
# MIELOMA MULTIPLO

## La complessità genomica come *pabulum* per la resistenza al trattamento

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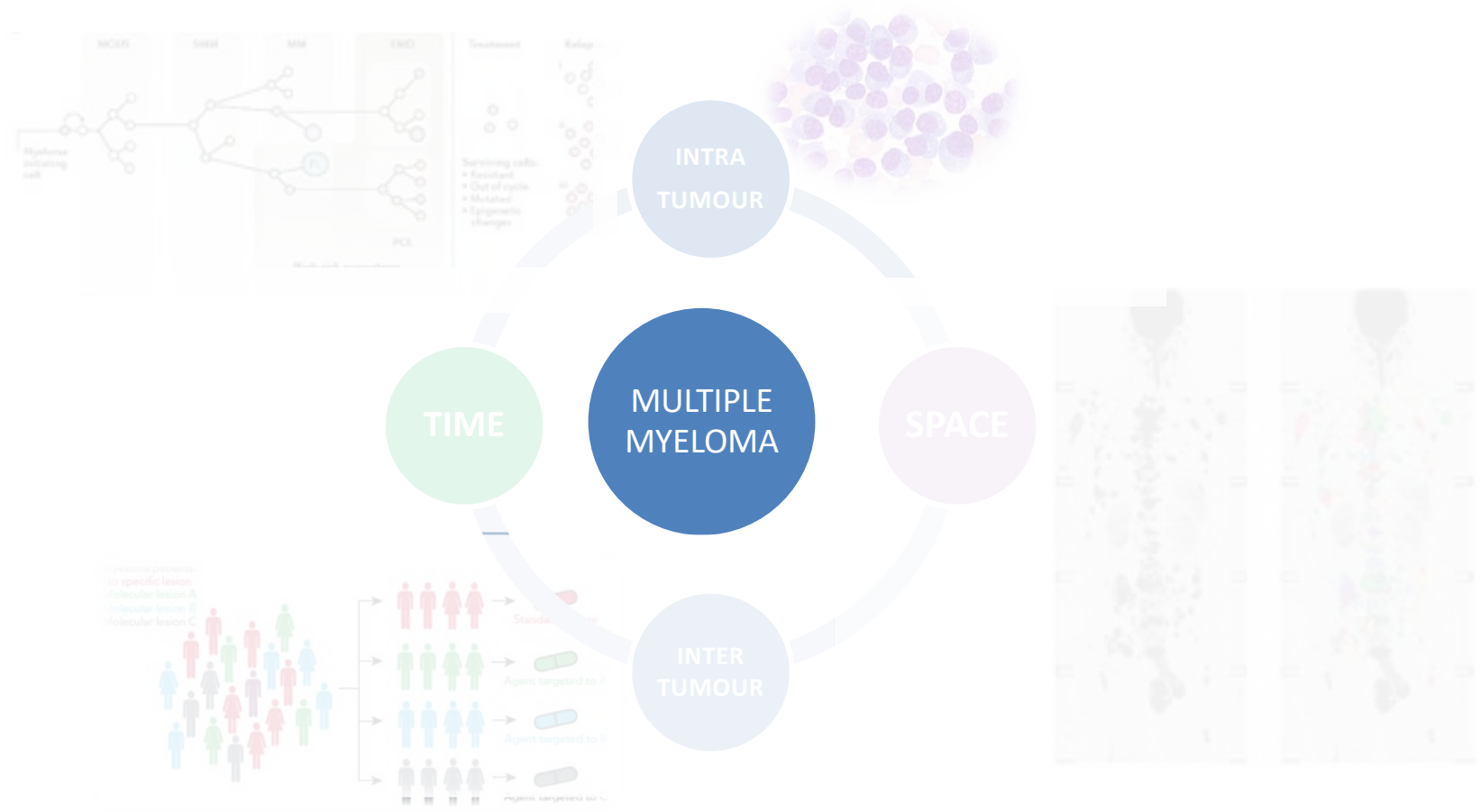
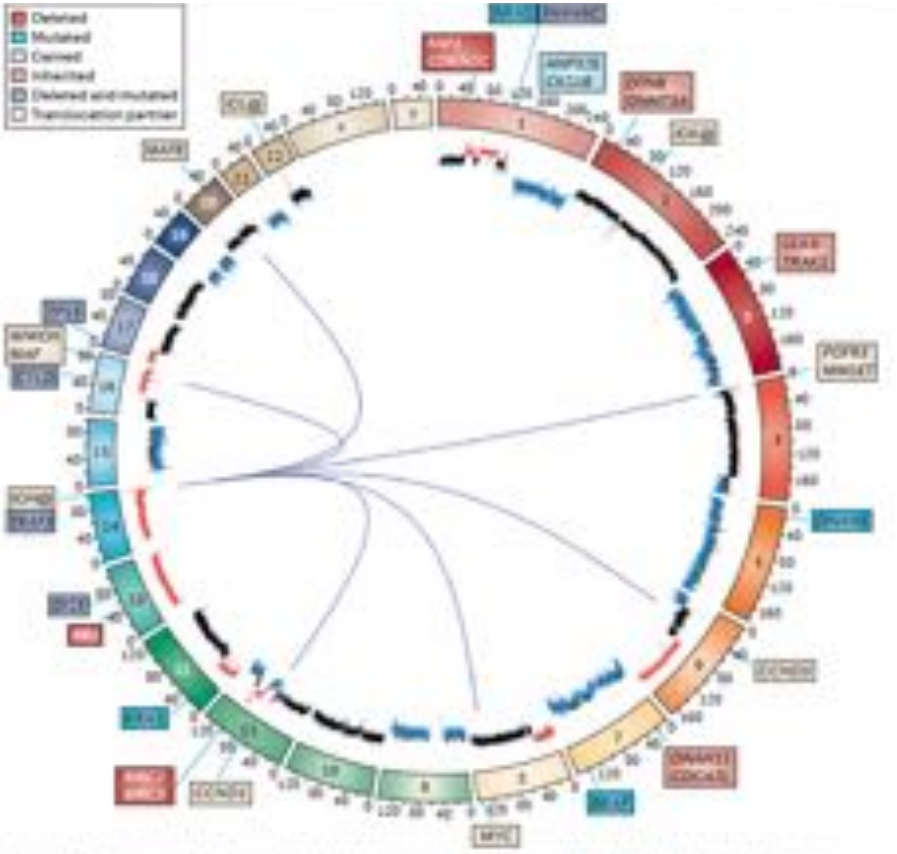
# Different LEVELS of BIOLOGICAL HETEROGENEITY in Multiple Myeloma (MM)



# INTRA-TUMOUR HETEROGENEITY
## Genomic complexity



- **Chromosome translocations**
- **Copy number abnormalities**
- **Mutations**

Clonal vs. Subclonal

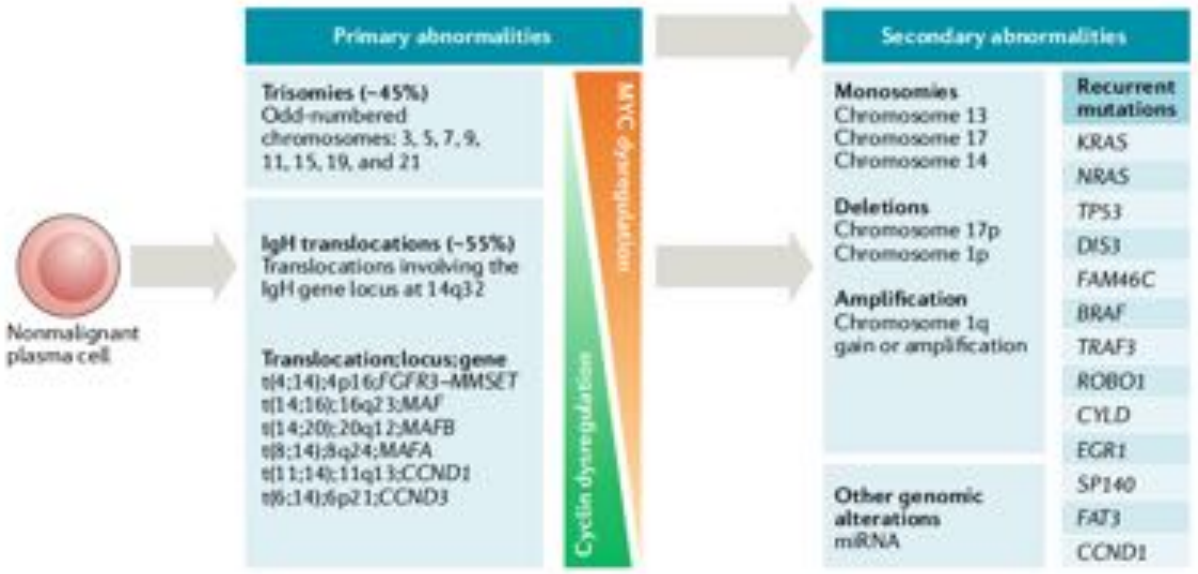
Drivers vs. Passengers

*Morgan GJ et al., Nat Rev Cancer 2012*

# INTER-TUMOUR HETEROGENEITY
## Primary and Secondary abnormalities

→**PRIMARY**: **Hyperdiployd pts 45% vs. IgH translocations pts 55%**

→**SECONDARY**: **monosomies, deletions, amplifications, recurrent mutations, others (e.g. miRNA)**

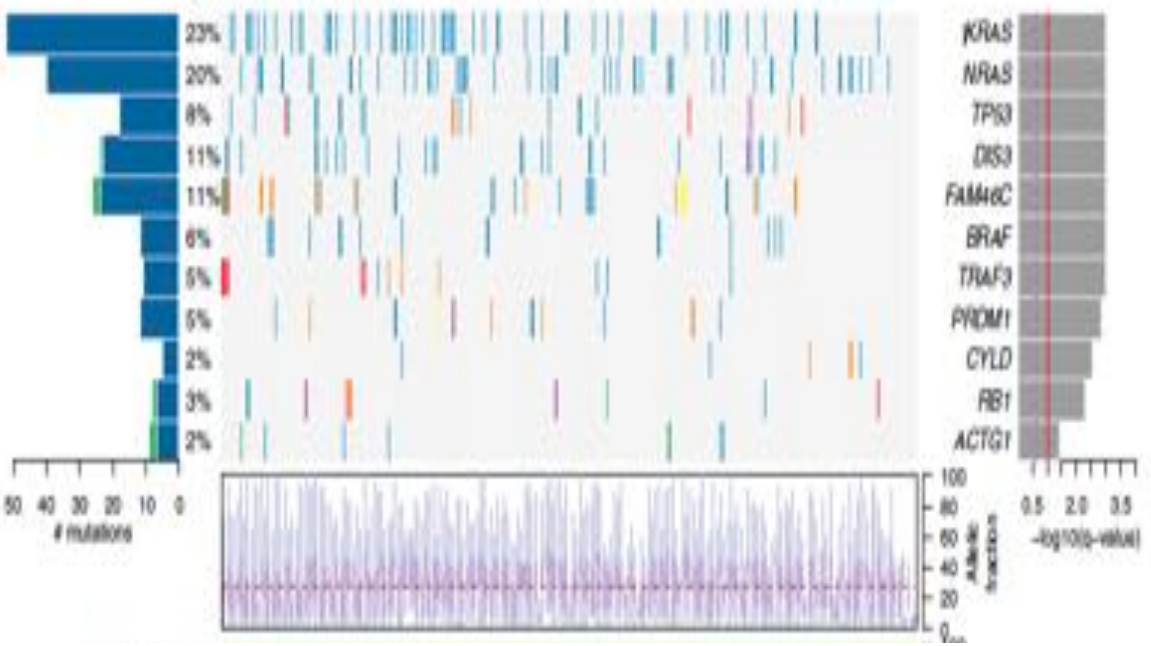


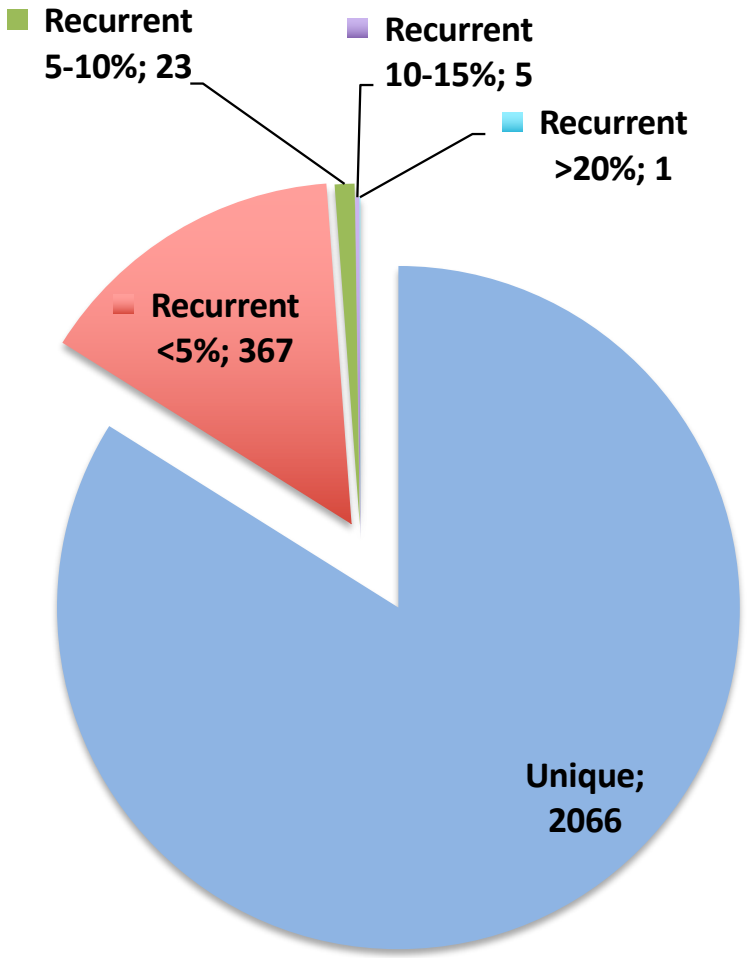

*Kumar SK et al., Nat Rev Clin Oncology 2018*

# Genetic heterogeneity in MM

| | |
|---|---|
| Total n. of genes found in screen | 2462 |
| Cancer Census* Genes | 83 |
| Non Cancer Census Genes | 2379 |

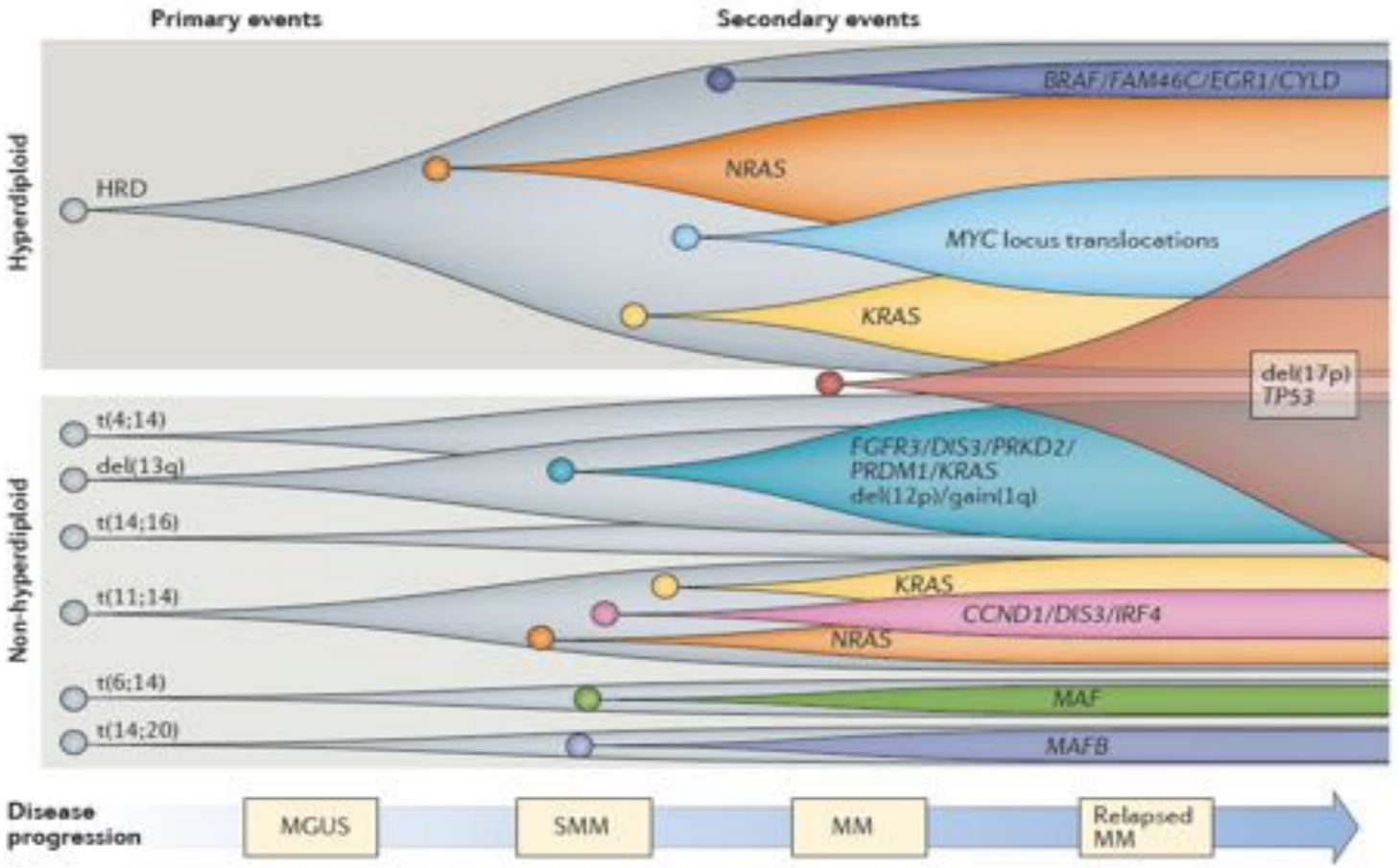
| | |
|---|---|
| Recurrent ≥2 | 396 |
| Unique | 2066 |



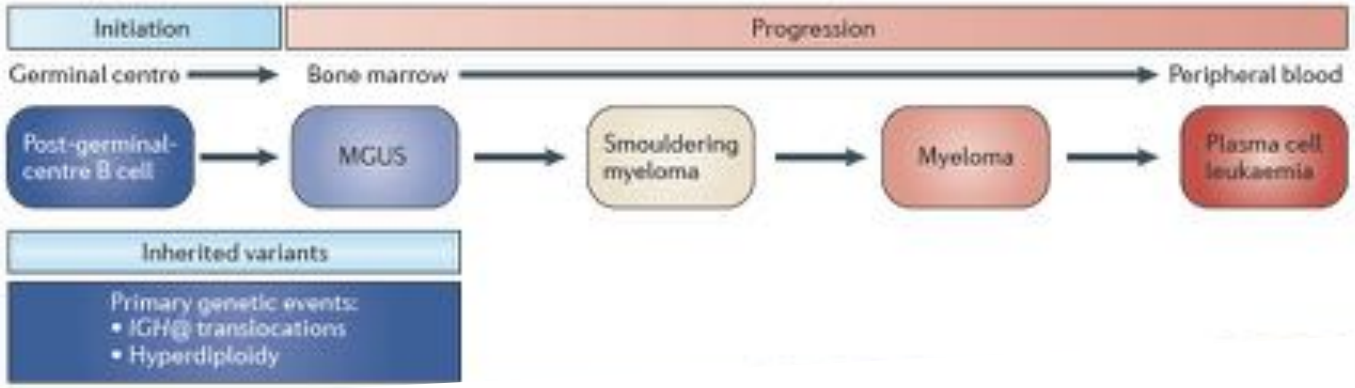

Bolli et al, Nat Comm 2014; Lohr et al, Cancer Cell 2014

# Molecular Pathogenesis and genetic architecture of MM




*Manier S. et al, Nature Reviews 2016*

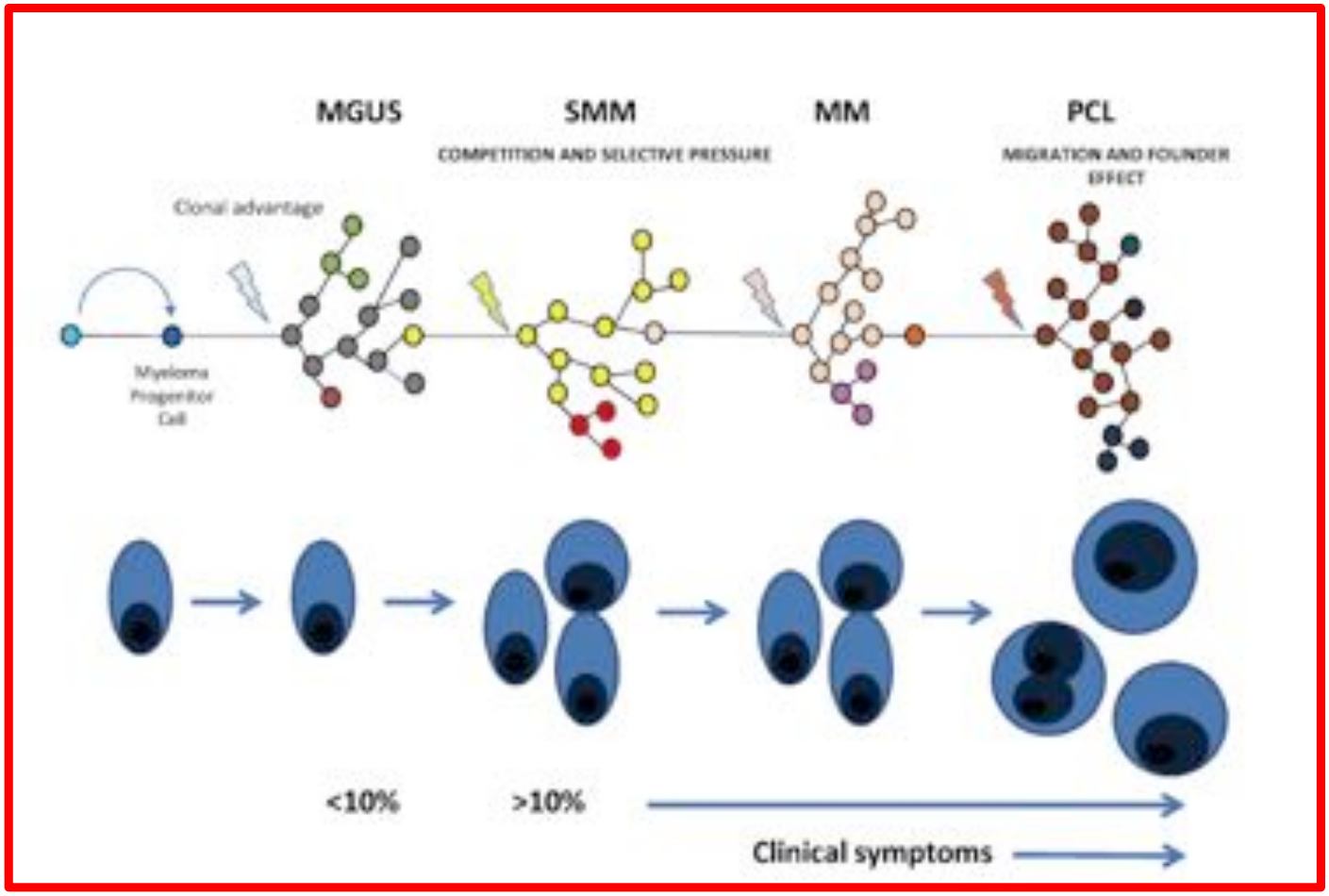
# TEMPORAL HETEROGENEITY
## Multistep progression




→ **Relevance in order to PREVENT THE ONSET OF MM**

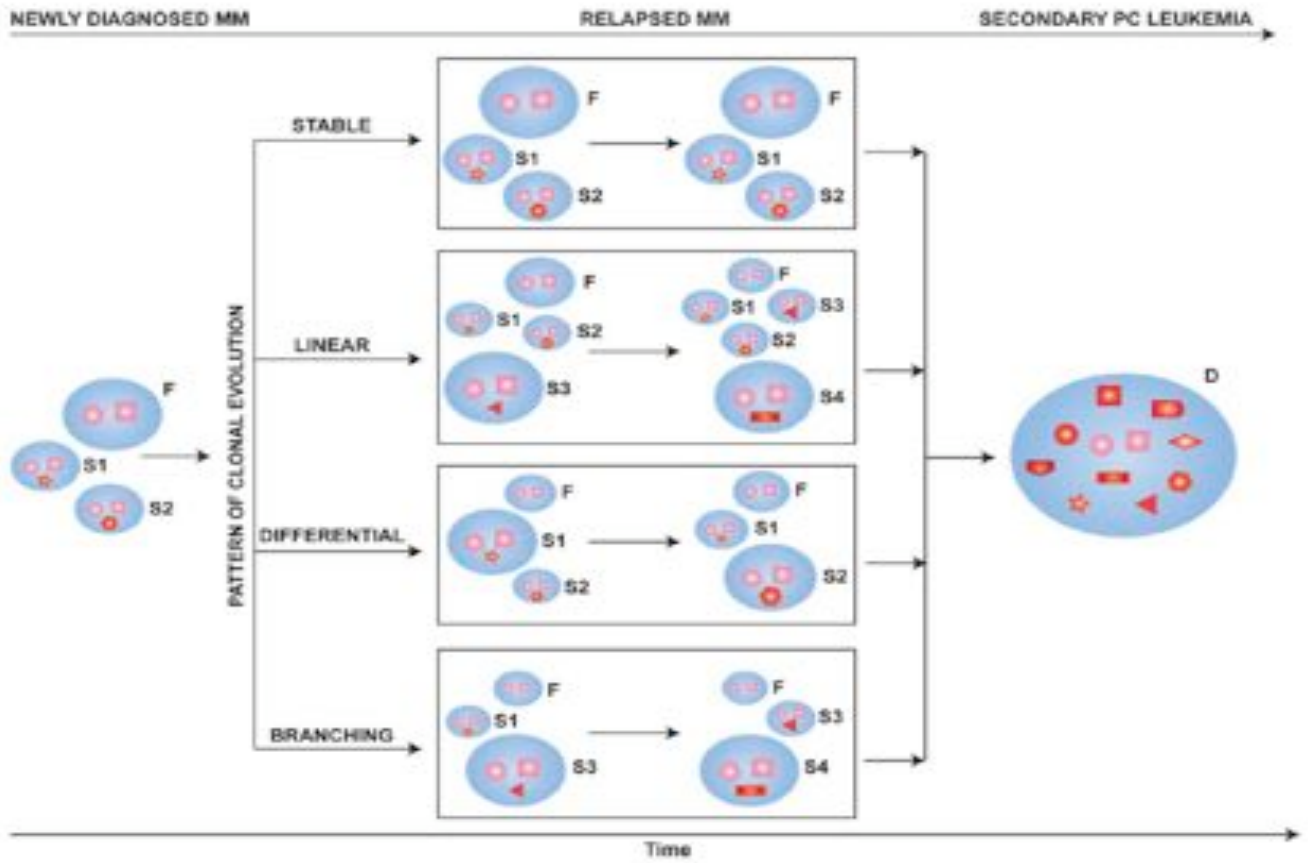
*Morgan GJ et al., Nat Rev Cancer 2012*
*Manier S et al., Nat Rev Clin Oncology 2017*

# TEMPORAL HETEROGENEITY in MM
## Clonal Evolution




*Morgan GJ, Hematology 2012*

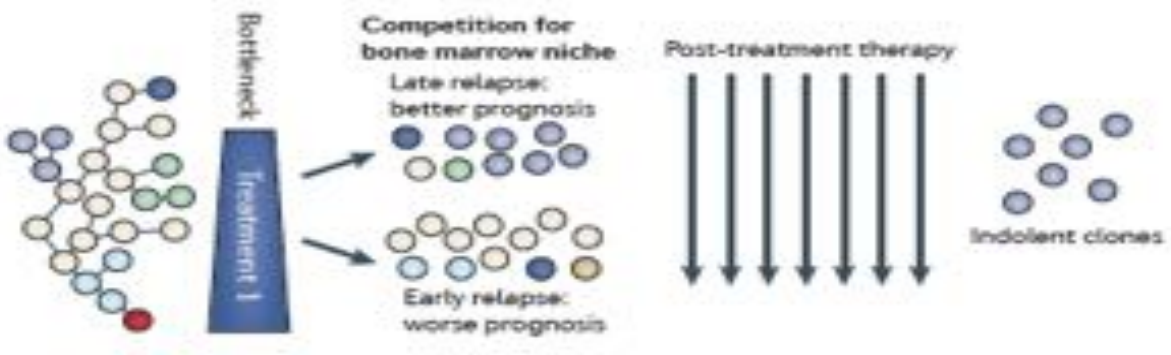
# Mechanisms of clonal evolution in MM




*Bianchi G et al. Ca Cancer J Clin 2014*

# Mechanisms involved in the sub-clonal selection in MM

- Therapeutic pressure

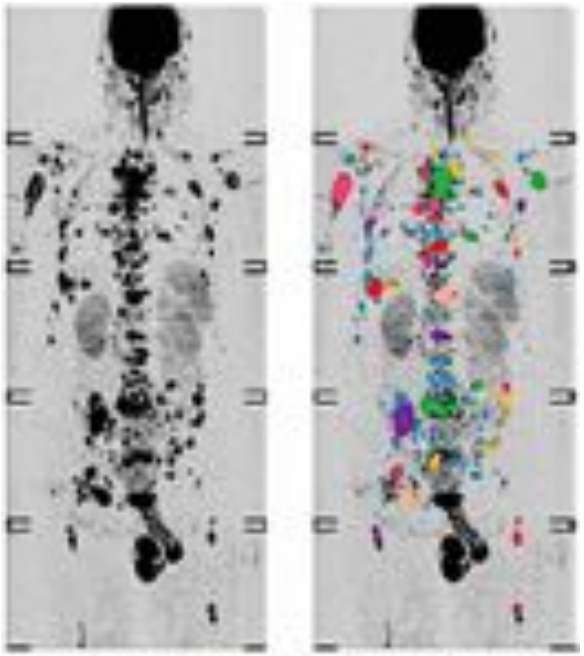



- Natural history of the disease
  - Different growth potential of the sub-clones
  - Effect of the microenvironment

*Gareth J., Nature Rev. 2012*

# SPATIAL HETEROGENEITY
# MM as a model for the process of metastasis

Presence of multiple myelomatous “omas” throughout the skeleton, indicating that there is continuous trafficking of tumor cells to multiple areas in the bone marrow niches

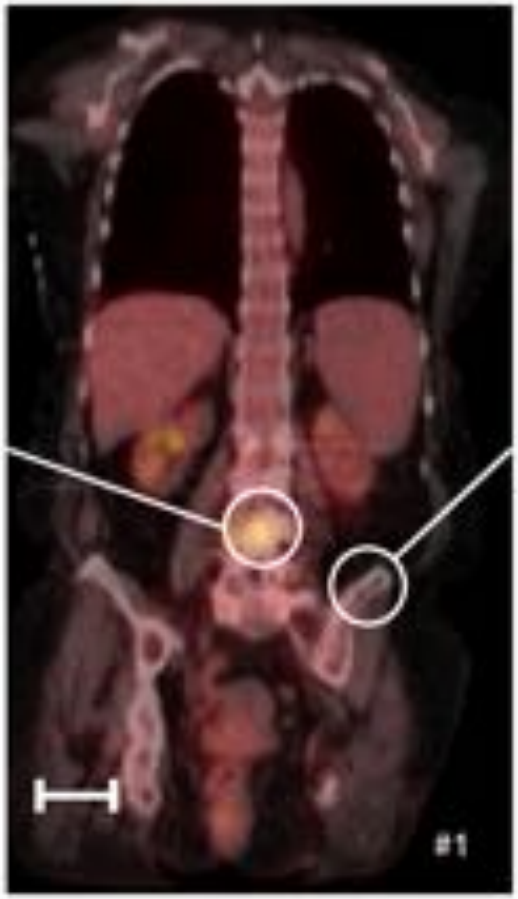
- According to recent multi-region sequencing studies spatial genomic heterogeneity is a common phenomenon in myeloma
- Tumor driver mutations and high-risk genomic aberrations can be restricted to one focal lesion and absent at other FLs or the iliac crest

*Ghobrial I. et al, Blood 2012*
*Rasche L et al., Int Journal of Mol Sciences 2019*

# SPATIAL HETEROGENEITY
## Genomically different focal lesions

Focal lesion at 4th lumbal vertebra:

- GEP70 high risk
- Non-Hyperdiploid
- Del(1p12)
- Del(1p32)
- Del(13q)
- Biallelic *TP53* del




Left iliac crest:

- GEP70 low risk
- Hyperdiploid
- t(MYC)
- $BRAF^{V600E}$

**38/51 (75%) pts**

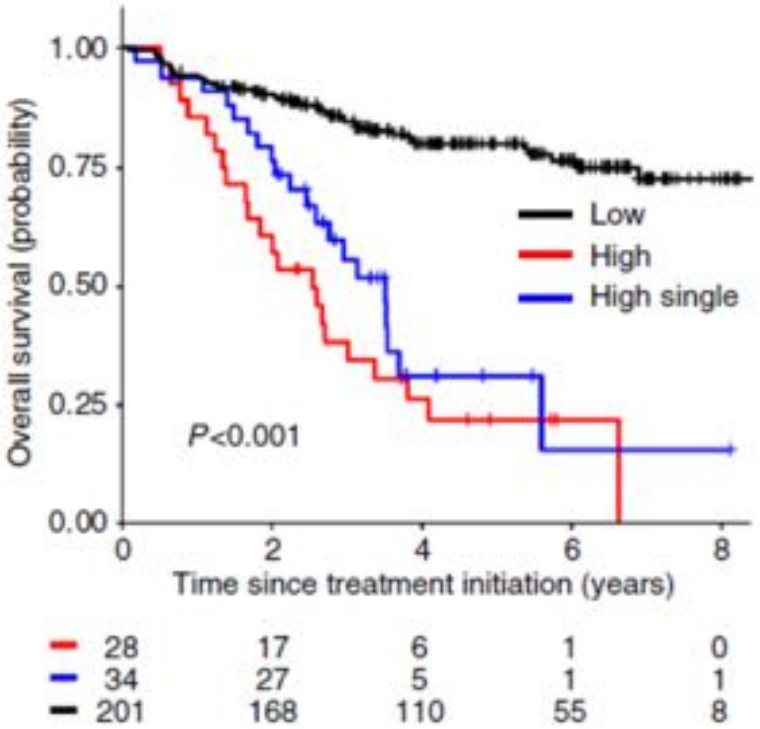
**EVIDENCE OF SPATIAL HETEROGENEITY**

**Equally distributed over all genome**

**Both primary and secondary abnormalities**

*Rasche L et al., Nat Comm 2017*

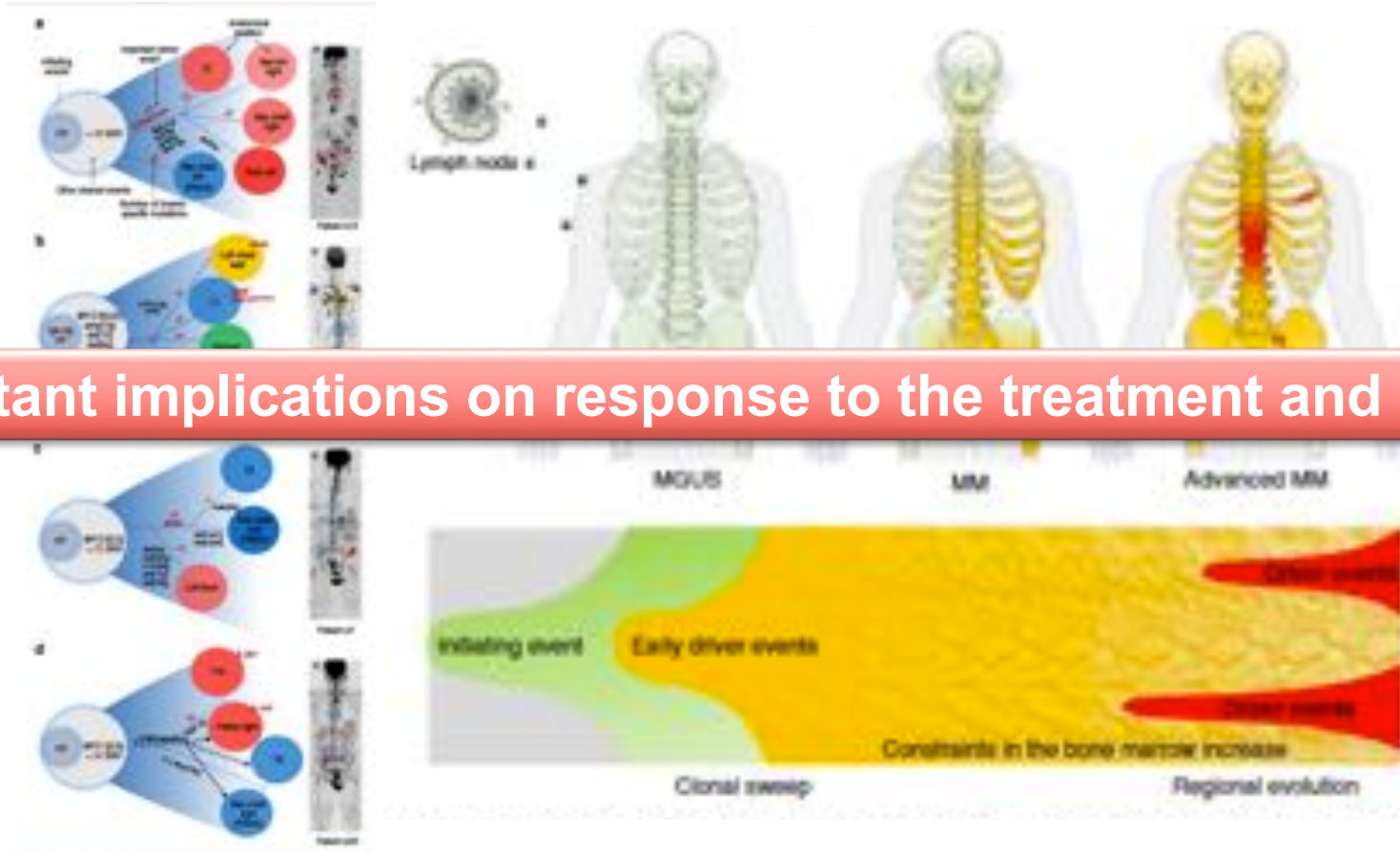
# Impact of regional high risk diseas on survival




- ✓ A **worse outcome** for cases with a **non-homogenous** distribution of GEP70 high-risk clones
- ✓ High-risk subclones **drive** prognosis even if they are not ubiquitously distributed

Rasche et al, Nature Comm 2017

# Regional evolution in MM




**Important implications on response to the treatment and MRD**

*Rasche L et al., Nat Comm 2017*

# Clinical hallmarks of high-risk MM

✓ 20-30% [1,2]

✓ Clinical **aggressive** behaviour → Early **relapse**

✓ Clinical **aggressive** behaviour → Treatment **Refractory**

✓ **PFS** < 18 months or **OS** < 2-3 years[1,2]

1. Barlogie B et al, Blood 2014; 2. Kumar S et al, Leukemia 2013

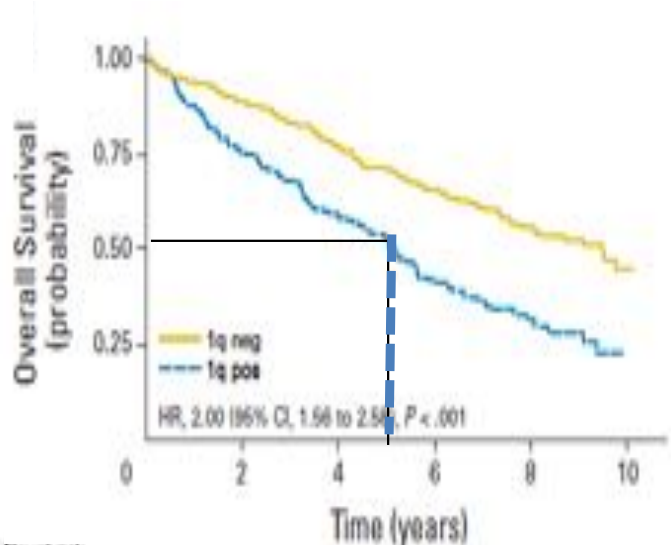
# Recognizing genetic high-risk feature in MM

Table 1 A comparison between the genetic and molecular markers in the RISS and mSMART stratification

| Stage | RISS | | mSMART | | Risk |
|---|---|---|---|---|---|
| I | Serum albumin $\geq 3.5$ g dL$^{-1}$<br>Serum β2M $< 3.5$ mg L$^{-1}$<br>No high-risk cytogenetics<br>Normal LDH level | 5 year OS 82% | t(11;14)<br>t(6;14) | Median OS<br>8–10 years | Standard |
| II | Not fitting stage I or III | 5 year OS 62% | t(4;14)<br>del(13)<br>Hypodiploidy<br>PCLI $\geq$ 3% | Median OS<br>4–5 years | Intermediate |
| III | Serum β2M $> 5.5$ mg L$^{-1}$<br>High-risk cytogenetics: t(4;14), t(4;16), or del(17p) or elevated LDH | 5 year OS 40% | del(17p)<br>t(14;16)<br><br>t(14;20)<br>GEP high-risk signatures | Median OS<br>3 years | High |

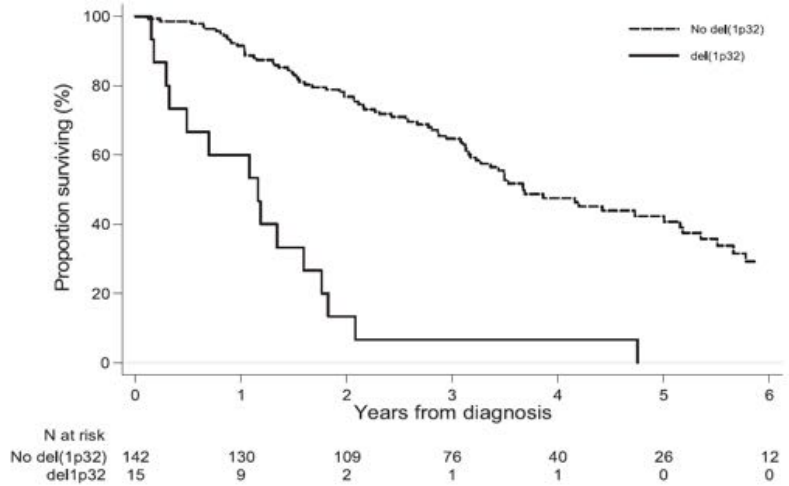
# Recognizing high-risk: Chr1 abnormalities?

1q gains: 30-35% of patients



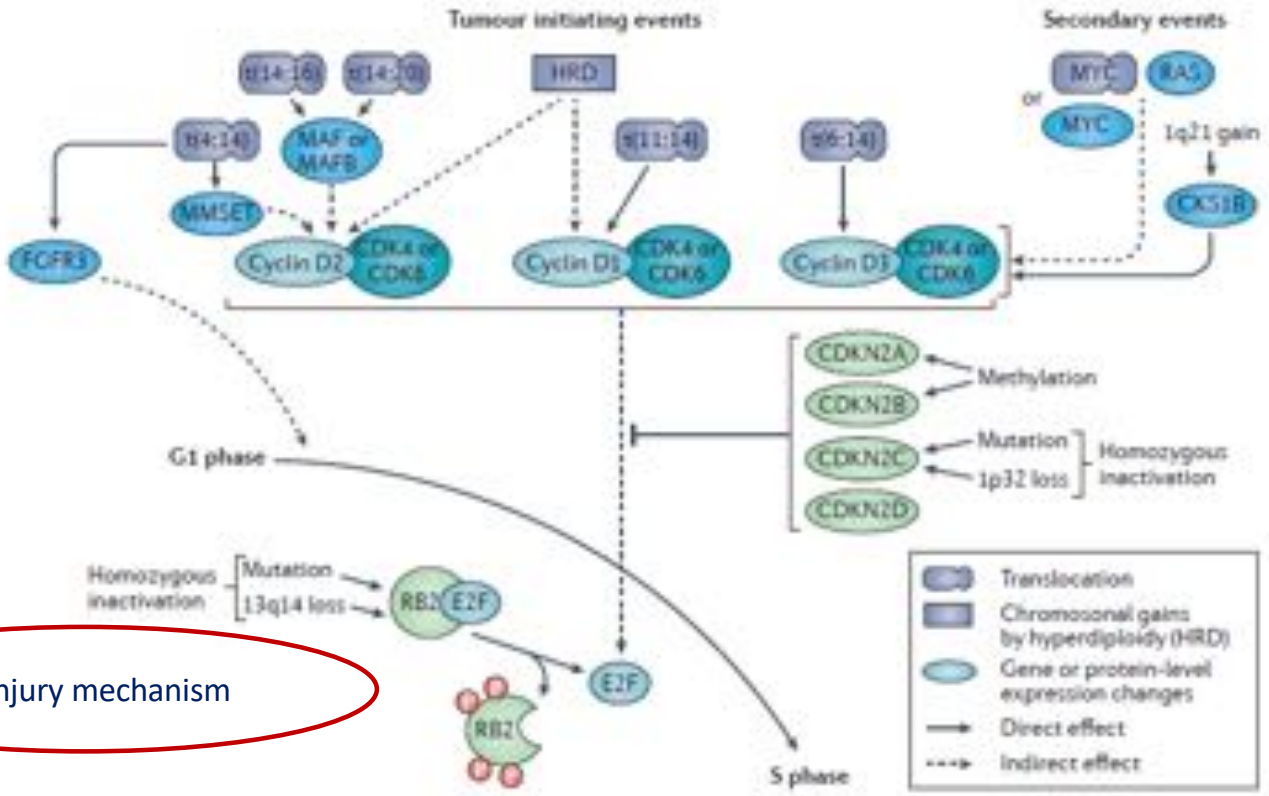

Avet-Loiseau et al, J Clin Oncol 2012

Del(1p32)




Hebraud et al, Blood 2015

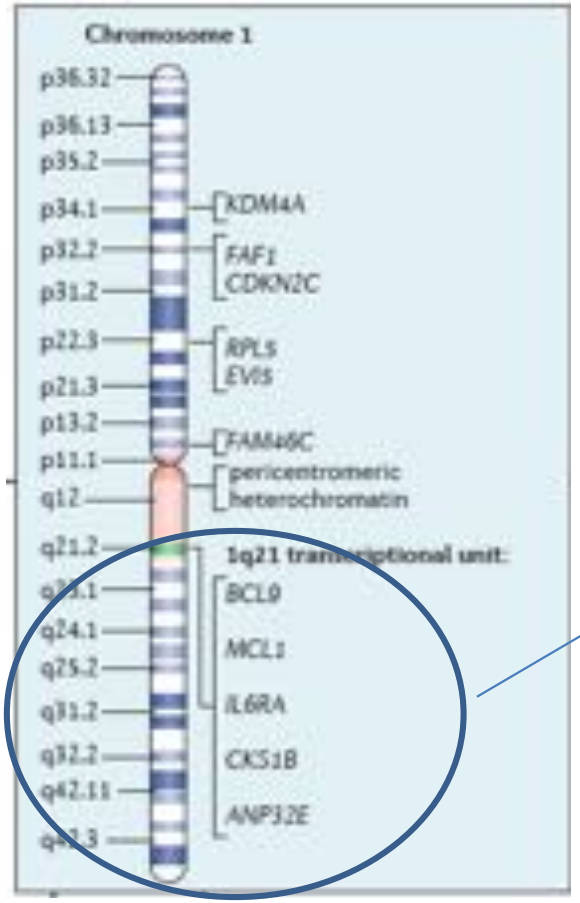
# Convergent evolutionary route to high-risk MM via cell cycle dysregulation




Multiple-injury mechanism

Pawlyn et al, Nature Reviews 2017

# Copy Number Abnormalities: Chr1




**amp or gain(1q21)**

MCL1
BCL9 → Anti-apoptotic , proliferation

CSK1B → Cellular proliferation through p27 degradation and cyclin-dependent kinases

Pawlyn et al, Nature Reviews 2017

# Copy Number Abnormalities: del(17p)

✓Whole arm-level **aberrations** and whole-arm **jumping translocations** are associated with **high-risk** myeloma

✓**TP53** gene at the short arm of chromosome 17

**Small sub-clones** with monoallelic inactivation of TP53 (<20%)

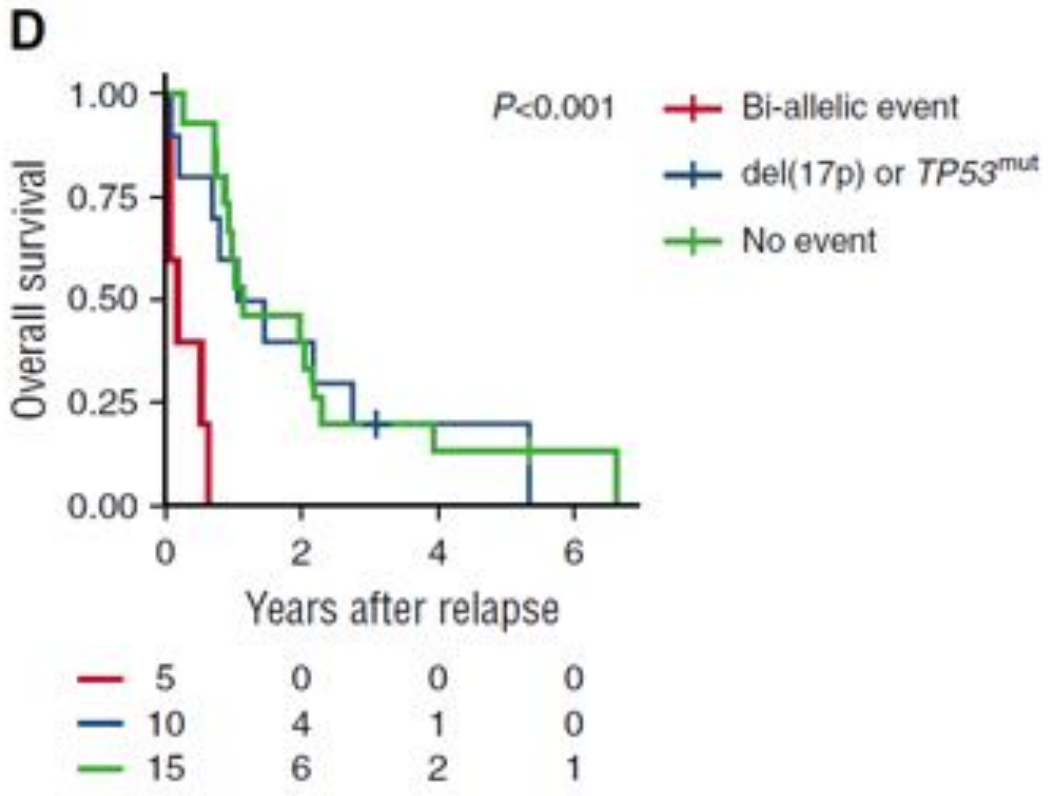
Vs.

**Larger clones** with monoallelic inactivation of TP53 (50-60%)

→ **High-risk**

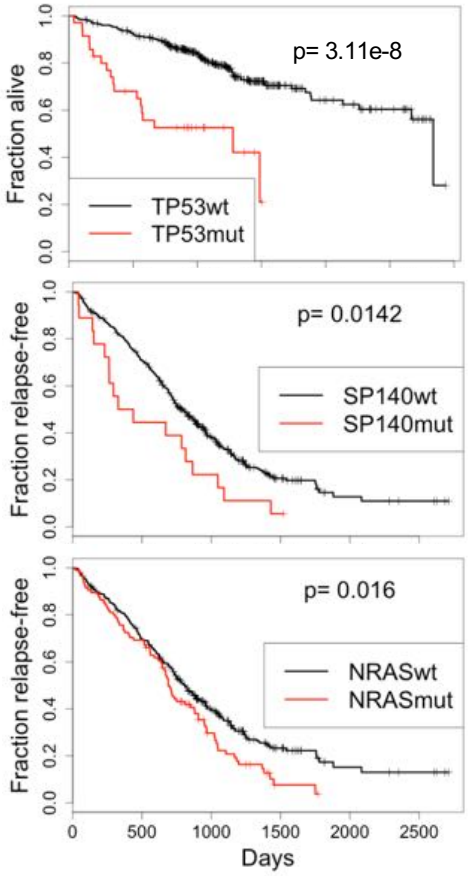
Lionetti et al, Oncotarget 2016; Pawlyn et al, Nature Reviews, 2017

# TP53 bi-allelic events identify aggressive clinical course in relapsed MM patients

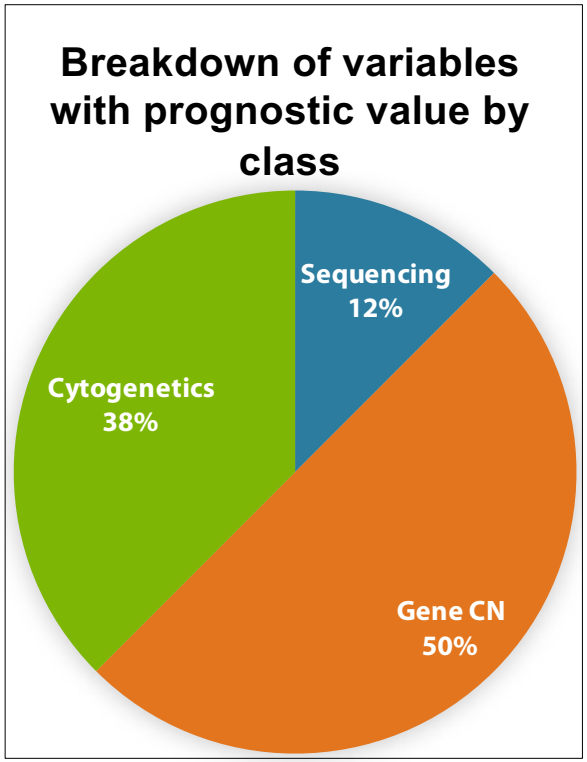



Weinhold et al , Blood 2016

# Copy number and karyotype dominate the landscape of negative prognostic variables



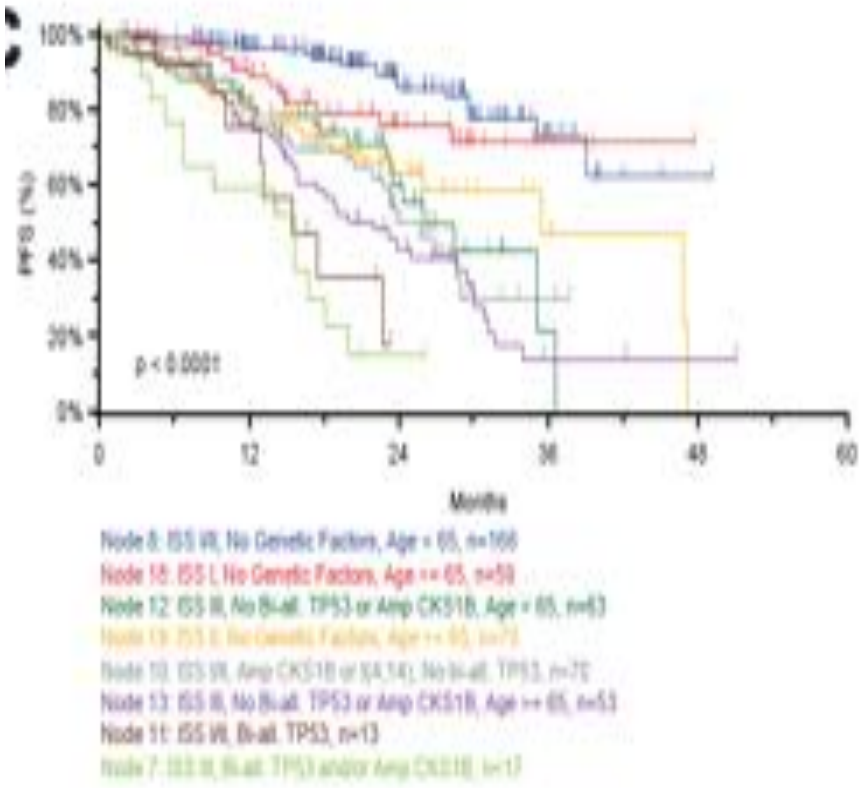

| | PFS | OS |
|---|---|---|
| TP53 | | |
| NRAS | | × |
| SP140 | | × |
| APC_del | × | |
| CYLD_del | | |
| FAM46C_del | × | |
| FAT1_del | | |
| FAT3_del | | |
| SNX7_del | | |
| TP53_del | | |
| CDKN2C_del | | × |
| MYC_amp | | × |
| PRDM1_del | | × |
| SP140_del | | × |
| del1p | | |
| amp1q | | |
| del12p13.31 | × | |
| del13 | | |
| del16q | | |
| del17p13 | | |
| t(14:20) | | |
| t(4:14) | | |
| t(8:14) | | × |

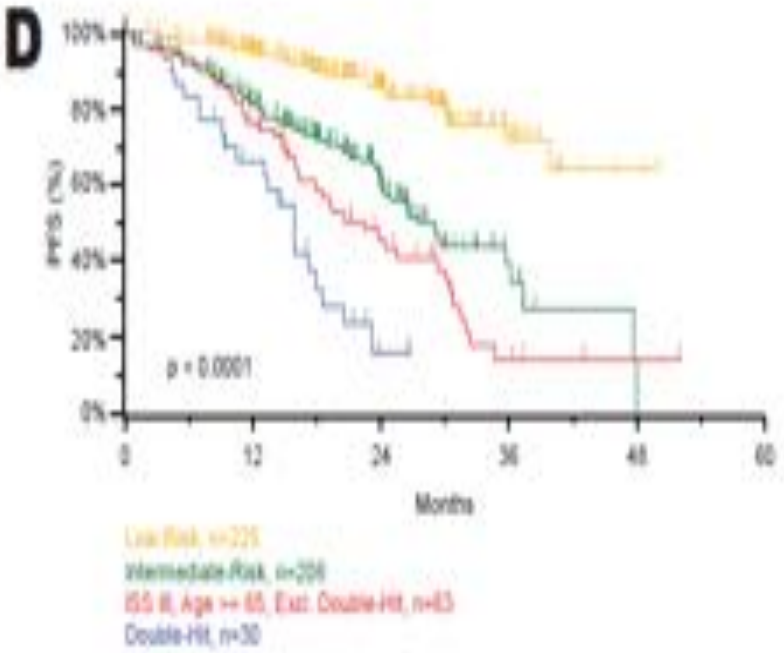



= $p < 0.05$ on univariate analysis

Bolli et al, Leukemia 2017

# Redefining High-risk MM




**High-risk segment defined by ISS III and either**

a) **biallelic *TP53* inactivation or**

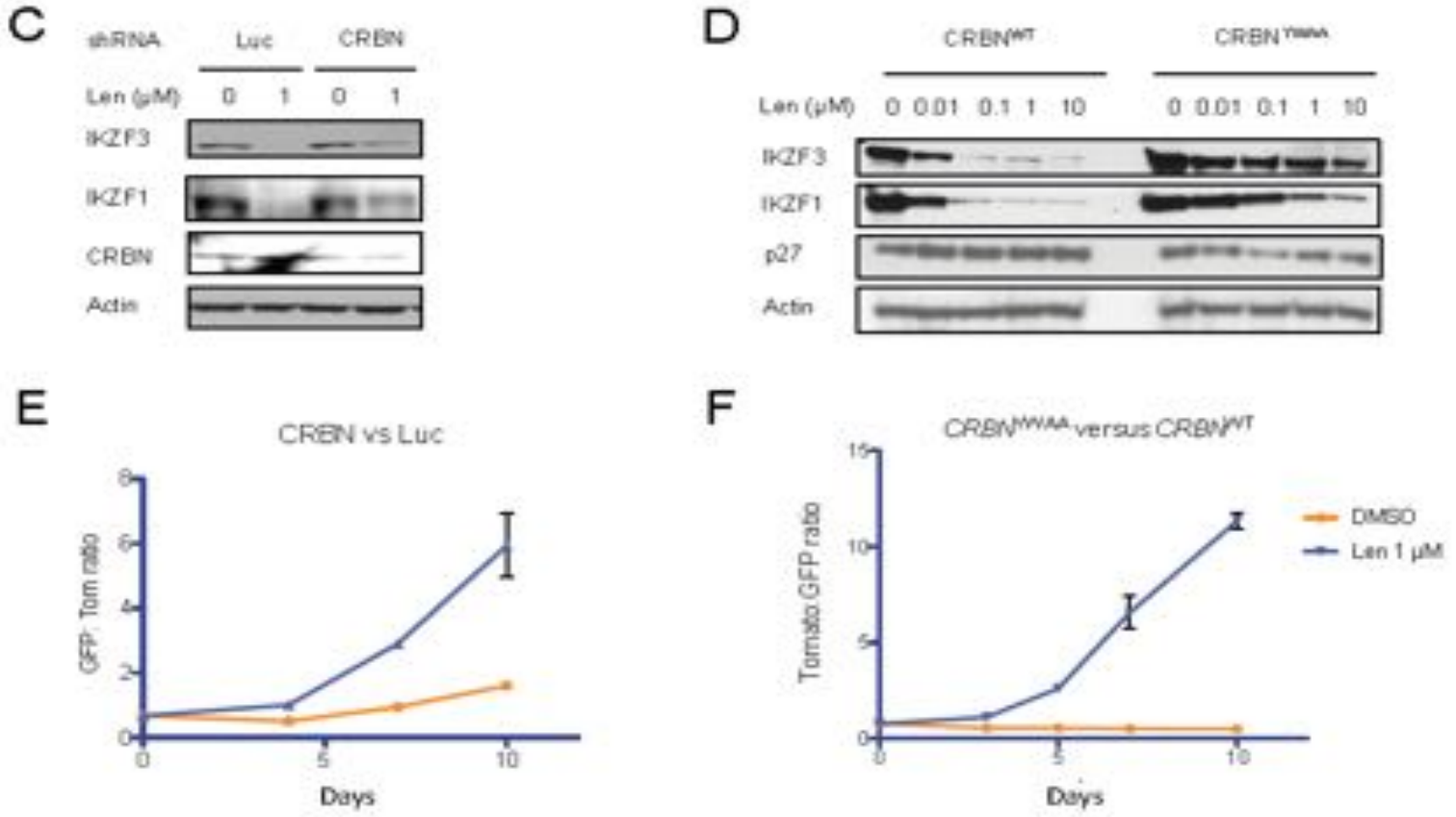
b) **amplification of *CKS1B* (1q21)**

Walker et al. Leukemia 2019

# Mechanisms of drug resistance in the “era” of new drugs

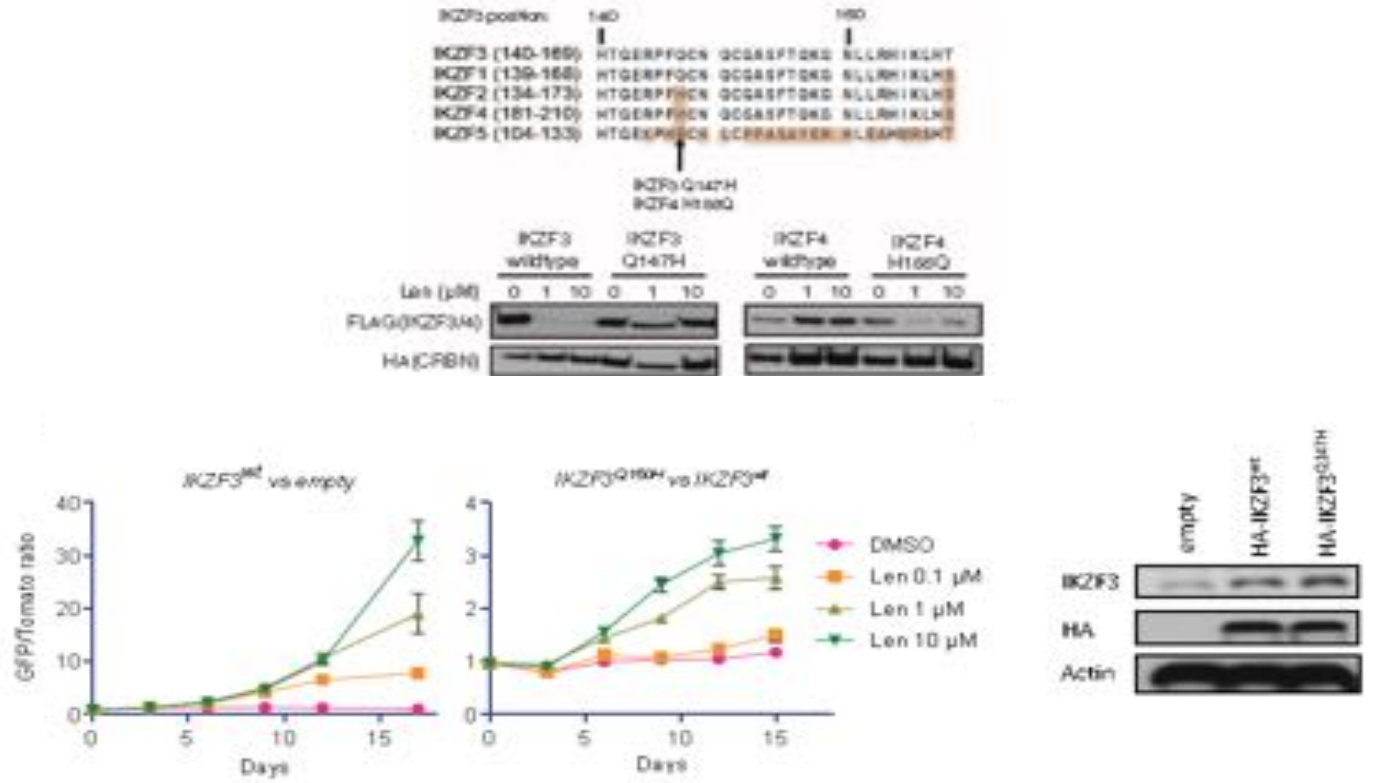
- **Cytogenetics and epigenetic alterations**
- **Clonal evolution and escape**
- **Mutational changes**
- Microenvironment changes
- MM cancer stem cells
- Metabolic reprogramming

# CRBN down-regulation or mutations induce LEN resistance in MM cells



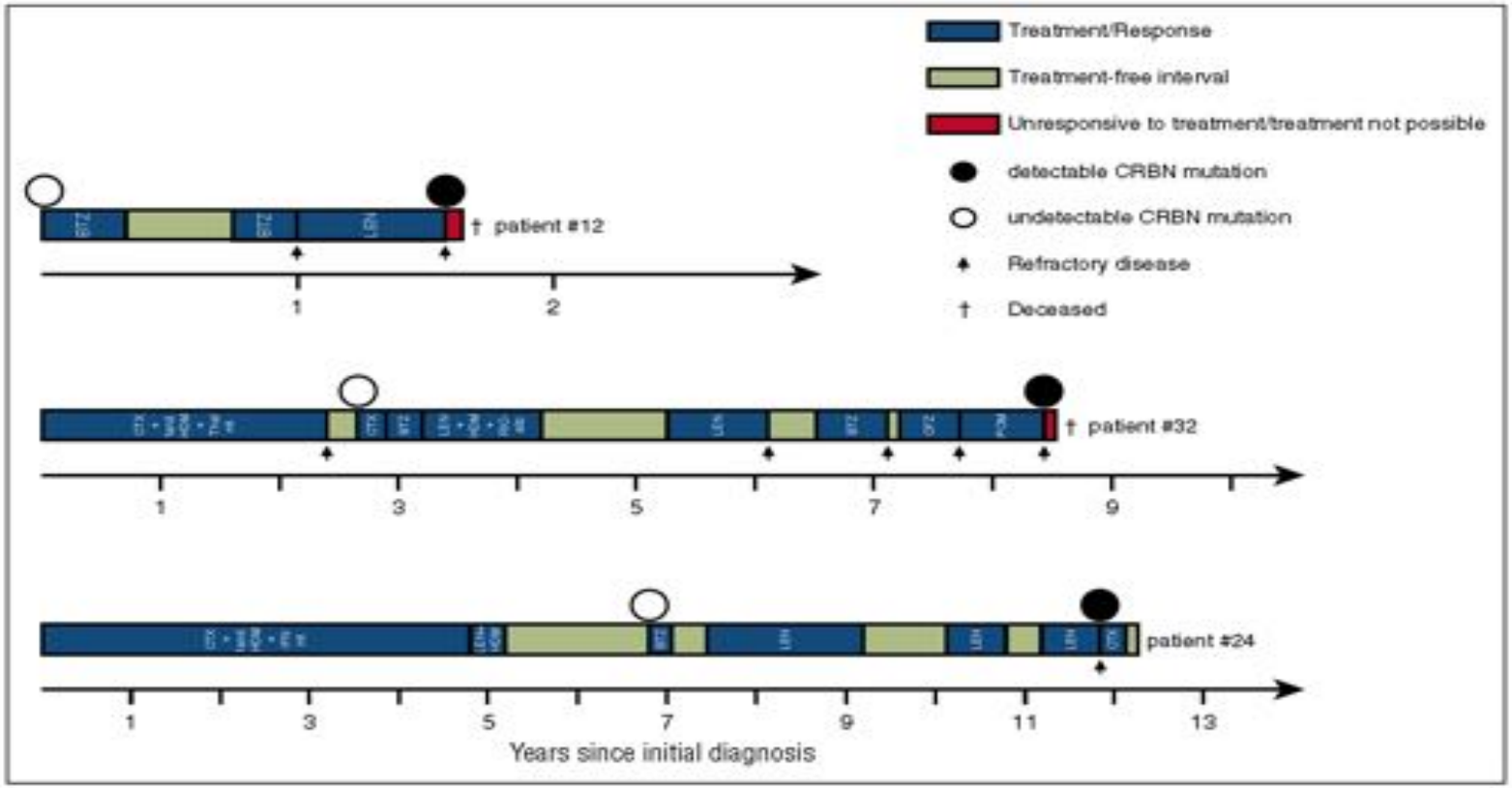

*KrÖnke J et al, Science 2014*

# IKZF3 mutations or overexpression induce LEN resistance in MM cells



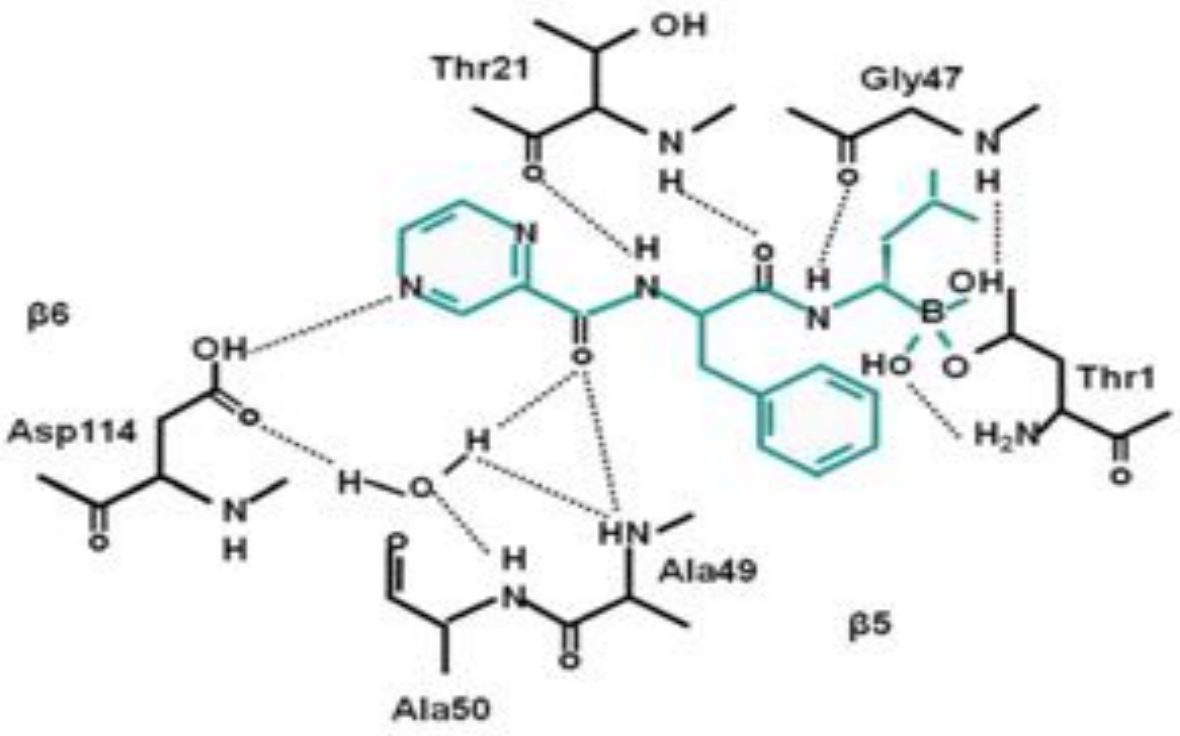

*KrÖnke J et al, Science 2014*

# CRBN mutations and clinical course of MM patients



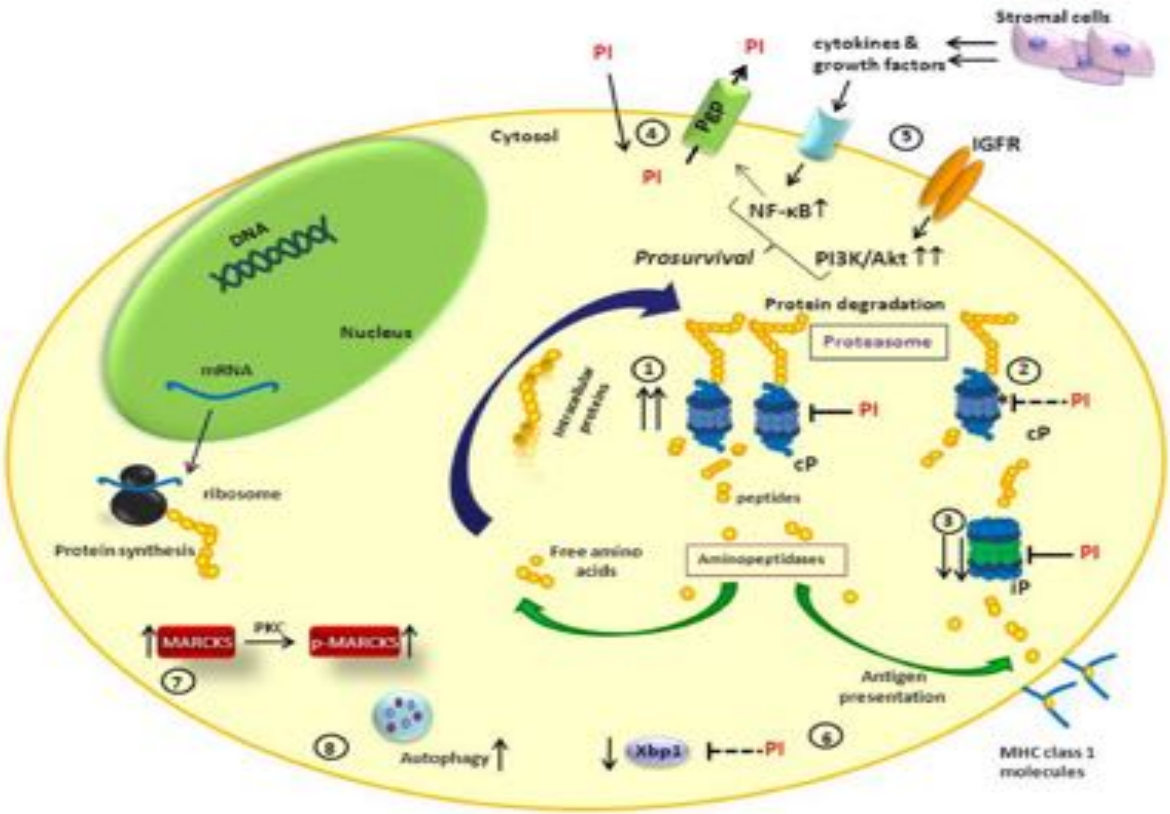

*Kortum KM et al, Blood 2016*

# Interaction of Bortezomib (BOR) and the proteasome subunit β5




Lü S. et al, Biomark Res 2013

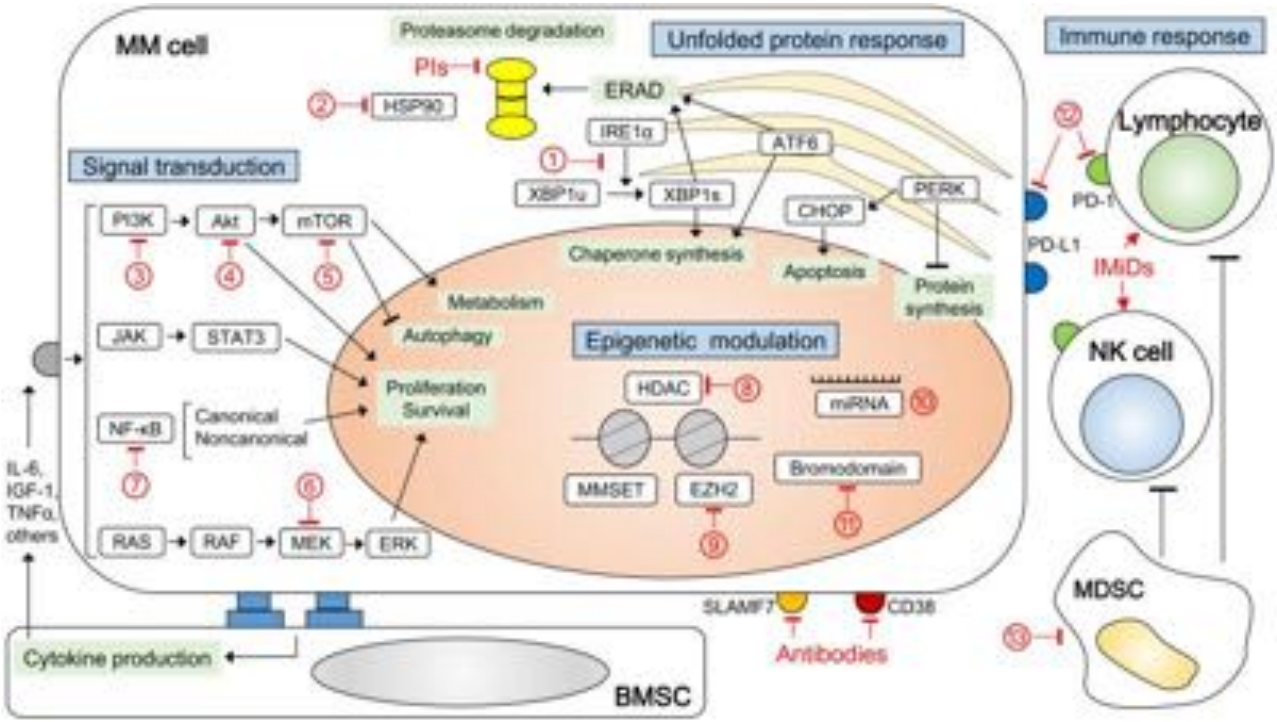
# Molecular mechanisms involved in Proteasome inhibitors (PI)s resistance




1. Up-regualtion of costitutive Proteasome (cP) subunit: β5
2. Point mutations in *PSMB5*
3. Down-regulation of the immunoproteasome (iP) subunit: β5i
4. Cellular extrusion of Pi by the transporter Pgp
5. Activation of pro-survival pathways (i.e NF-kB)
6. Loss of XBP1
7. Increased expression of phosphorylated MARCS
8. Autophagy up-regulation

Niewerth D. et al, Drug Resist Uptdat 2015

# New drugs to overcome drug resistance in MM




**Antibodies**:

- Anti SLAMF7: Elotuzumab,
- Anti CD38: Daratumumab Isatuzimab
- Anti BCMA

1. **IRE1α inhibitors** (MKC-3946, STF-083010 )
2. **HSP90 inhibitors** (17-AAG, TAS-116)
3. **PI3K inhibitors** (CAL-101)
4. **Akt inhibitors** (perifosine, afuresertib, TAS-117, MK-2206)
5. **mTOR inhibitors** (rapamycin, everolimus, temsirolimus)
6. **MEK inhibitors** (selumetinib)
7. **NF-κB inhibitors** (PBS-1086)
8. **HDAC inhibitors** (vorinostat, panobinostat, ricolinostat, BG45)
9. **EZH2 inhibitors** (UNC1999)
10. **Synthetic miRNAs** (miR-29b, miR-34a)
11. **Bromodomain inhibitors** (JQ1)
12. **PD-1/PD-L1 antibodies** (CT-011)
13. **PDE5 inhibitors**

# Take home messages…

- ✓ MM is characterized by high intra-, inter-clonal and spatial genetic heterogeneity.
- ✓ No unique genetic and pathogenetic mechanisms define the high-risk MM.
- ✓ Copy number and karyotype dominate the landscape of negative prognostic variables.
- ✓ Prognostic impact of TP53 mutations but not of the most frequent mutations (KRAS, NRAS, DIS3, BRAF, FAM46C).
- ✓ Several mechanisms are involved in IMiDs and PIs resistance including mutations of CRBN-related pathways and PSMB5.