Citogenética del mieloma

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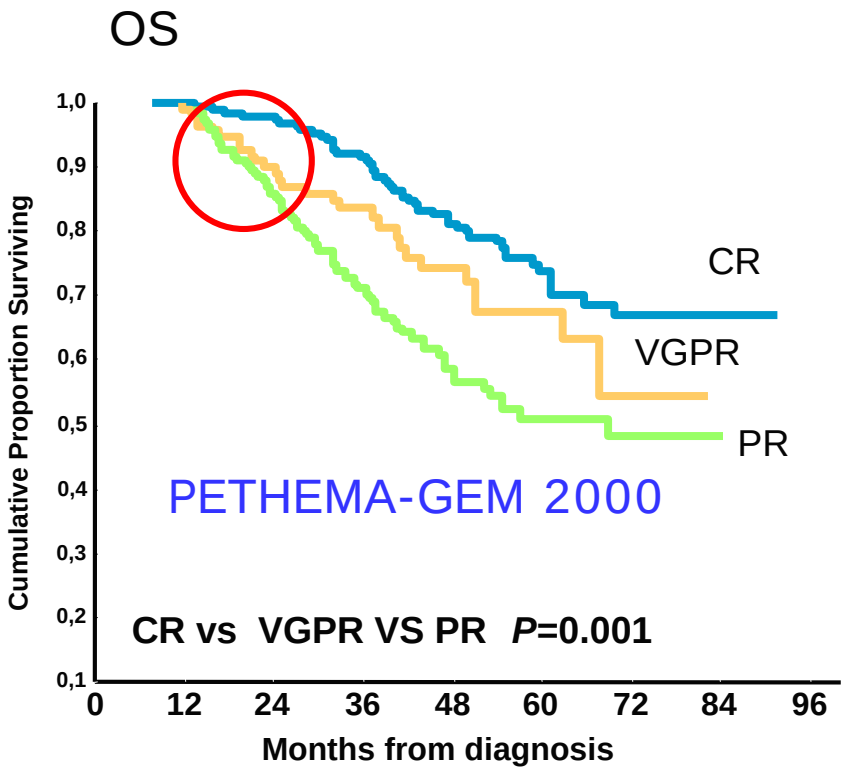
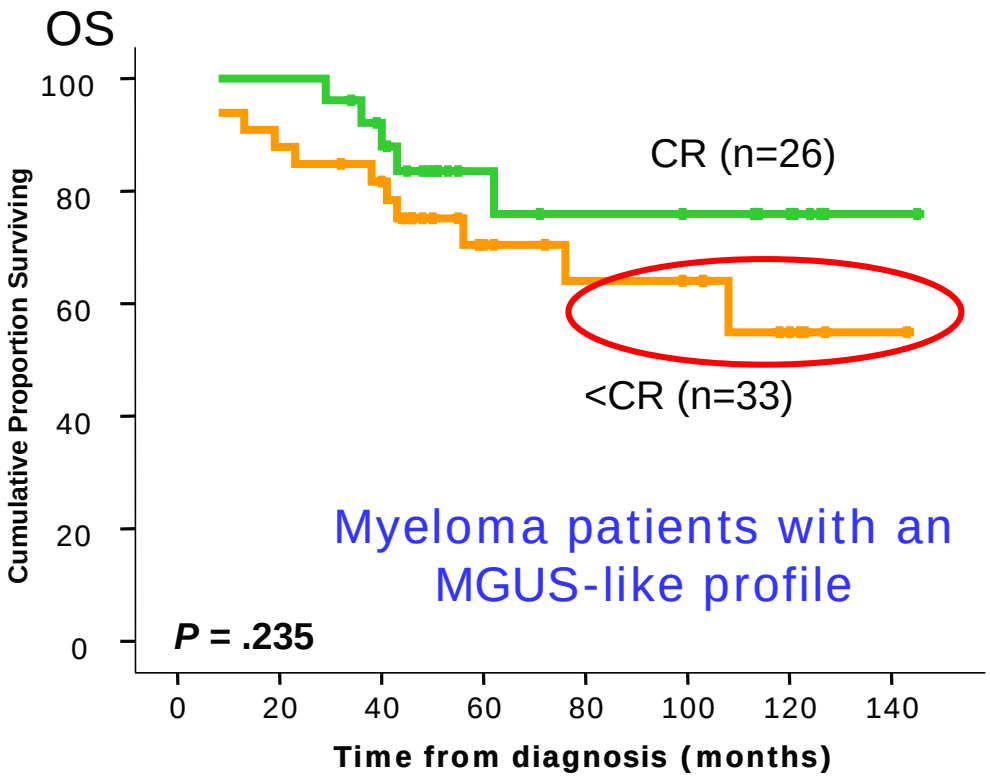
MM is a heterogeneous disease

Median survival around 6 years

1-2 years

More than 15 years

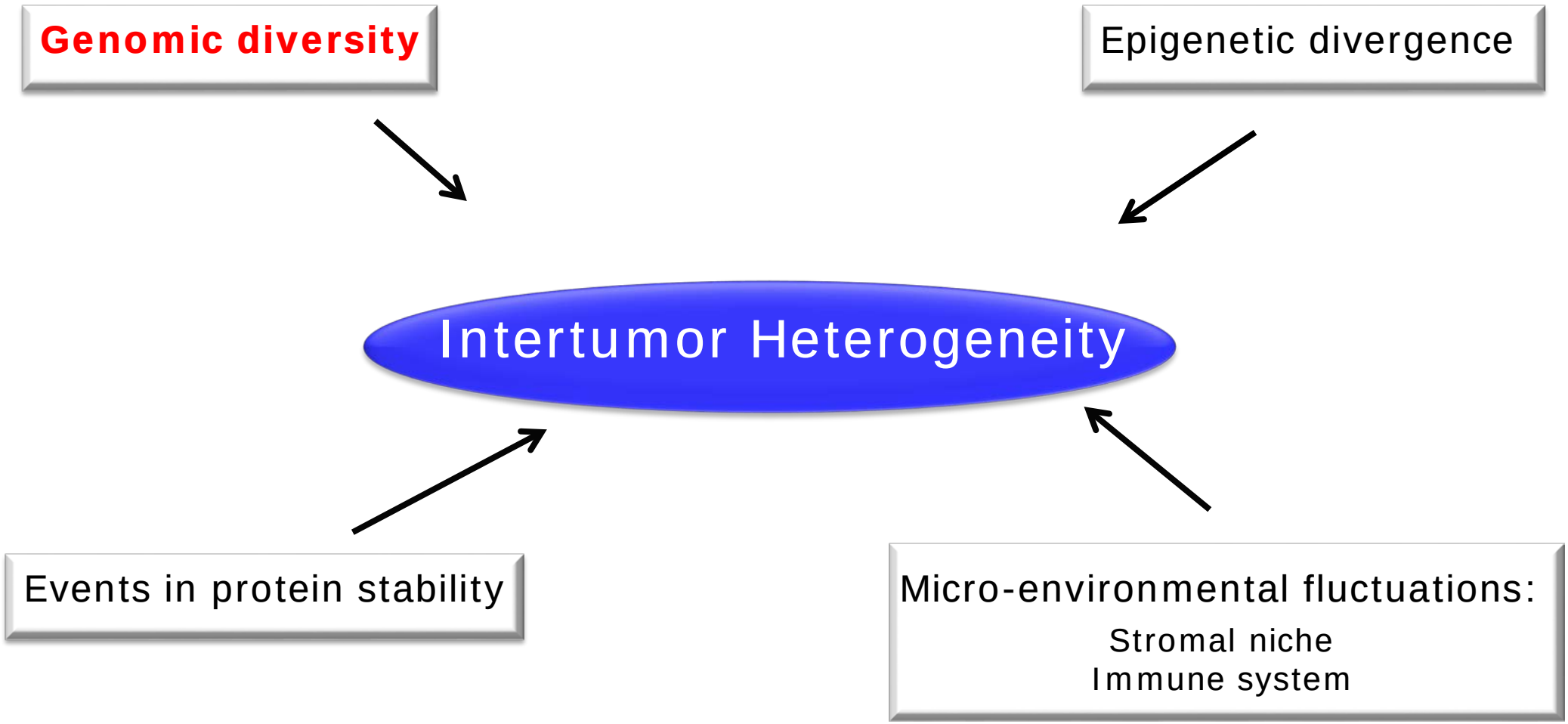




Lahuerta JJ et al, JCO 2008

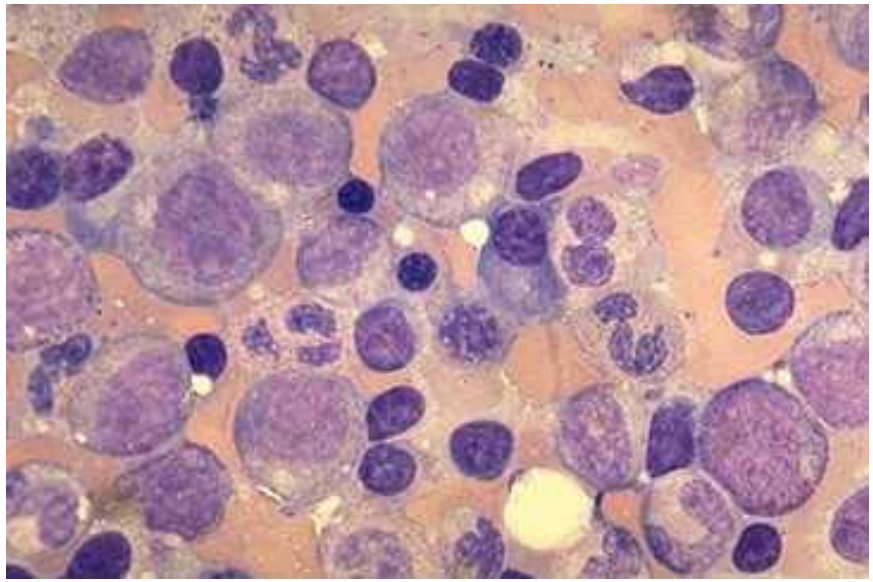
Paiva B et al, Leukemia 2013

Different entities with distinct clinical evolution

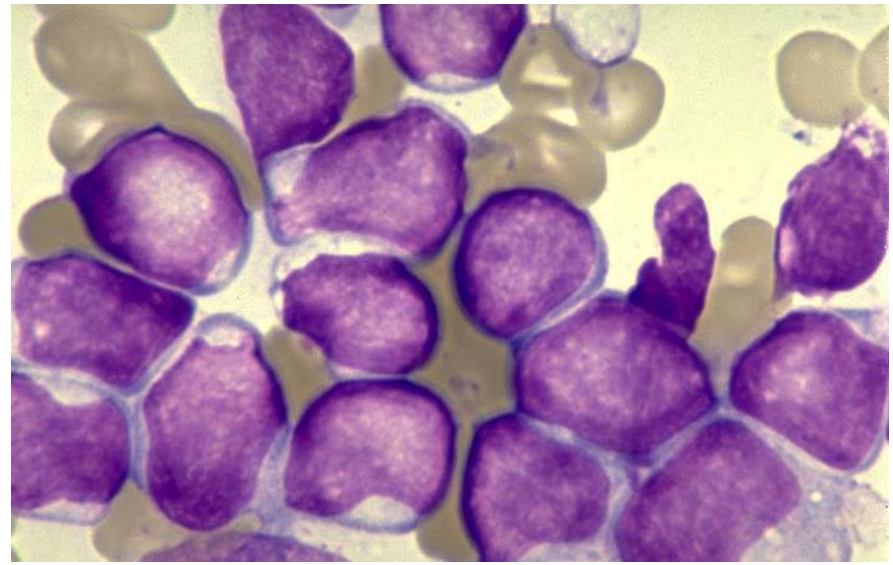


Burrel RA et al, Nature 2013

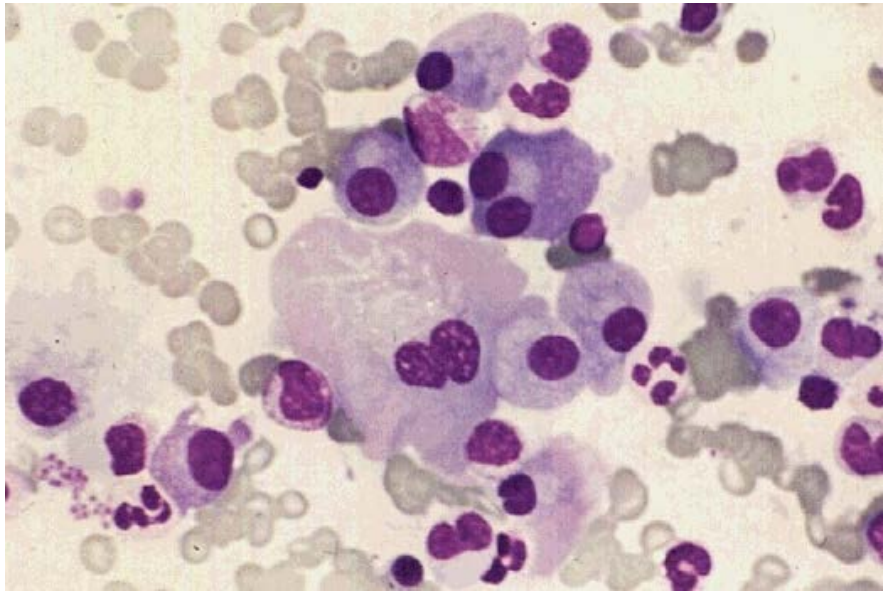
Normal BM



Acute leukemia

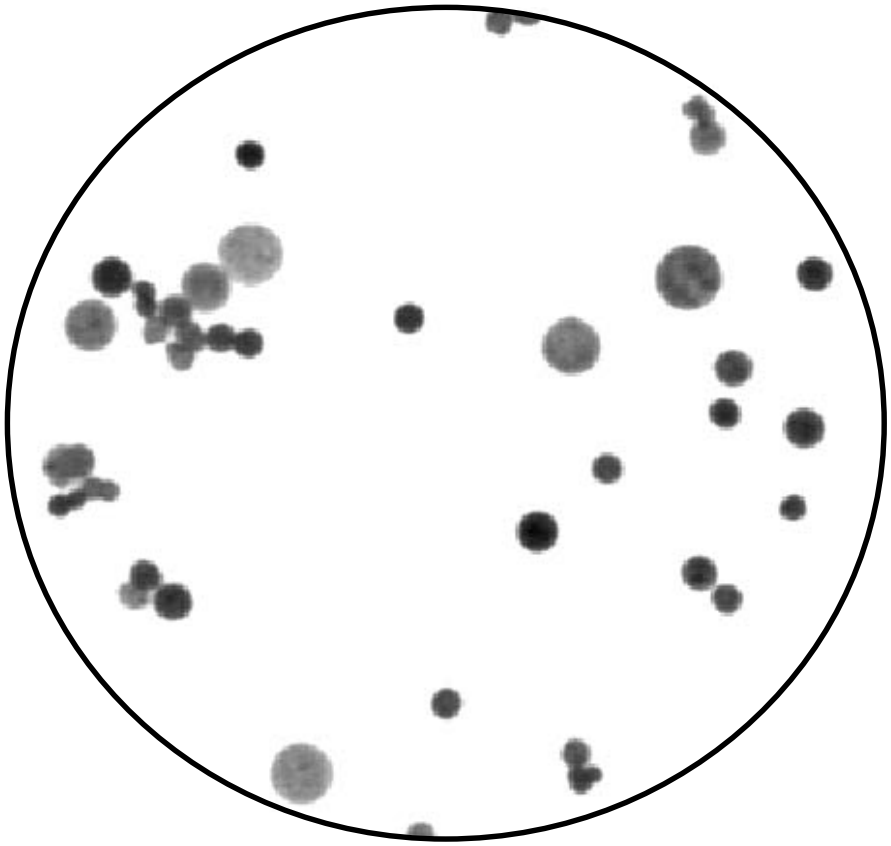


Myeloma

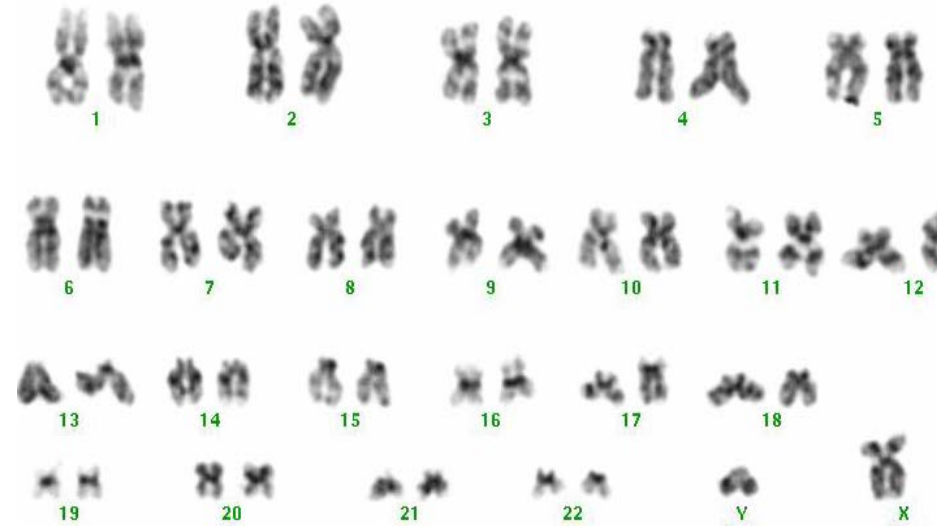


Low infiltration of tumoral cells in the bone marrow

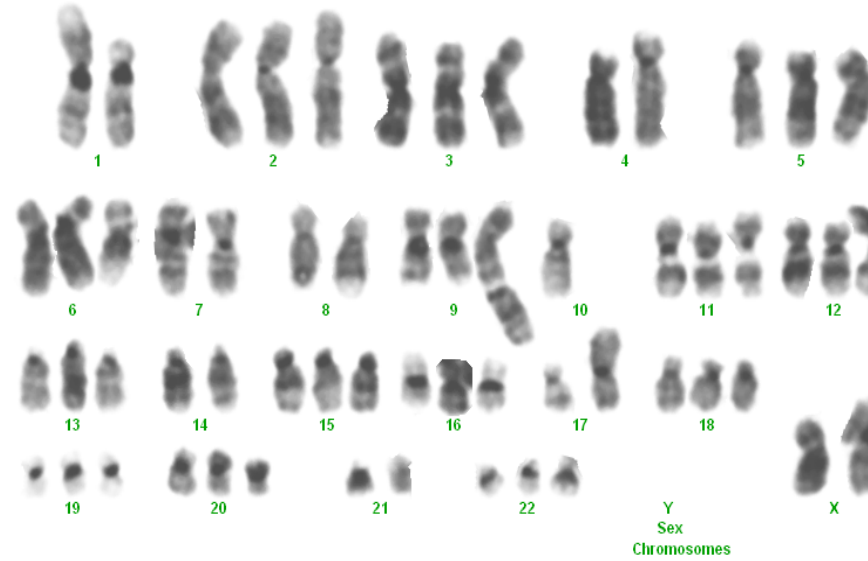
Low mitotic index



Failure ~ 10-25%



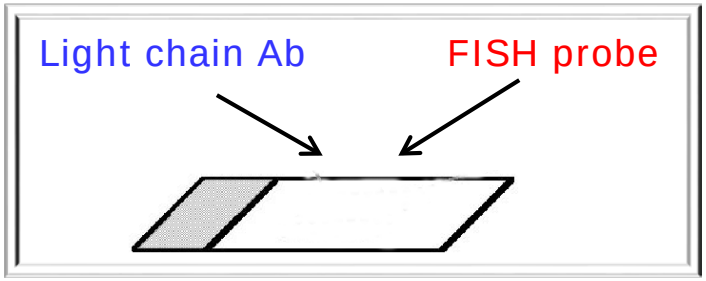
Normal Karyotype ~ 40-50%



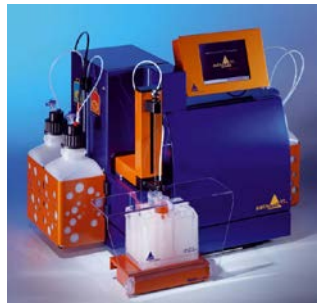
Abnormal Karyotype ~ 30-40%

The plasma cells need to be selected enabling an unambiguous identification

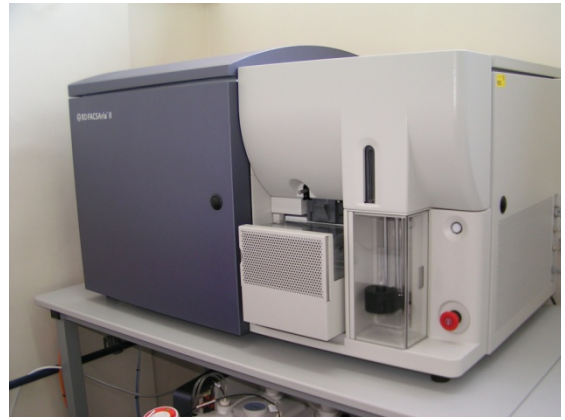
Concomitant labeling of the cytoplasmic immunoglobulin light chain



Immunomagnetic separation



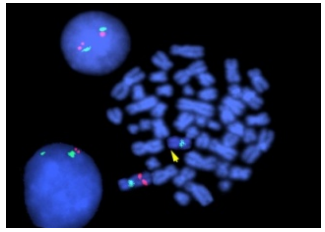
Flow cytometry, FACSAria II cell sorter



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

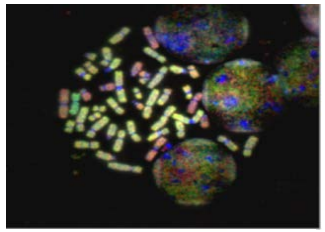
Conventional Cytogenetics

5-10 Mb



Fluorescence *in situ* hybridization

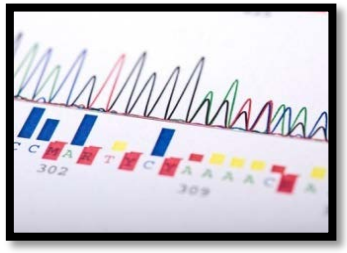
100 Kb-5 Mb



Comparative genomic hybridization (CGH)

SNP-arrays

50 Kb



NGS

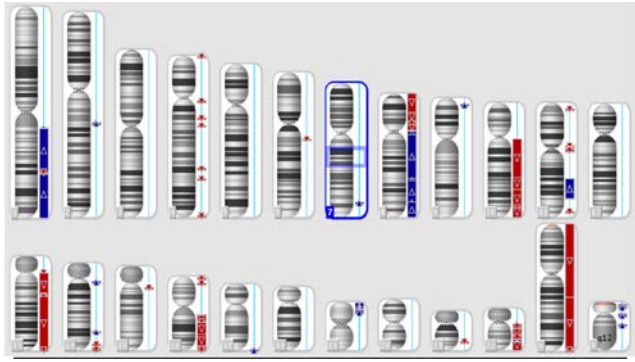
base-pair

DNA

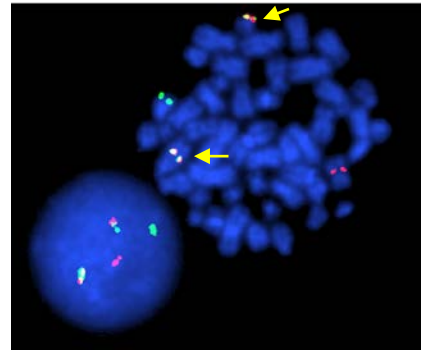
Copy number abnormalities

Translocations

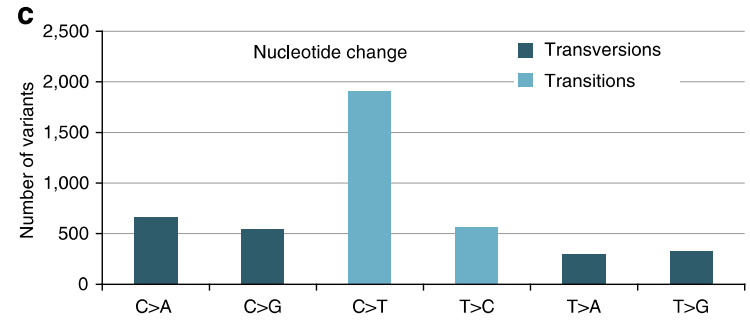
Point mutations



Array comparative genomic hybridization (aCGH)
SNP-arrays

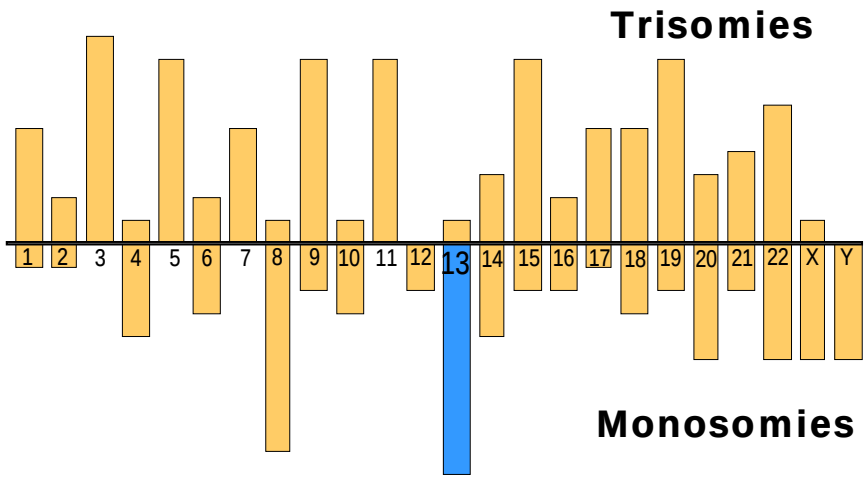


FISH

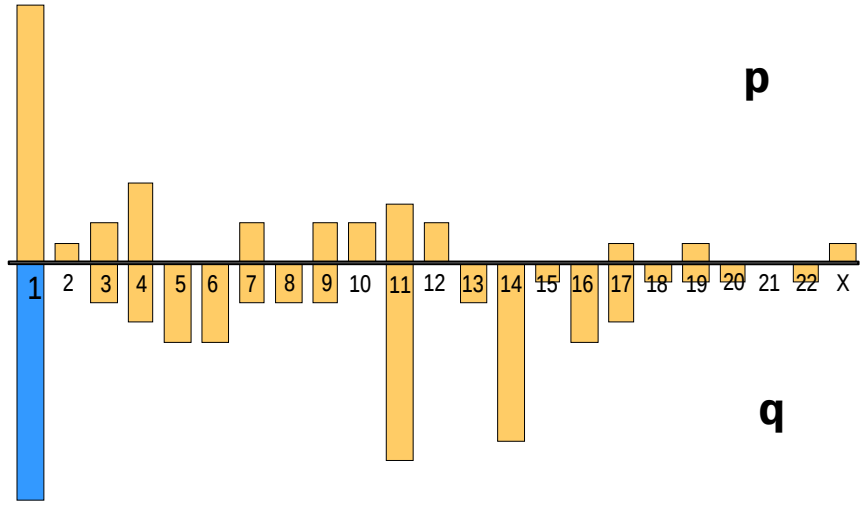


Next-generation sequencing

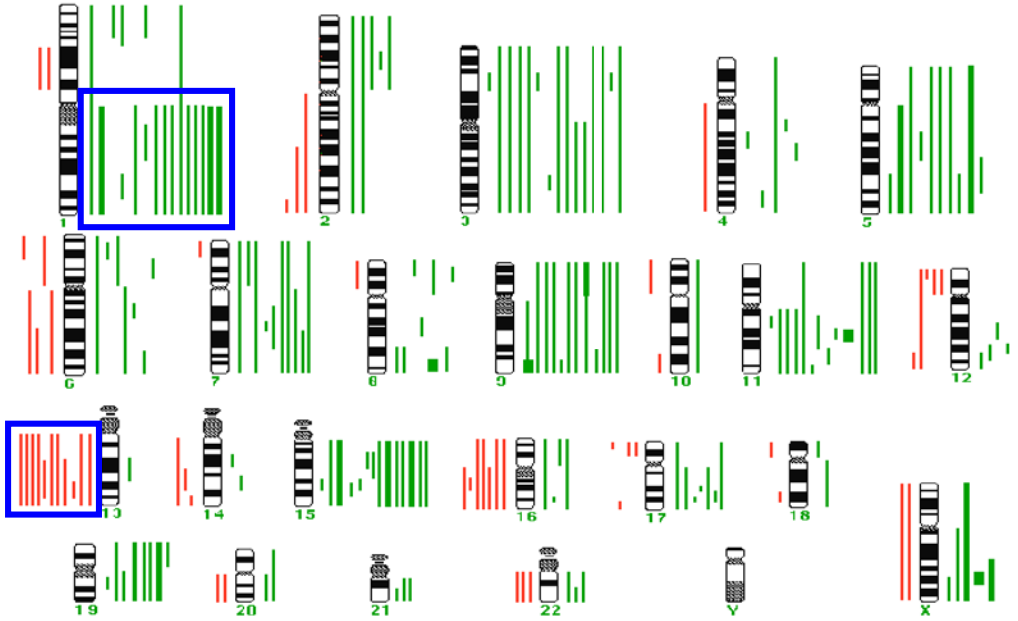
Numerical abnormalities in MM



Structural abnormalities in MM

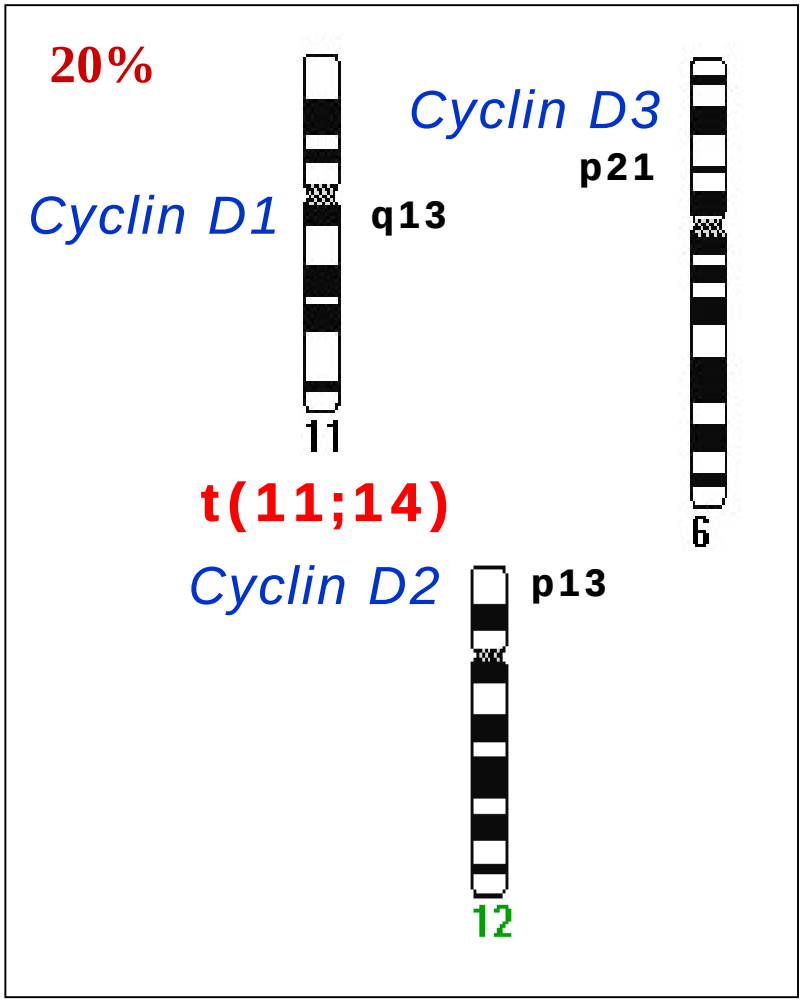


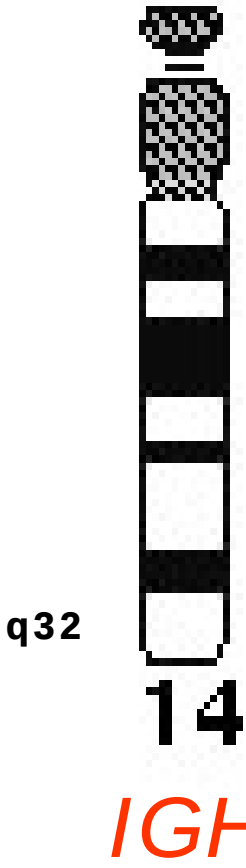
Almost all MM cases are cytogenetically abnormal

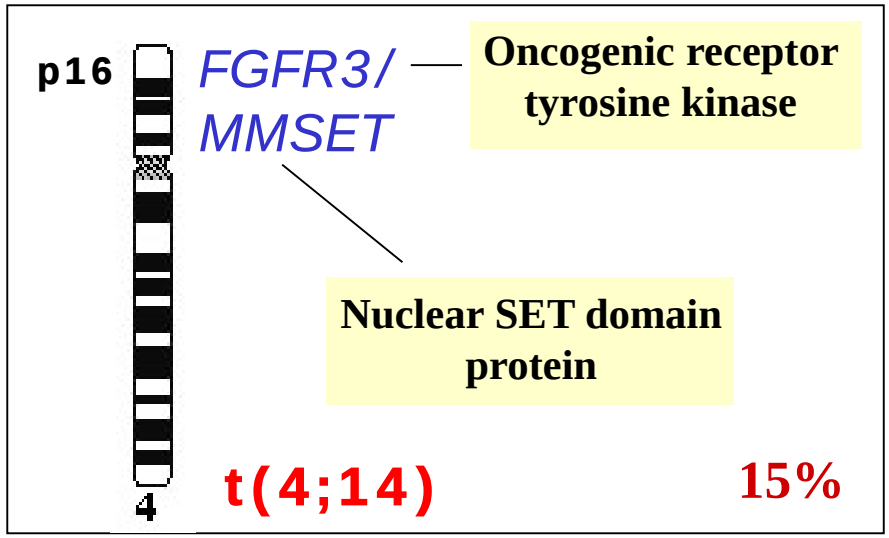


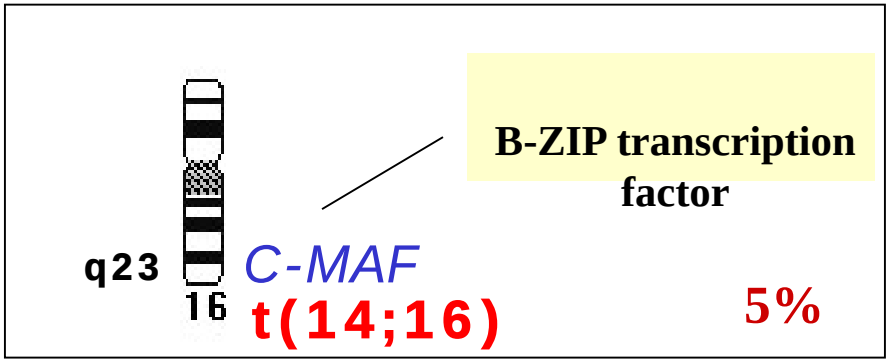
Gutiérrez et al, Blood 2004

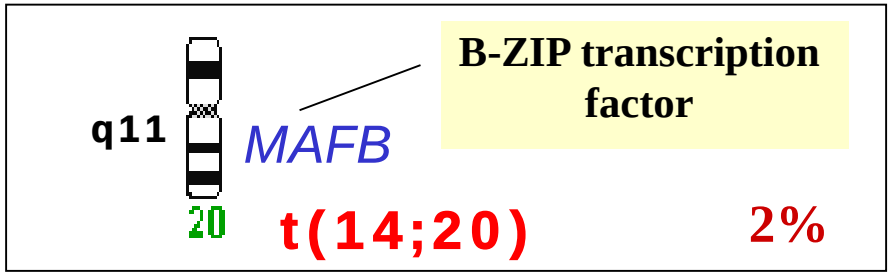
IGH translocations











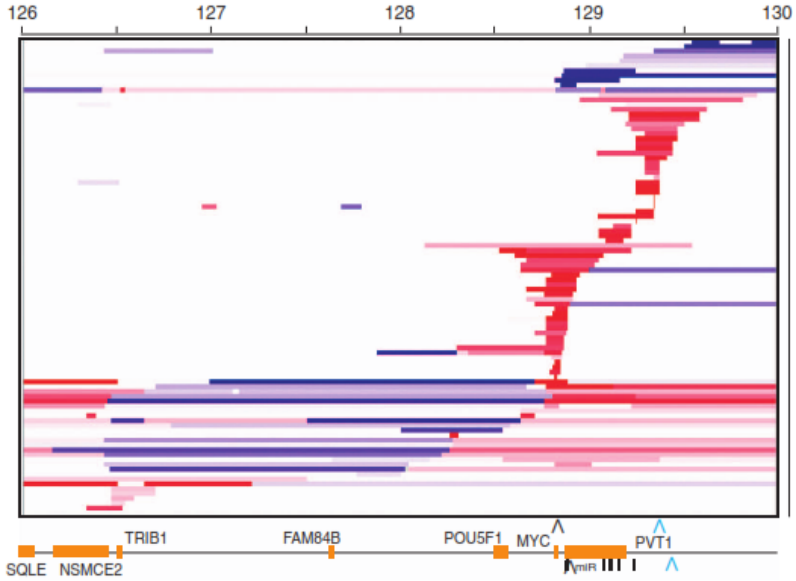
Chesi, Blood 1998; Avet-Loiseau, GCC 1999

MYC rearrangements in myeloma

Rearrangements of the *MYC* detected by FISH are present in 15% of primary human multiple myeloma tumors and more than half of HMCLs (Avet-Loiseau et al, Blood 2001)

218 patients

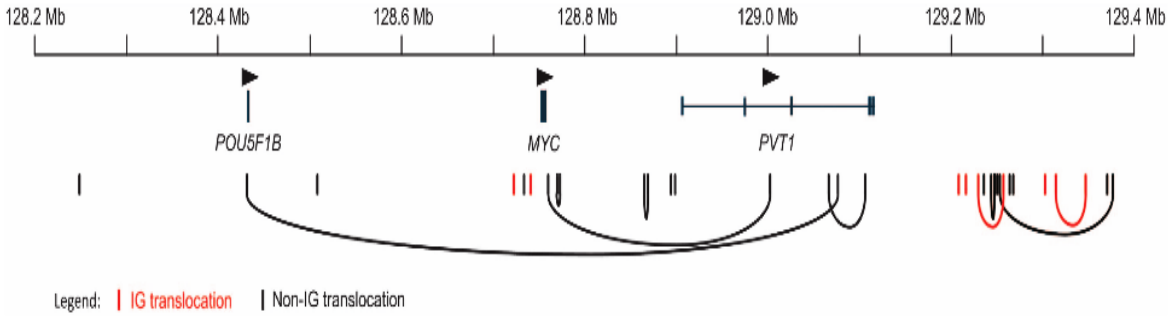
FISH
CGH arrays



MYC locus rearrangements: 49%

104 patients

Capture assay followed
by massively parallel sequencing

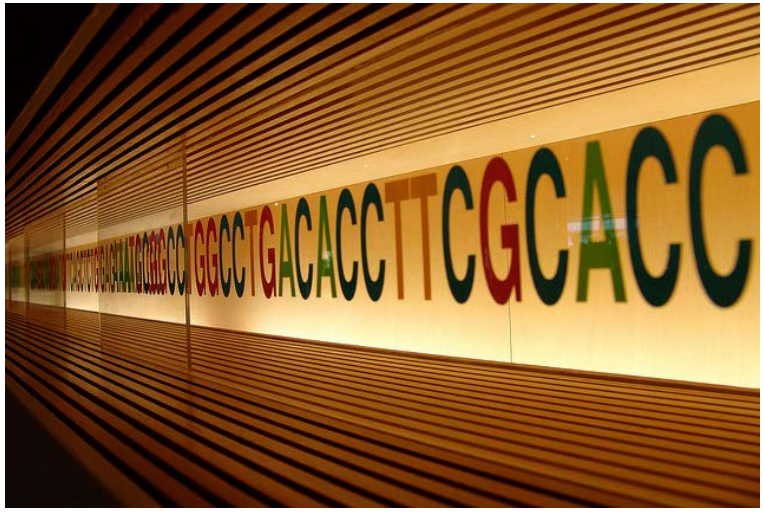


MYC translocation in 21% of samples

Affer et al, Leukemia 2014

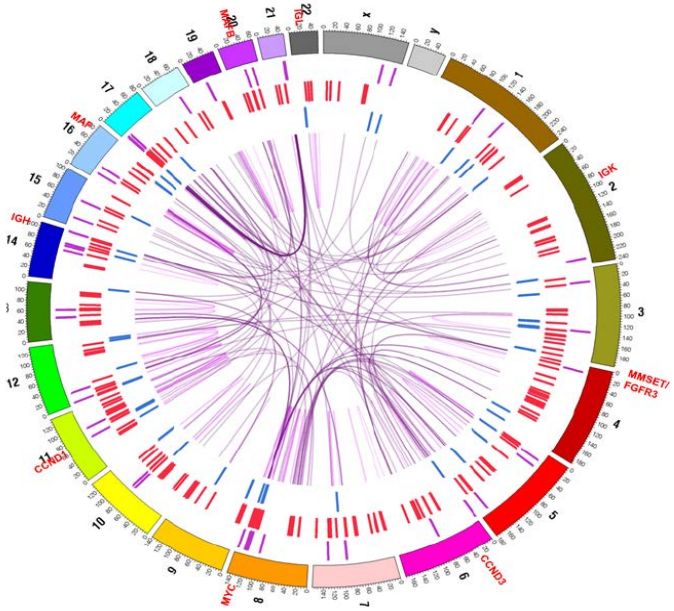
Walker et al, Blood Cancer Journal 2014

Whole-exome/genome sequencing



Hairy-cell leukemia

BRAF mutations ~ 100%



Waldenstrom's Macroglobulinemia

MYD88 mutations ~ 90%

Multiple myeloma

?

Massively parallel sequencing

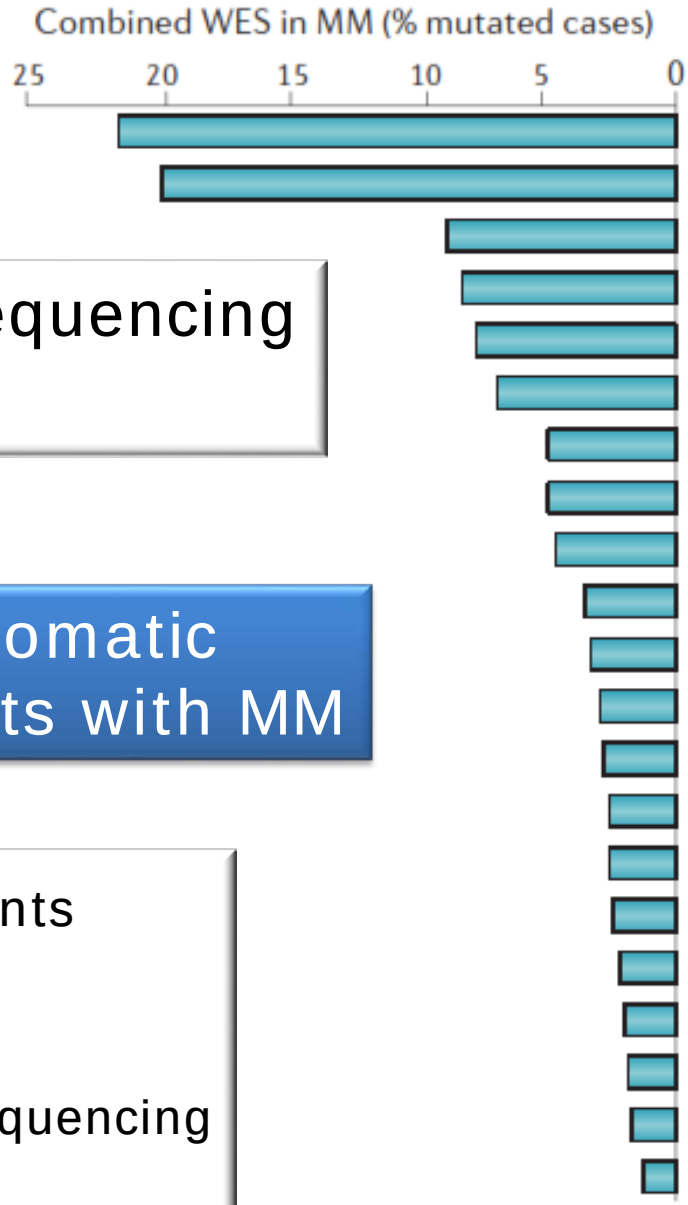
Paired tumor/normal samples

Most frequent somatic mutations in patients with MM

733 MM patients

↓

Whole-exome sequencing

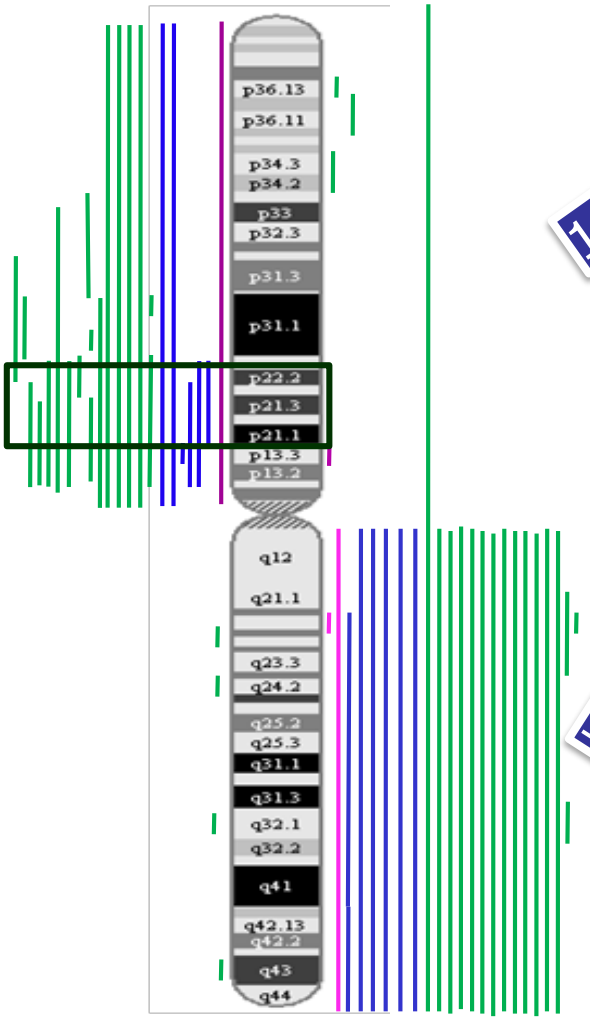


	Walker *et al.* % ($n = 463$)	Lohr *et al.* % ($n = 203$)	Boli *et al.* % ($n = 67$)
KRAS	21*	23*	20*
NRAS	19*	20*	20*
FAM46C	6*	11*	10*
BRAF	7*	6*	12*
TP53	3*	8*	12*
DIS3	9*	11*	1
PRDM1	–	5*	–
SP140	–	4	6*
EGR1	4*	4	6
TRAF3	4*	5*	2
ATM	3	4	3
CCND1	2*	3	4
HISTH1E	3*	–	–
LTB	3*	1	4*
IRF4	3*	2	–
FGFR3	3*	2	–
RB1	2	3*	–
ACTG1	–	2*	–
CYLD	2*	2*	1
MAX	2*	1	–
ATR	1	1	2

*Mutations reaching significance

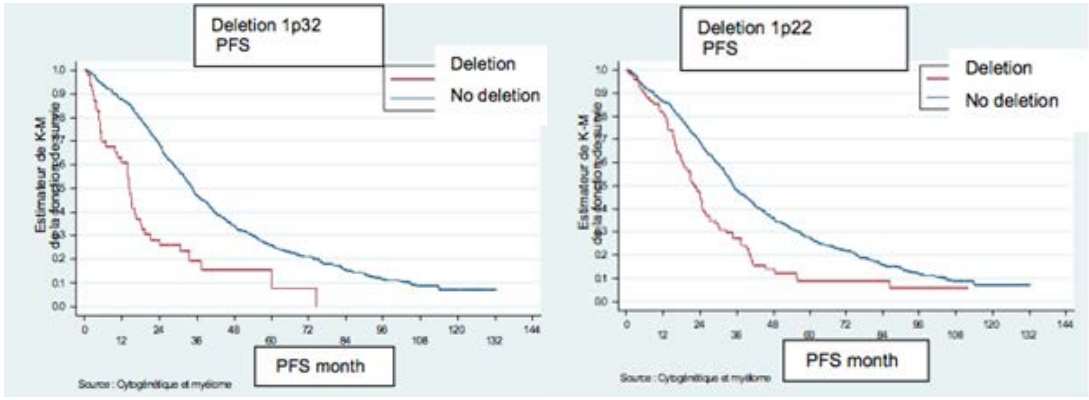
Manier et al, Nature Reviews 2016

Chromosome 1 abnormalities



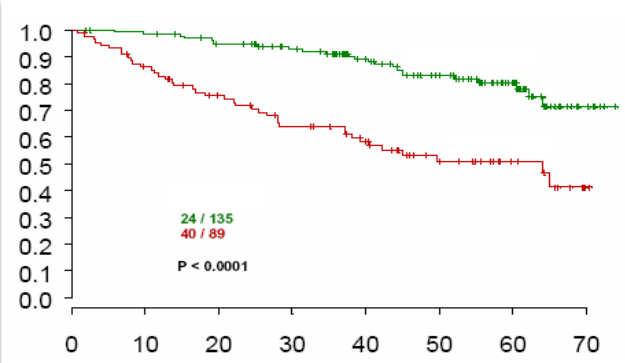
1

1p deletions

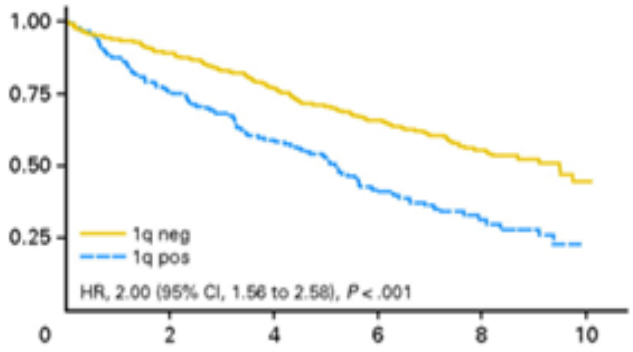


- 1195 patients in IFM trial
- Major negative prognostic factors for OS and PFS
- Confirms importance of 1p deletion from previous studies

1q gains

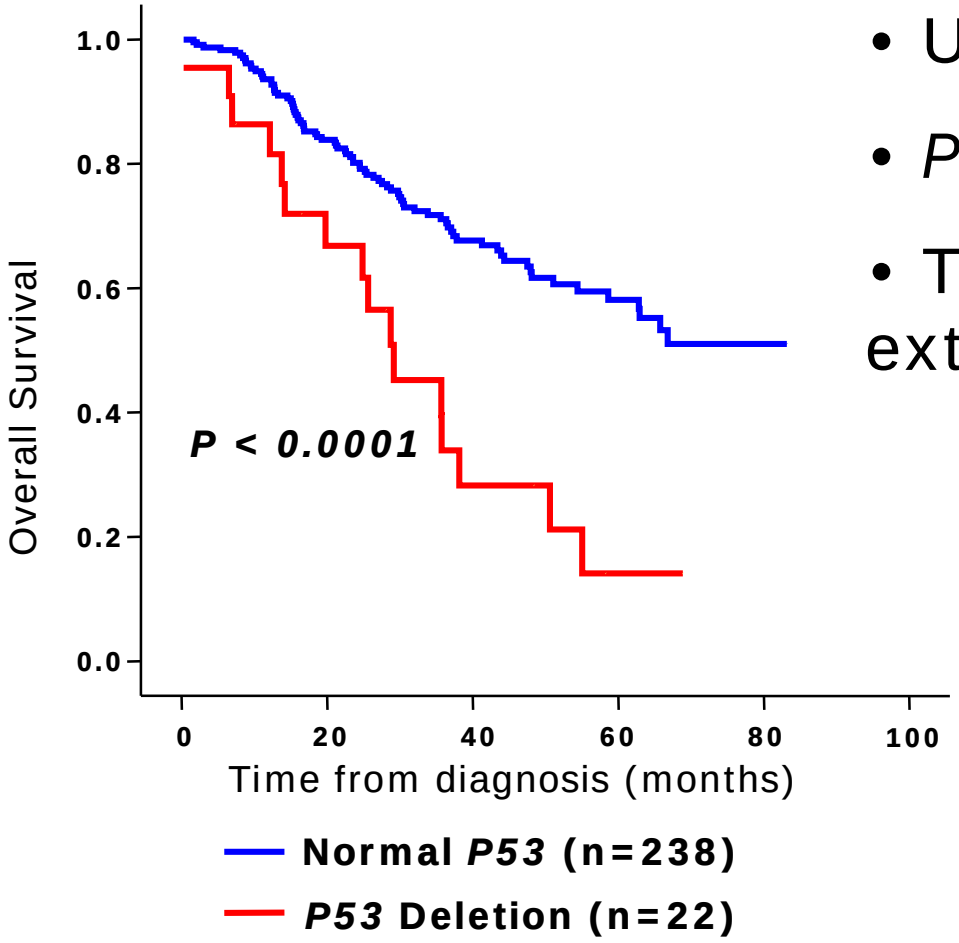


Total Therapy 2

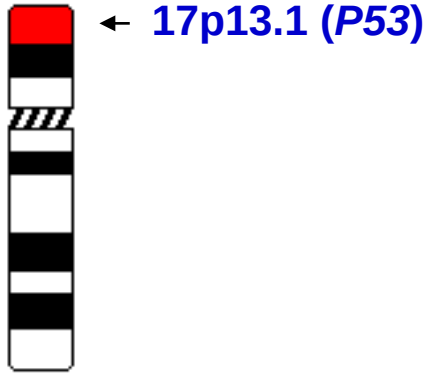


IFM 99-02 and 99-04 trials

17p (*P53*) deletion is associated with adverse prognosis



- Uncommon in newly diagnosed MM
- *P53* mutations*:* rare
- The lack of P53 may promote the extramedullary disease

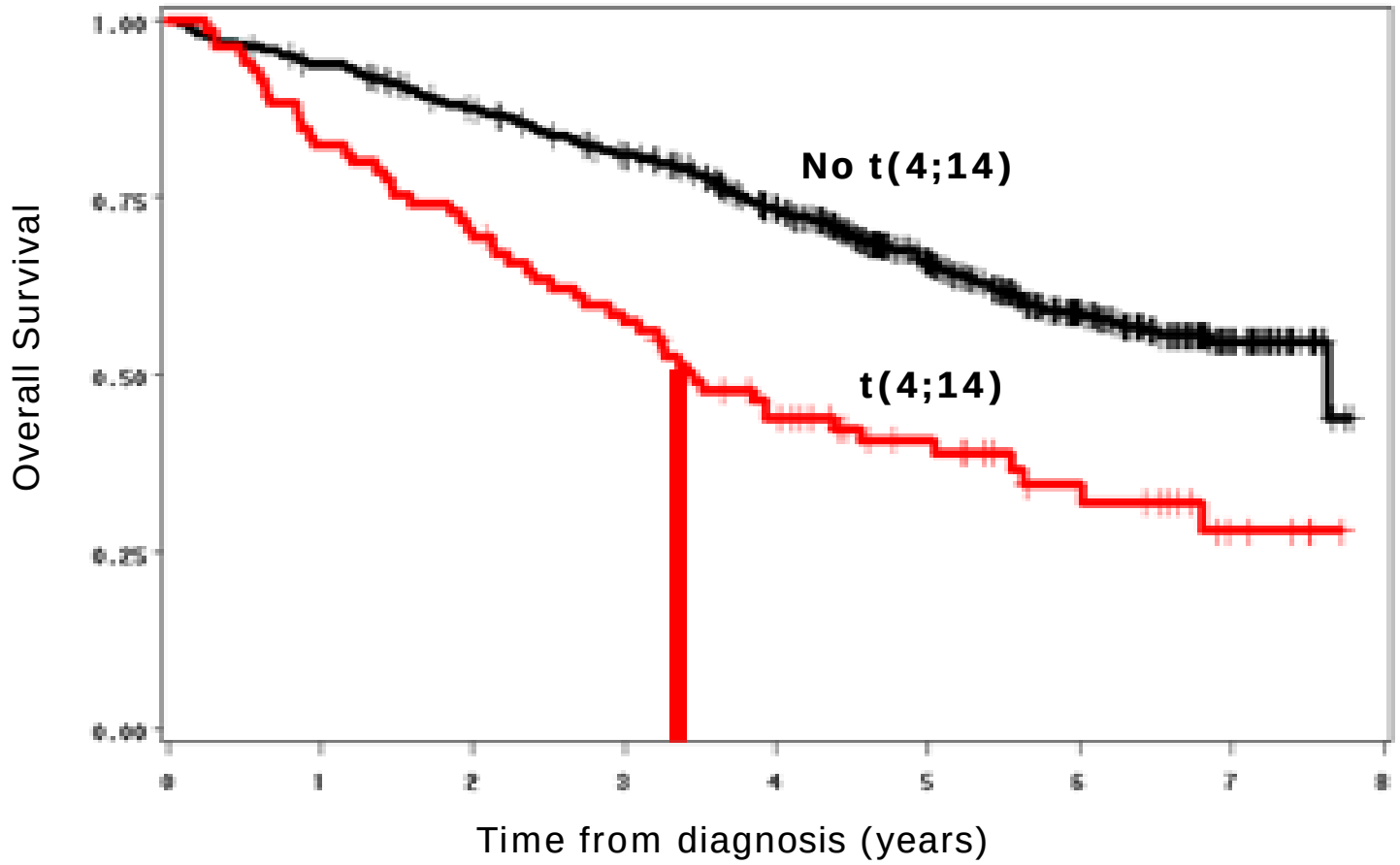


PETHEMA/GEM (Spanish MM group): 260 patients undergoing autologous transplantation

Chang et al, Blood 2005; Chng et al, Leukemia 2007; Tiedemann, Leukemia 2008; López-Anglada, Eur J Haematol 2009

Prognostic implications of *IGH* translocations

t(4;14)

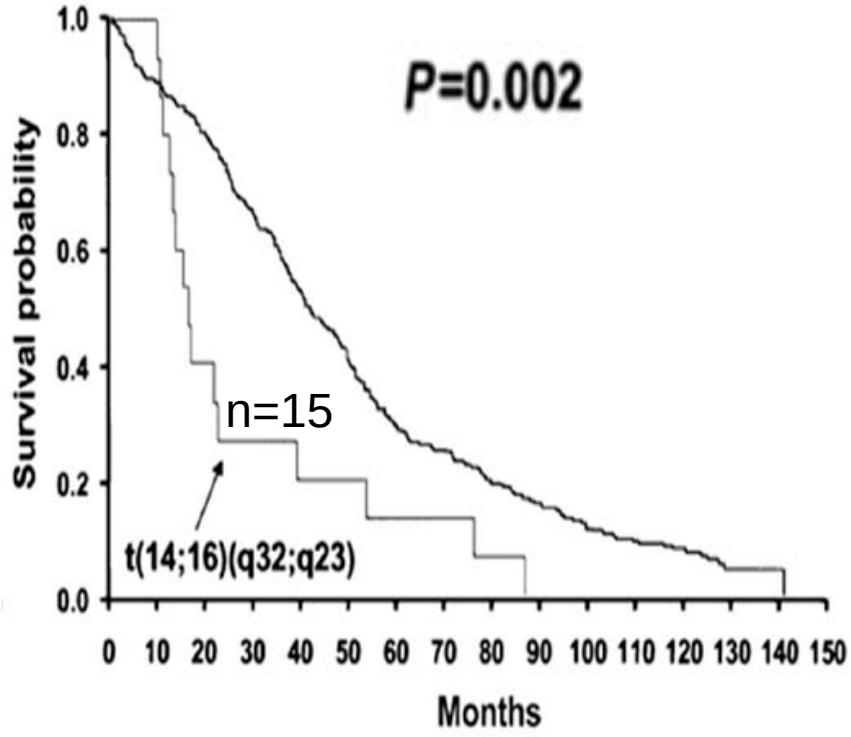


Patients with t(4;14) respond to treatment but early relapses

Kindly provided by Avet-Loiseau

Prognostic implications of *IGH* translocations

t(14;16)



Conventional Chemotherapy

Parameter	n=32	Univariate analysis	
		HR (95% CI)	*P*
Age (n = 697)		1.03 (1.02-1.05)	< .0001
β_2-microglobulin ≥ 4 vs < 4		2.02 (1.65-2.47)	< .0001
t(4,14) positive vs negative		2.24 (1.72-2.92)	< .0001
del(17p) ≥ 60 vs < 60		2.57 (1.88-3.50)	< .0001
del13 > 0 vs 0		1.63 (1.34-1.97)	< .0001
t(14,16) positive vs negative		1.28 (0.82-2.01)	.281

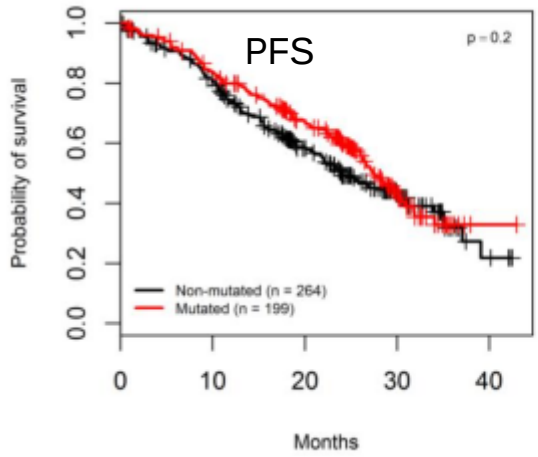
Double-intensive regimen

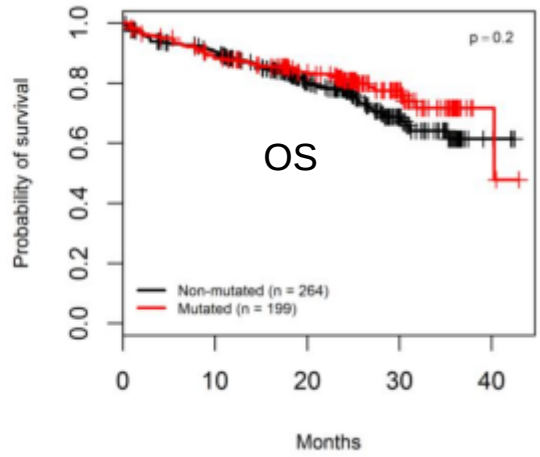
Fonseca et al, Blood 2003 *Avet-Loiseau et al, Blood 2011*

Mutations in the RAS and NF-kB pathways are pronostically neutral

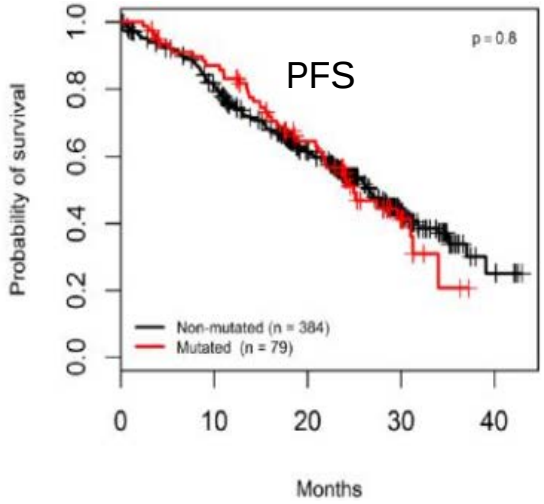
Mutations

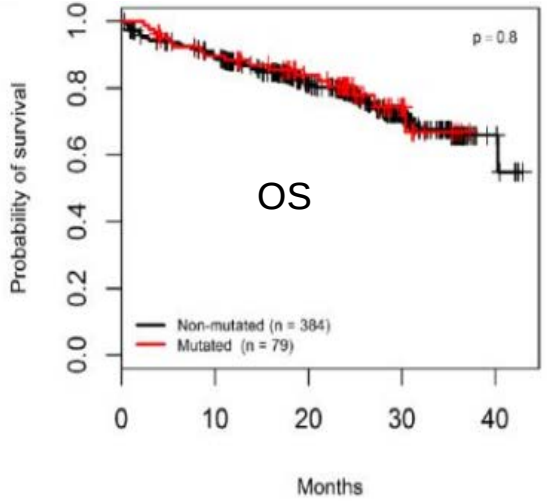
KRAS
NRAS
BRAF





NF-κB pathway mutations



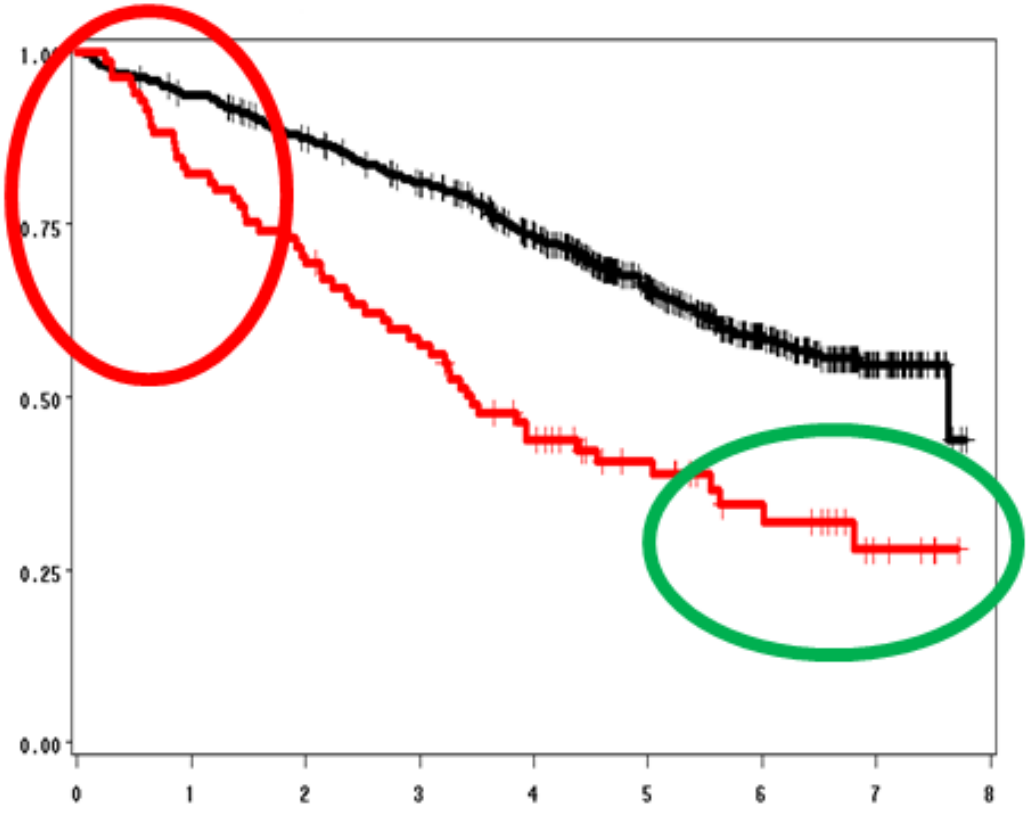


The mutational spectrum is dominated by mutations in the RAS (43%) and NF-κB (17%) pathways, but although they are prognostically neutral, they could be targeted therapeutically

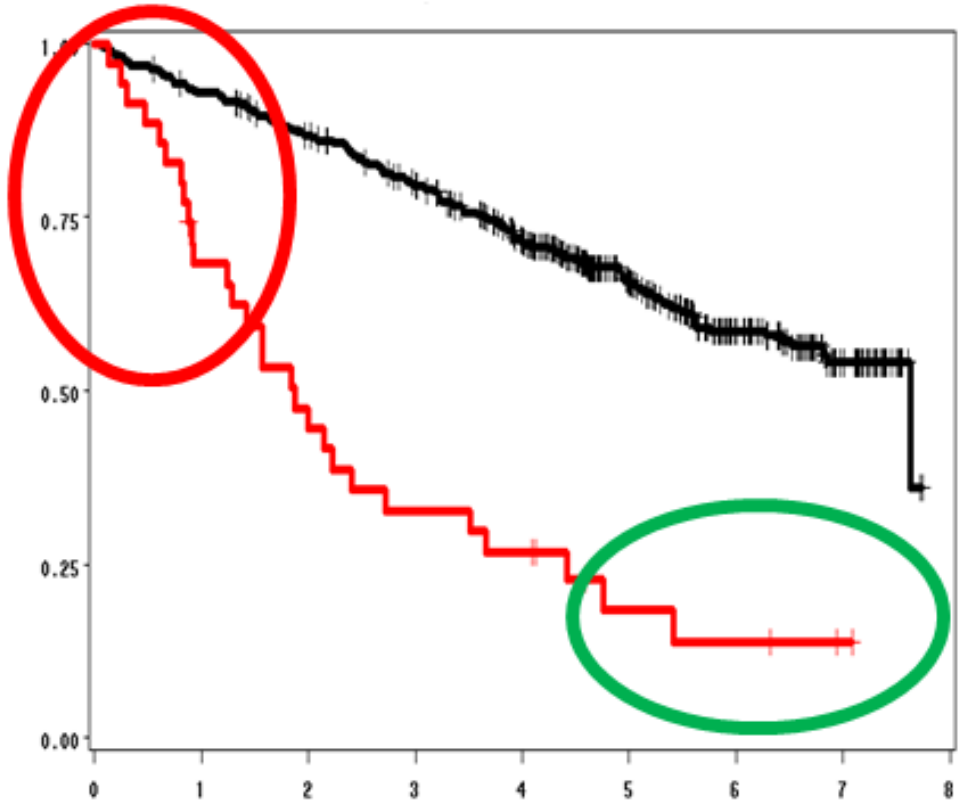
Walker BA, JCO 2015

Heterogeneity in high-risk patients

t(4;14) overall survival



del(17p) overall survival



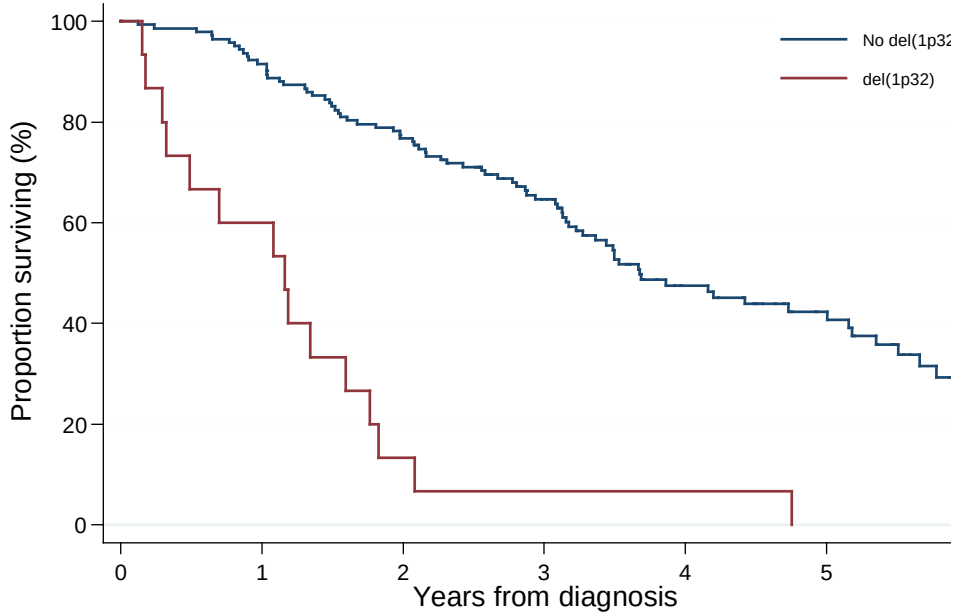
Patients < 65 years, double HDM/ASCT

Do other chromosomal changes impact the outcome?

Hebraud et al, Blood 2015

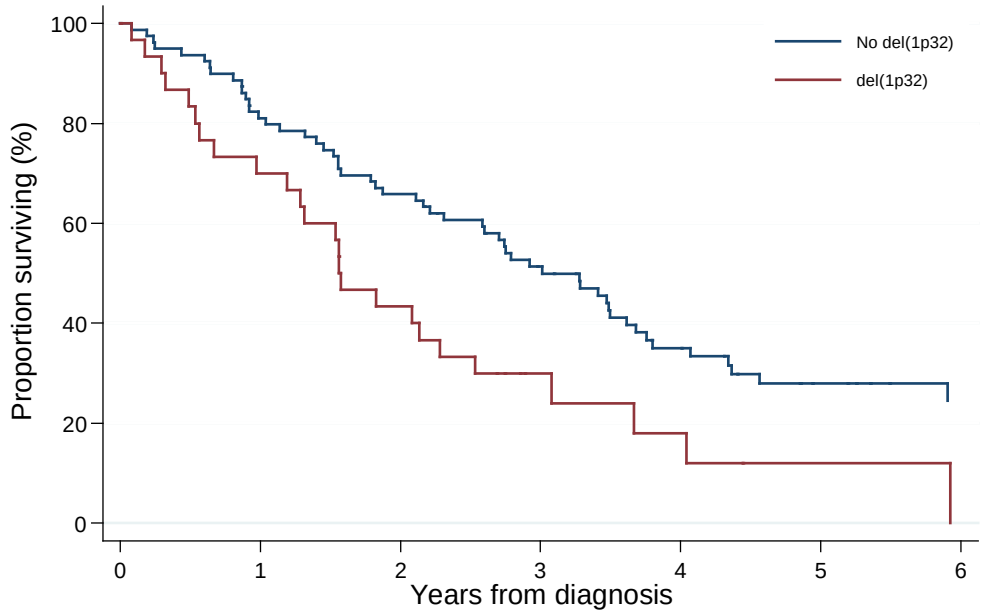
Heterogeneity in high-risk patients

Overall survival according to del(1p32)



N at risk						
No del(1p32)	142	130	109	76	40	26
del1p32	15	9	2	1	1	0

t(4;14)

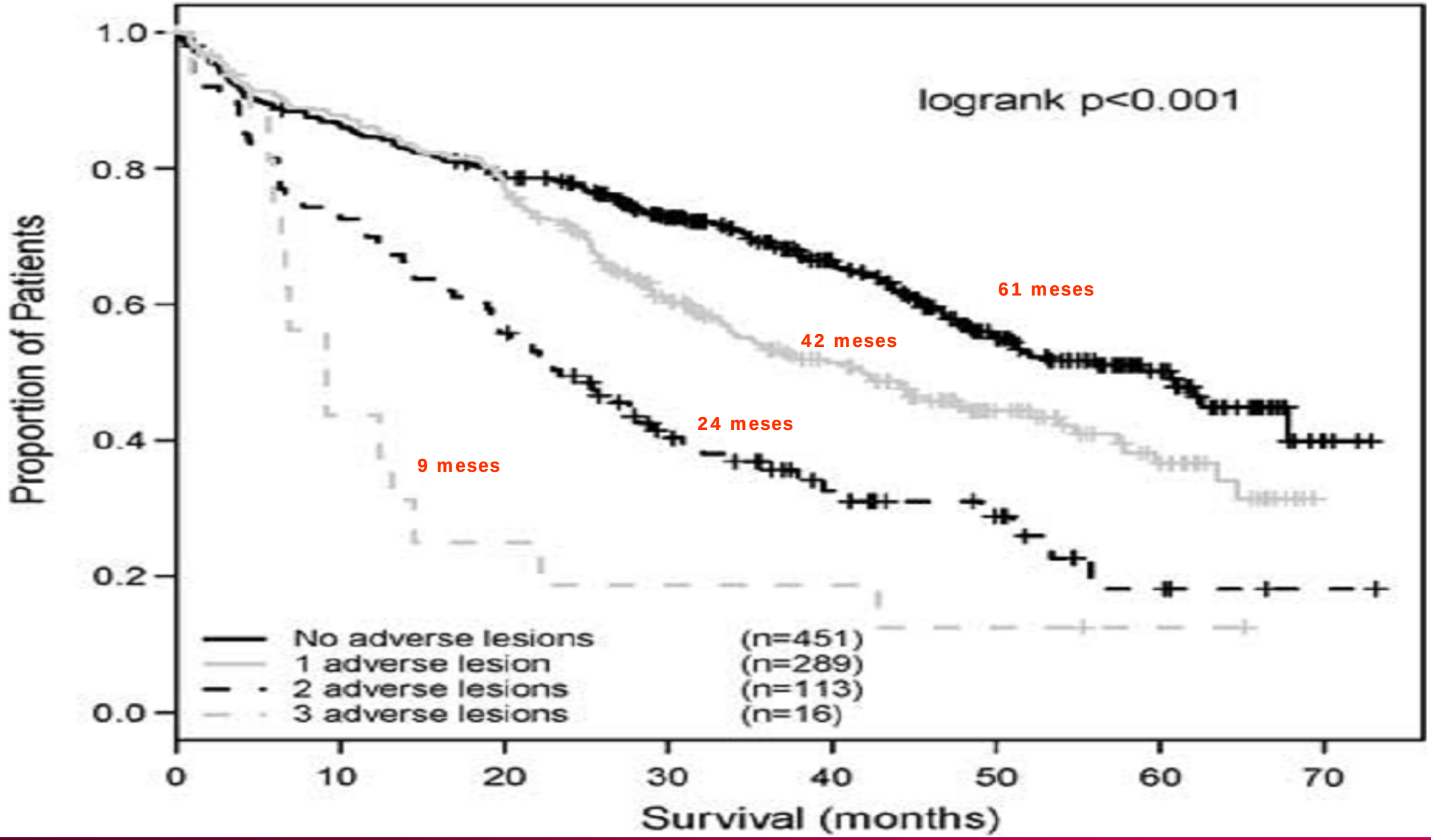


N at risk							
No del(1p32)	79	64	52	37	22	12	7
del1p32	31	21	13	5	3	1	0

del(17p)

Hebraud et al, Blood 2015

Overall survival graded by number of adverse lesions

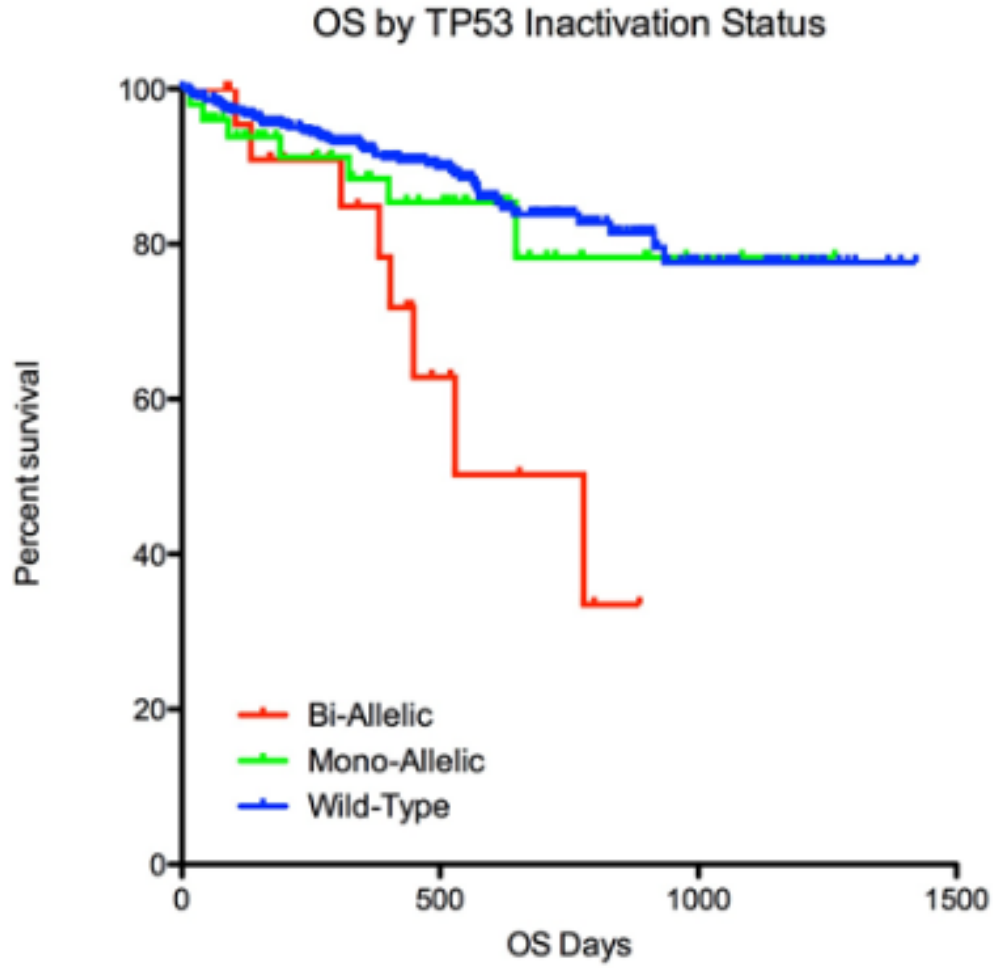


Boyd et al, Leukemia 2011

Bi-allelic TP53 inactivation is associated with poorer prognosis

CoMMpass Trial

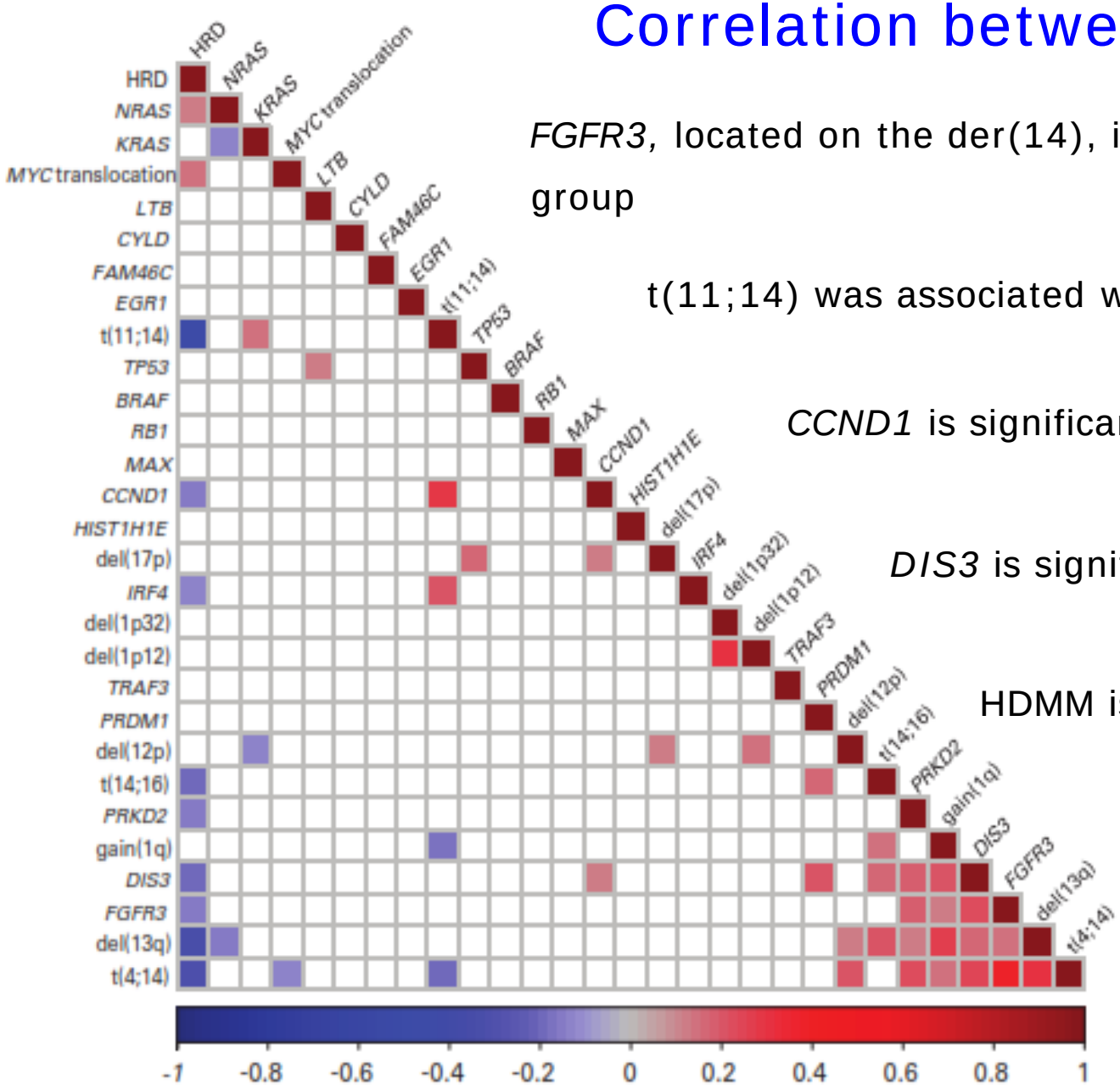
Over 1,000 MM patients analyzed by NGS:
- Whole Exome Sequencing (WES)
- RNA sequencing (RNA-seq)



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

ASCO 2016

Correlation between genomic events



FGFR3, located on the der(14), is only mutated in the t(4;14) group

t(11;14) was associated with *KRAS* and *IRF4* mutations

CCND1 is significantly mutated in the t(11;14) group

DIS3 is significantly mutated in the t(4;14) group

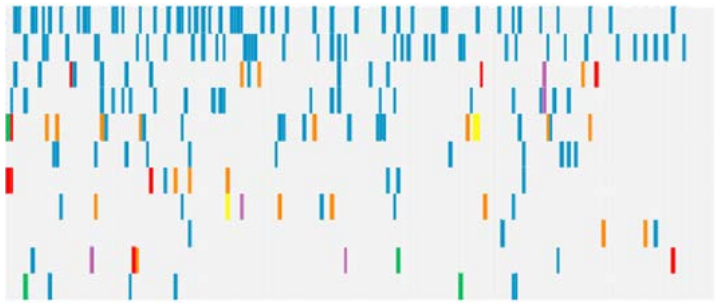
HDMM is enriched for FAM46C mutations

KRAS and NRAS mutations were mutually exclusive between each other but not with BRAF mutations

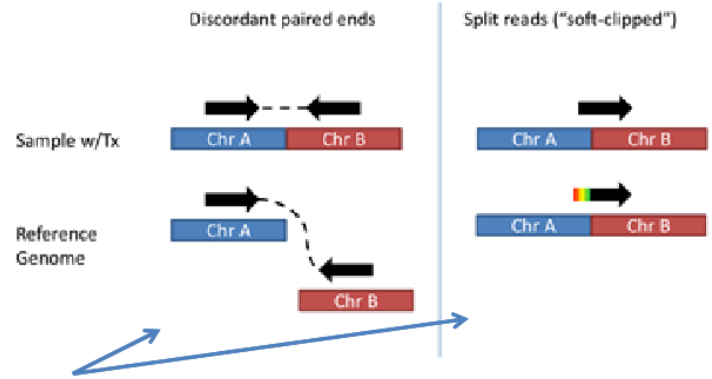
Walker et al, JCO 2015

Bolli et al, Leukemia 2018

Oportunity for detection of mutations, translocations and CNAs using a single NGS assay

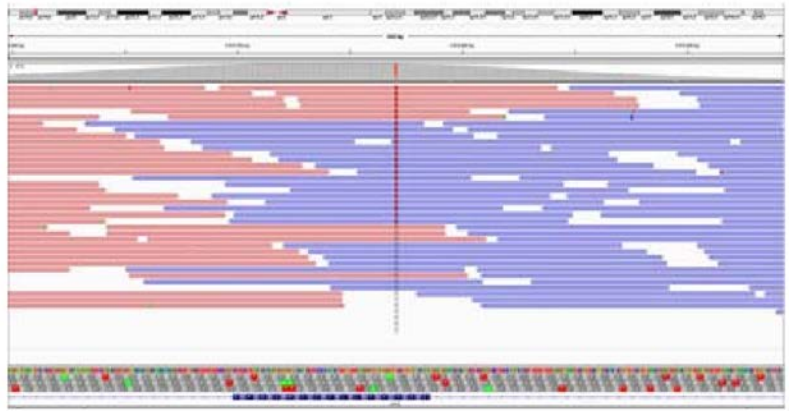


NGS



Detection of mutations

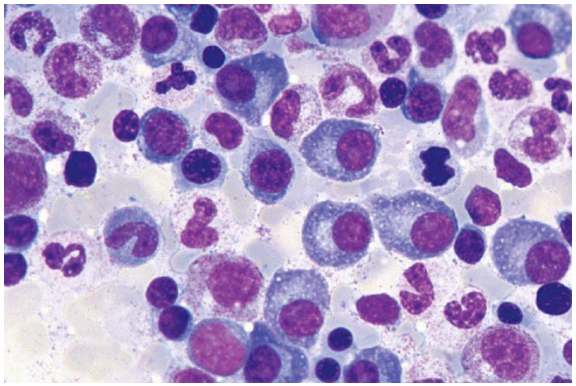
Detection of translocations



Detection of CNAs

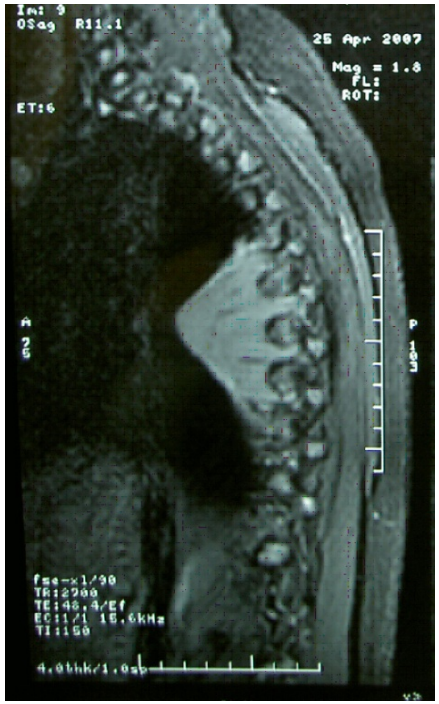
Translocations and CNAs have a preponderant contribution over gene mutations in defining the genotype and prognosis of each case.
Bolli et al, Leukemia 2018

Intratumor Heterogeneity



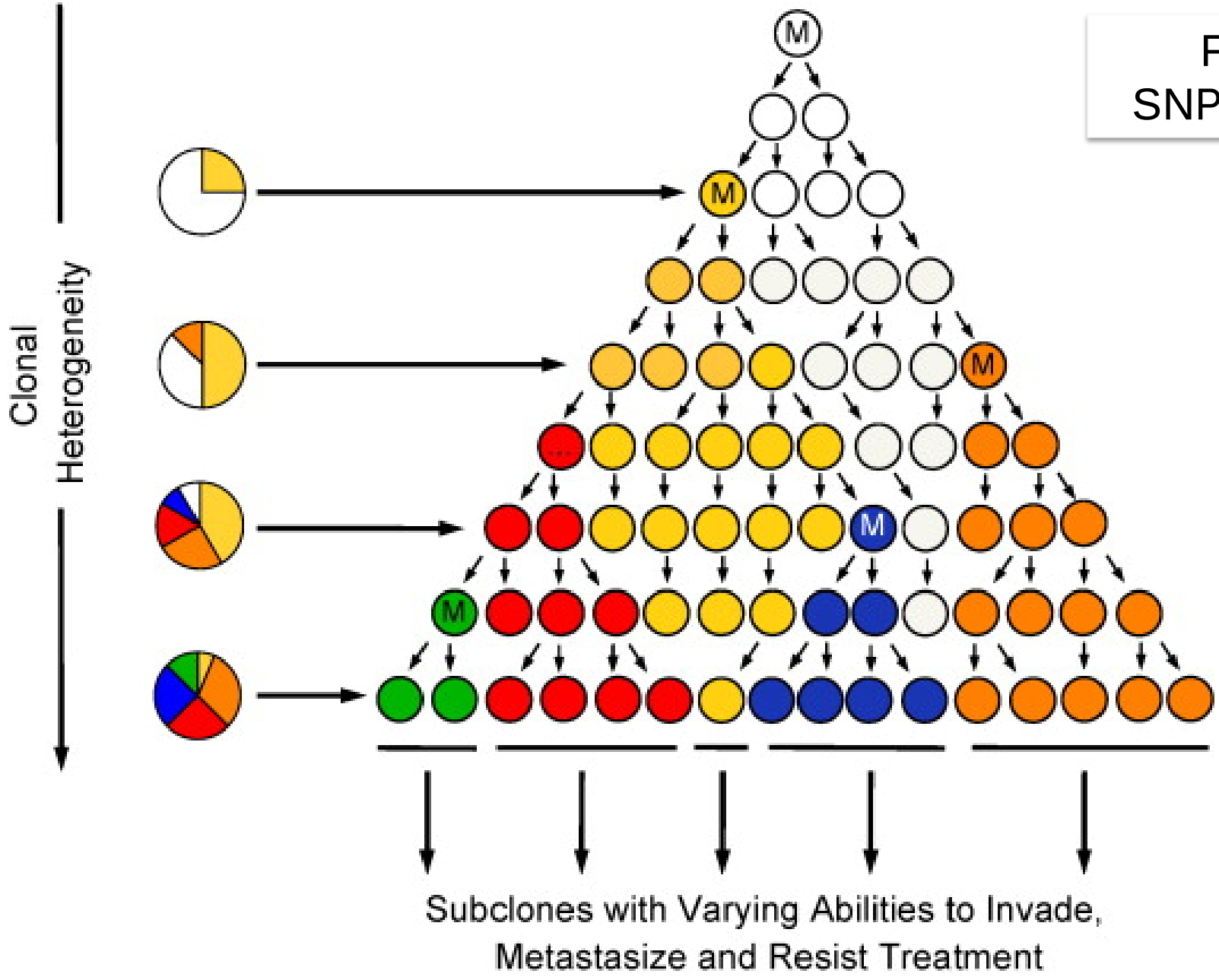
Complete remission at BM → t(4;14) Monosomy 13

MM IgG lambda with light chain proteinuria and a paraspinal plasmacytoma



Extramedullary plasmacytomas appeared during therapy and were refractory to every line of treatment → t(4;14) Monosomy 13 **17p deletion**

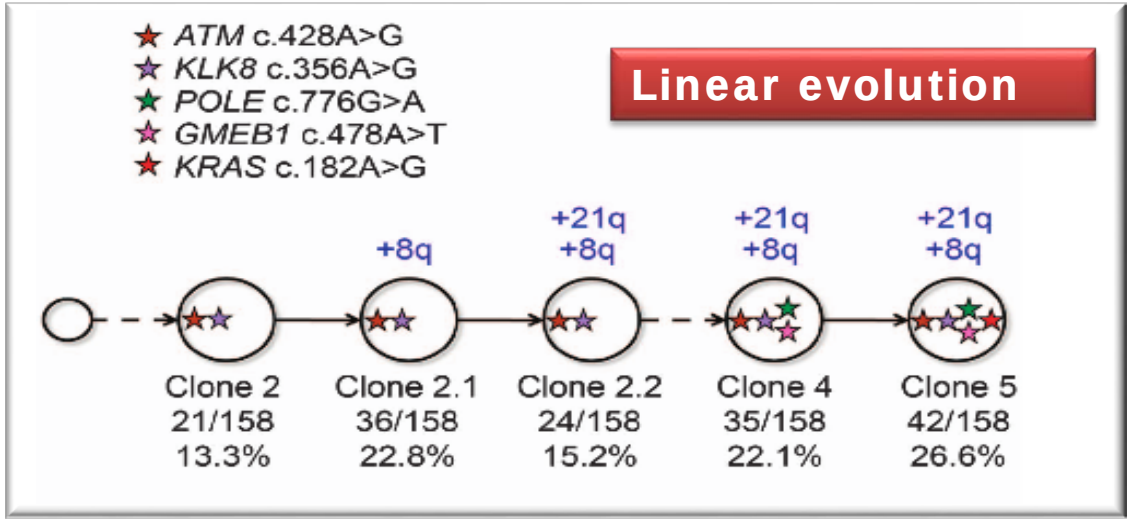
López-Anglada, Eur J Haematol 2009


FISH
SNP arrays
Clonal
Heterogeneity
M
Subclones with Varying Abilities to Invade,
Metastasize and Resist Treatment

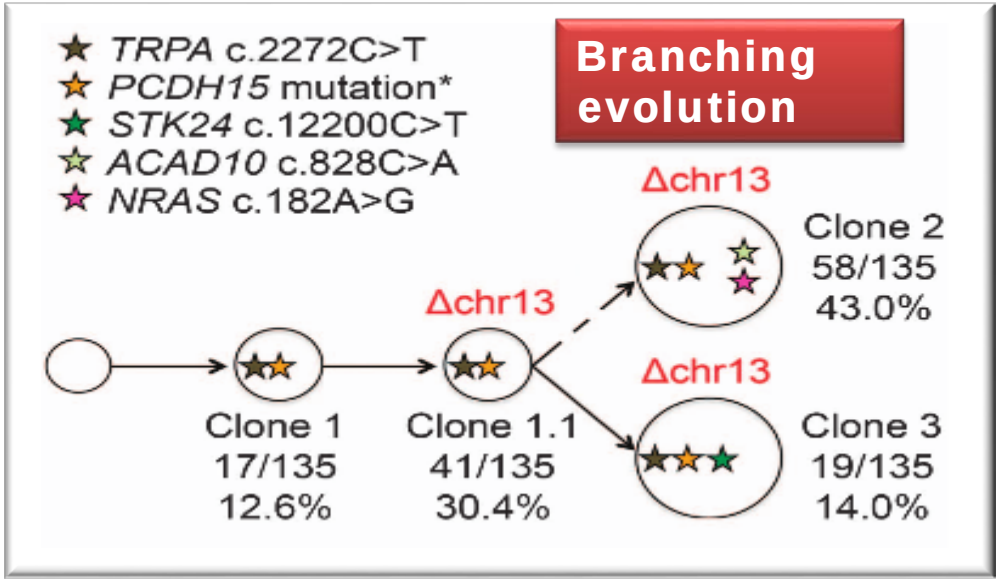
Intraclonal heterogeneity demonstrated by Massively Parallel Sequencing

Single cell genetic analysis

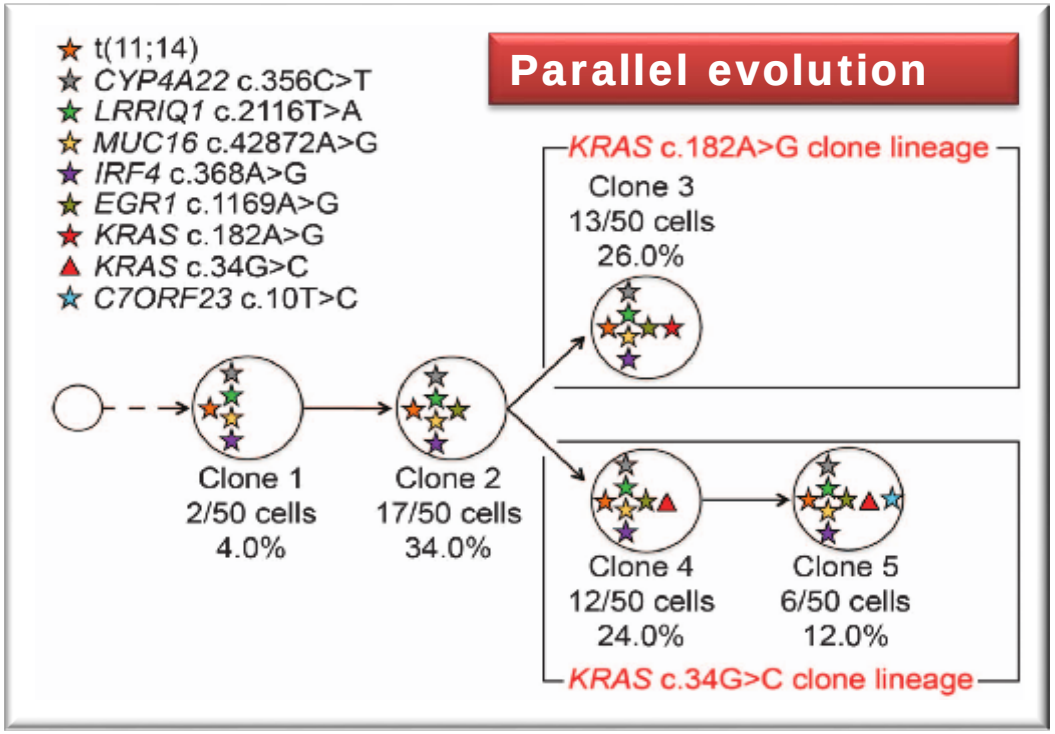
Linear evolution



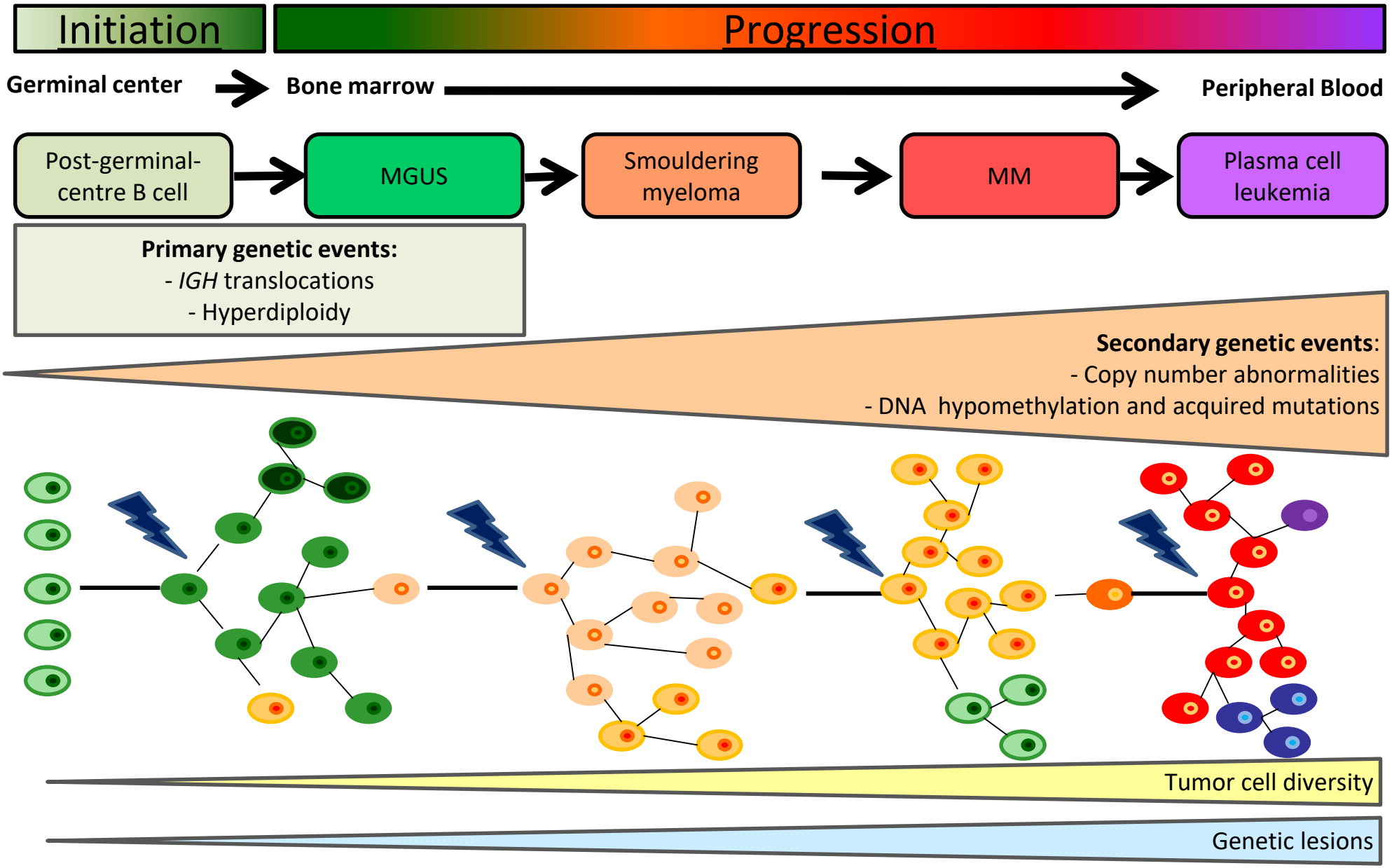
Branching evolution



Parallel evolution



Melchor et al, Leukemia 2014



Morgan G (modified), Nature 2012

The impact of genomic diversity and intra-clonal heterogeneity on the treatment of myeloma

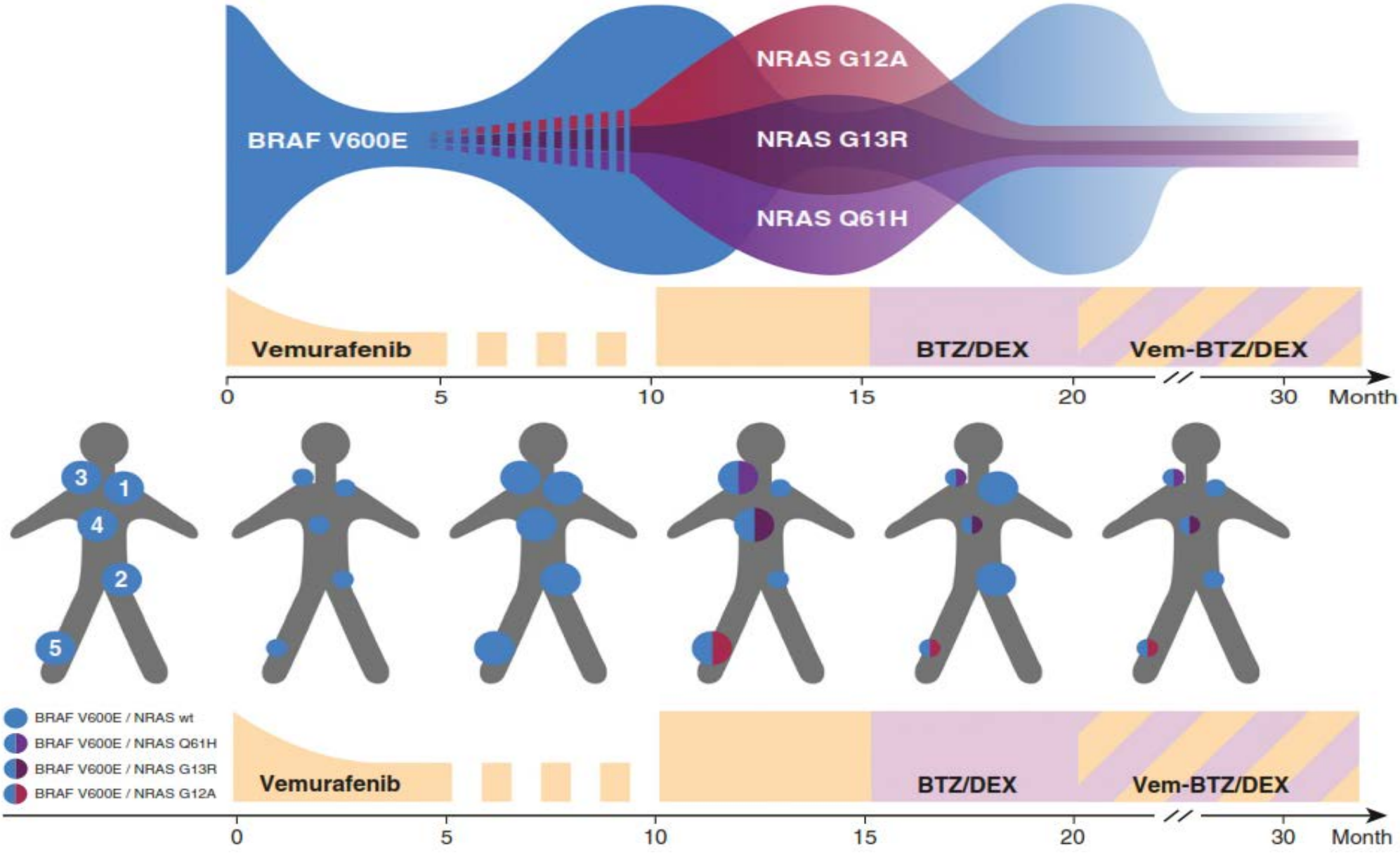
Challenge for cancer therapy

✓ Targeted therapy might have a paradoxically stimulatory effect on the subclones lacking the relevant mutation.

✓ Multi-drug combination with different mechanism of action in order to eradicate dominant as well as minor clones.

✓ Limitations of basing treatment decisions on the findings derived from a single bone marrow biopsy.

The clinical course and management of a patient with BRAF V600E-mutant MM developing resistance to treatment with vemurafenib



Raab et al, Blood 2016

The impact of genomic diversity and intra-clonal heterogeneity on the treatment of myeloma

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DNA — Genome

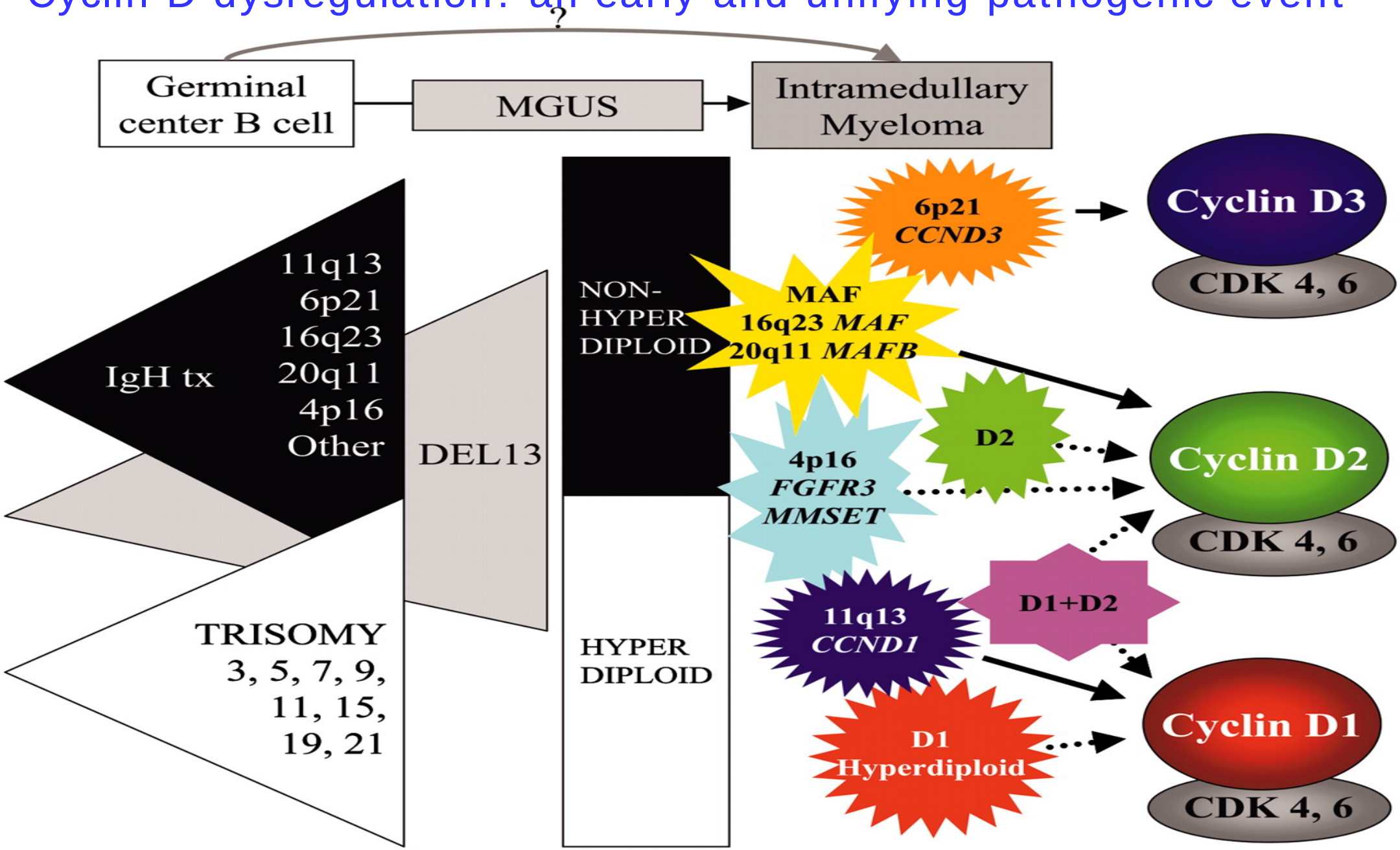
Transcription

mRNA — Transcriptome

Translation

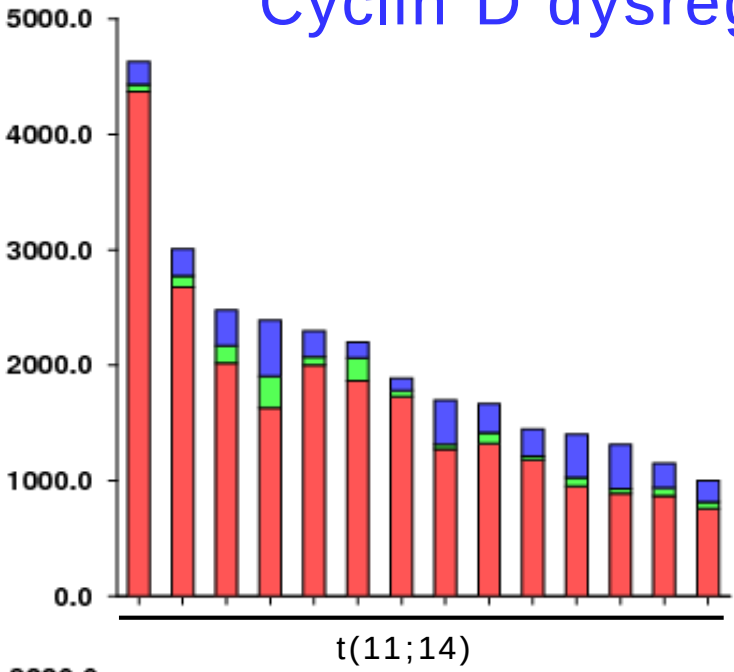
Protein — Proteome

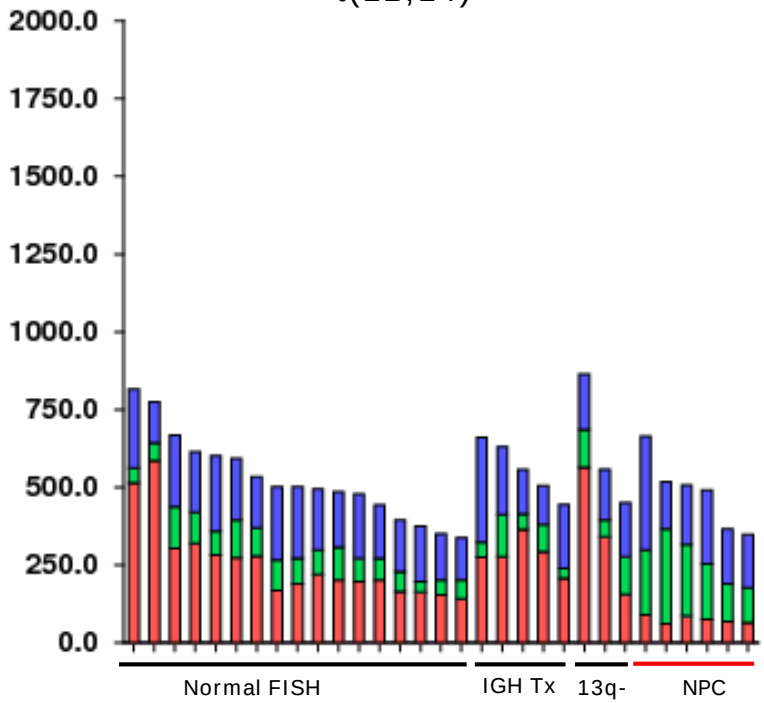
Cyclin D dysregulation: an early and unifying pathogenic event

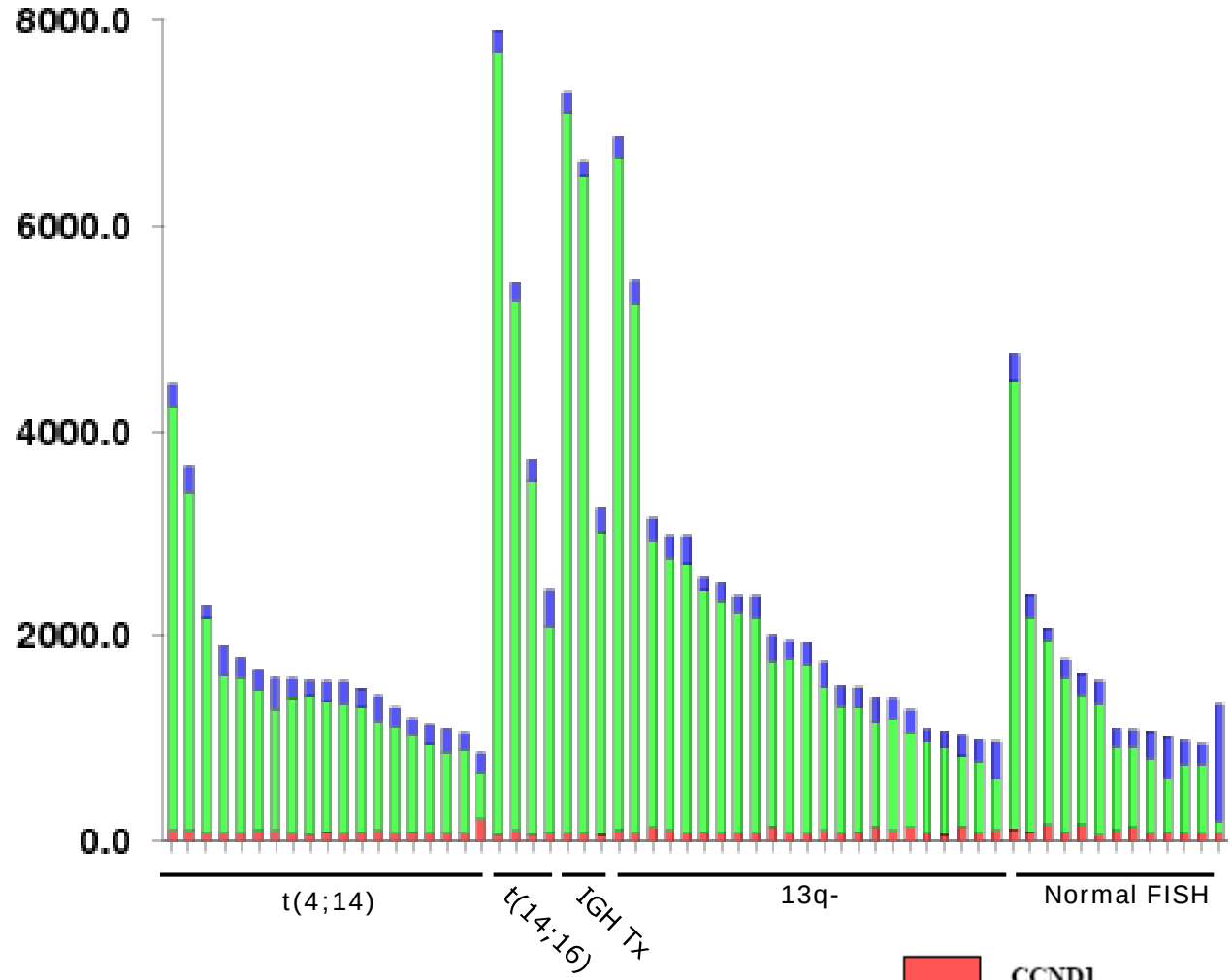


Bergsagel et al, Blood 2005

Cyclin D dysregulation in 100 myeloma samples







Expression by microarray analysis

Post-Transcriptional Modifications Explain the Overexpression of CCND2 in Multiple Myeloma

✓ CCND2 is highly expressed in most of the multiple myeloma samples without CCND1 or CCND3 overexpression

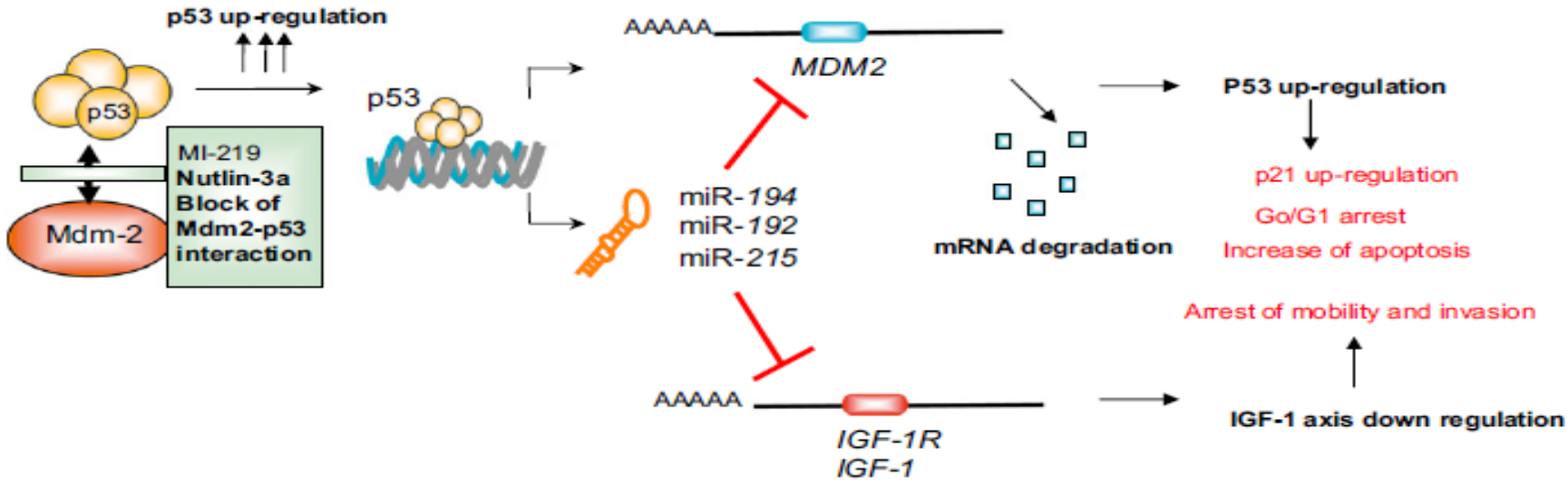
✓ The mechanisms by which CCND2 is upregulated in a set of MMs are not completely deciphered

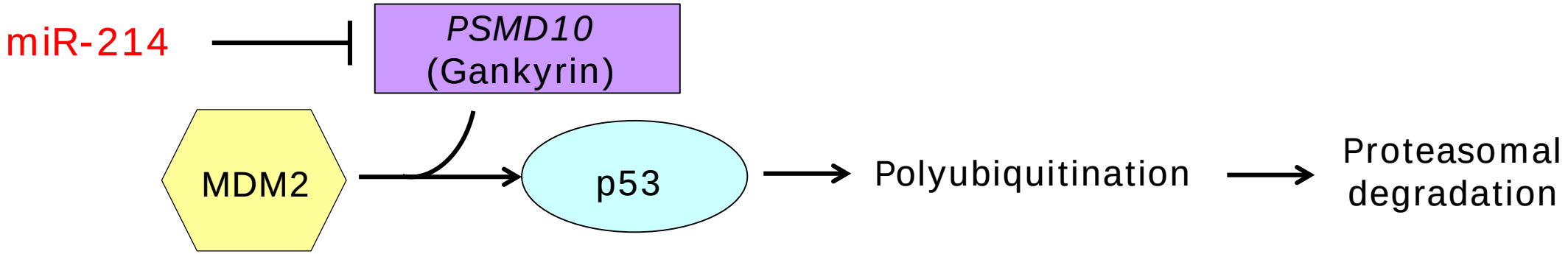
✓ Role of post-transcriptional regulation through the interaction between miRNAs and their binding sites at 3'UTR in CCND2 overexpression in MM

Misiewicz-Krzeminska et al, Clin Cancer Res 2016

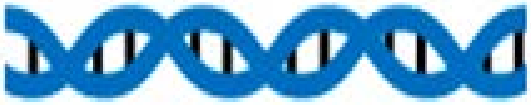
P53 inactivation induced by the deregulation of miRNAs targeting P53

miR-192, 215, and 194 Impair the p53/MDM2 Autoregulatory Loop





Pichiorri, Cancer Cell 2010; Misiewicz-Krzeminska, Haematologica 2012; Herrero, Int J Mol Sci. 2016



DNA — Genome

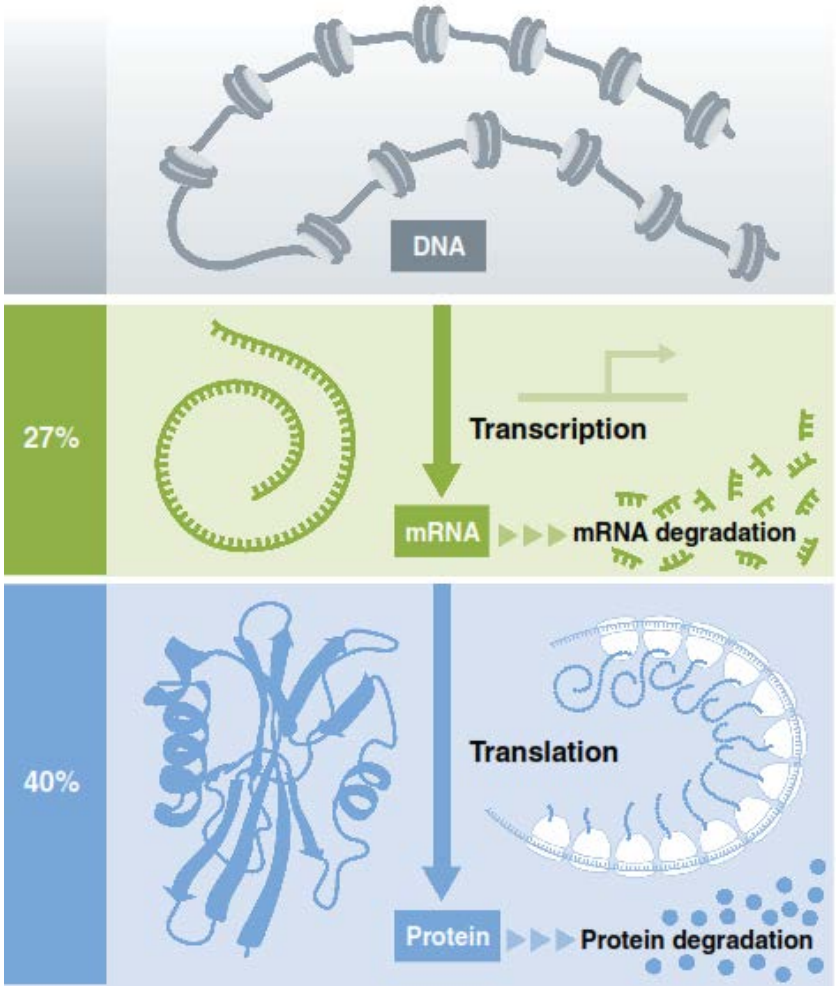
Transcription

mRNA — Transcriptome

Translation

Protein — Proteome

Relative influence of transcriptional and translational regulation on protein abundance



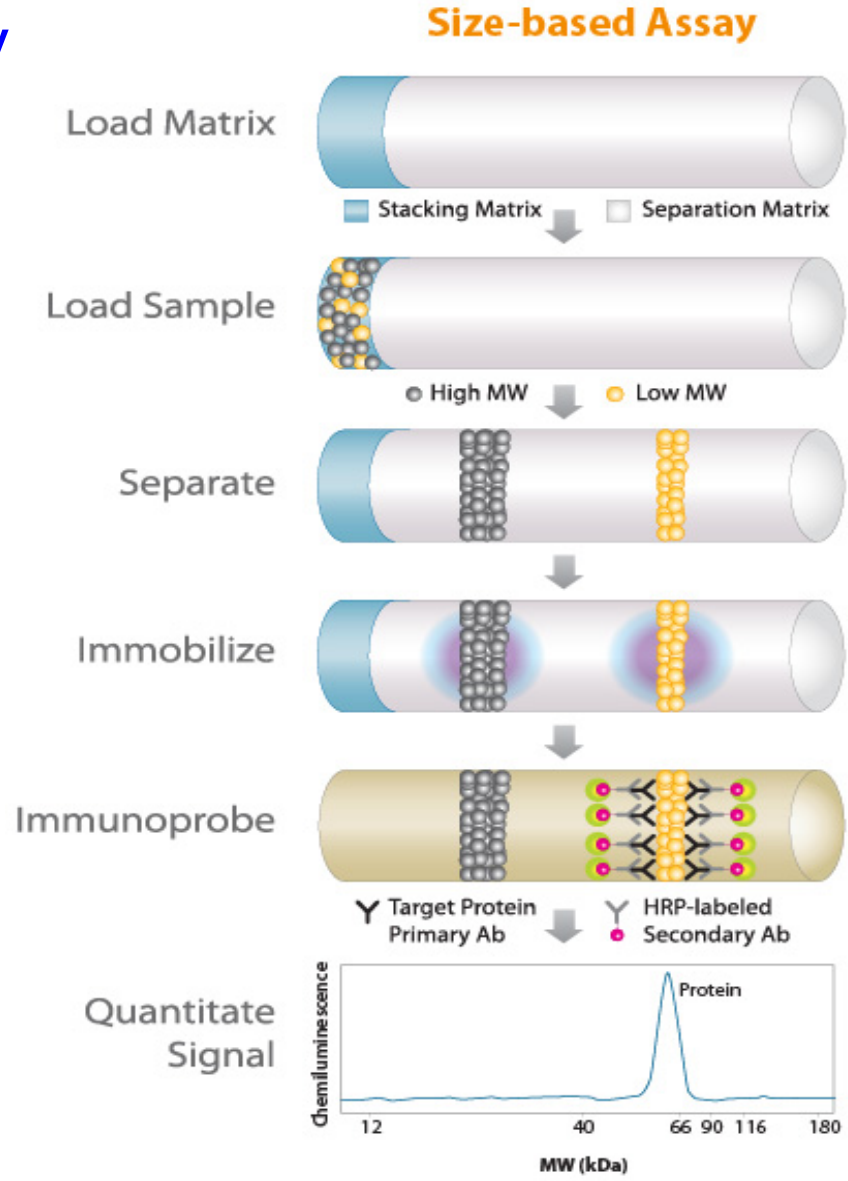
The unresolved difficulties in studying the proteome have made the quantification of messenger RNA (mRNA) an indirect measure of protein expression, although many studies have shown that protein levels cannot be predicted from mRNA measurements

mRNA levels cannot be used as surrogates for protein levels

Plotkin, Molecular Systems Biology 2010

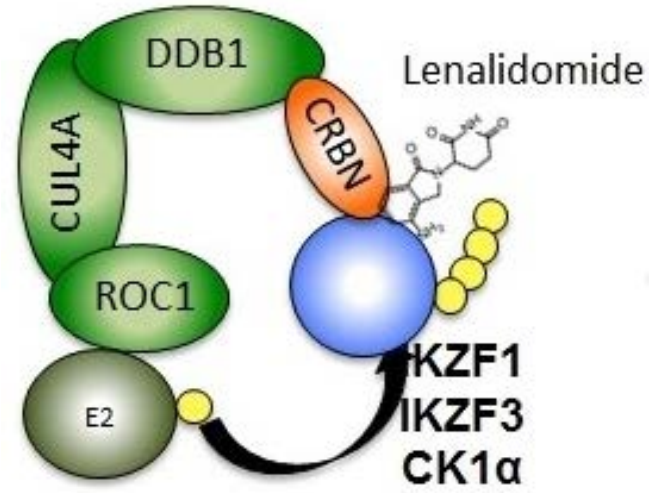
Combination of capillary electrophoresis with immunoassay

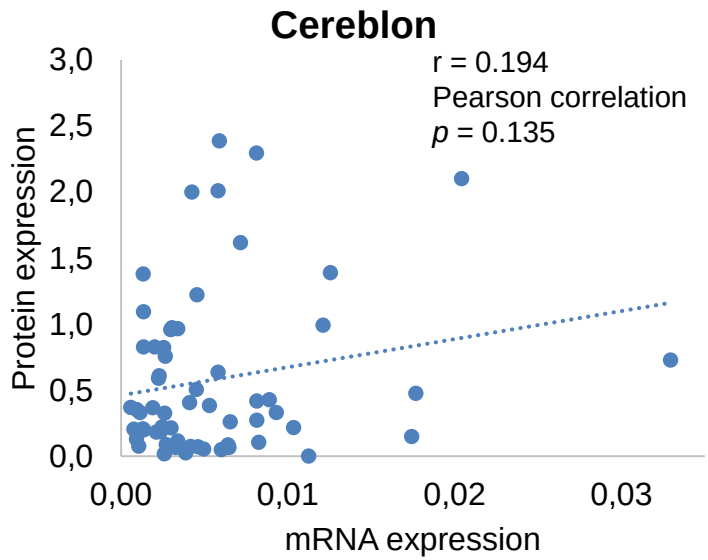
Simple Western



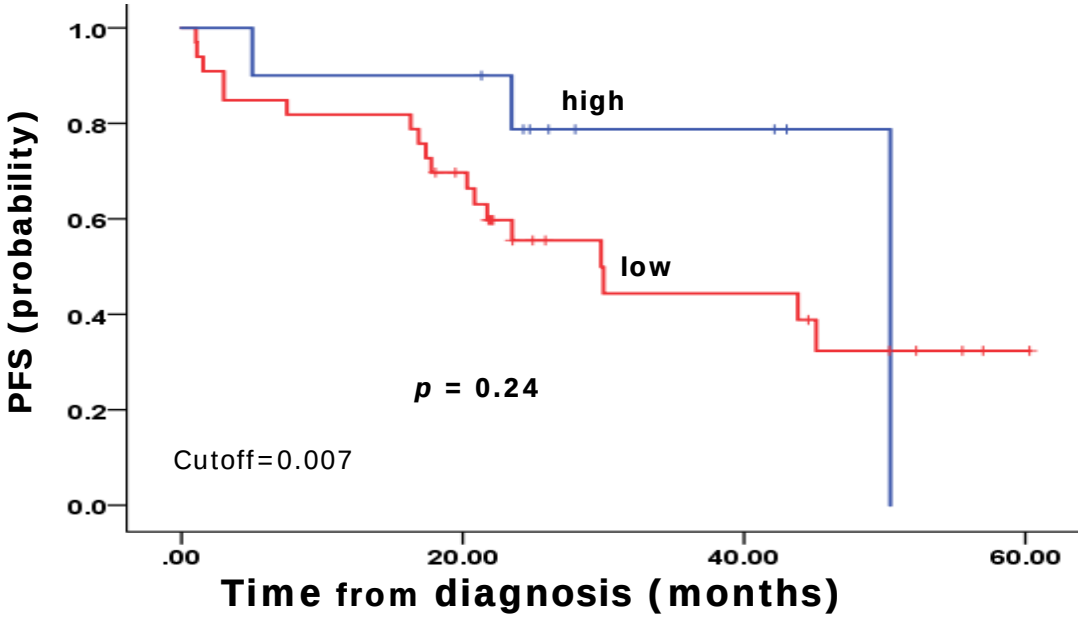
Sufficient protein to analyze over 50 proteins from one single MM sample.

Cereblon (*CRBN*)

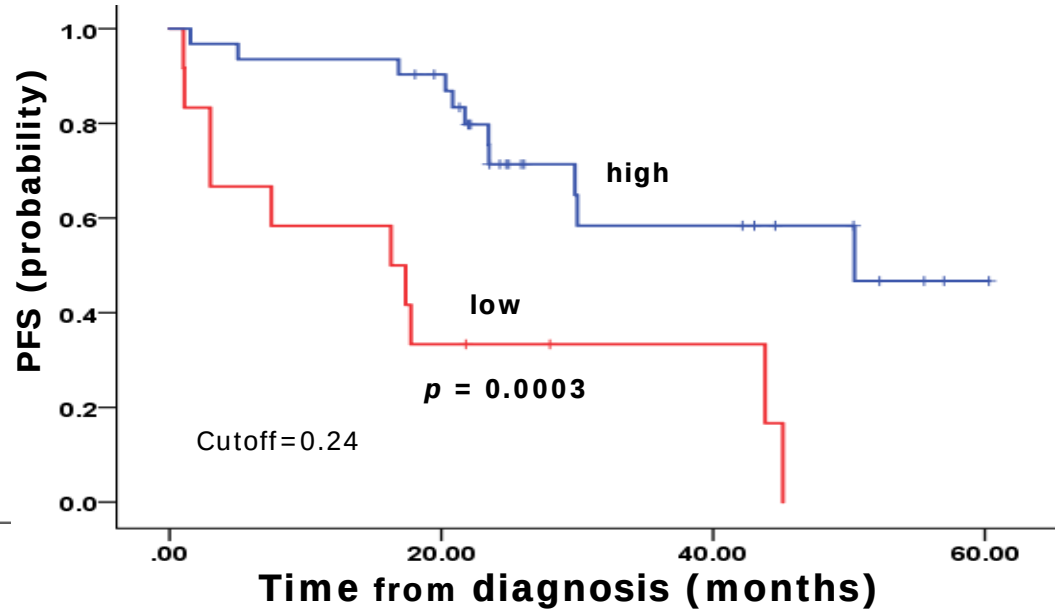




CRBN mRNA



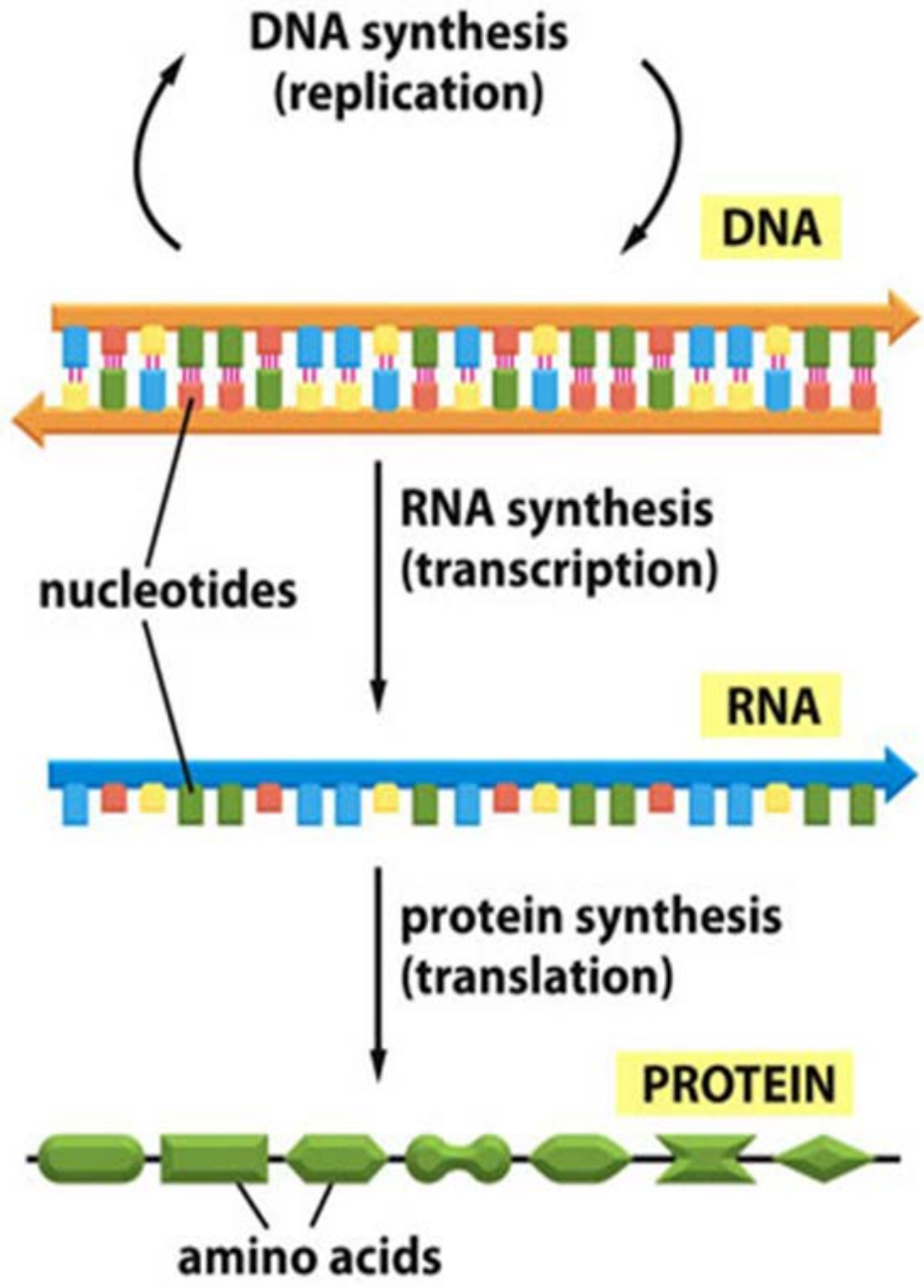
CRBN protein



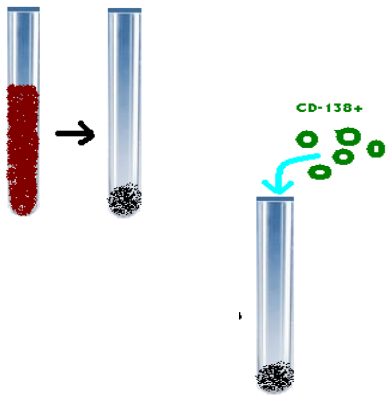
Misiewicz-Krzeminska et al, Haematologica 2018

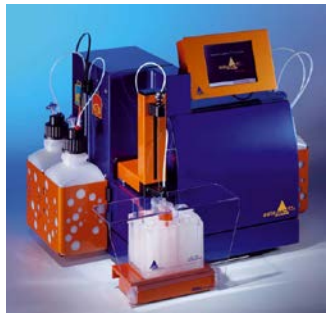
Central Dogma of Molecular Biology

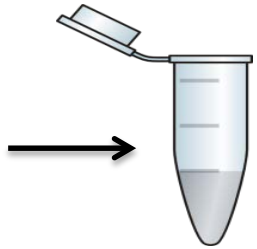
"Genomics involves the study of all genes at the DNA, mRNA, and proteome level as well as the cellular or tissue level"



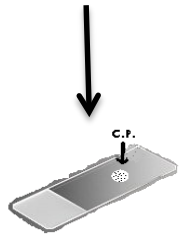
Methodology overview: everything from the same ONE sample







CD138+
in RLT+
-80°C
for years



FISH

AllPrep
(Qiagen)

DNA

SNP arrays,
NGS, epigenetic
analysis

RNA

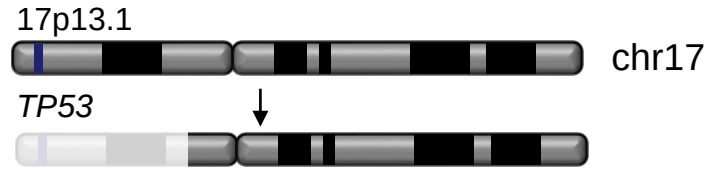
GEP, RNA seq, qRT-PCR, mRNA isoforms analysis, posttranscriptional modifications

In-house
protocol

protein

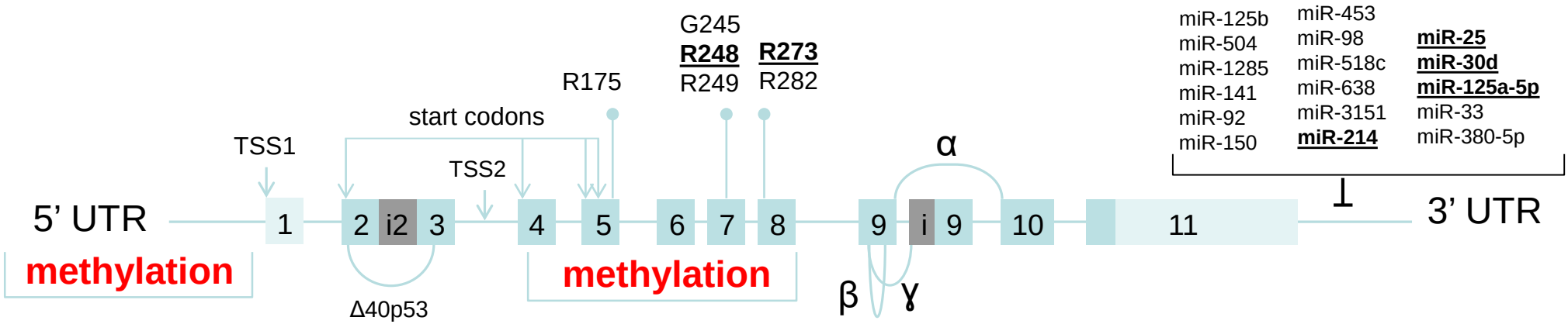
Simple western for expression level and isoform identification

deletions



mutations

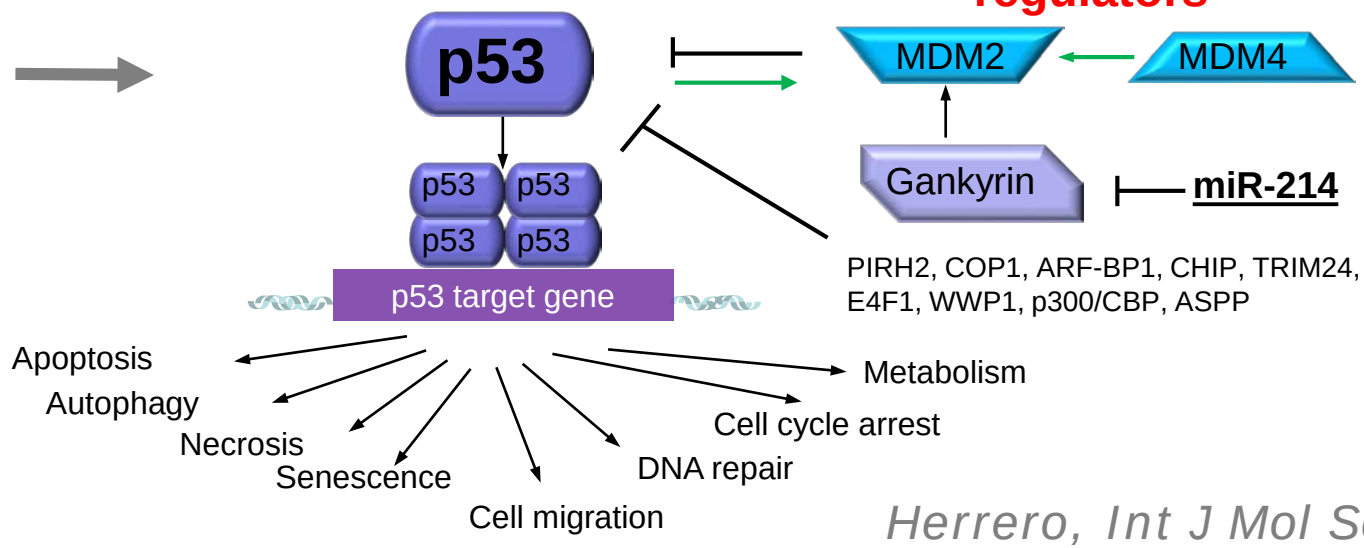
MicroRNAs



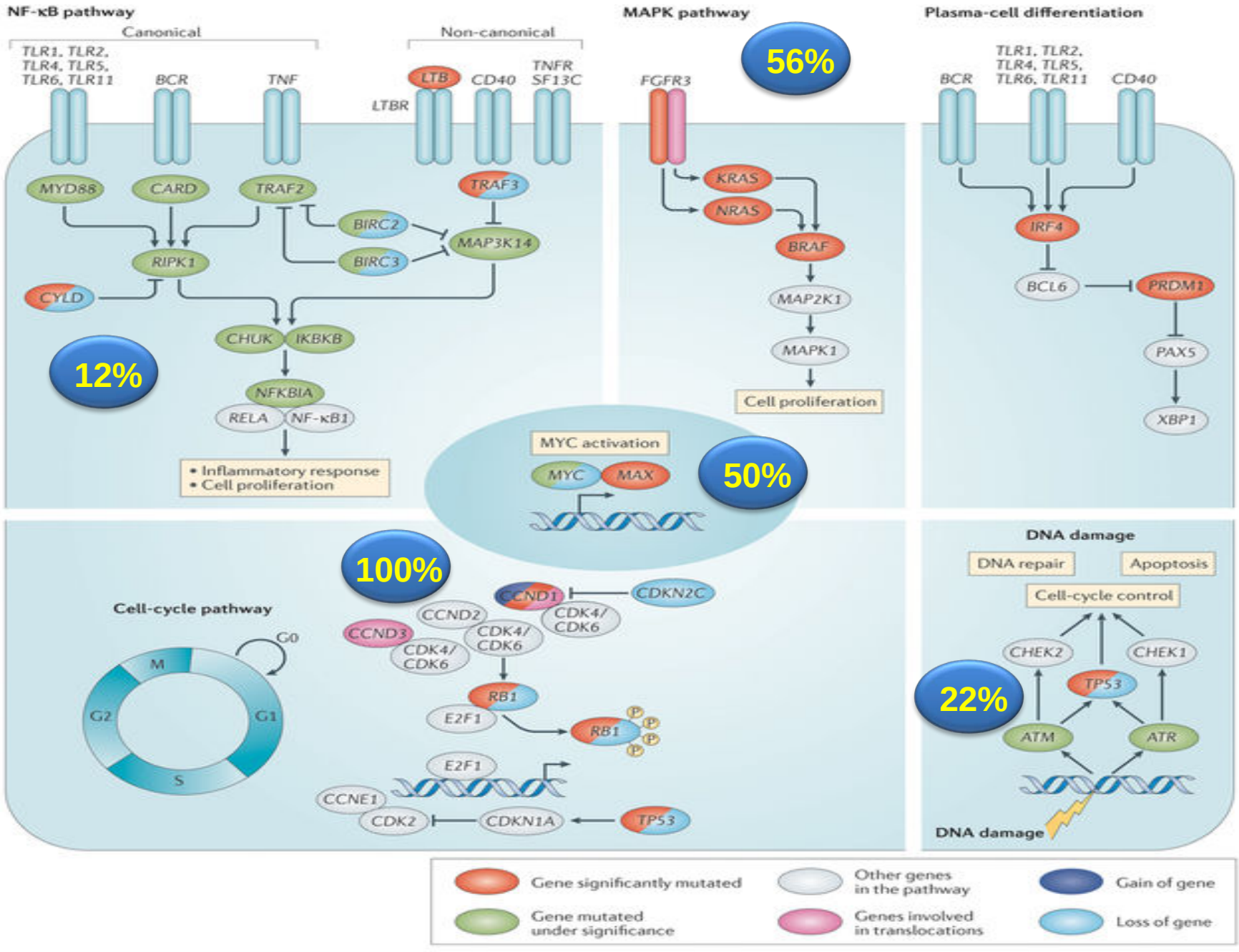
p53 isoforms

p53α (p53 wt)
Δ40p53α
Δ133p53α
Δ160p53α
p53β
Δ40p53β
Δ133p53β
Δ160p53β
p53γ
Δ40p53 γ
Δ133p53γ
Δ160p53γ

regulators

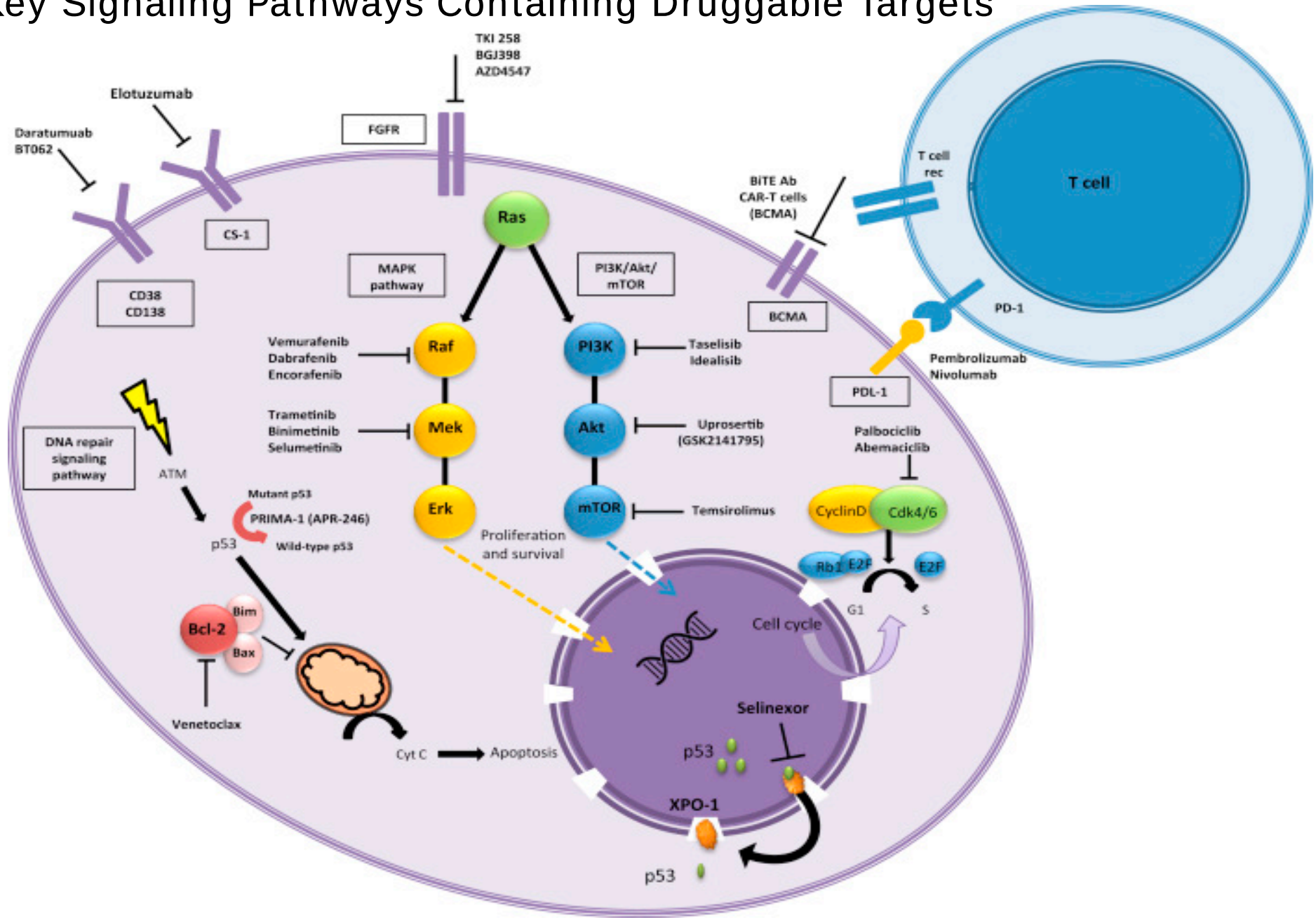


Herrero, Int J Mol Sci. 2016



Manier, Nature Reviews 2016; Bolli, Leukemia 2018

Key Signaling Pathways Containing Druggable Targets



González-Calle et al, Clin Lymphoma Myeloma Leuk. 2017

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GEM
GRUPO ESPAÑOL DE MIELOMA

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Instituto de Salud Carlos III
Unión Europea
Fondo Europeo de Desarrollo Regional
"Una manera de hacer Europa"

International Myeloma Foundation
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