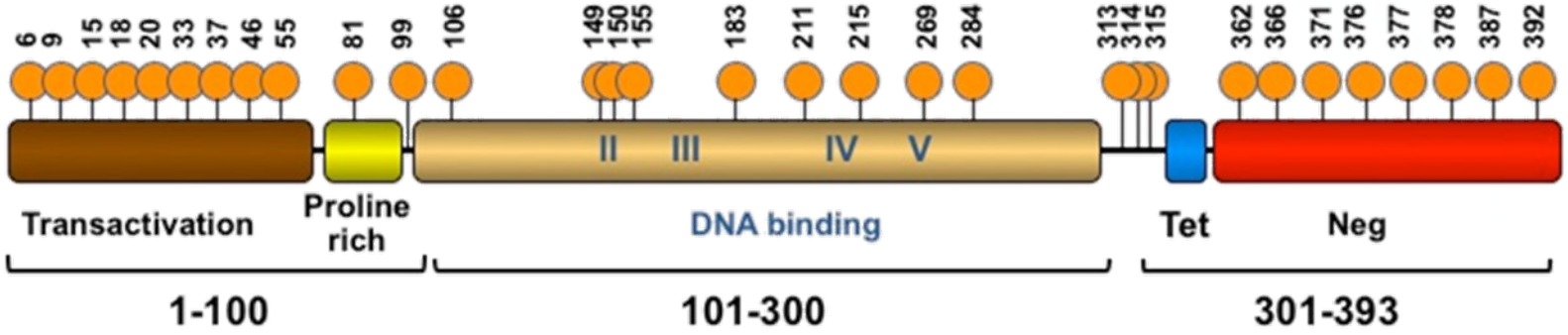
TP53 gene

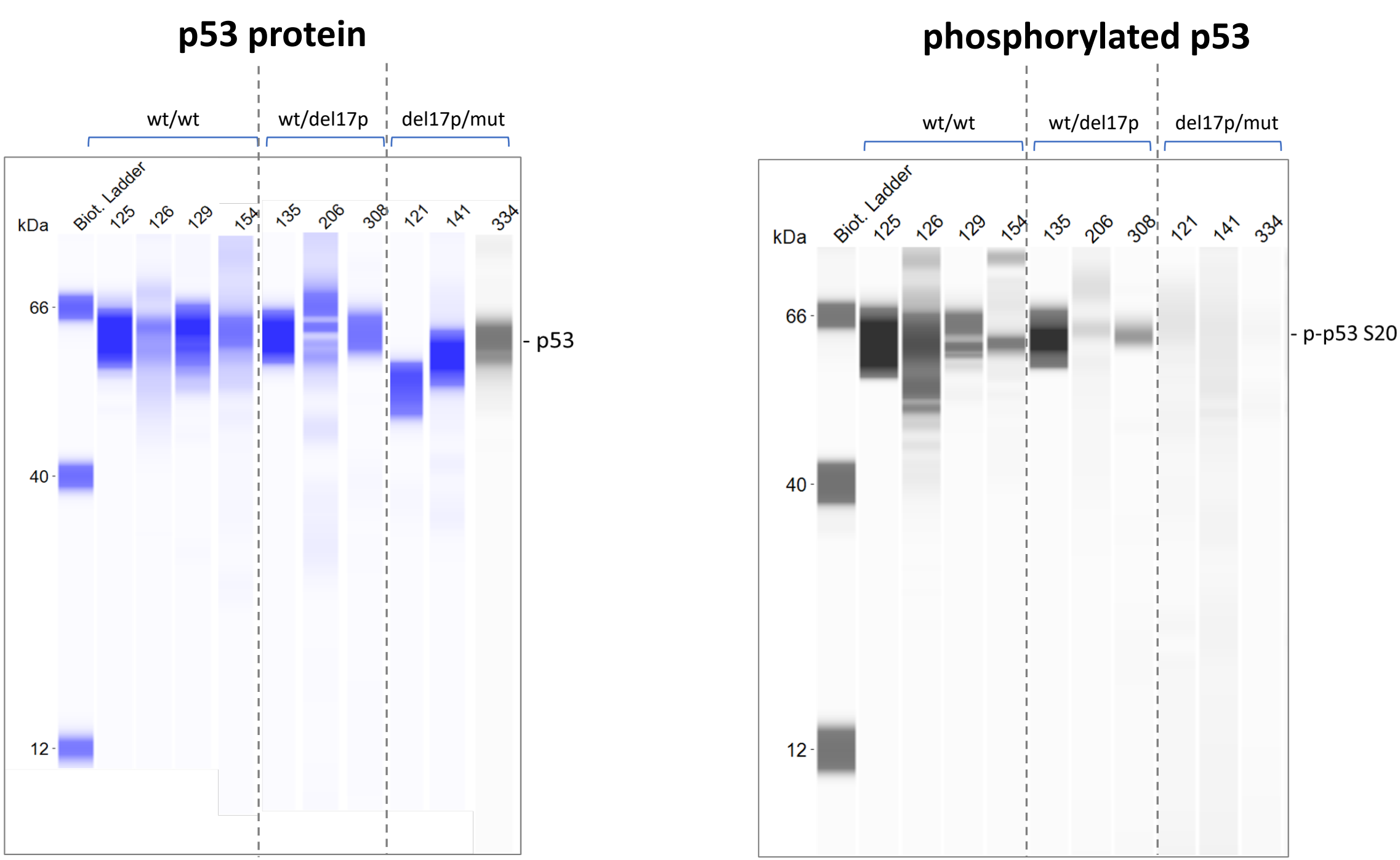


p53 protein

TAp53α 53 kDa

phosphorylation




p53 protein
wt/wt
wt/del17p
del17p/mut
kDa
Biot. Ladder
125
126
129
154
135
206
308
121
141
334
66
40
12
- p53
phosphorylated p53
wt/wt
wt/del17p
del17p/mut
kDa
Biot. Ladder
125
126
129
154
135
206
308
121
141
334
66
40
12
- p-p53 S20

To define the transcriptional signature of DH-*TP53* and to find out if it was present in other patients who did not have biallelic inactivation of *TP53*

CoMMpass study

660 PATIENTS

- Clinical data
- Biological data

① Selection of patients with del(17p) + *TP53* mutation

② Gene expression profile of genetic abnormalities present in MM

③ Identification of expression pattern of *TP53* biallelic inactivation

"Whole genome sequencing"

SeqFISH

- t(11;14)
- t(4;14)
- t(14;16)
- Del(17p)
- 1q+

RNA-Seq

- t(11;14) profile
- t(4;14) profile
- t(14;16) profile
- del(17p) profile
- 1q+ profile

DESeq2

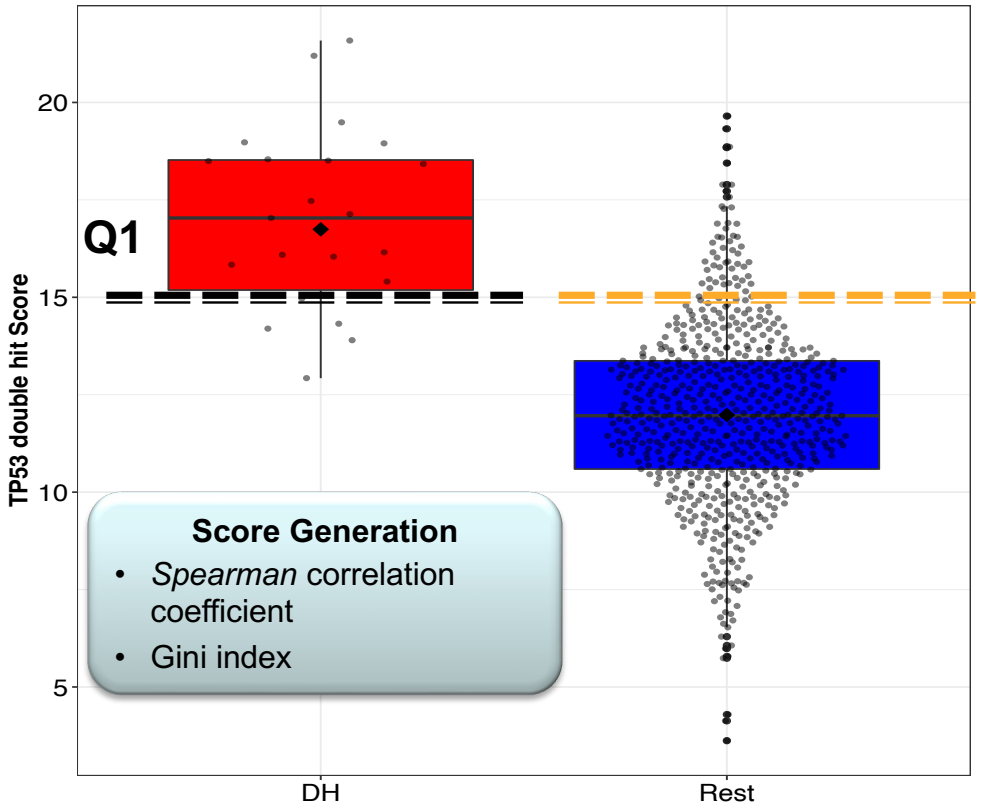
Exclusive DH-*TP53* signature

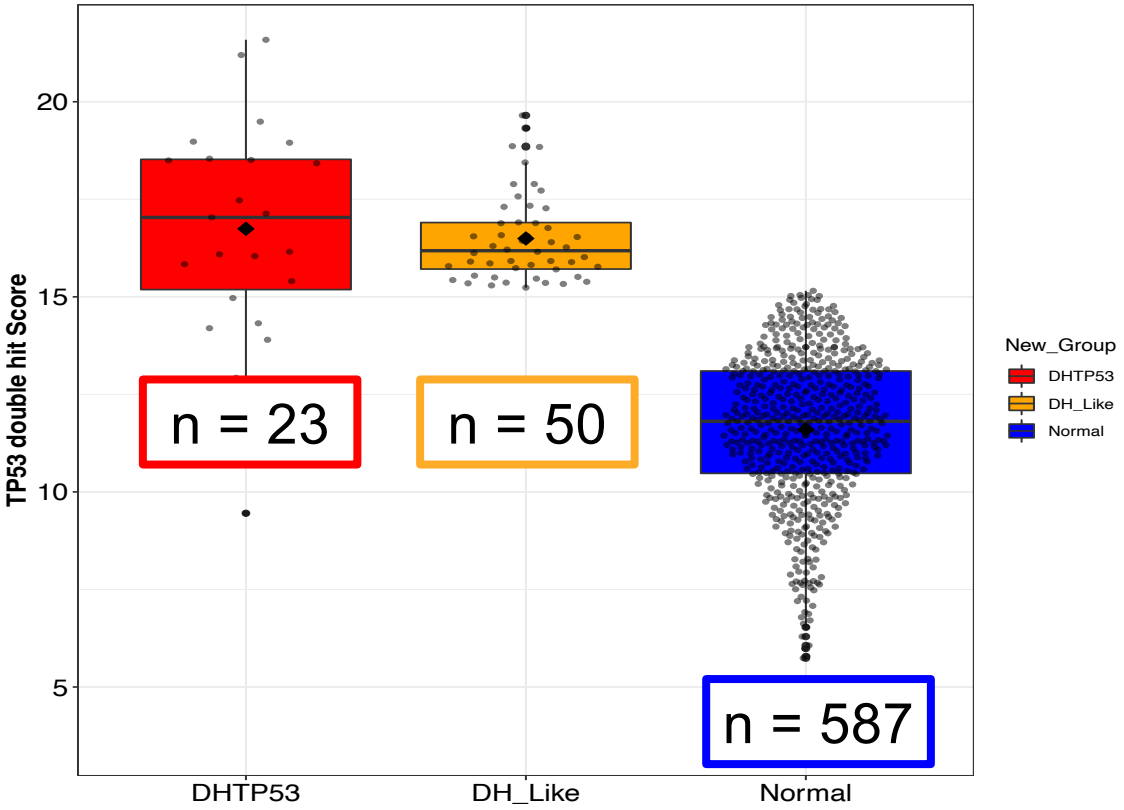
removing

- (11;14) profile
- t(4;14) profile
- t(14;16) profile
- del(17p) profile
- 1q+ profile

Patients with a score above the first quartile of the DH-*TP53* group were assigned to a new subgroup named DH-*TP53*-like

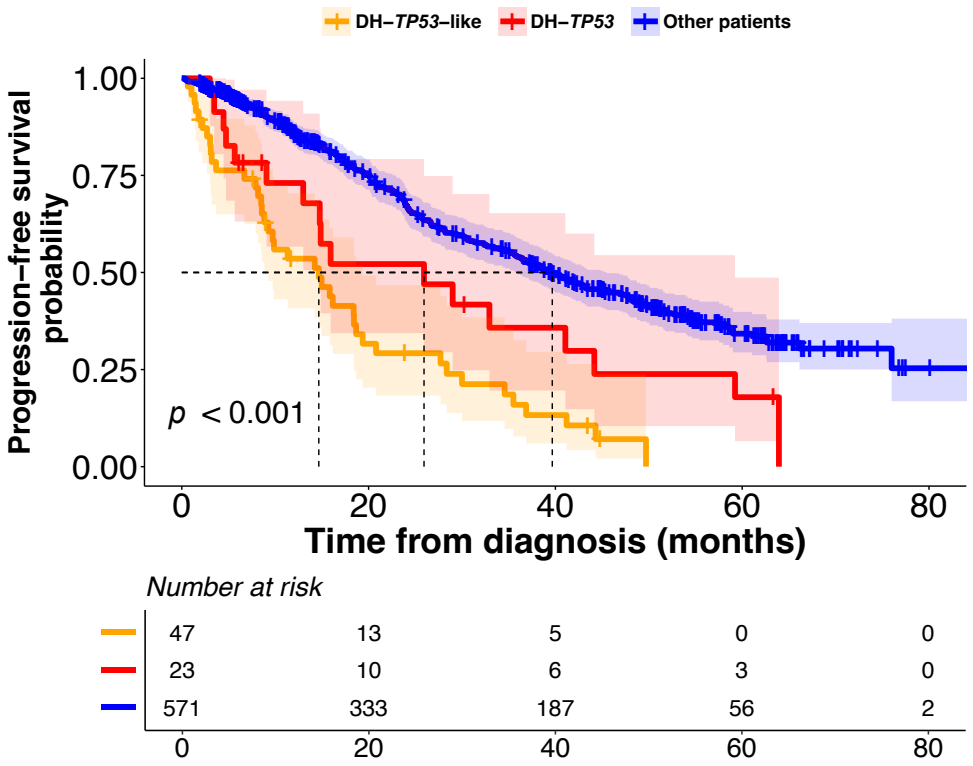
DH-*TP53*-like subgroup: 50 out of 660 (7.5%) MM patients without the combination of del(17p) and *TP53* mutation

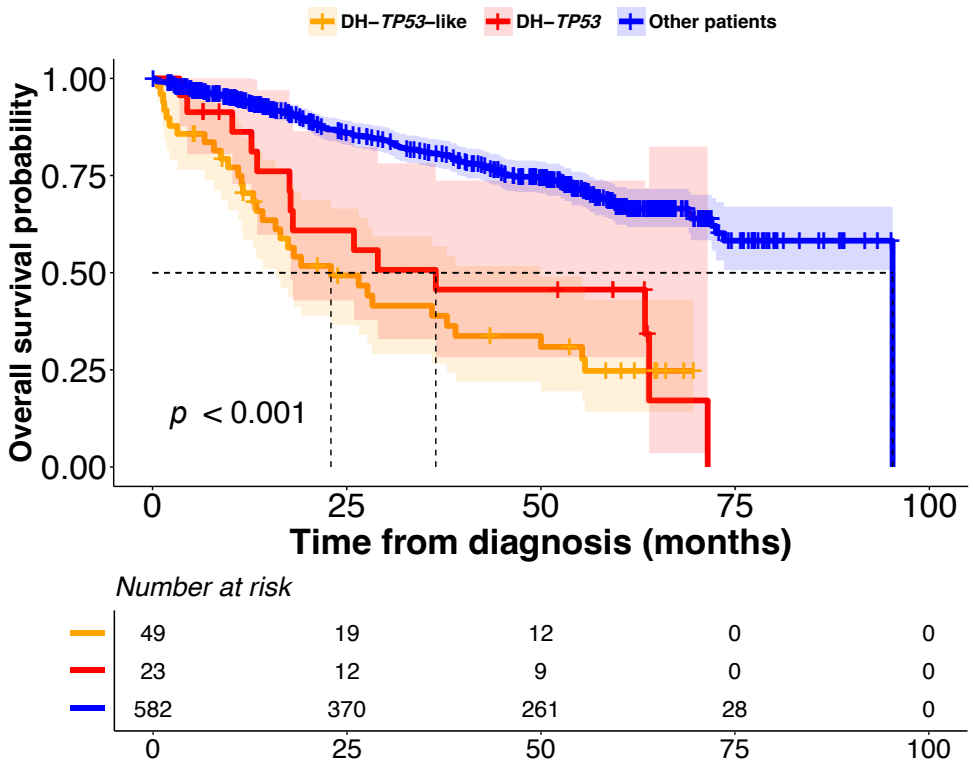




De Ramón C. Br J Haematol. 2022

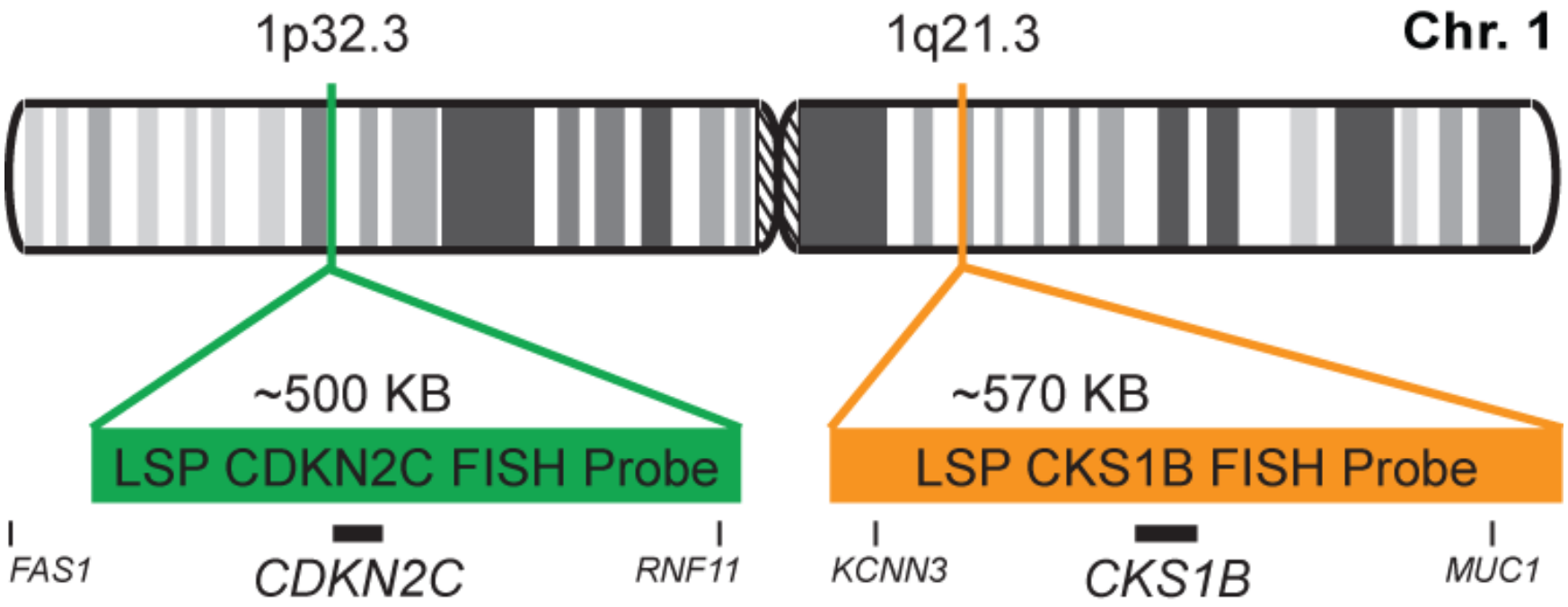
Transcriptional signature of *TP53* biallelic inactivation identifies a group of MM patients without this genetic condition but with dismal outcome



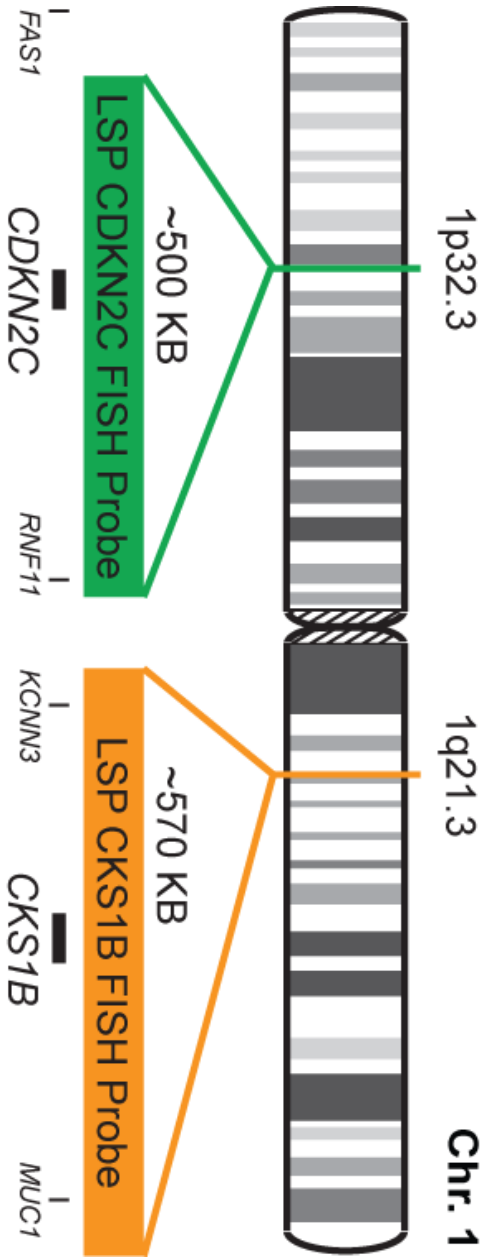


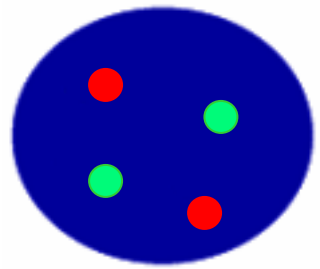
De Ramón C. Br J Haematol. 2022

1p/1q probe

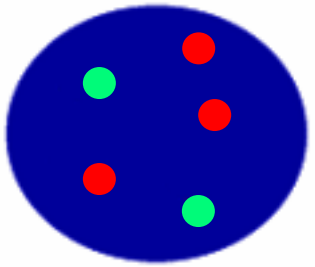


CytoTest

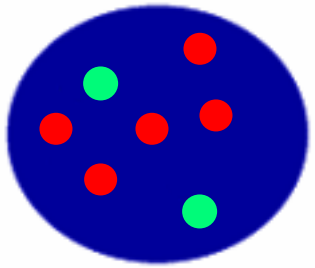




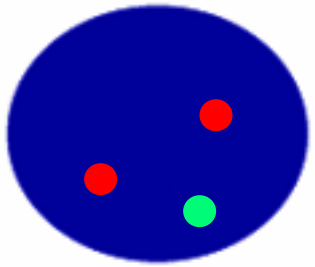
Normal



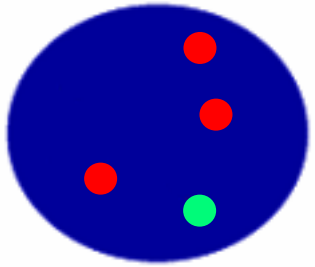
1q gain



1q amplification

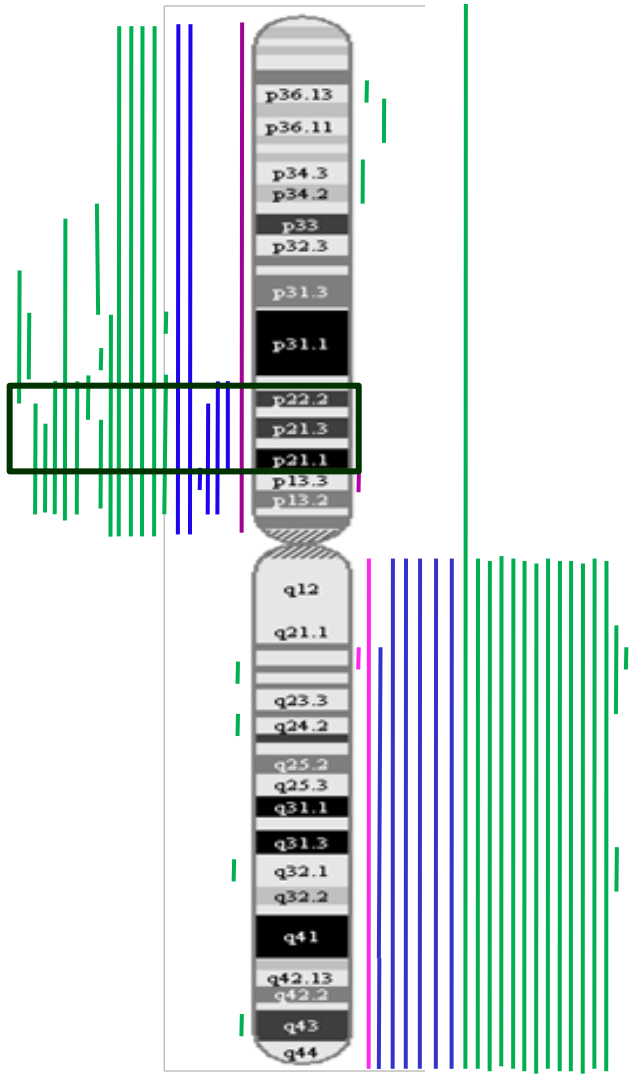


1p loss



1 q gain/1p loss

Low incidence of 1p deletion by comercial FISH



1

López-Corral L, Leukemia 2012

Incidence in 1195 patients

1p deletion: 23%

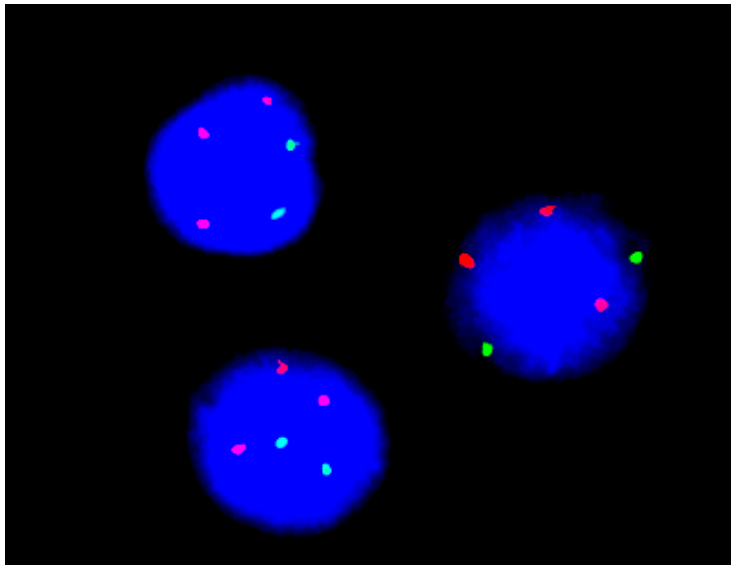
1p22 : 15%

1p32 : 7%

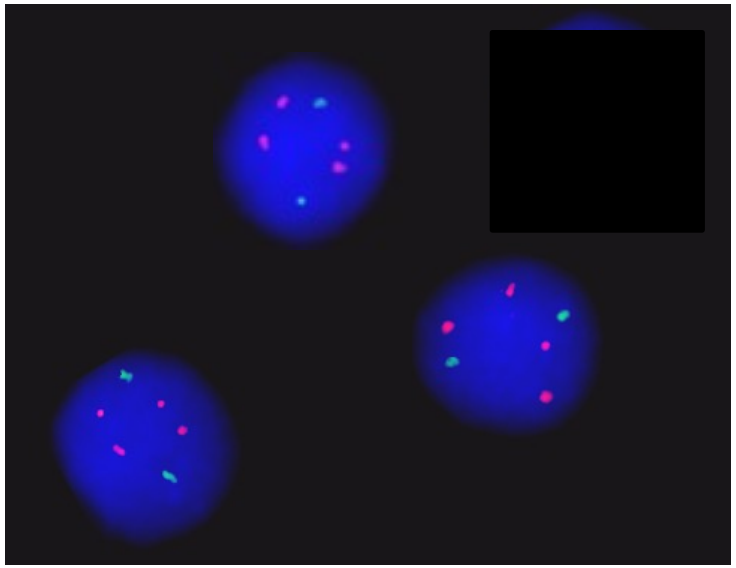
Hebraud et al. ASH 2012

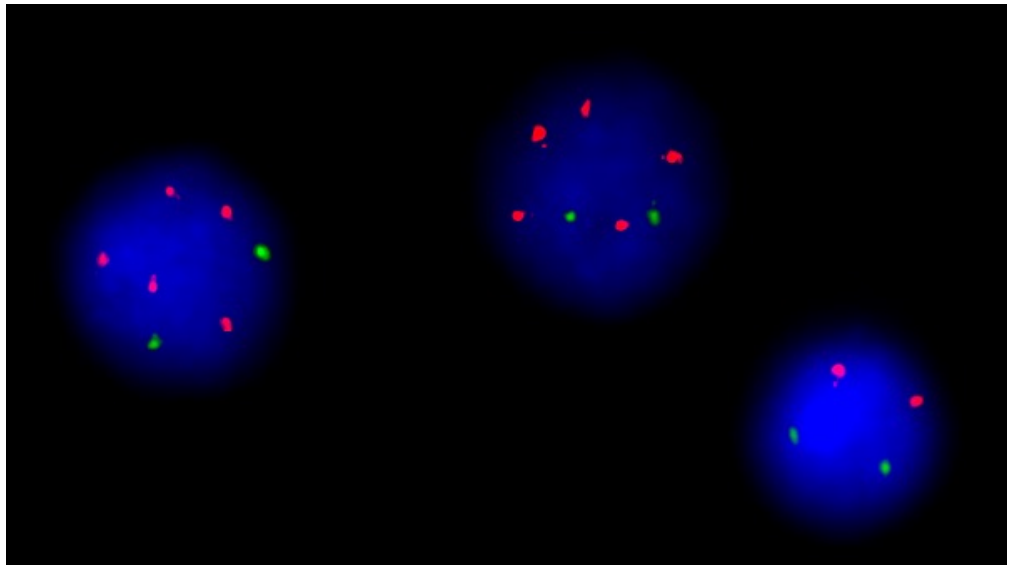
- Custom FISH probes
- SNP-array

1q gain/amplification



Gain of 1q (3 copies)

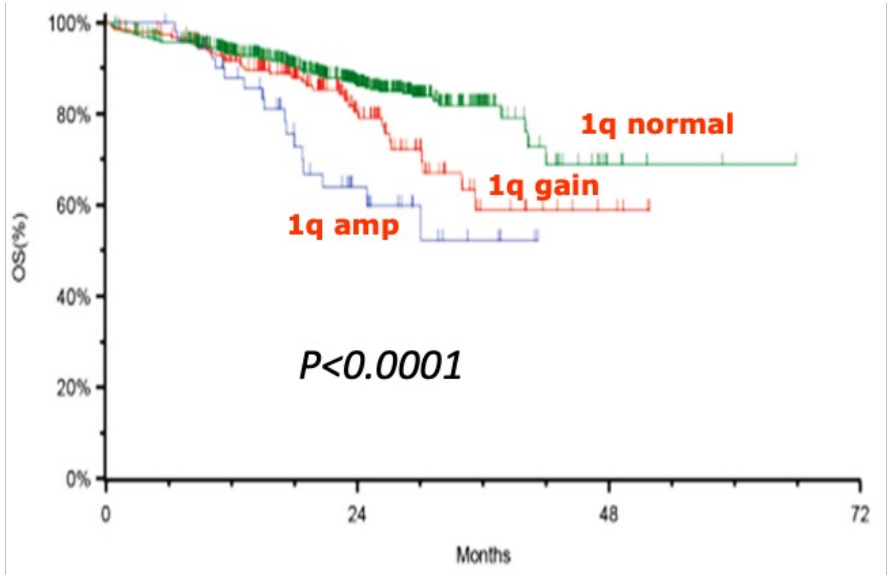




Amplification of 1q21 (≥4 copies)

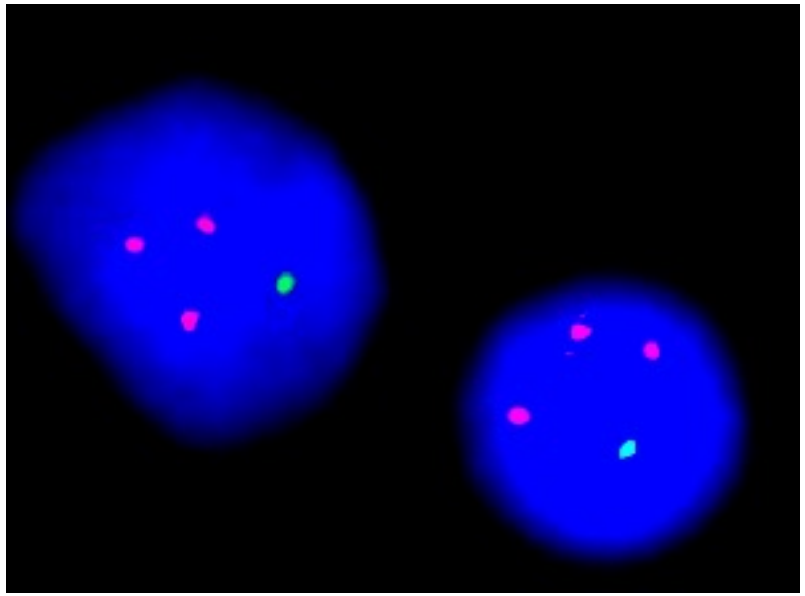
WGS/WES data from
1273 NDMM patients

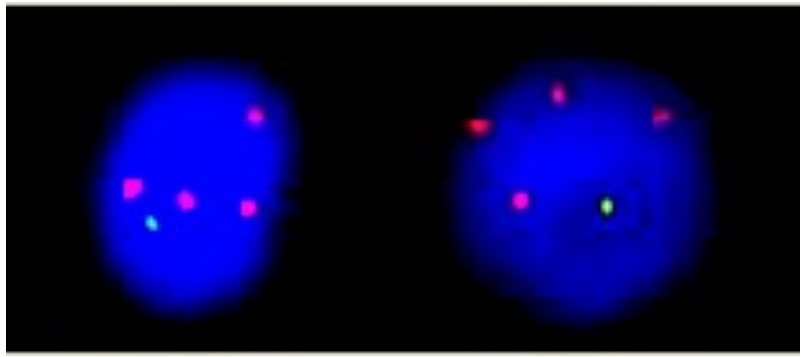
Amplification of 1q + ISS 3 = double hit MM

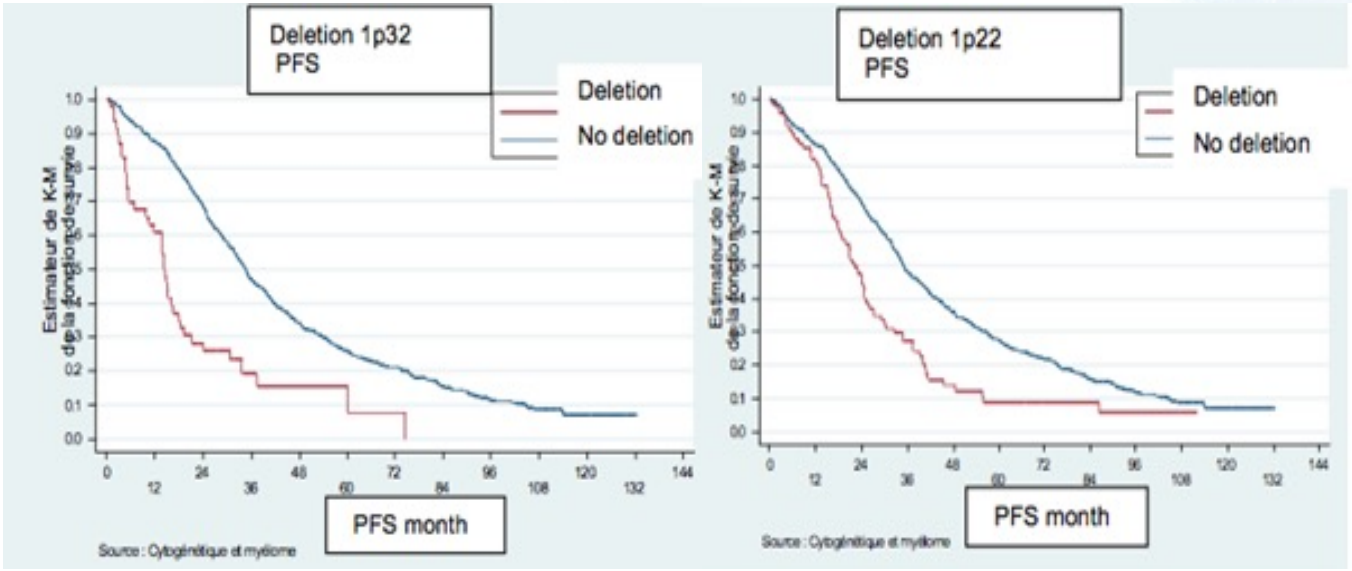


Walker BA, Leukemia 2019

1p deletion

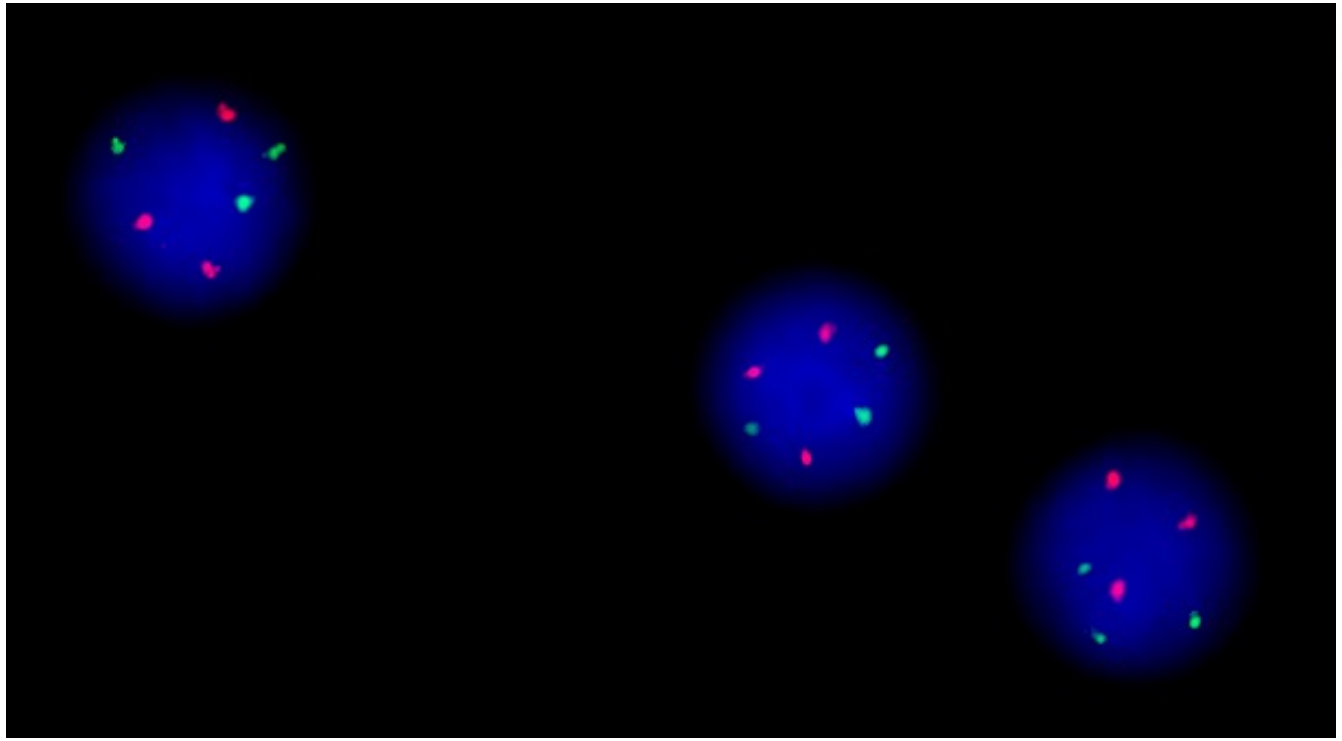




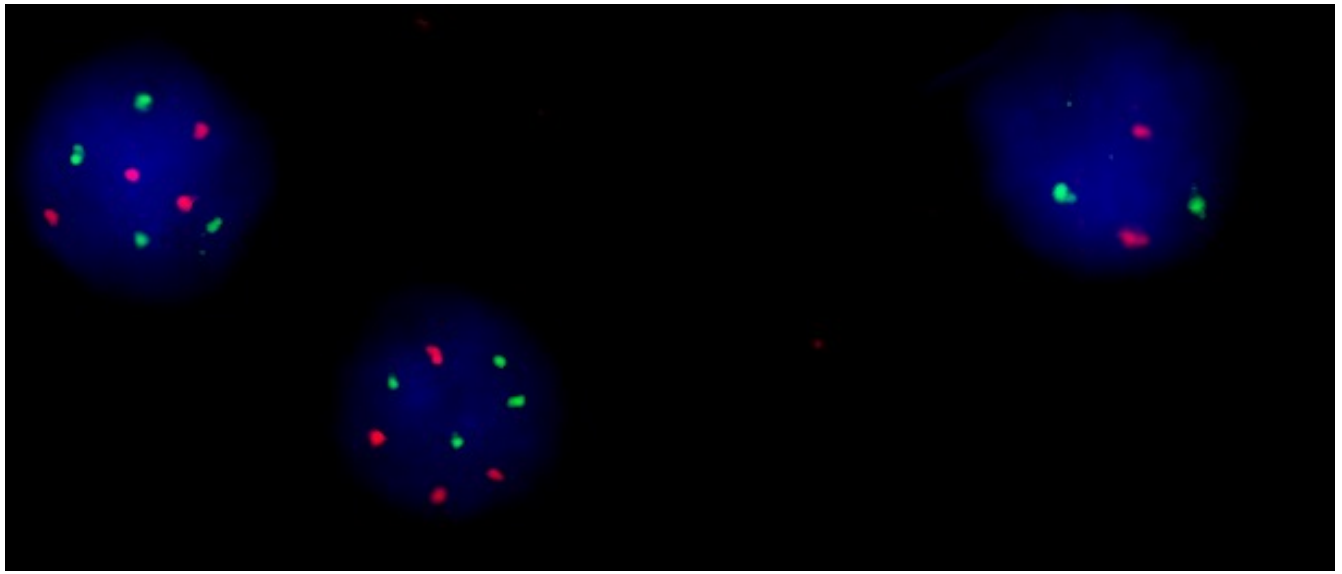


- 1195 patients in IFM trial
- Major negative prognostic factors for OS and PFS
- Confirms importance of 1p deletion from previous studies

Hebraud B, Leukemia 2013



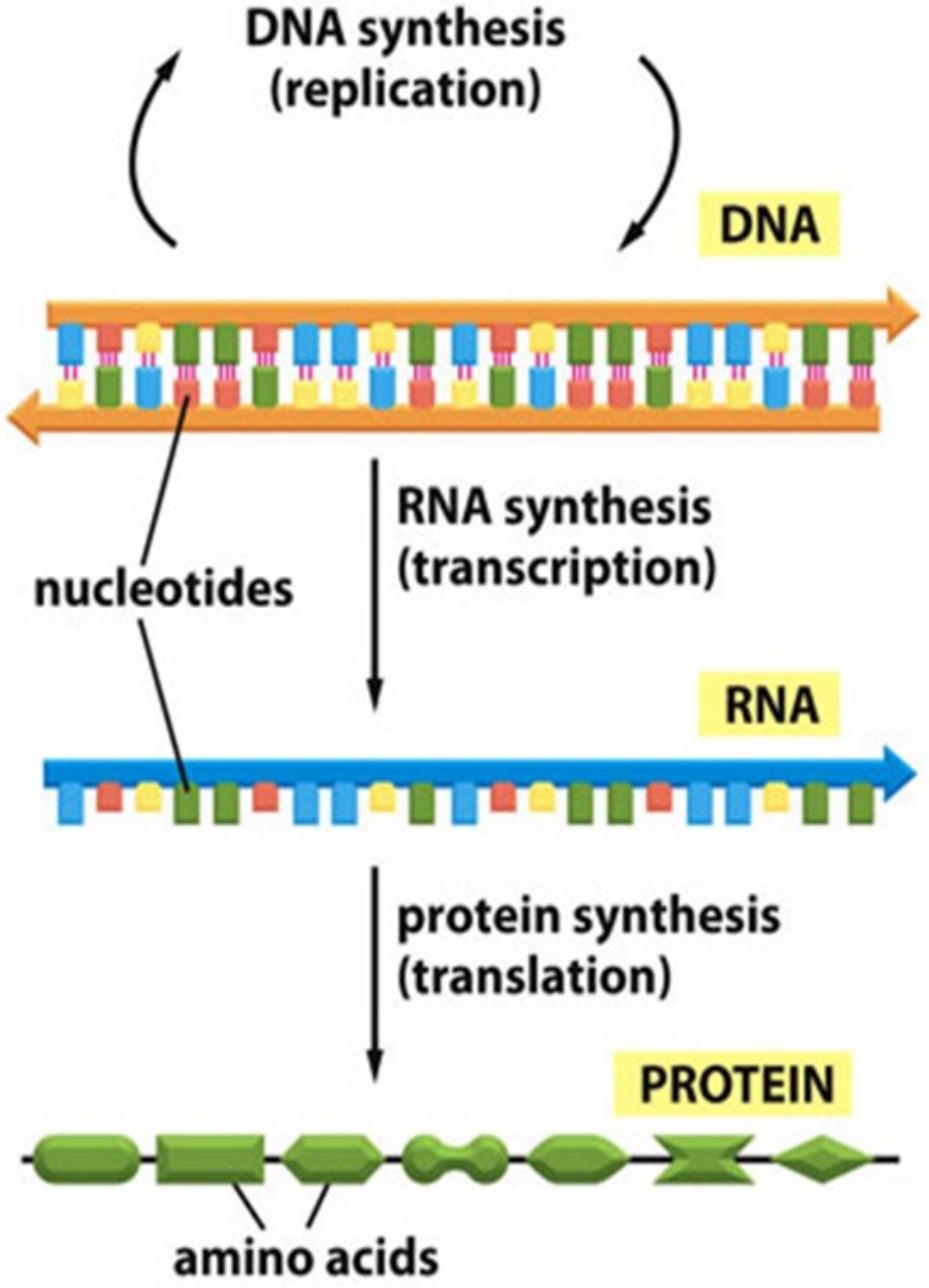
Trisomy 1 N = 8



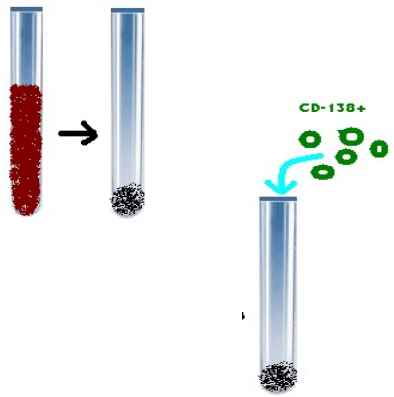
Tetrasomy 1 N = 3

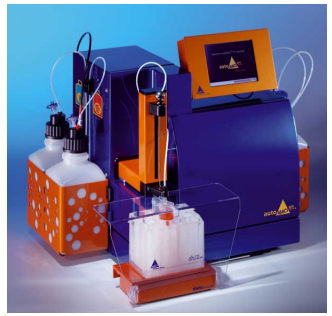
Central Dogma of Molecular Biology

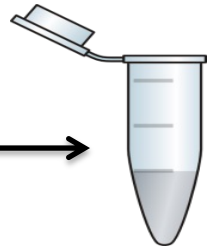
"Genomics involves the study of all genes at the DNA, mRNA, and proteome level as well as the cellular or tissue level"



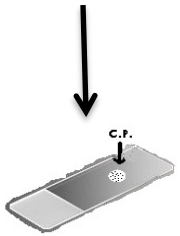
Methodology for Genomics: everything from the same ONE sample







CD138+
in RLT+
-80°C
for years



FISH

AllPrep
(Qiagen)

DNA

SNP arrays,
NGS, epigenetic
analysis

RNA

GEP, RNA seq, qRT-PCR, mRNA isoforms analysis, posttranscriptional modifications

In-house
protocol

protein

Simple western for expression level and isoform identification

AGRADECIMIENTOS

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asociación española contra el cáncer