Unmet challenges in high risk hematological malignancies: from benchside to clinical practice

Scientific Board:
Marco Ladetto (Alessandria)
Umberto Vitolo (Turin)

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Biology of high risk multiple myeloma

Nicola Giuliani, PhD MD
Associate Professor of Hematology
University of Parma

Hallmarks of high-risk multiple myeloma (MM)

- ✓ 20-30% [1,2]
- ✓ Clinical **aggressive** behaviour
- ✓ **PFS** < 18 months or **OS** < 2-3 years[1,2]

→ Early **relapse**

→ Treatment **Refractory**

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

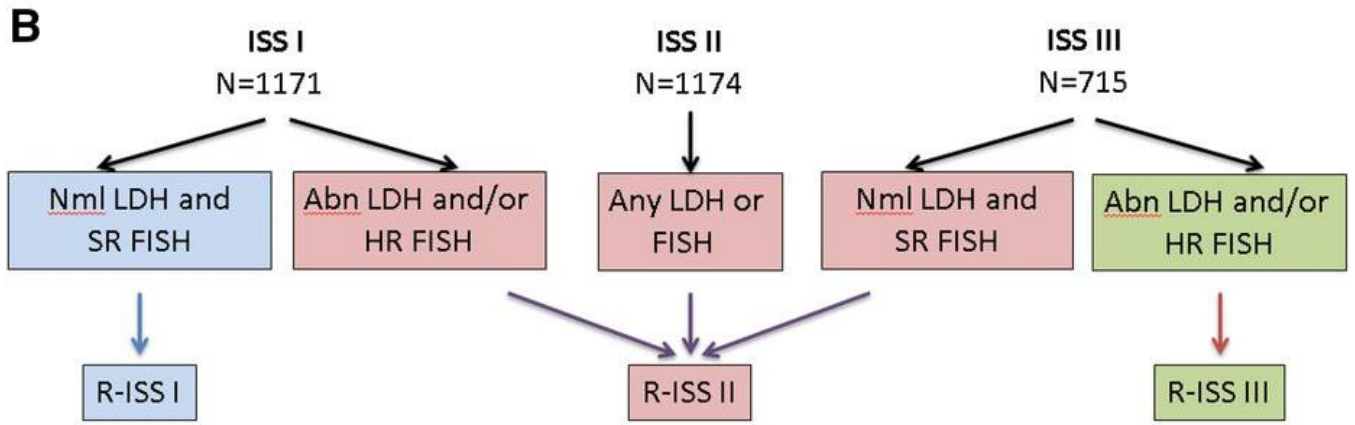
Defining Myeloma Staging

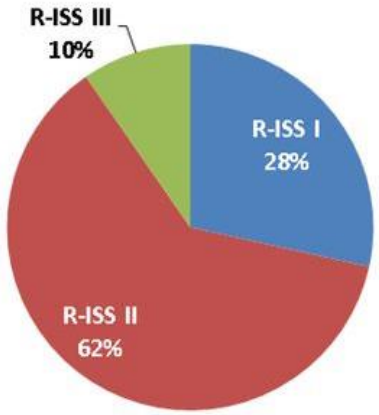
- **Durie Salmon Staging**
- **b2-Microglobulin**

- ISS identifies 3 groups with validated and confirmed survival differences
  - **Stage 1 (b2M <3.5 mg/L & albumin 3.5+ g/dL) - 62 Mos**
  - **Stage 2 (Neither Stage 1 or 3*) - 44 Mos**
  - **Stage 3 (b2M 5.5+ mg/L) - 29 Mos**
- ISS prognostic regardless of
    - Age, geographic region, individual institution or cooperative group, standard or transplant therapy, method of albumin

Recognizing high-risk: R-ISS

R-ISS 1
ISS I
Normal LDH
Normal FISH





	R-ISS I (N=871)	R-ISS II (n=1894)	R-ISS III (n=295)
5-year PFS, % (n=3060)	54	36	22
5-year OS, %			
All (n=3060)	81	60	40
ASCT (n=1998)	83	62	39
No ASCT (n=1062)	75	52	47

R-ISS 3
ISS III
AbN LDH &/or
AbN FISH

Adverse FISH; t(4;14), t(14;16), del17p

Palumbo A et al J Clin Oncol 2015; *Dispenzieri et al, Hematology Am Soc Hematol Educ Program. 2016*

mSMART 2.0: Classification of Active MM

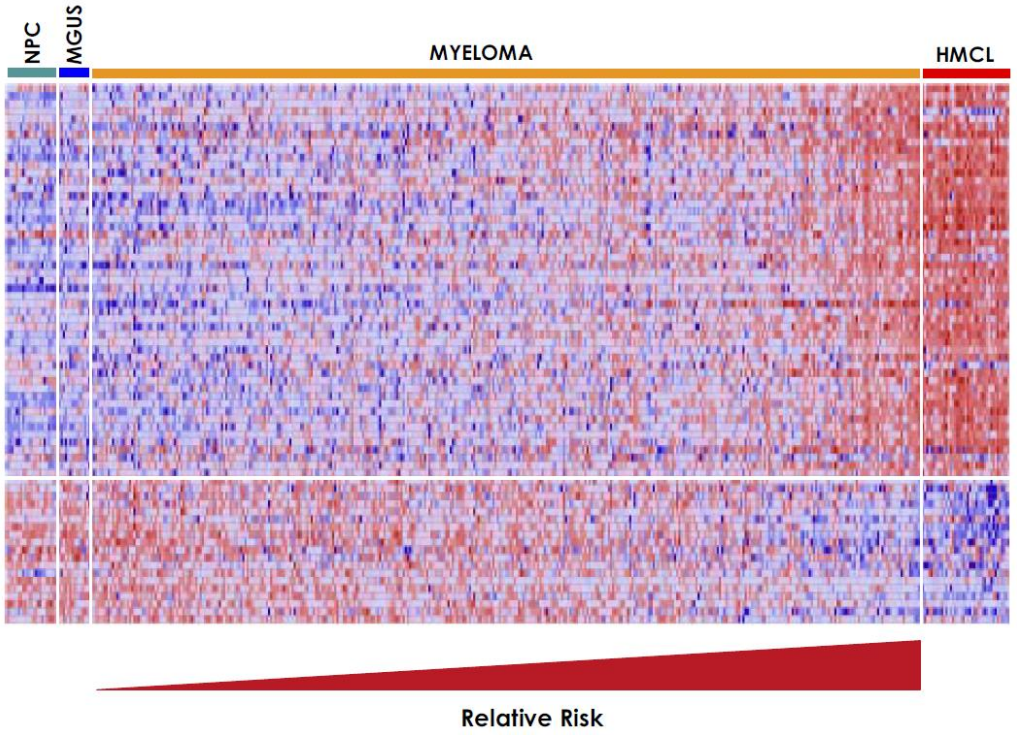
20%	20%	60%
High-Risk	**Intermediate-Risk***	**Standard-Risk*†**

▪ FISH
  ▪ Del 17p
  ▪ t(14;16)
  ▪ t(14;20)
▪ GEP
  ▪ High risk signature
3 ys
	▪ FISH
	  ▪ t(4;14)‡
	▪ Cytogenetic Deletion 13 or hypodiploidy
	▪ PCLI ≥3%
	4-5 ys
		All others including:
		▪ Hyperdiploid
		▪ t(11;14)**
		▪ t(6;14)
		10 ys

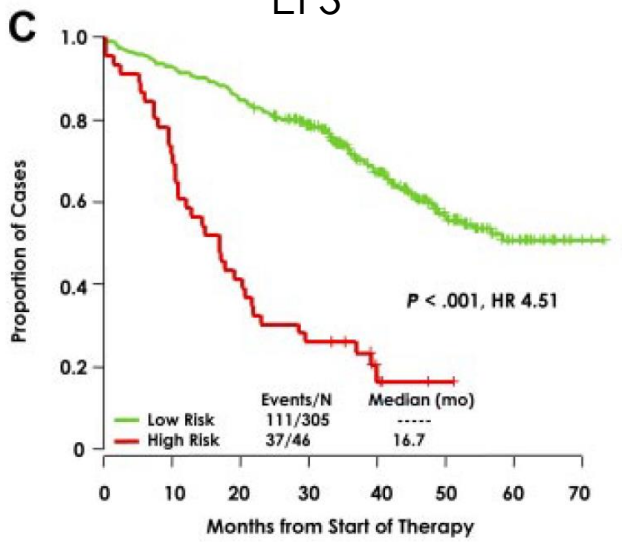
* Note that a subset of patients with these factors will be classified as high-risk by GEP
† LDH >ULN and beta-2 M > 5.5 may indicate worse prognosis
‡ Prognosis is worse when associated with high beta-2 M and anemia
**t(11;14) may be associated with plasma cell leukemia

Dispenzieri et al. Mayo Clin Proc 2007;82:323-341; Kumar et al. Mayo Clin Proc 2009 84:1095-1110
v8 Revised and updated: Feb 2011

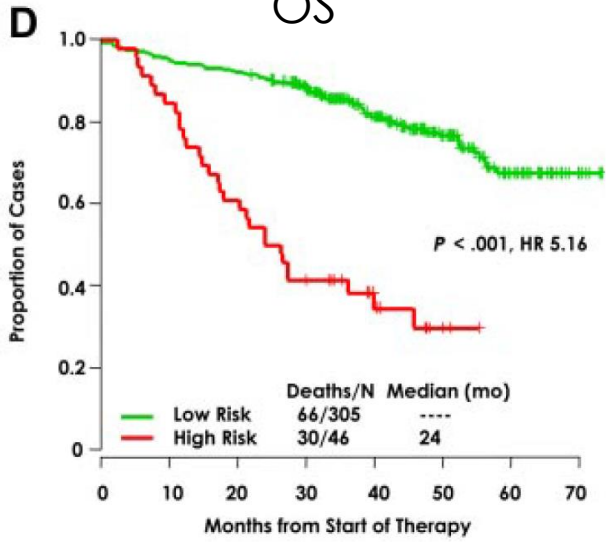
Expression-based Definition of Aggressive Disease (GEP70)



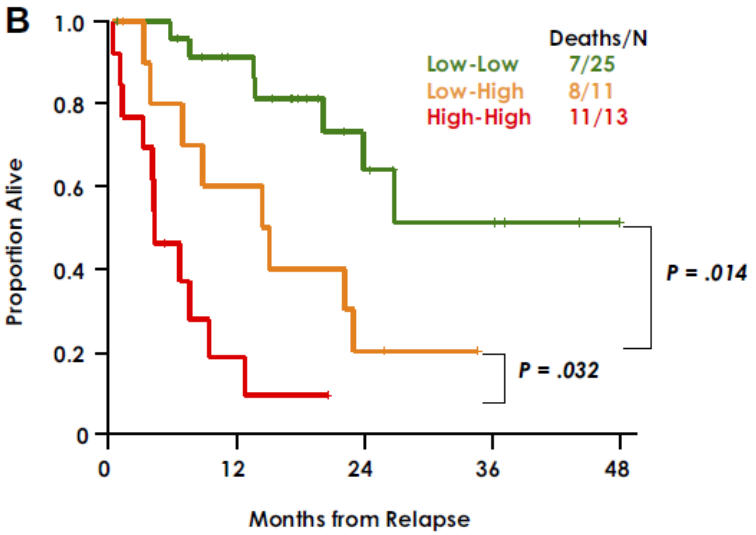
EFS



OS



Diagnosis-relapse risk



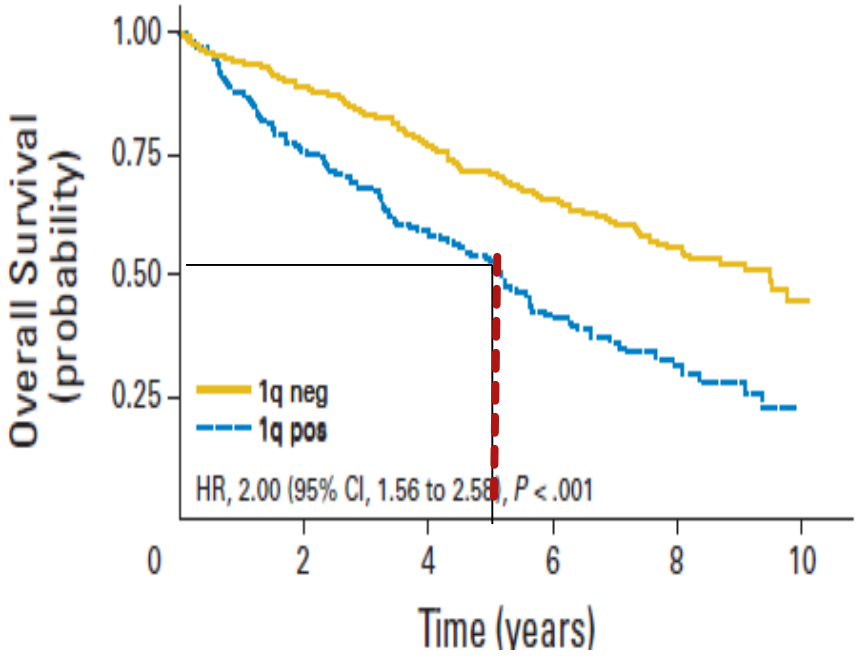
Shaughnessy et al, Blood 2007

Expression-based Definition of Aggressive Disease

	Signature	No of genes	Genes common with 70 Gene	No of Genes common with 92 Gene
1	**UAMS**	**70 genes**	**--**	**2 genes (BIRC5, LTBP1)**
2	**HOVON-65/GMMG-HD4 (EMC92)**	92 genes	2 genes (BIRC5, LTBP1)	--
3	**IFM**	15 genes	None	1 gene (FAM49A)
4	**Chromosome instability signature**	214 genes	7 genes	15 genes
5	**Centrosome index signature (CNTI)**	4 genes	None	None
6	**Cell death signature implicated by homozygous deletion (HZDCD)**	6 genes	None	None
7	**7-gene prognostic signature HMCL MM cell lines study**	7 genes 6 genes	None None	None None
8	**Proliferation signature**	50 genes	3 genes (BIRC5, ASPM, CKS1B)	6 genes (ESPL1, MCM6, NCAPG, SPAG5, ZWINT, BIRC5)

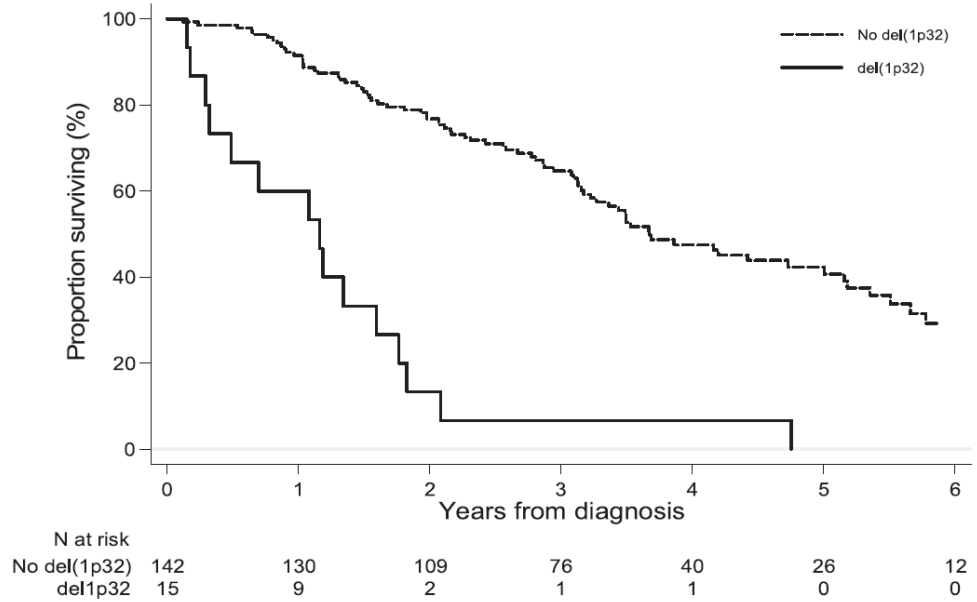
Recognizing high-risk: Chr1 abnormalities?

1q gains: 30-35% of patients



Avet-Loiseau et al, J Clin Oncol 2012

Del(1p32)



Hebraud et al, Blood 2015

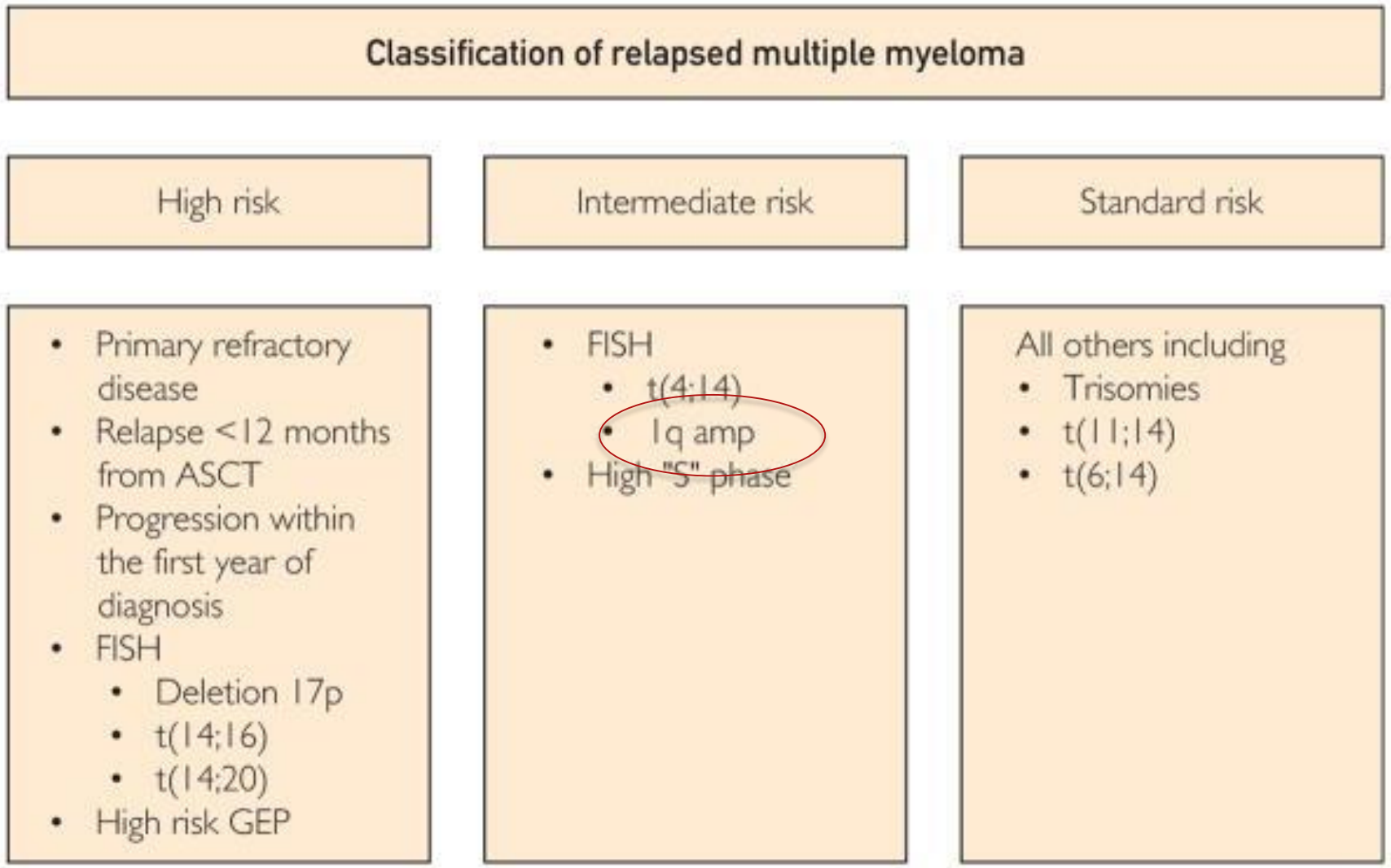
TABLE 4. Mayo Clinic Risk Stratification for Multiple Myeloma

Risk group	Percentage of newly diagnosed patients with the abnormality
Standard risk	75
Trisomies	
t(11;14)	
t(6;14)	
Intermediate risk	
t(4;14)	10
gain(1q)	
High risk	
t(14:16)	
t(14;20)	15
del(17p)	

Adapted from *Am J Hematol.*[2]

Rajkumar et al., Mayo Clin Proc 2016

Recognizing high-risk MM at relapse



Dingli et al., Mayo Clin Proc 2017

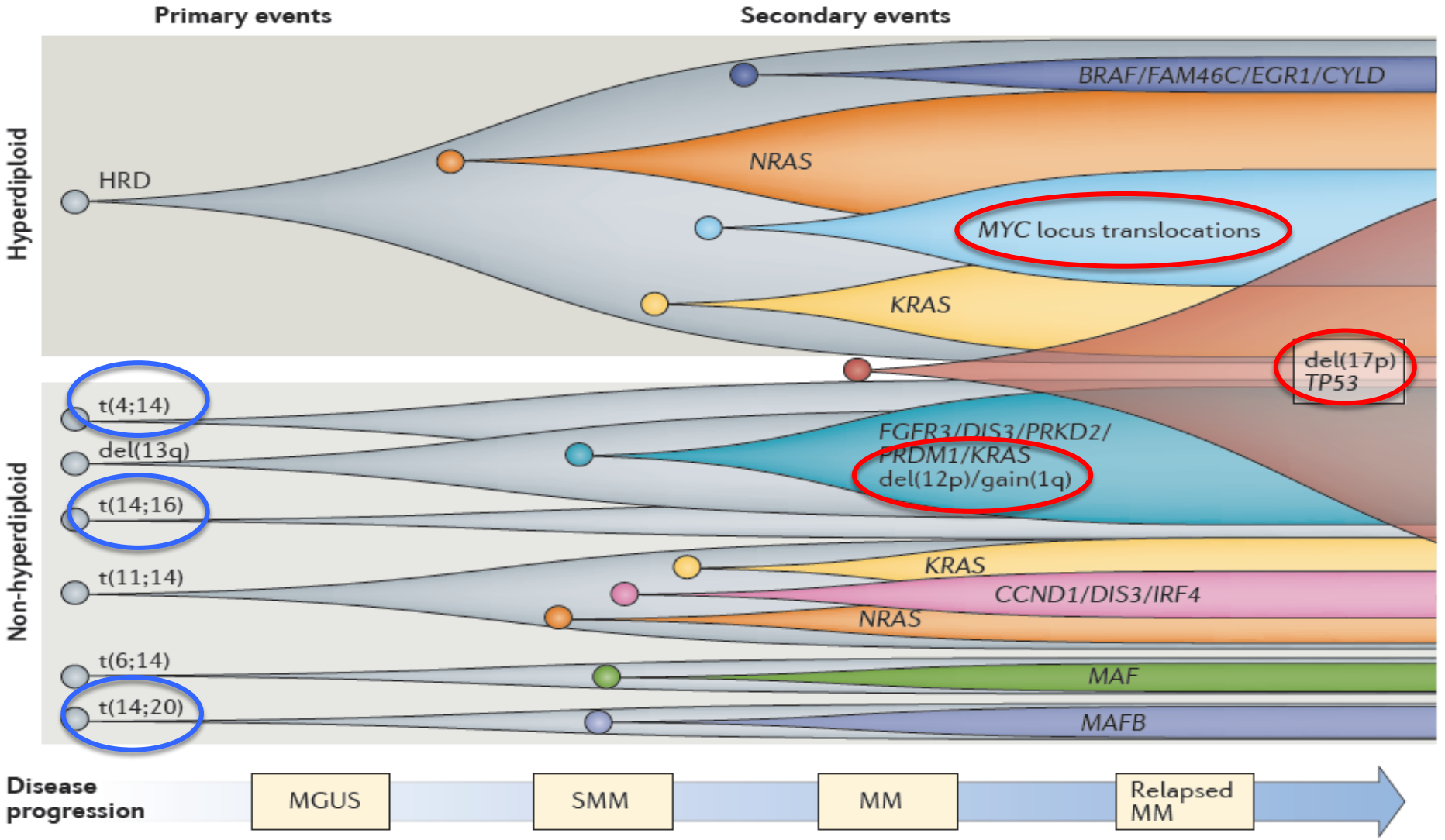
Which biological processes drive the high risk phenotype ?

- ✓ Many genetic **drivers** of the high risk disease
- ✓ High potential to acquire **additional changes**
- ✓ Higher ability to **evolve**

- ✓ High **proliferative** rate
- ✓ Anti-**apoptosis**
- ✓ Drug **resistance**
- ✓ Microenvironment **independency**

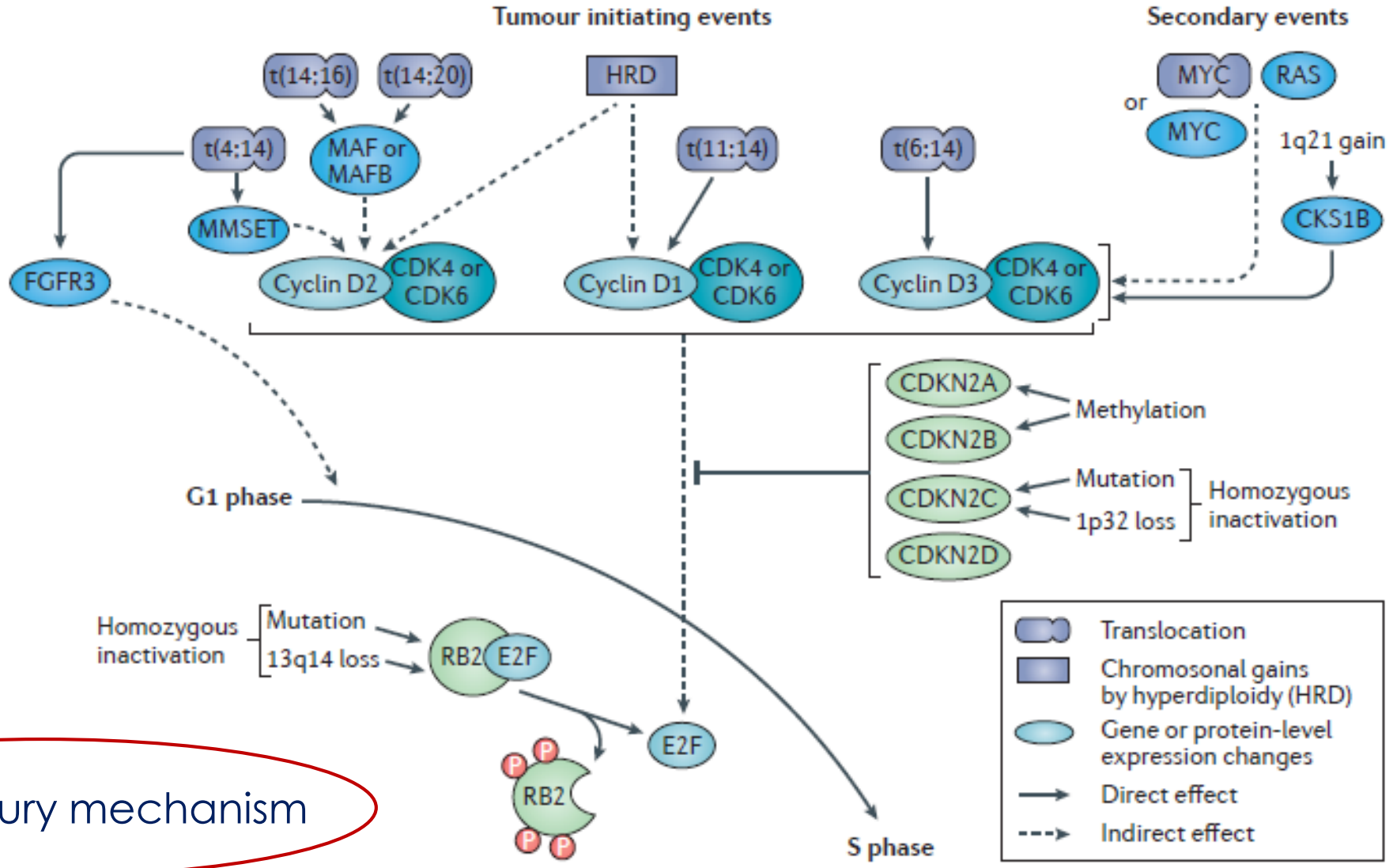
1. Barlogie et al, Blood 2014; 2. Kumar et al, Leukemia 2013

Molecular Pathogenesis and genetic architecture of MM



Manier S. et al, Nature Reviews 2016

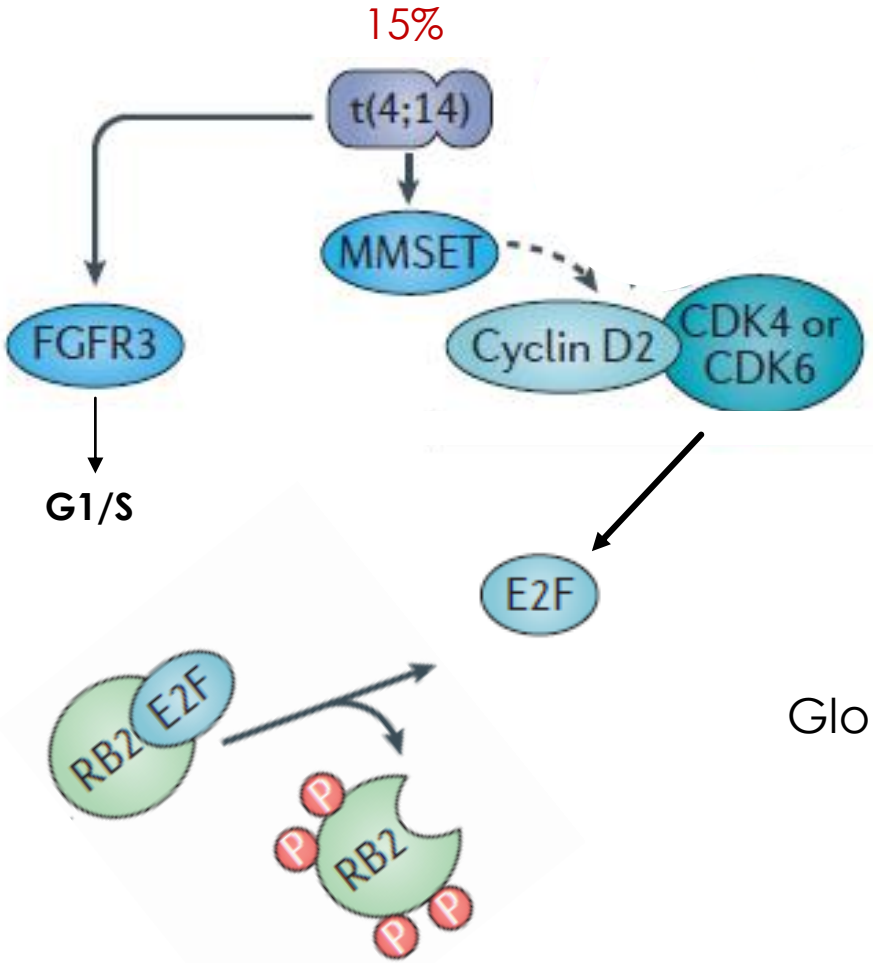
Convergent evolutionary route to high-risk MM via cell cycle dysregulation



Multiple-injury mechanism

Pawlyn et al, Nature Reviews 2017

IgH traslocations: t(4;14)



✓ This traslocation results in overexpression of **FGFR3** and **MMSET**;

Encodes a histone methyltransferase

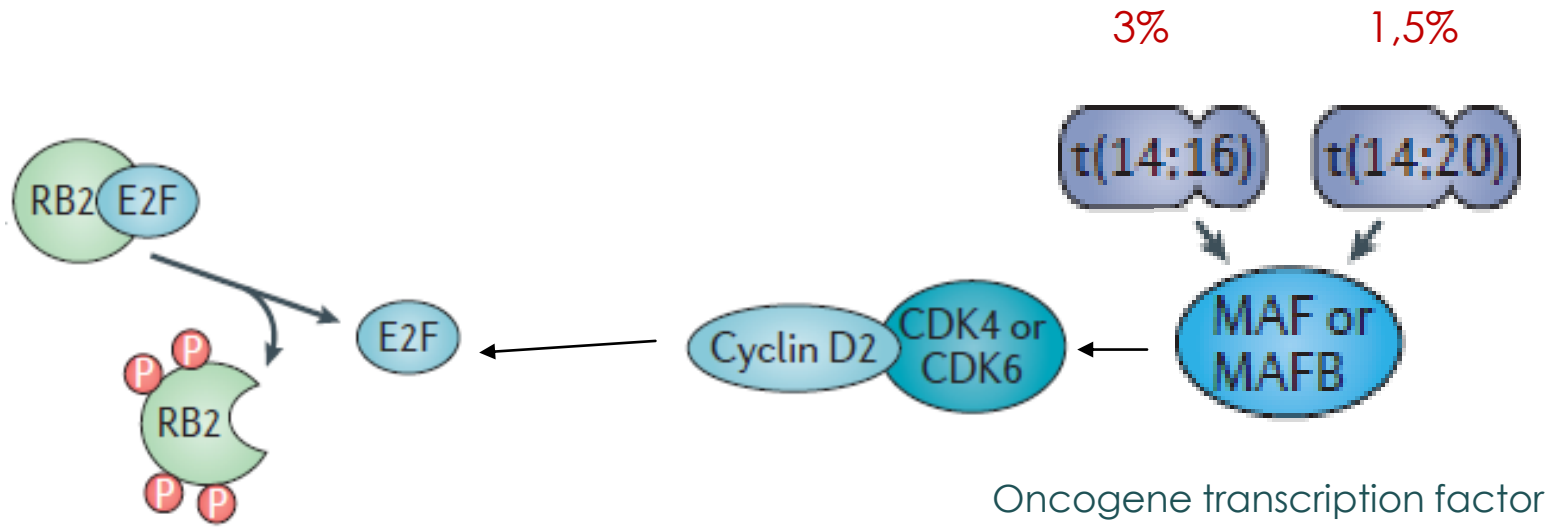
Global **epigenetic dysregulation**

Indirectly drives the cell through the **G1/S** checkpoint

Pawlyn et al, Nature Reviews 2017

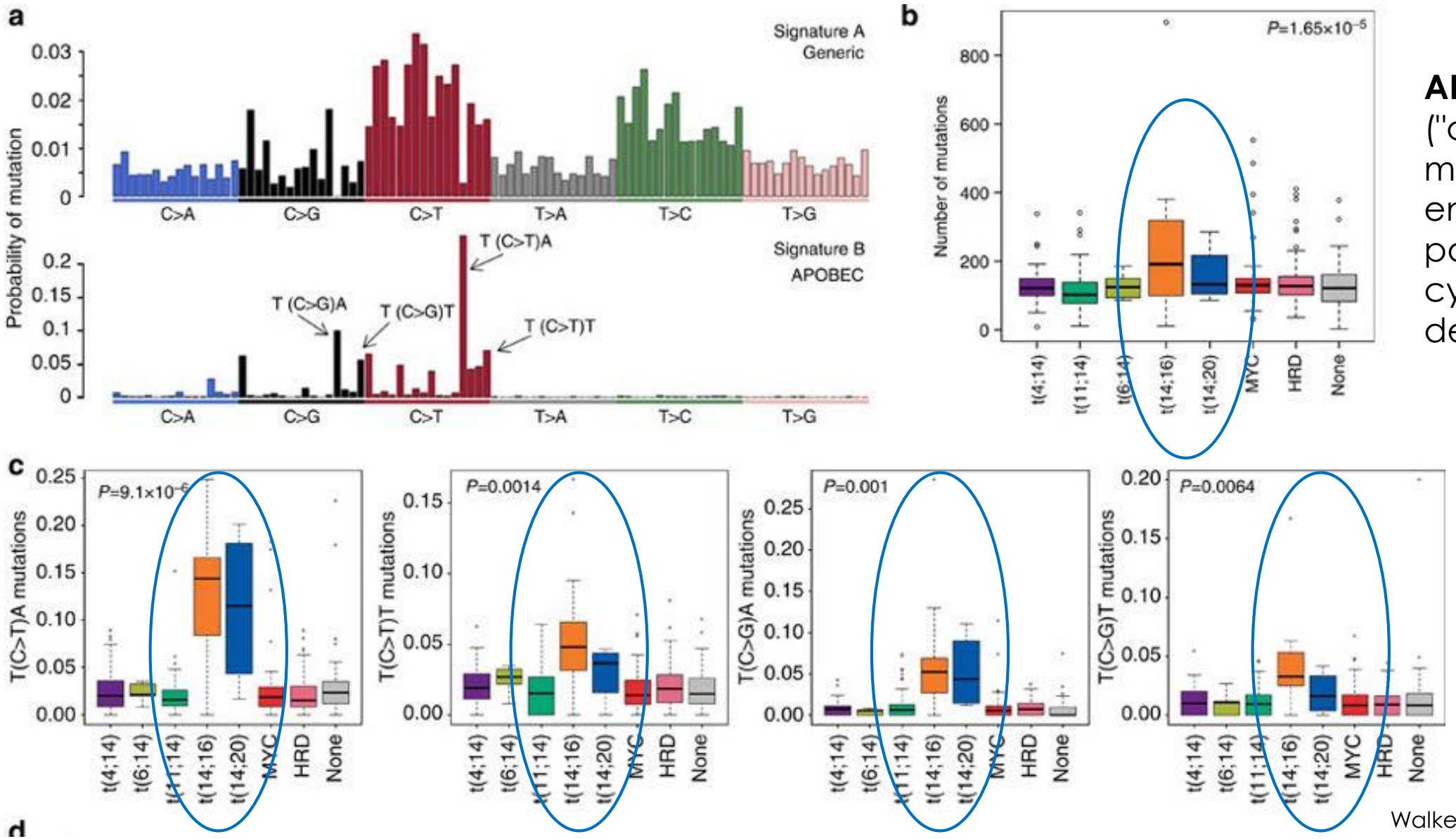
Walker et al, Blood 2011

IgH traslocations: t(14;16) t(14;20)



Pawlyn et al, Nature Reviews 2017

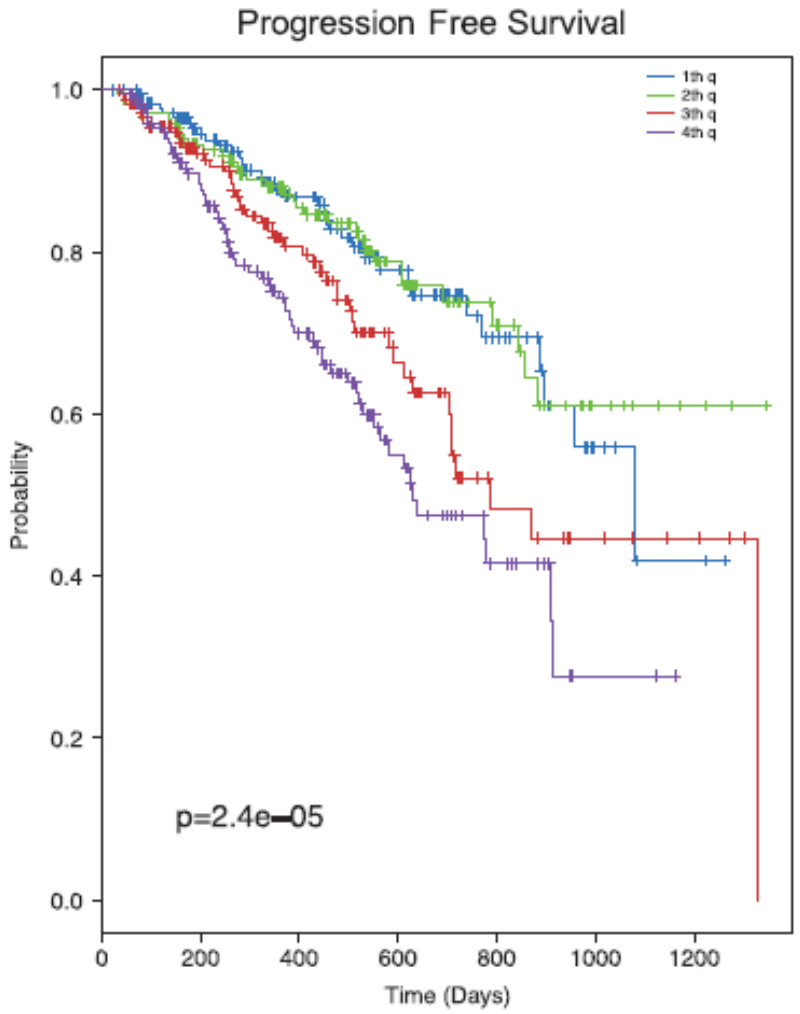
APOBEC is An Important Mutational Processes in Myeloma Associated with t(14;20) t(14;16)



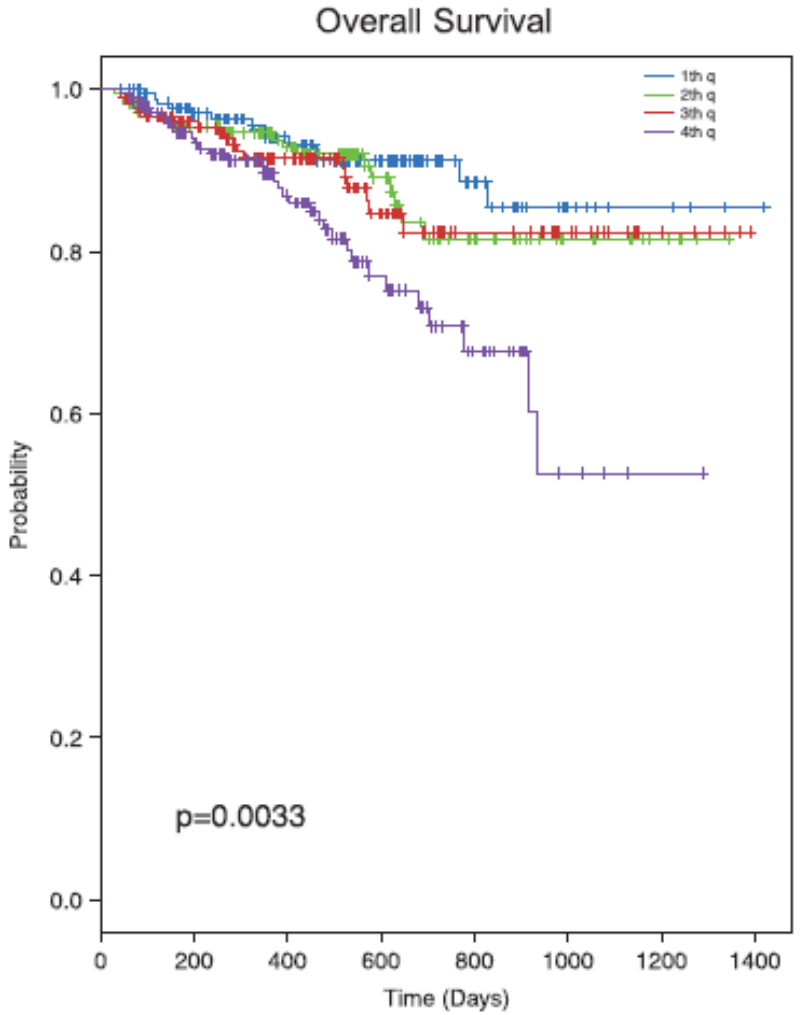
APOBEC ("apolipotrein B mRNA editing enzime catalytic polypeptide-like") cytidine deaminases.

Walker BA, Nat commun 2015

Prognostic Impact of APOBEC signature



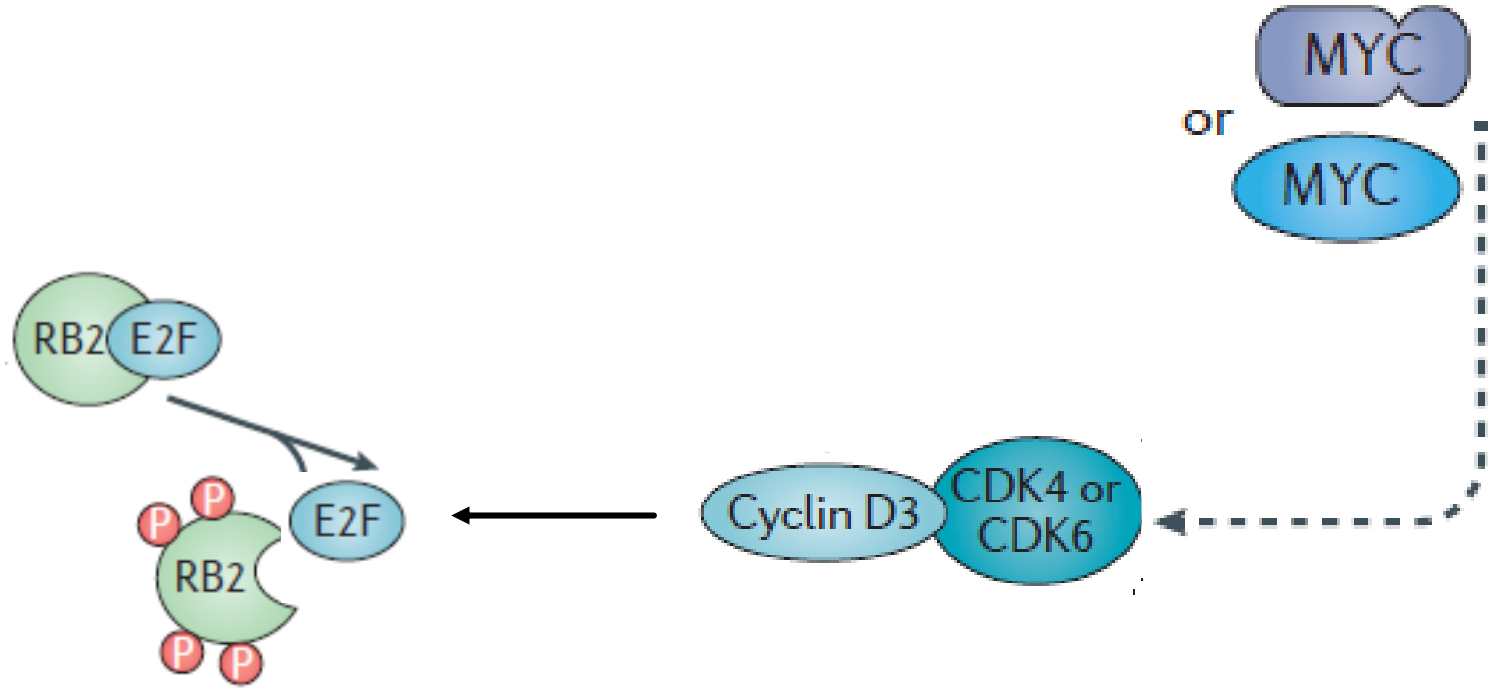
Patients with an absolute **APOBEC contribution** in the fourth quartile had **shorter** 2-year PFS and 2-year OS than patients in the first-third quartile



Maura et al, Leukemia 2018

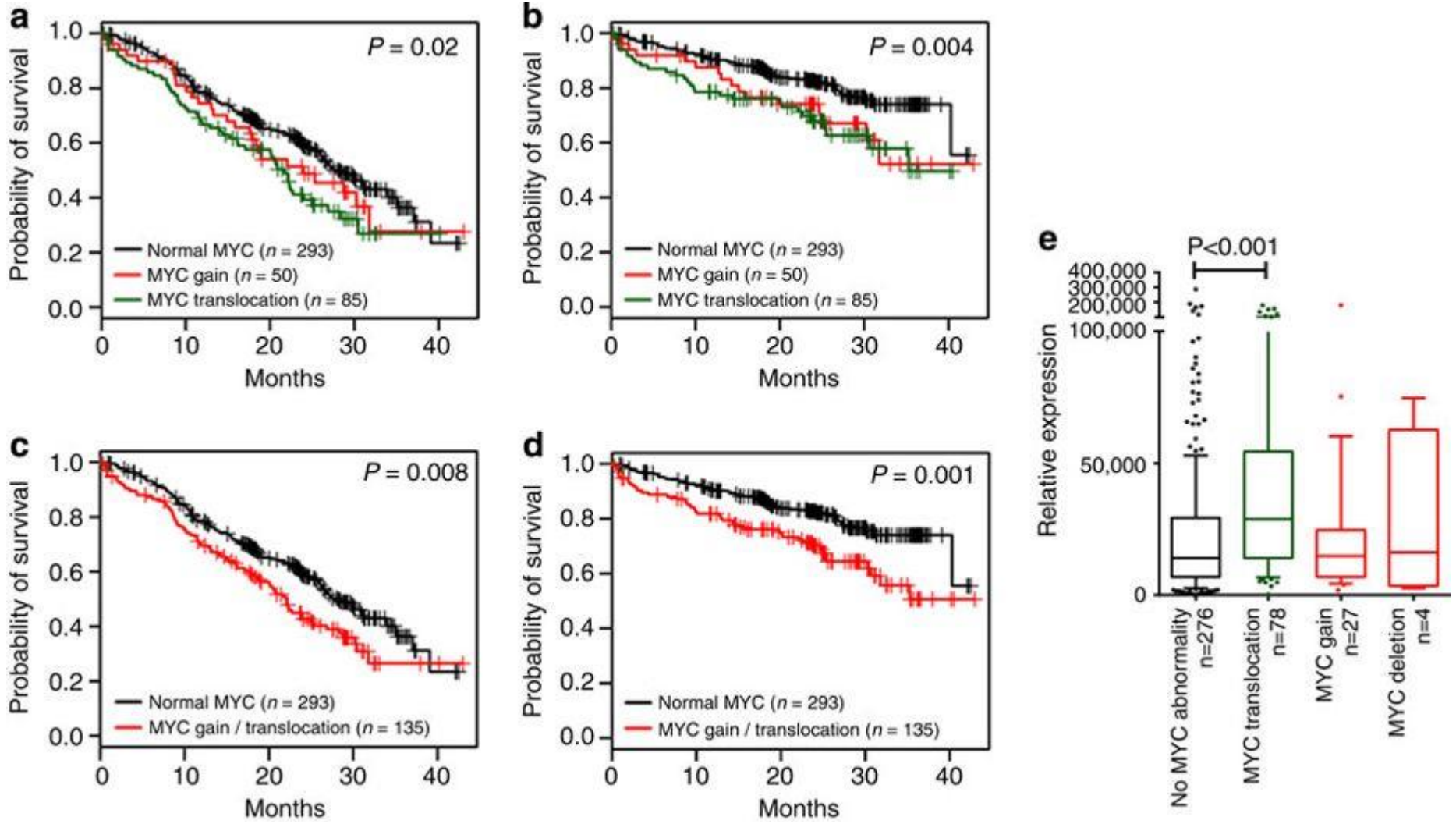
MYC traslocation/amplification

- ✓ Associated with the mutational signatures **APOBEC**
- ✓ Occur in **20%** of newly diagnosed cases of multiple myeloma



Walker et al , Nature Comm 2015

Prognostic Impact of MYC traslocation/amplification



Walker et al, Nature Comm 2015

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

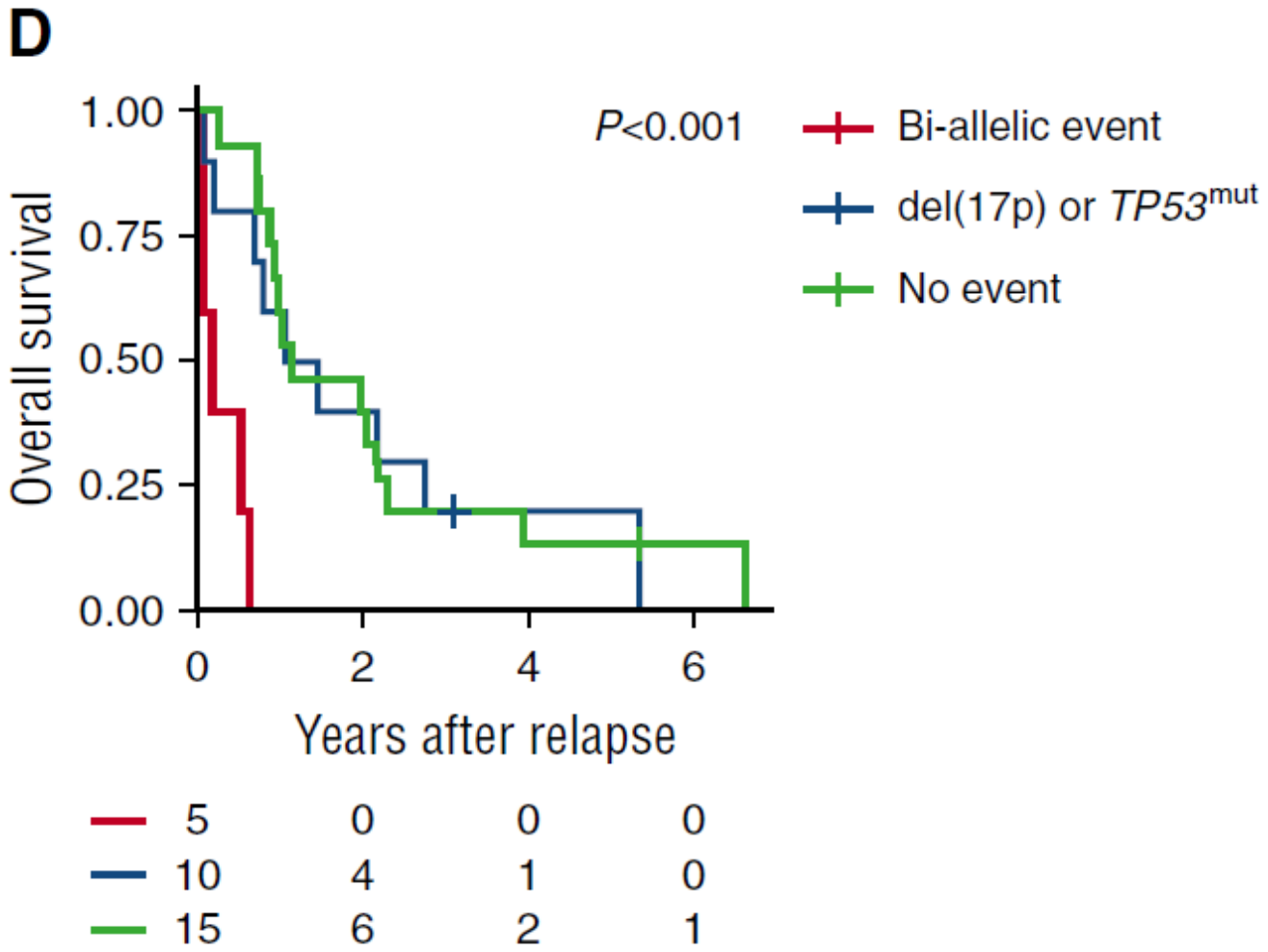
Small sub-clones with monoallelic inactivation of TP53 (<20%)

Vs.

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

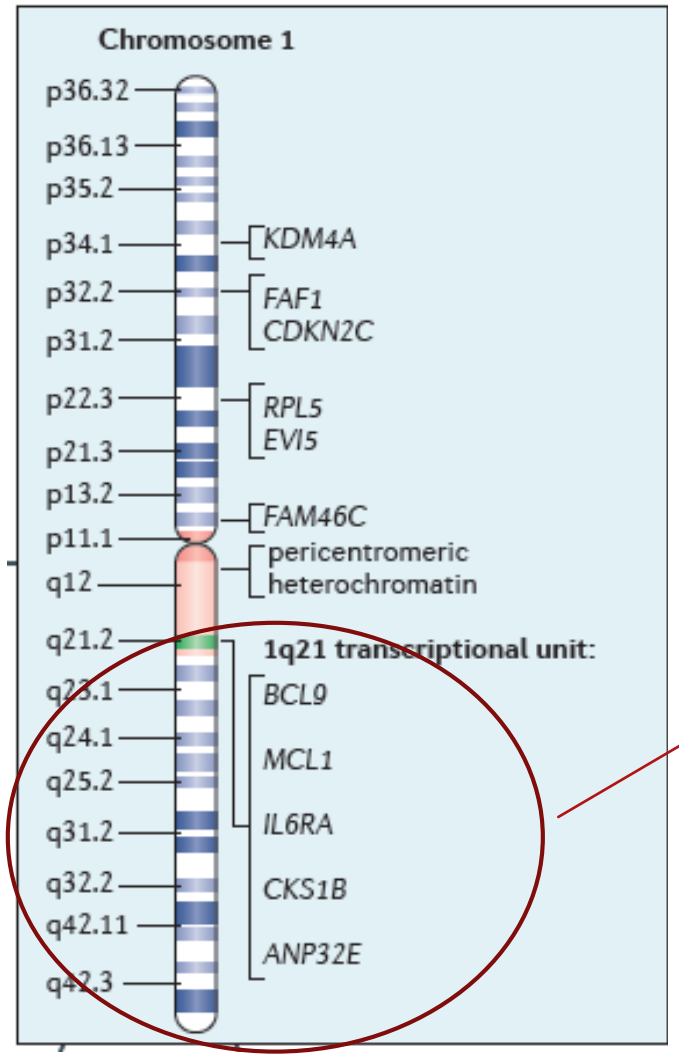
Lionetti et al, Oncotarget 2016; Pawlyn et al, Nature Reviews, 2017

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



Weinhold et al , Blood 2016

Copy Number 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

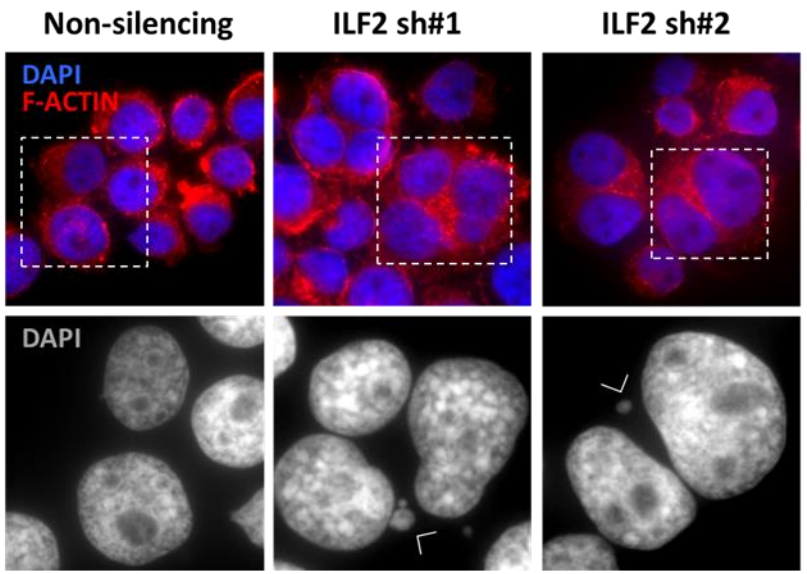
Copy Number Abnormalities (gain 1q21)

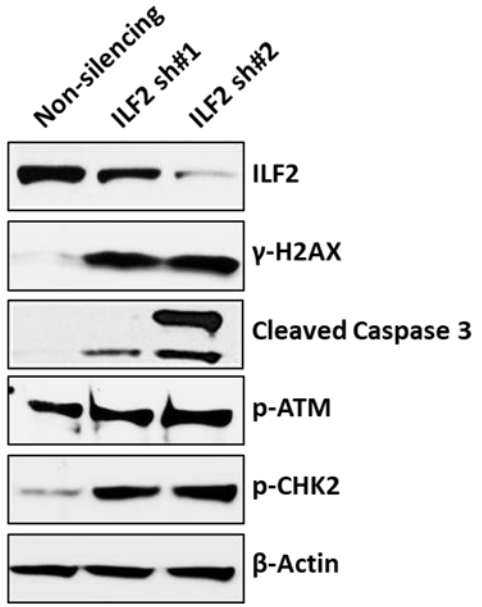
- ✓ ILF2 is required for the splicing of genes involved in **DNA damage repare** after DNA damage activation
- ✓ ILF-2 **overexpression** induce **tolerance** of genome instability

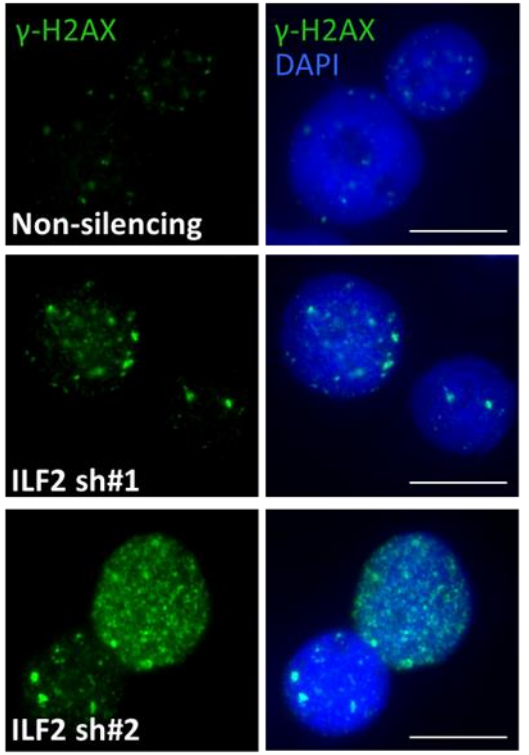
ILF2 Is a Regulator of RNA Splicing and DNA Damage Response in 1q21-Amplified Multiple Myeloma

Matteo Marchesini,[1] Yamini Ogoti,[1] Elena Fiorini,[1] Anil Aktas Samur,[2] Luigi Nezi,[3] Marianna D'Anca,[1] Paola Storti,[4] Mehmet Kemal Samur,[2] Irene Ganan-Gomez,[1] Maria Teresa Fulciniti,[5] Nipun Mistry,[6] Shan Jiang,[3] Naran Bao,[1] Valentina Marchica,[4] Antonino Neri,[7] Carlos Bueso-Ramos,[8] Chang-Jiun Wu,[3] Li Zhang,[6] Han Liang,[6] Xinxin Peng,[6] Nicola Giuliani,[4] Giulio Draetta,[3] Karen Clise-Dwyer,[9] Hagop Kantarjian,[1] Nikhil Munshi,[5] Robert Orlowski,[10] Guillermo Garcia-Manero,[1] Ronald A. DePinho,[11] and Simona Colla[1,12,*]

✓ ILF2 **downregulation** induces **Genomic Instability**, **apoptosis** and increases **sensitivity to DNA damage agents** of MM cells



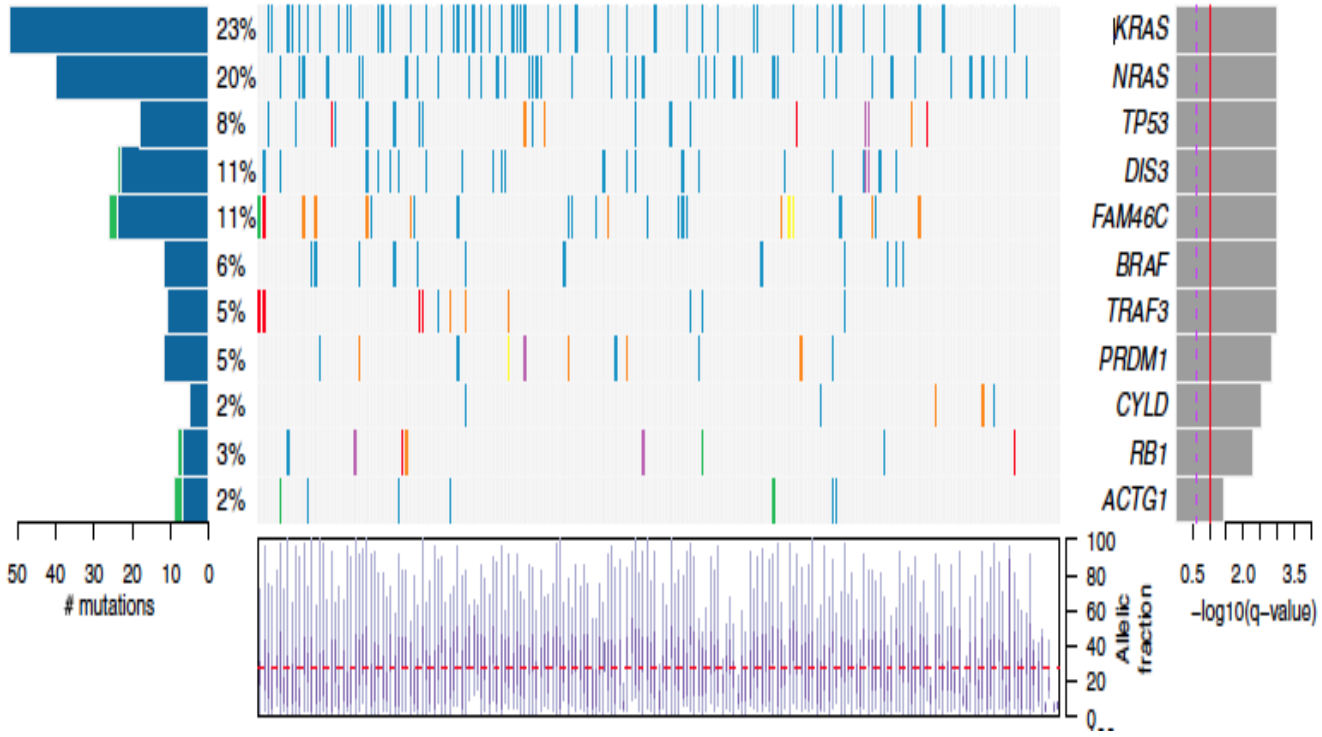




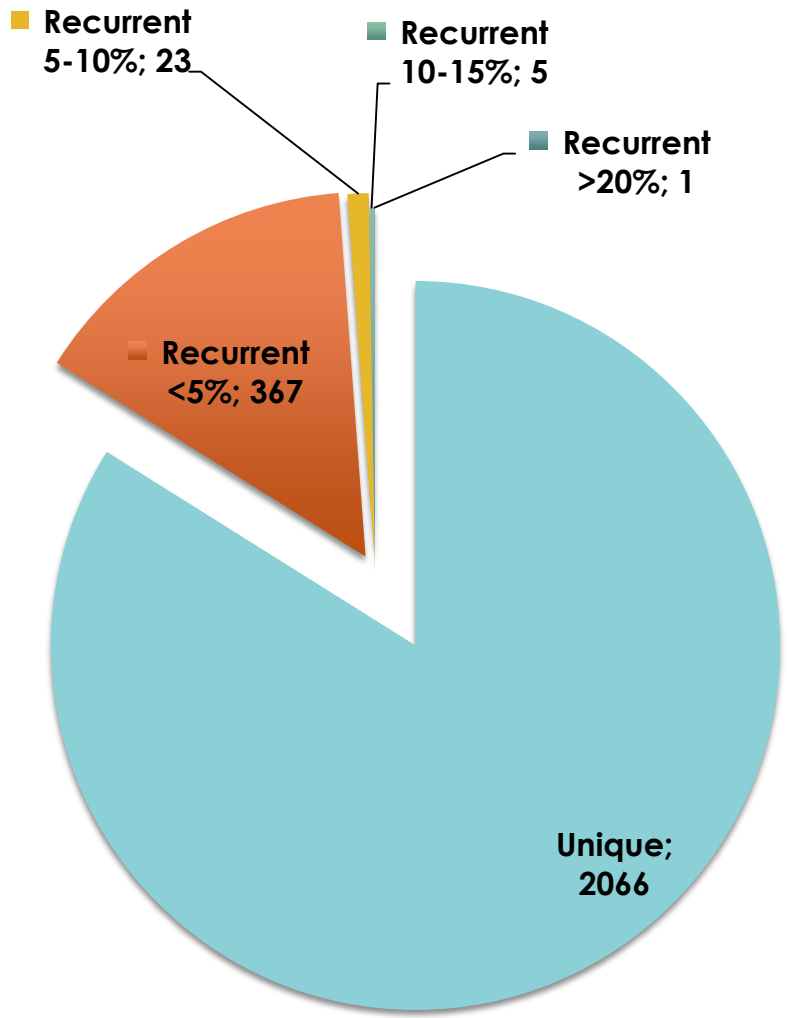
Marchesini M. et al, Cancer Cell 2017

Heterogeneity of Somatic Variants

Total n. of genes found in screen	2462
Cancer Census* Genes	83
Non Cancer Census Genes	2379
Recurrent ≥2	396
Unique	2066



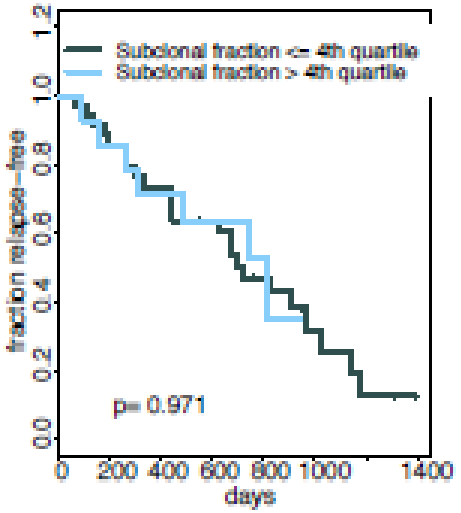
Distribution of genes

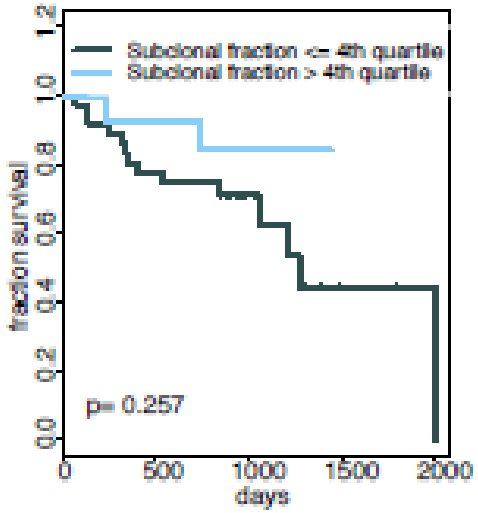


Bolli et al, Nat Comm 2014; Lohr et al, Cancer Cell 2014

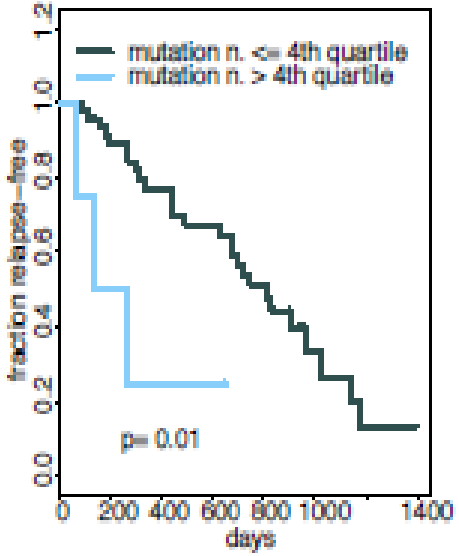
Genome Sequencing: Prognostic Implications of Mutations in Myeloma

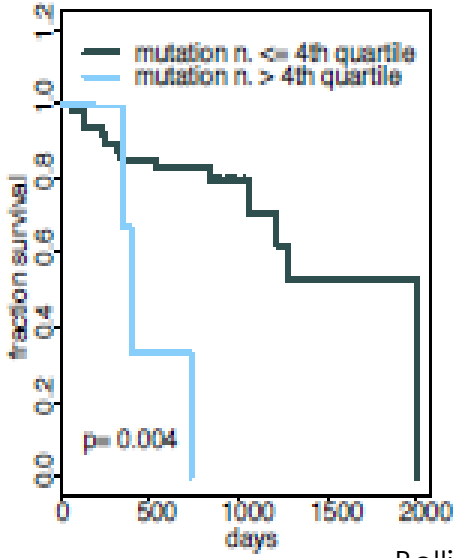
Subclonal Fraction





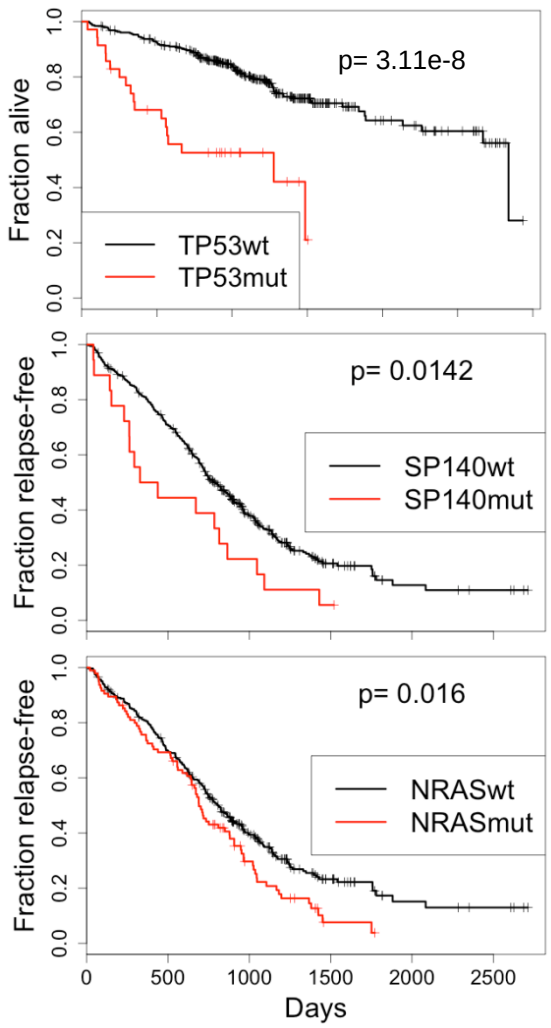
Frequency of Mutation



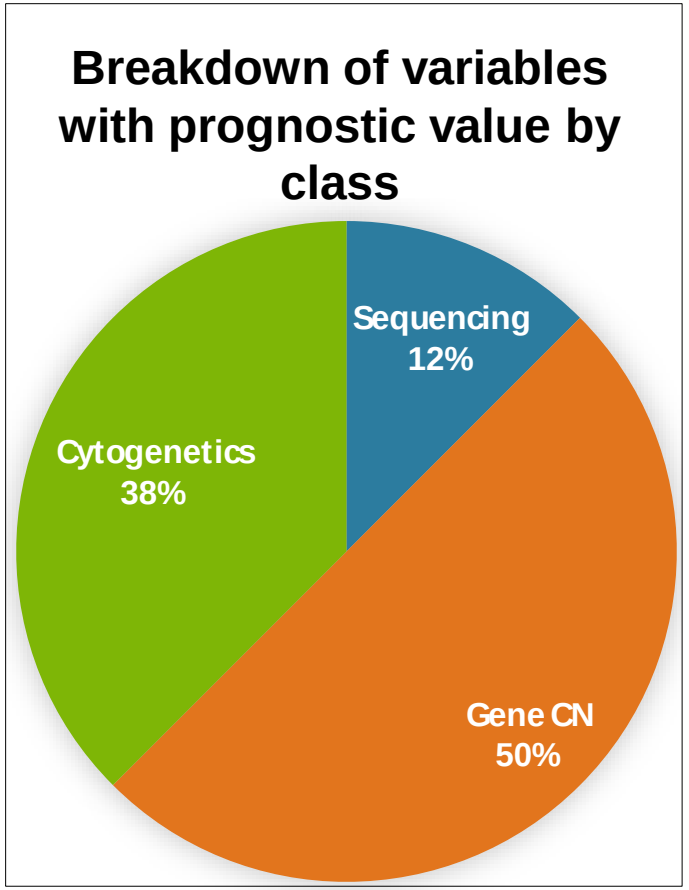


Bolli et al. Nature Comm 2014

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

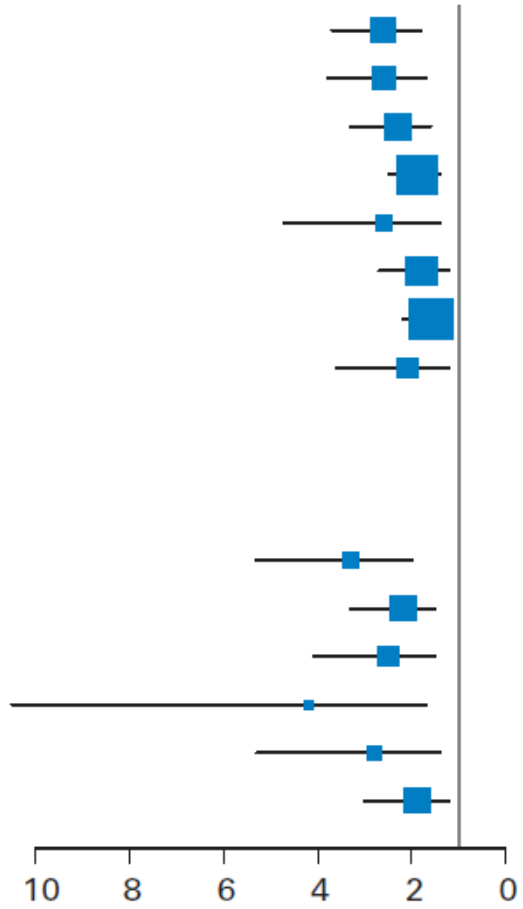
Impact of mutations on survival in a multivariate analysis

Progression-Free Survival

	HR	LCI	UCI	*P*	Sign.
TP53 signal	2.6	1.8	3.7	< .0001	***
ISS III	2.6	1.7	3.8	< .0001	***
t(4;14)	2.3	1.6	3.3	< .0001	***
Age > 70 years	1.9	1.4	2.5	< .0001	***
ZFHX4	2.6	1.4	4.7	< .0001	**
ISS II	1.8	1.2	2.7	.004	**
MYC translocation	1.6	1.2	2.2	.005	**
ATM/ATR	2.1	1.2	3.6	.008	**

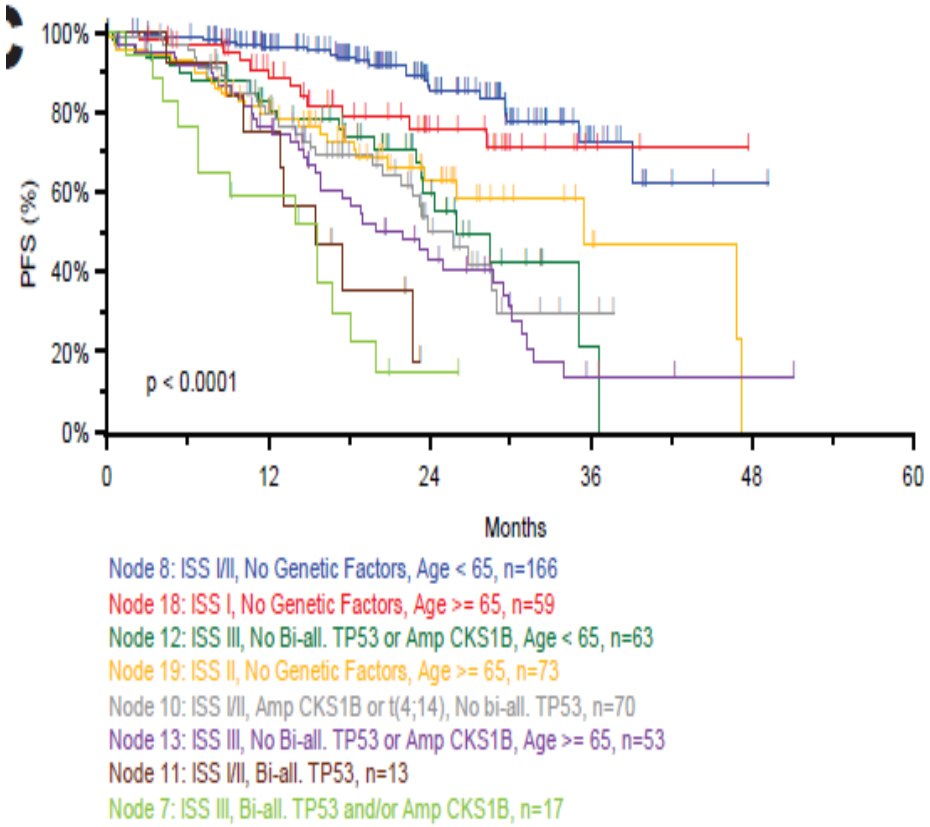
Overall Survival

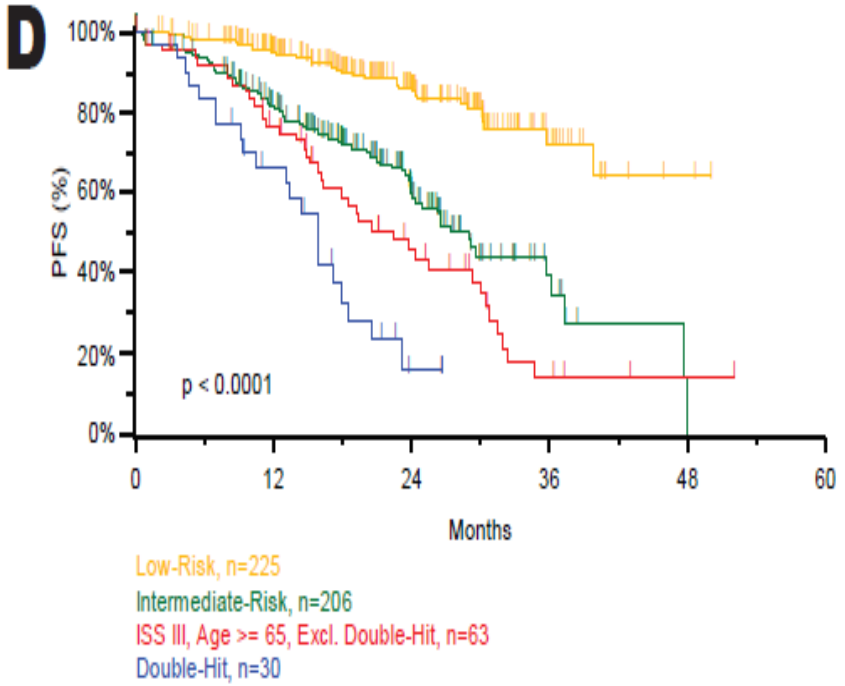
TP53 signal	3.3	2	5.3	< .0001	***
ISS III	2.2	1.5	3.3	.0001	***
amp(1q)	2.5	1.5	4.1	.0008	***
CCND1	4.2	1.7	10.5	.0025	**
ATM/ATR	2.8	1.4	5.3	.0029	**
MYC translocation	1.9	1.2	3	.0036	**



Walker BA et al. J Clin Oncol 2015

Redefining High-risk Myeloma
N = 784





High-risk segment defined by either

a) biallelic *TP53* inactivation or

b) ISS III and amplification of *CKS1B* (1q21)

Walker et al. ASH 2017

Prognostic Implications of Mutations in Myeloma

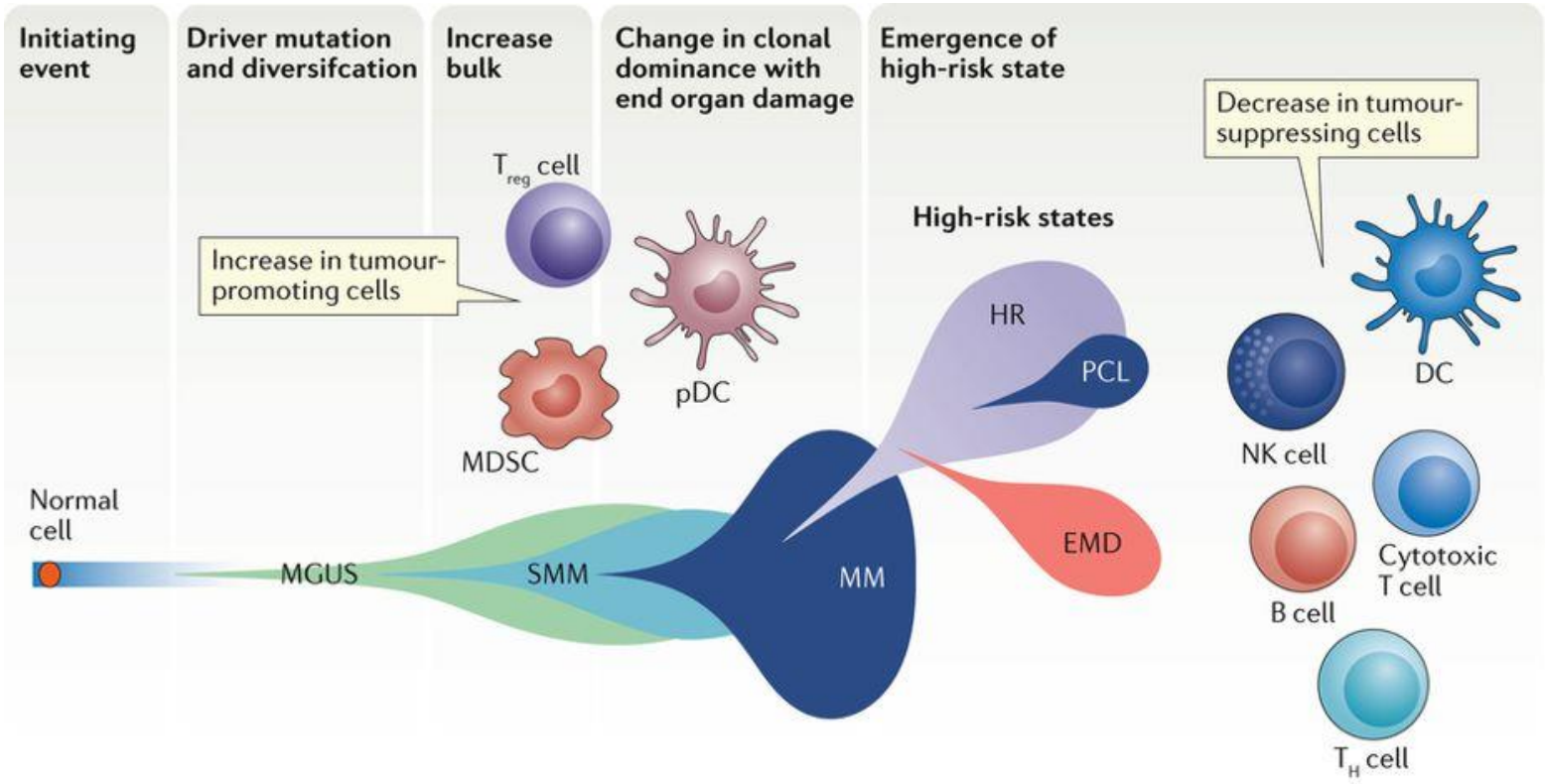
More data are needed… but,

No prognostic impact of the most frequent mutations such as KRAS, NRAS, DIS3, BRAF, FAM46C

Prognostic impact of TP53 mutations

Others are very rare, thus probably not any major pronostic value

The interaction between genetic drivers and microenvironment changes drives high-risk disease states



Pawlyn et al, Nature Reviews 2017

Tumor Morphology and Phenotypic Evolution Driven by Selective Pressure from the Microenvironment

Alexander R.A. Anderson,[1,*] Alissa M. Weaver,[4] Peter T. Cummings,[2,3] and Vito Quaranta[4,*]

Cell *127*, 905–915, December 1, 2006

Darwinian Dynamics of Intratumoral Heterogeneity: Not Solely Random Mutations but Also Variable Environmental Selection Forces

Mark C. Lloyd[1,2], Jessica J. Cunningham[3], Marilyn M. Bui[3,4], Robert J. Gillies[3], Joel S. Brown[2], and Robert A. Gatenby[5,6]

Cancer Res; 76(11) June 1, 2016

Harsh tumor microenvironment conditions (e.g., hypoxia, heterogenous extracellular matrix) exert a dramatic selective force on the tumor, which grows as an invasive mass with fingering margins, dominated by a few clones with aggressive traits.

Scale	Microenvironment	
	Mild	Harsh
Molecular/subcellular	Aggressive traits selected	Aggressive traits selected
Cellular	Many phenotypes selected and coexisting	1-3 phenotypes selected and dominating
Tumor	No invasive morphology	Invasive morphology

Anderson ARA et al. Cell 2006

Hypoxic bone marrow niche

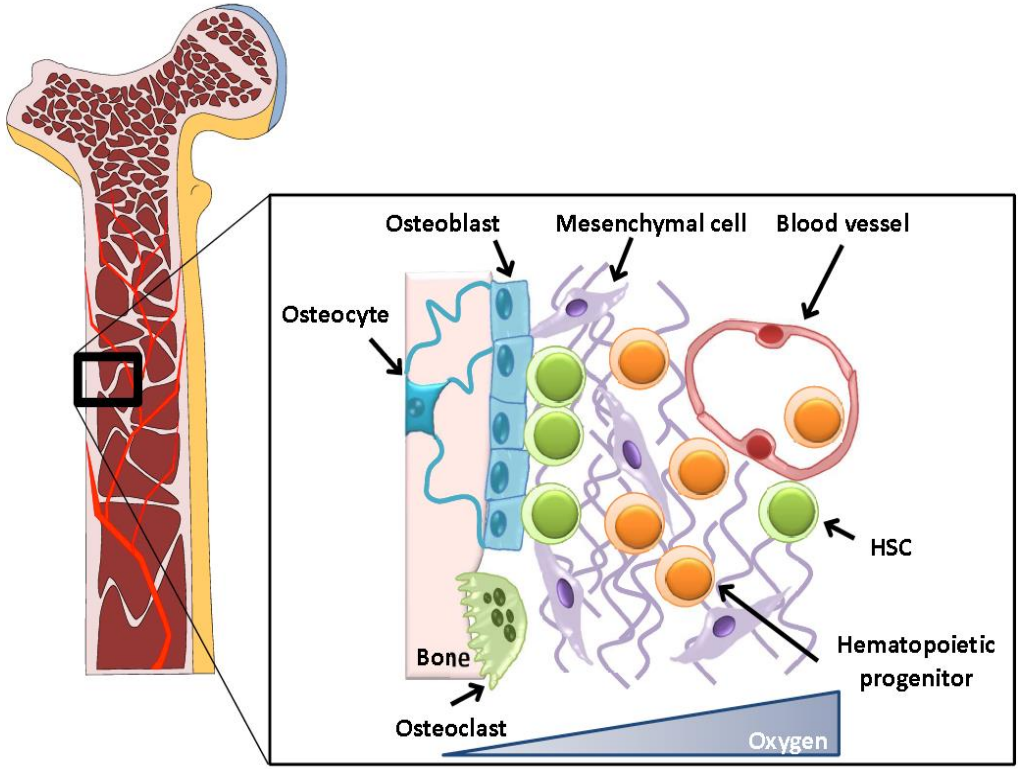
BLOOD, 1 JANUARY 2002 • VOLUME 99, NUMBER 1

To the editor:

Oxygen saturation in the bone marrow of healthy volunteers

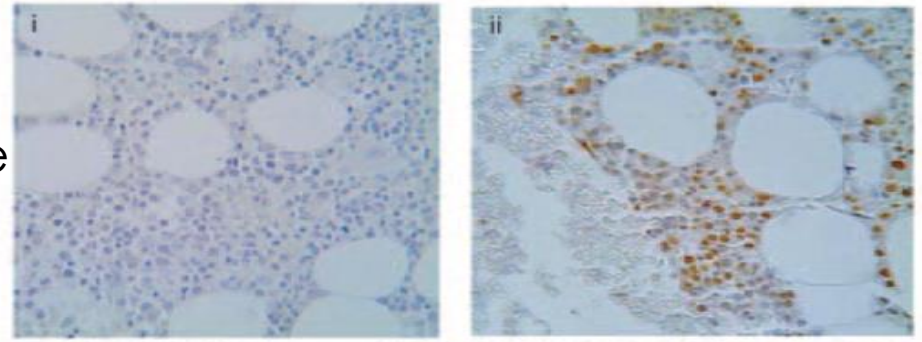
Jonathan S. Harrison, Pranela Rameshwar, Victor Chang, and Persis Bandari

- mean $pO_2 \pm$ SD: ***54.9 ± 0.98 mmHg***
- mean $sO_2 \pm$ SD: ***87.5 ± 1.1%***



Toscani D et al. *Ann N Y Acad Sci 2015*

MM cells over-express Hypoxia Inducible Factor (HIF)-1α

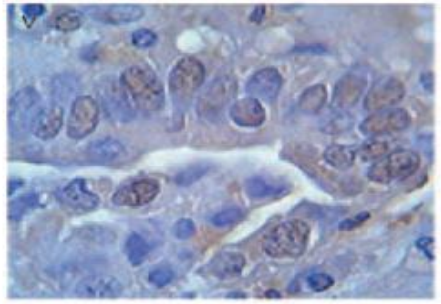


Negative control

MM bone biopsy

MM bone biopsy

MM bone biopsy



Positive control

PATIENTS	HIF-1α protein
MM1	-
MM2	-
MM3	-
MM4	+
MM5	-
MM6	+
MM7	-
MM8	-
MM9	+
MM10	-
MM11	+
MM12	+
MM13	-
MM14	-
MM15	-
MM16	-
MM17	-
MM18	-
MM19	-
MM20	+
MM21	+
MM22	-
MM23	-
MM24	-
MM25	-

Colla S et al. Leukemia 2010

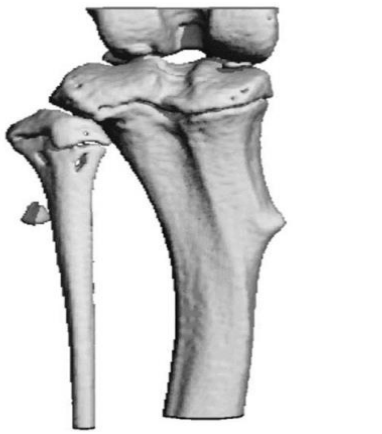
Published OnlineFirst June 9, 2009; DOI: 10.1158/0008-5472.CAN-08-4603

Research Article

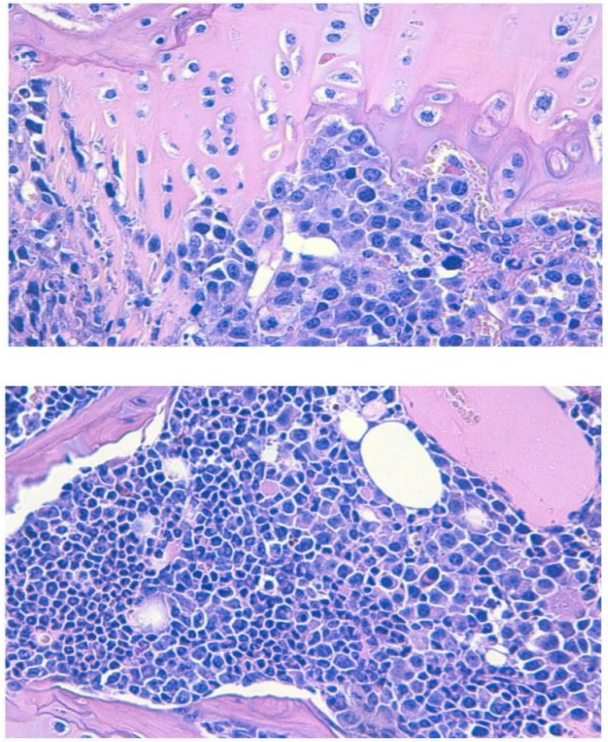
Targeting Angiogenesis via a c-Myc/Hypoxia-Inducible Factor-1α–Dependent Pathway in Multiple Myeloma

Jing Zhang,[1,2] Martin Sattler,[1] Giovanni Tonon,[3,8] Clemens Grabher,[4] Samir Lababidi,[6] Alexander Zimmerhackl,[1,2] Marc S. Raab,[1,2] Sonia Vallet,[5] Yiming Zhou,[7] Marie-Astrid Cartron,[7] Teru Hideshima,[1] Yu-Tzu Tai,[1] Dharminder Chauhan,[1,2] Kenneth C. Anderson,[1,2] and Klaus Podar[1,2,9]

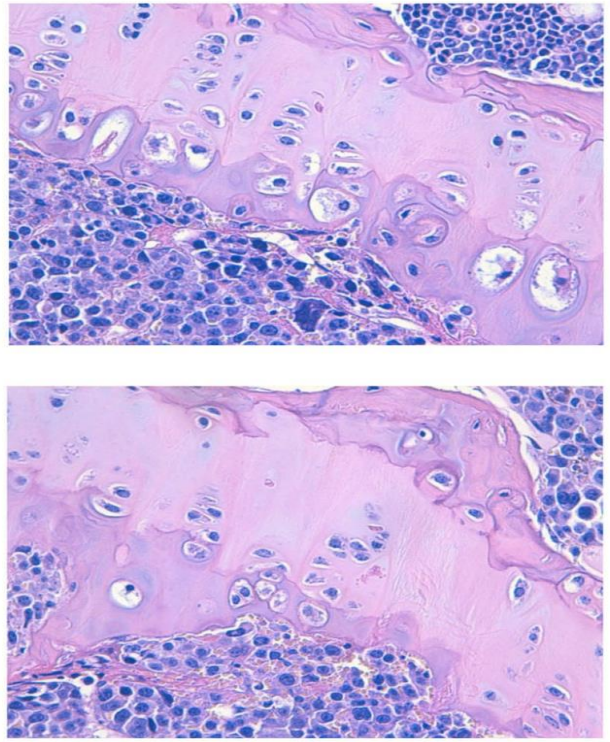
HIF-1α stable inhibition in MM cells suppress tumoral growth and bone destruction in vivo



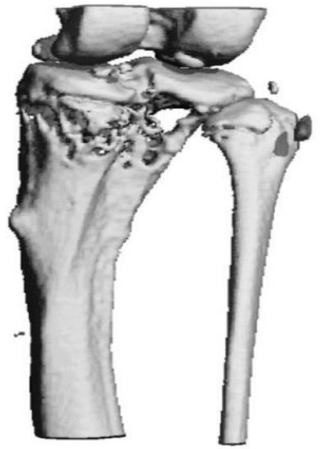
Saline



JJN3 pLKO.1



JJN3 anti-HIF-1α



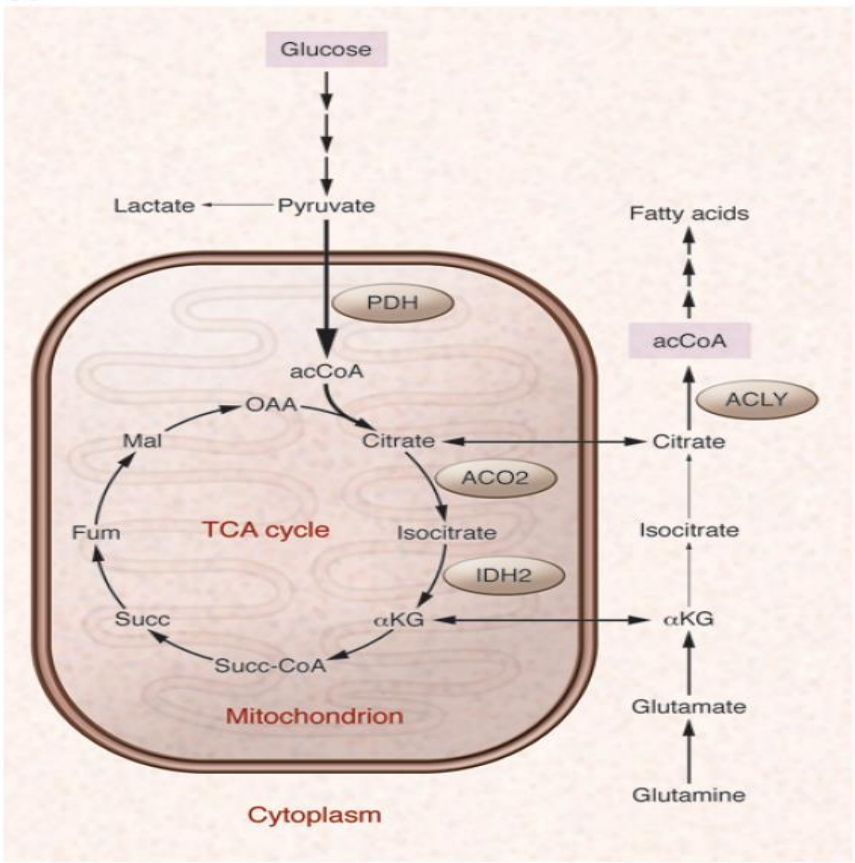
-HIF-1α

Storti P et al. Leukemia 2013

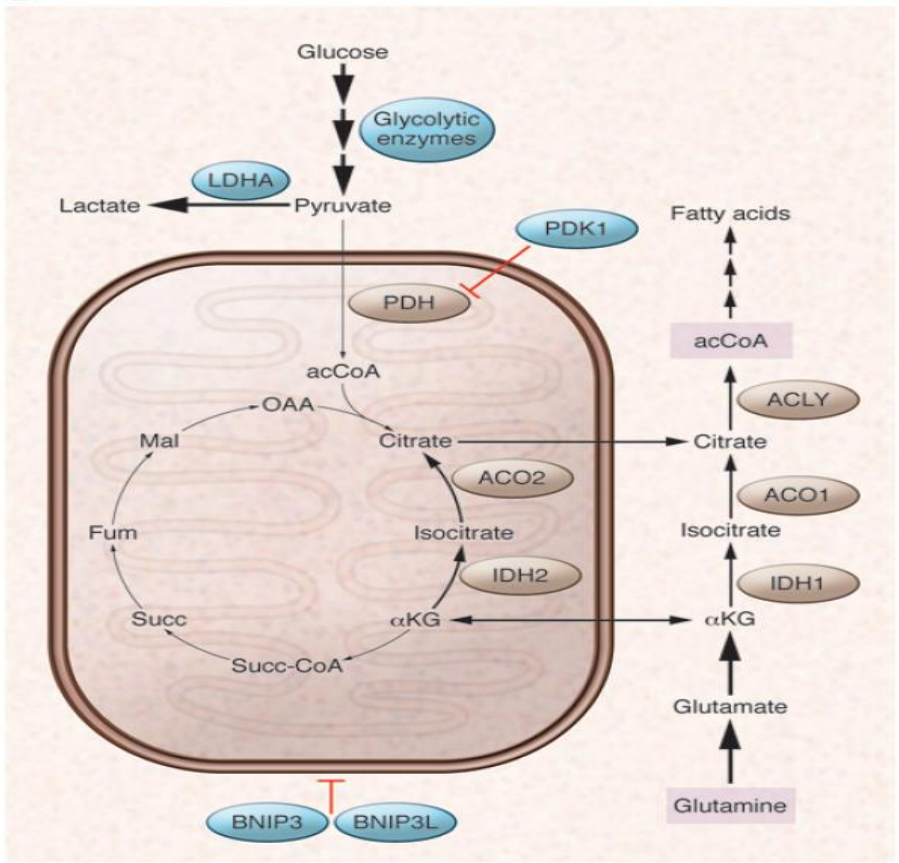
HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations

Gregg L. Semenza

The Journal of Clinical Investigation http://www.jci.org Volume 123 Number 9 September 2013

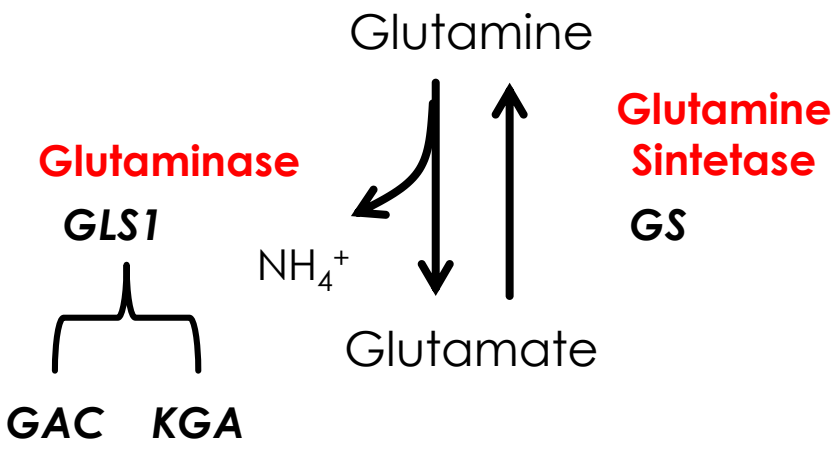


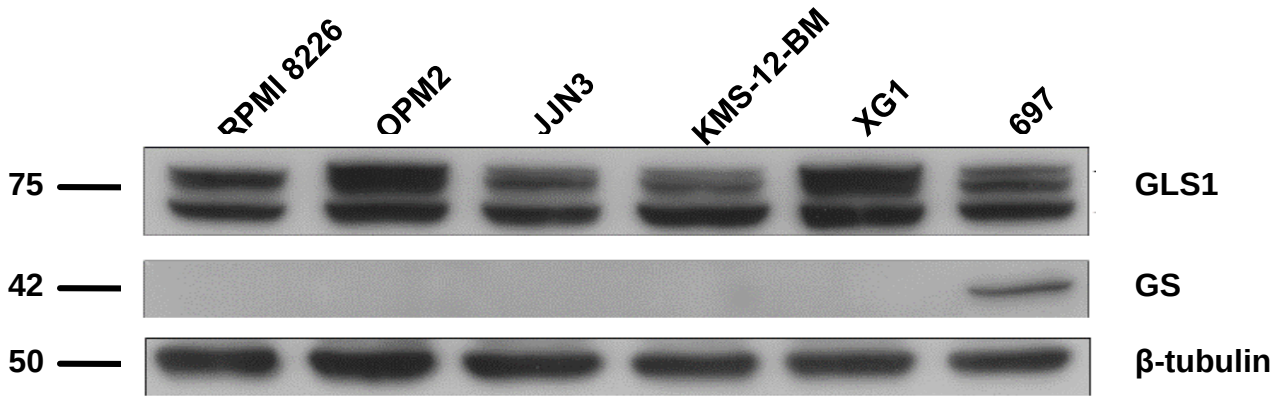
Well-oxygenated cells

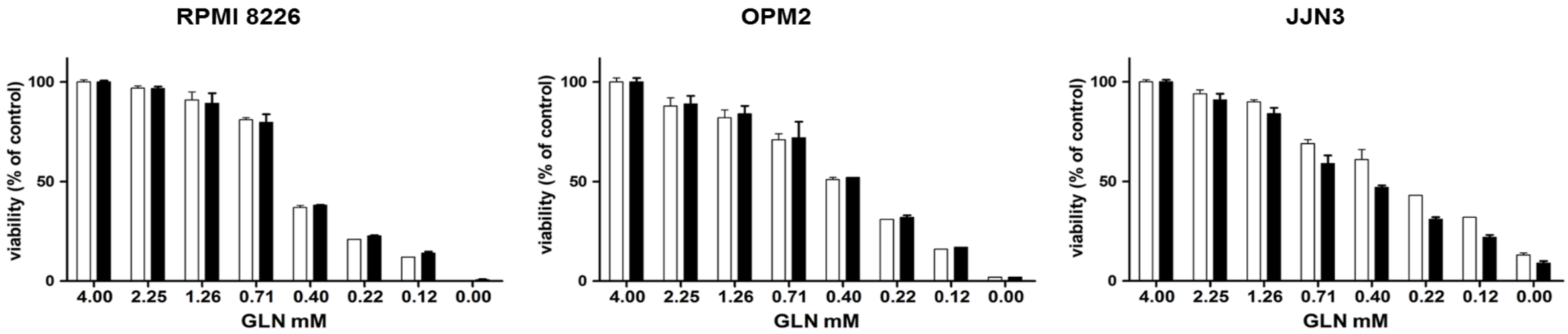


Hypoxic cells

High proliferative MM cells are glutamine (Gln) "addicted"

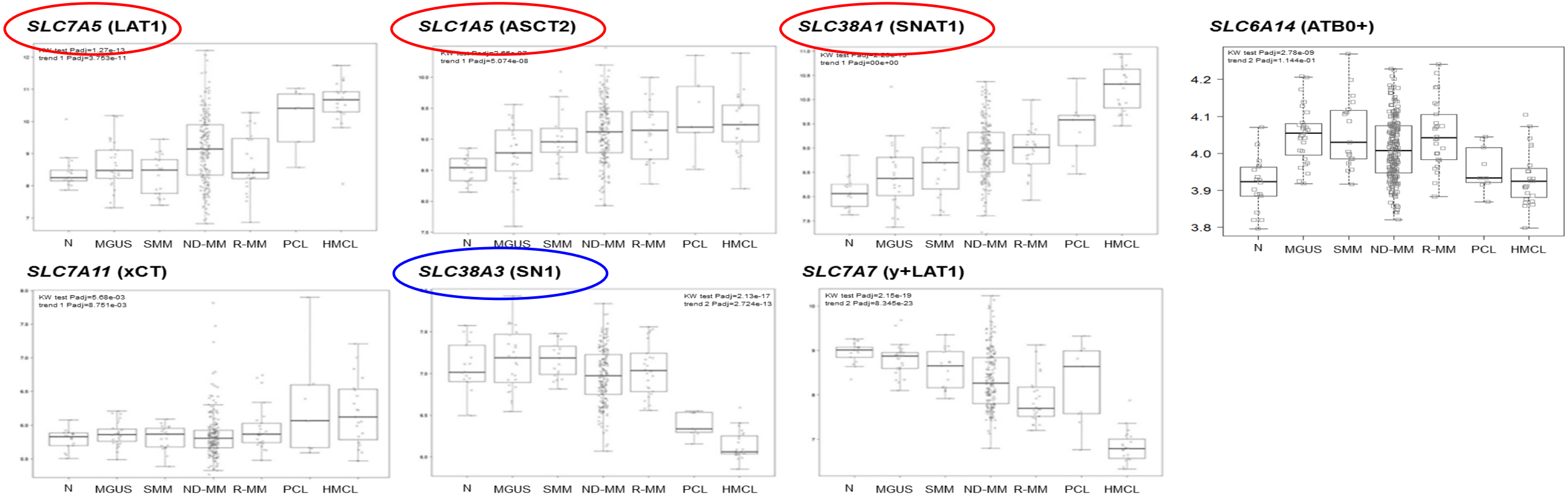






Bolzoni M et al, Blood 2016

GEP of the main Gln transporters by CD138+ cells

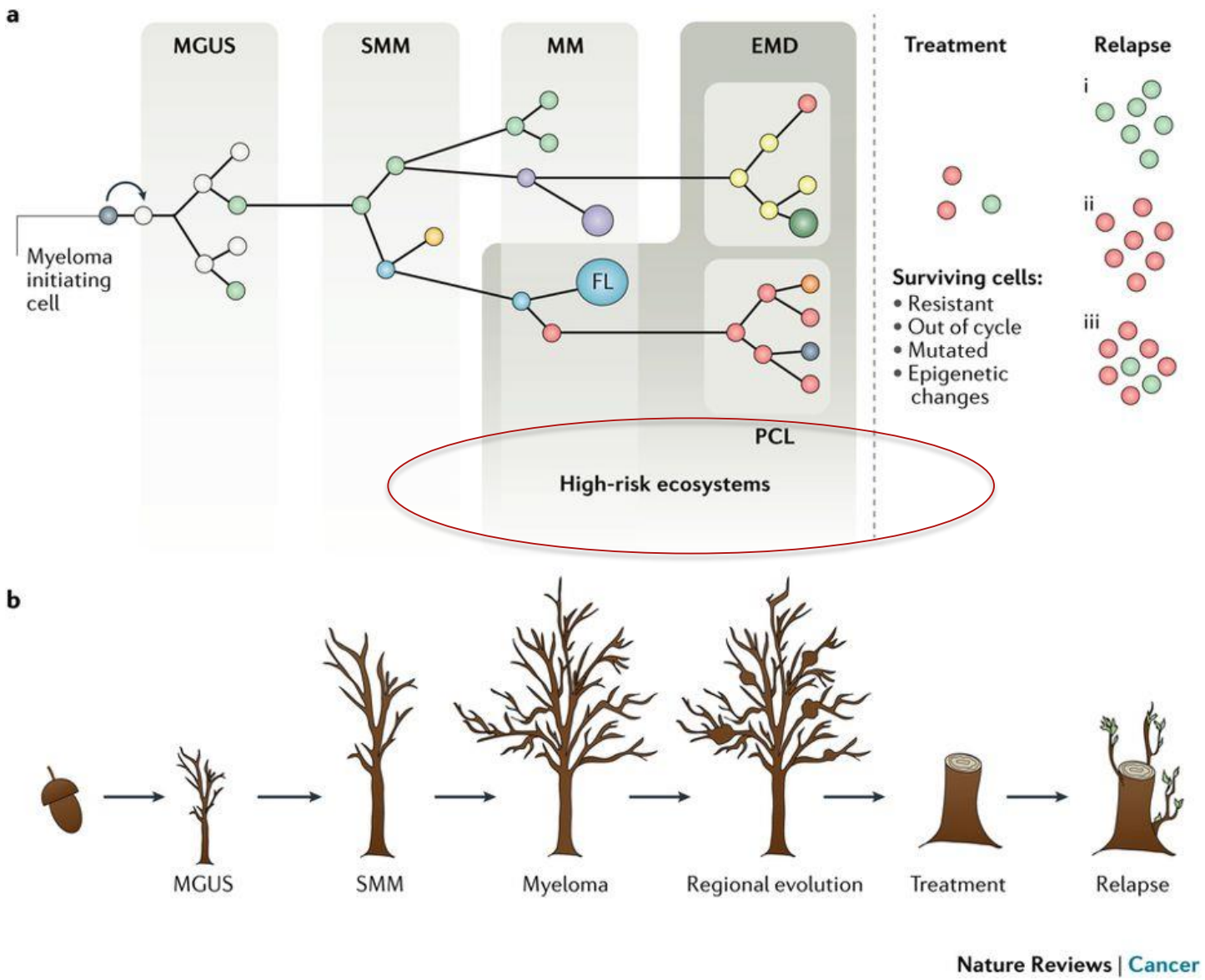


18 healthy donors (N), 28 MGUS, 19 SMM, 200 newly diagnosed MM (ND-MM), 26 relapsed MM (R-MM), 9 plasma cell leukemia (PCL) patients, together with 23 human myeloma cell lines (HMCLs)

GSE13591, GSE6205, GSE6477 and GSE6691 dataset, profiled on GeneChip® Human Genome U133A Arrays.

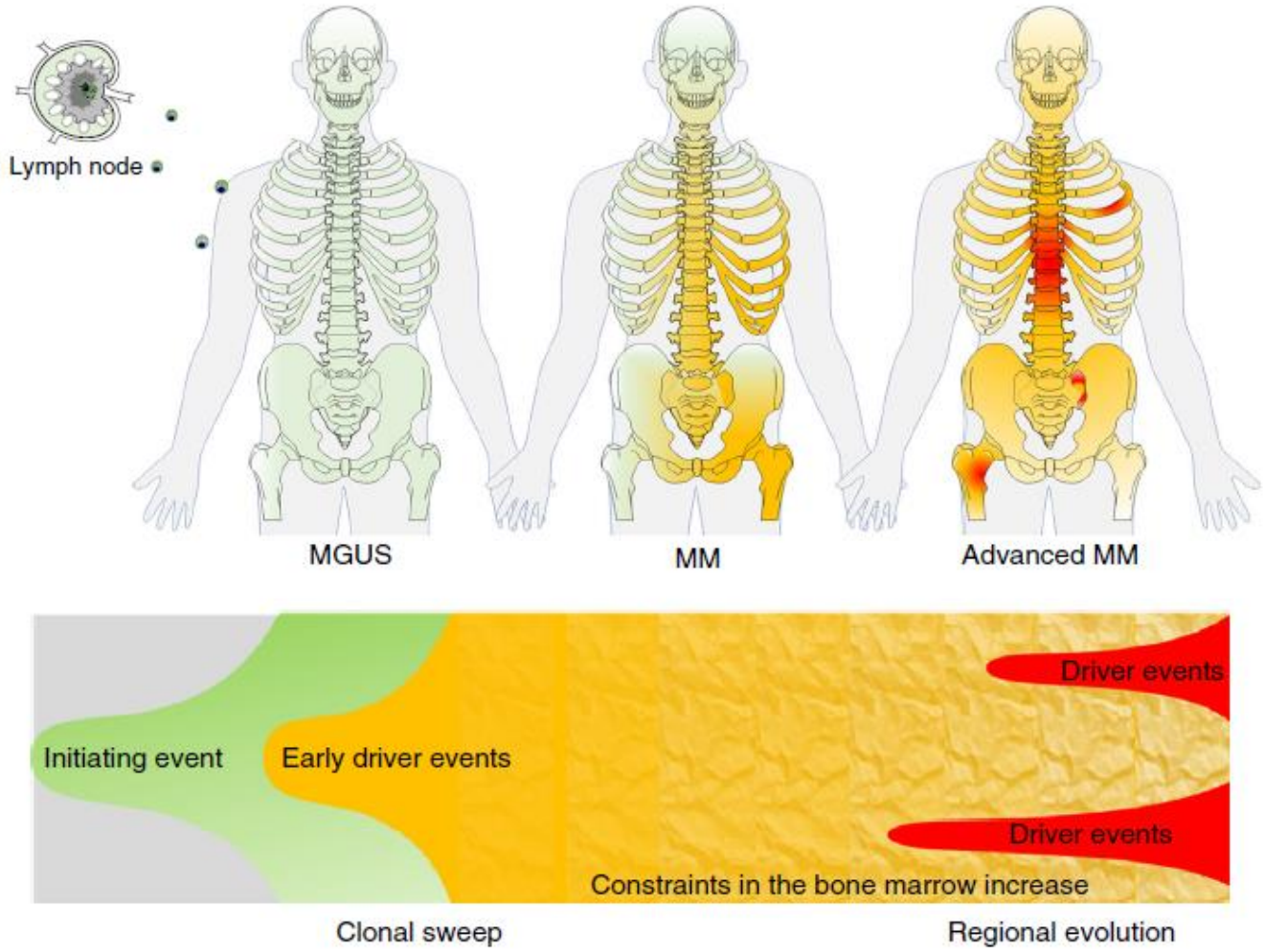
Bolzoni M et al, Blood 2016

Myeloma clonal evolution to high-risk



Pawlyn et al, Nature Reviews 2017

Regional evolution in multiple myeloma



Rasche et al, Nature Comm 2017

Spatial clonal architecture

Focal lesion at 4th lumbal vertebra:

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

Left liac crest:

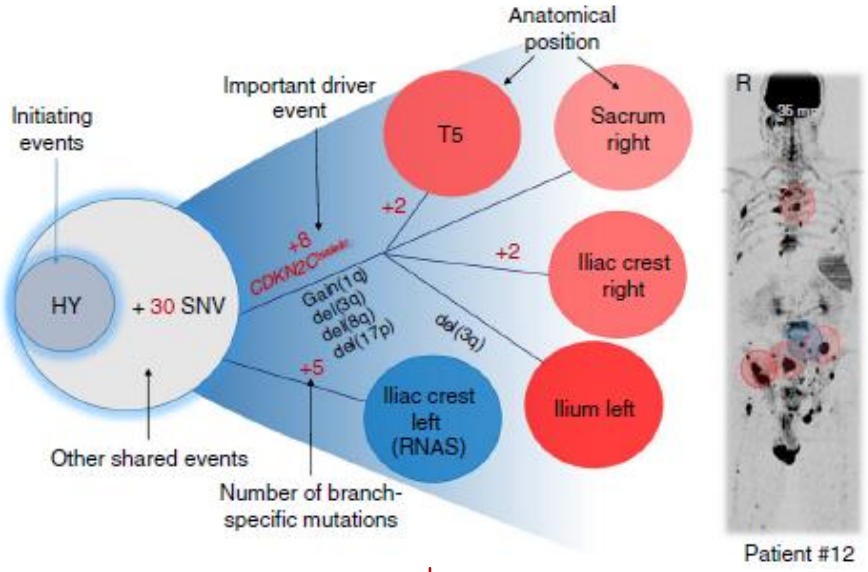
- GEP70 low risk
- Hyperdiploid
- t(MYC)
- BRAF^{V600E}

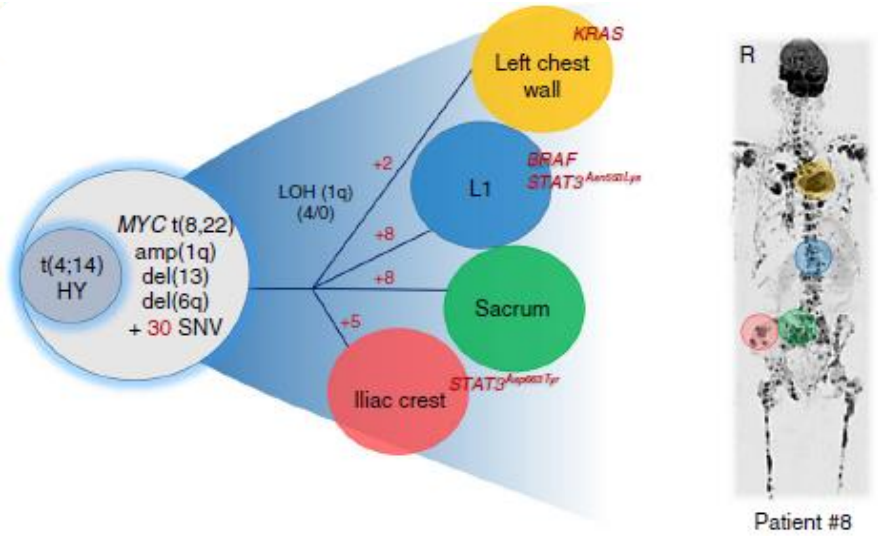
13 cases

- ✓ Medical imaging frequently shown an **imbalanced distribution** of **MM**
- ✓ Failure to detect clones that drives relapse may be explained by **regionally restricted** evolution
- ✓ Multi-region investigations are critical to understanding **intra-patients heterogeneity** in MM

Rasche et al, Nature Comm 2017

Multi-regional evolutionary events underlie disease progression



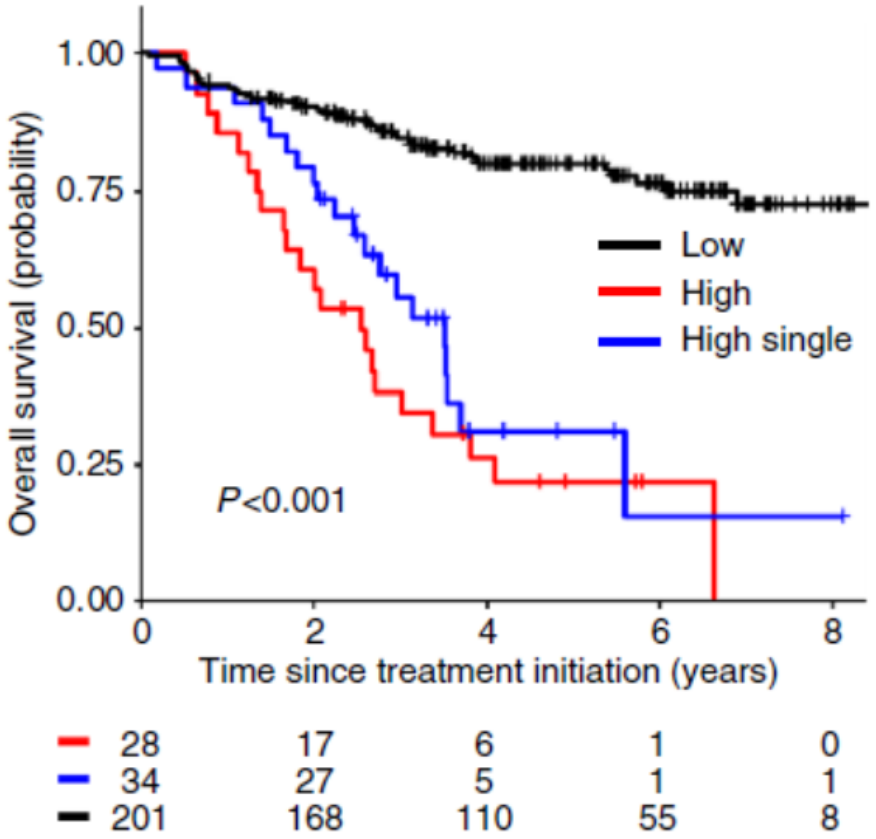


FLs have a **common high-risk ancestor** which disseminates in a metastatic way on a background of GEP70 low-risk disease

All sites have a common ancestor which was **further changed** during progression

Rasche et al, Nature 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

The biology of high risk MM: take home messages

- ✓ No unique pathogenic mechanism defines the high risk disease
- ✓ High proliferative rate
- ✓ Interaction between MM cells and microenviroment generate high risk ecosystem
- ✓ Metabolic adaptation to the hypoxic microenvironment
- ✓ Spatial genomic heterogeneity with a regional distrubution of high risk disease

Unmet challenges in high risk hematological malignancies: from benchside to clinical practice

Scientific Board:
Marco Ladetto (Alessandria)
Umberto Vitolo (Turin)

Turin, September 13-14, 2018
Torino Incontra Centro Congressi

Thanks for your attention…