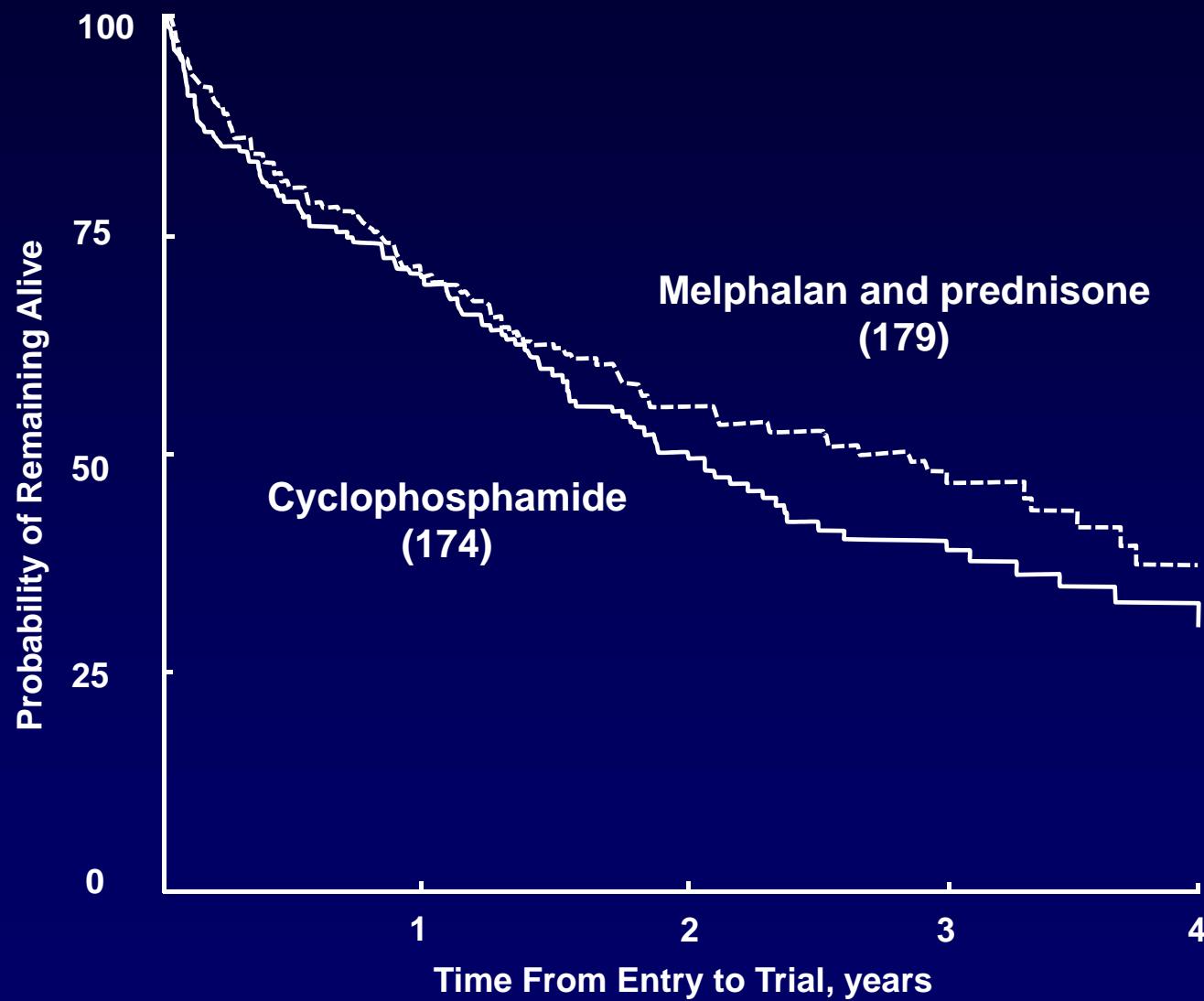


Relapsed or Refractory Multiple Myeloma (MM): What Lies Beneath?

Philippe Moreau, MD
University Hospital of Nantes
Nantes, France

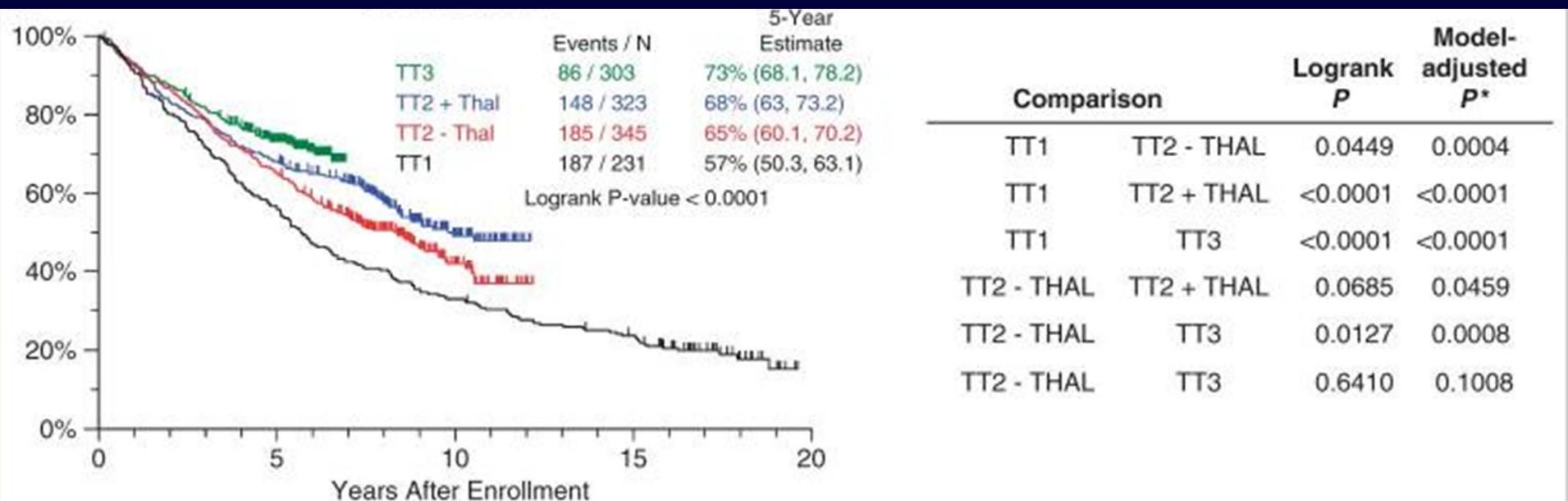
Resistance to Alkylators and Steroids



Medical Research Council III. *Br J Cancer*. 1980;42(6):823-830.

TT3: VTD – Tandem ASCT – VTD – VTD/TD

Overall survival

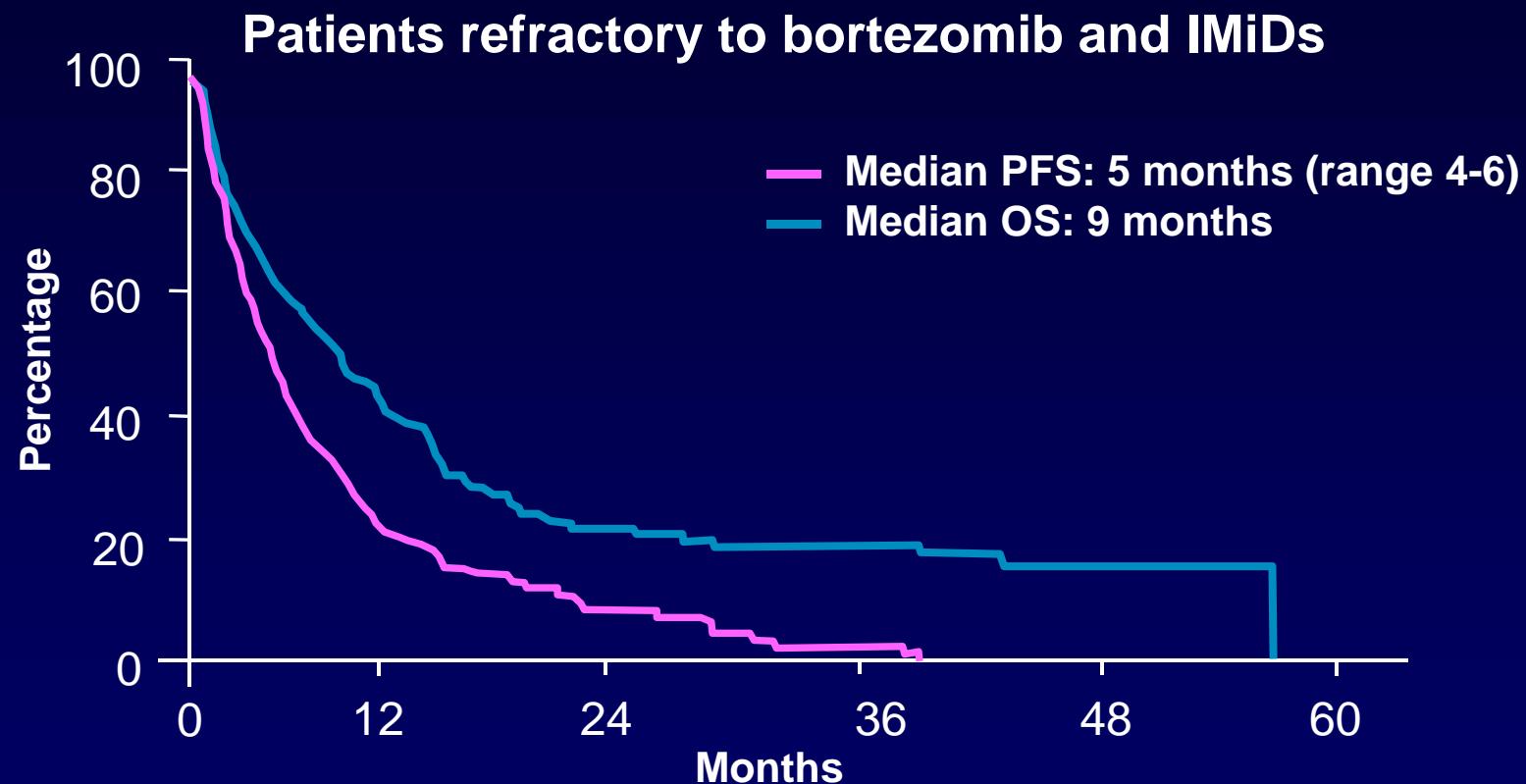


Resistance to PIs, IMiDs,
melphalan, and
dexamethasone

TT, total therapy; VTD, bortezomib, thalidomide, dexamethasone; ASCT, autologous stem-cell transplantation; PIs, proteosome inhibitors; IMiD, immunomodulatory drug

Usmani SZ, et al. *Leukemia*. 2013;27(1):226-232.

Prognosis for Patients Refractory to Novel Agents Remains Poor

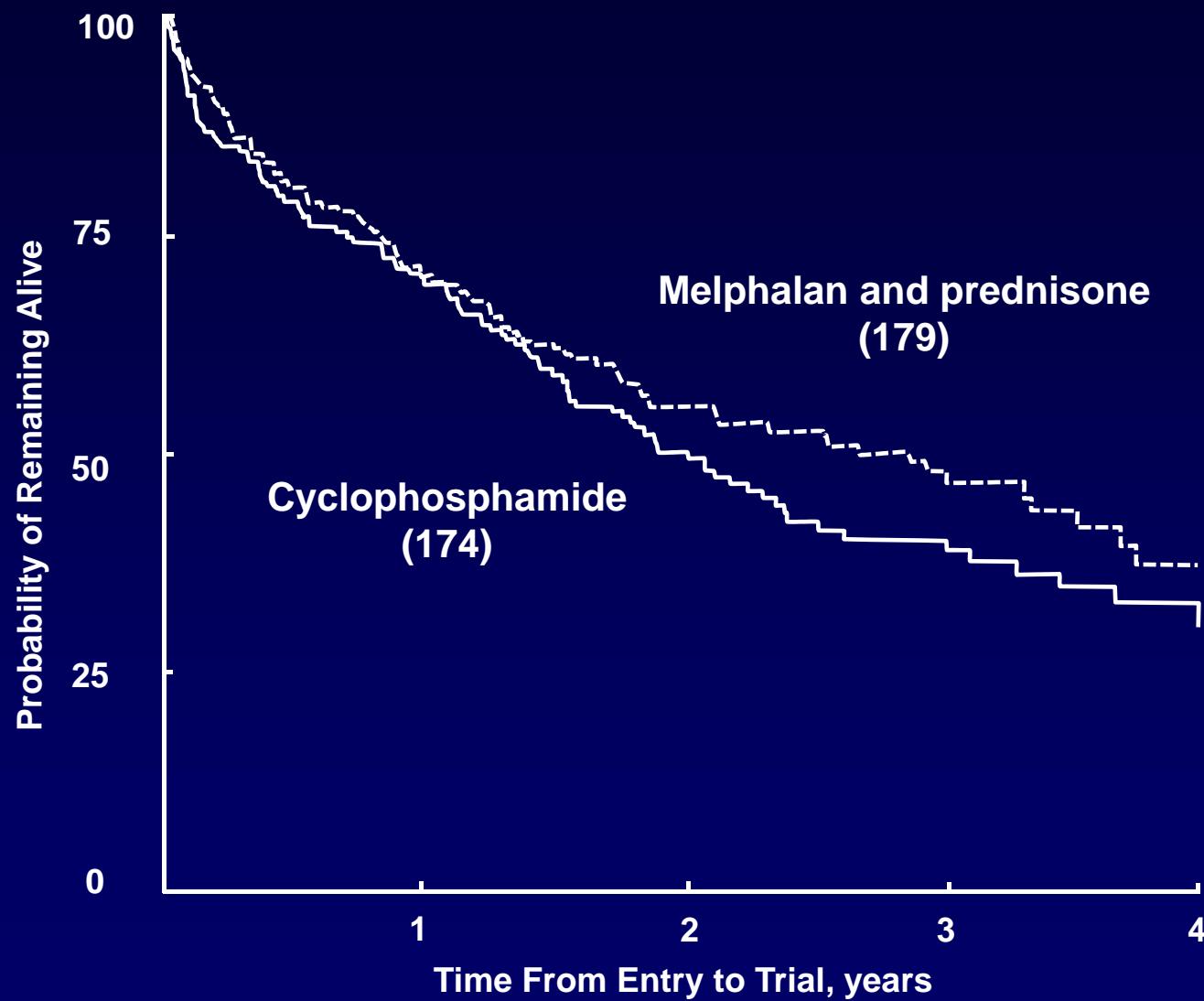


- Despite the benefit observed with novel agents in the last few years, new drugs are still needed for relapsed/refractory patients
- **Understanding resistance is KEY**

PFS, progression-free survival; OS, overall survival

Kumar SK, et al. *Leukemia*. 2012;26(1):149-157.

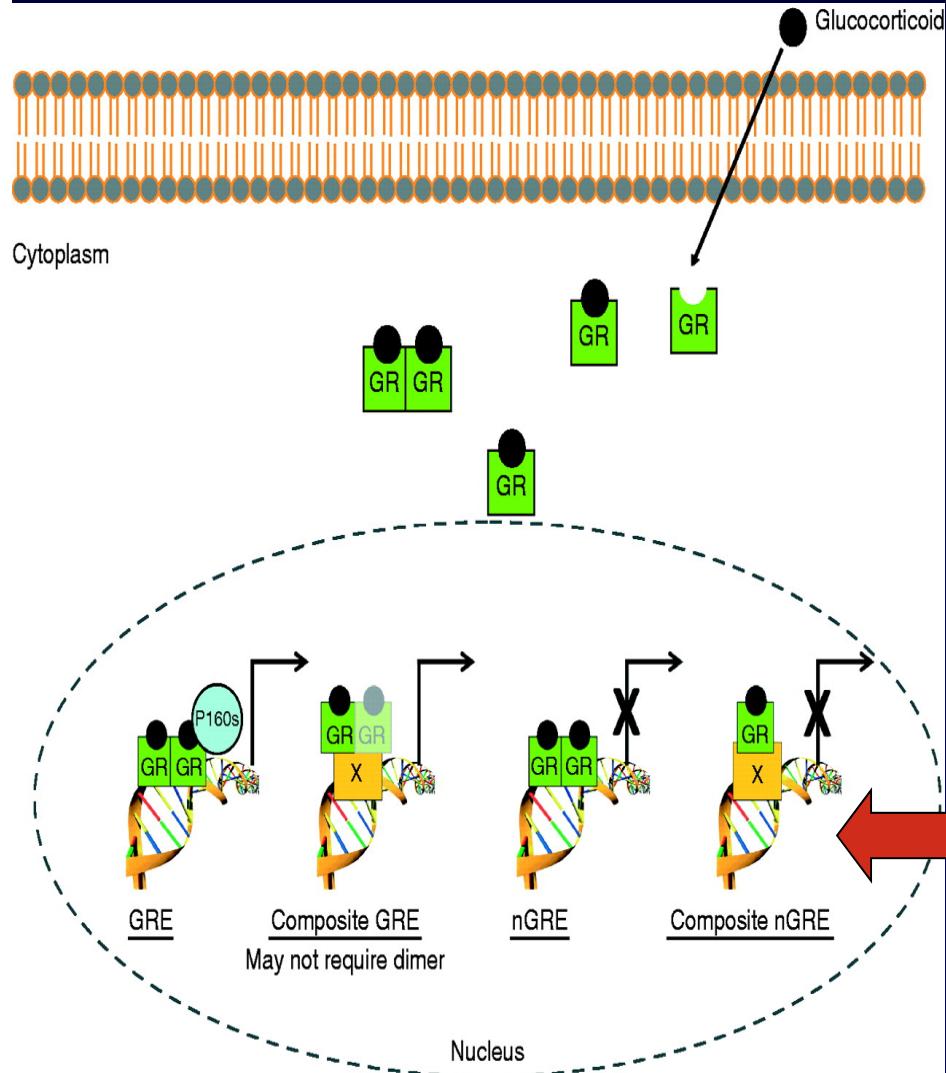
Resistance to Alkylators and Steroids



Medical Research Council III. *Br J Cancer*. 1980;42(6):823-830.

Resistance to Steroids/ Dexamethasone

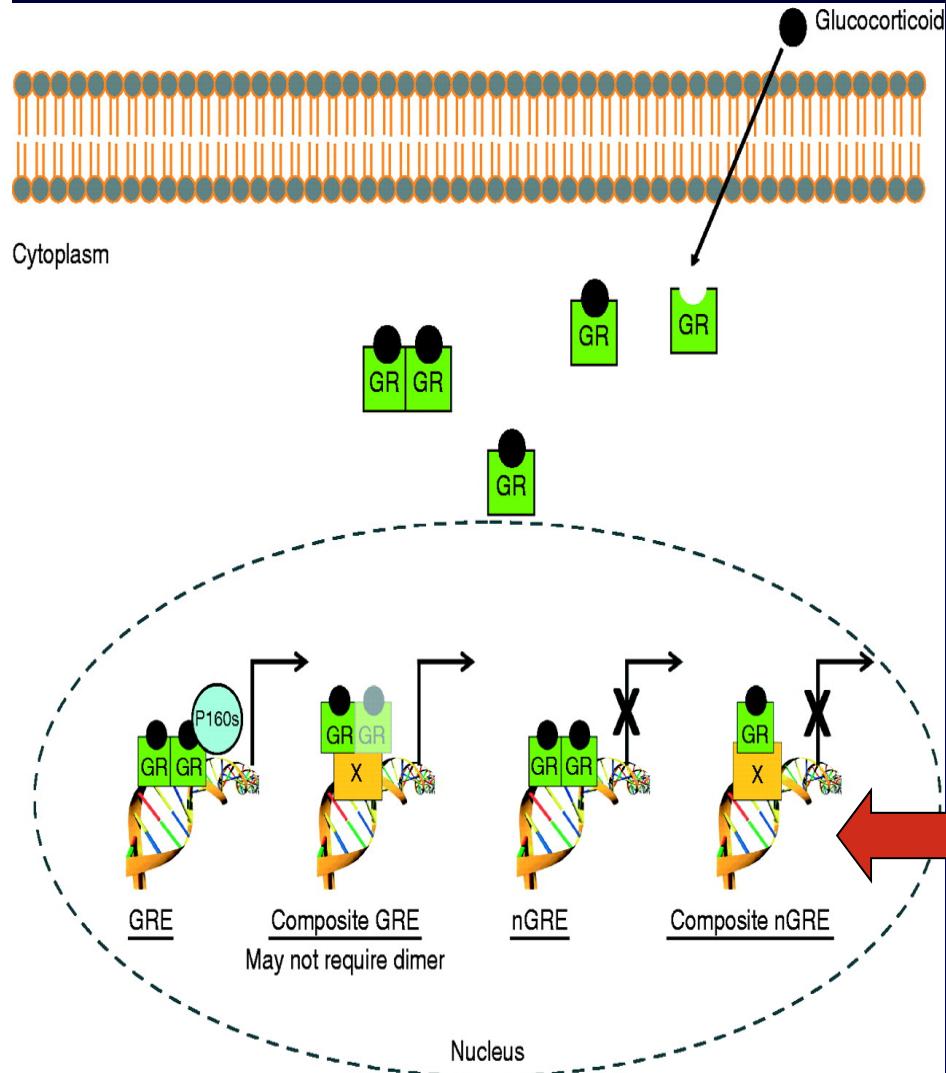
Resistance to Dexamethasone



Glucocorticoid receptor (GR) signaling

The glucocorticoid receptor translocates to the nucleus upon ligand binding where it will act as a homo- or heterodimer with or without other co-factors to either repress or drive transcription of target genes (>200 genes).

Resistance to Dexamethasone



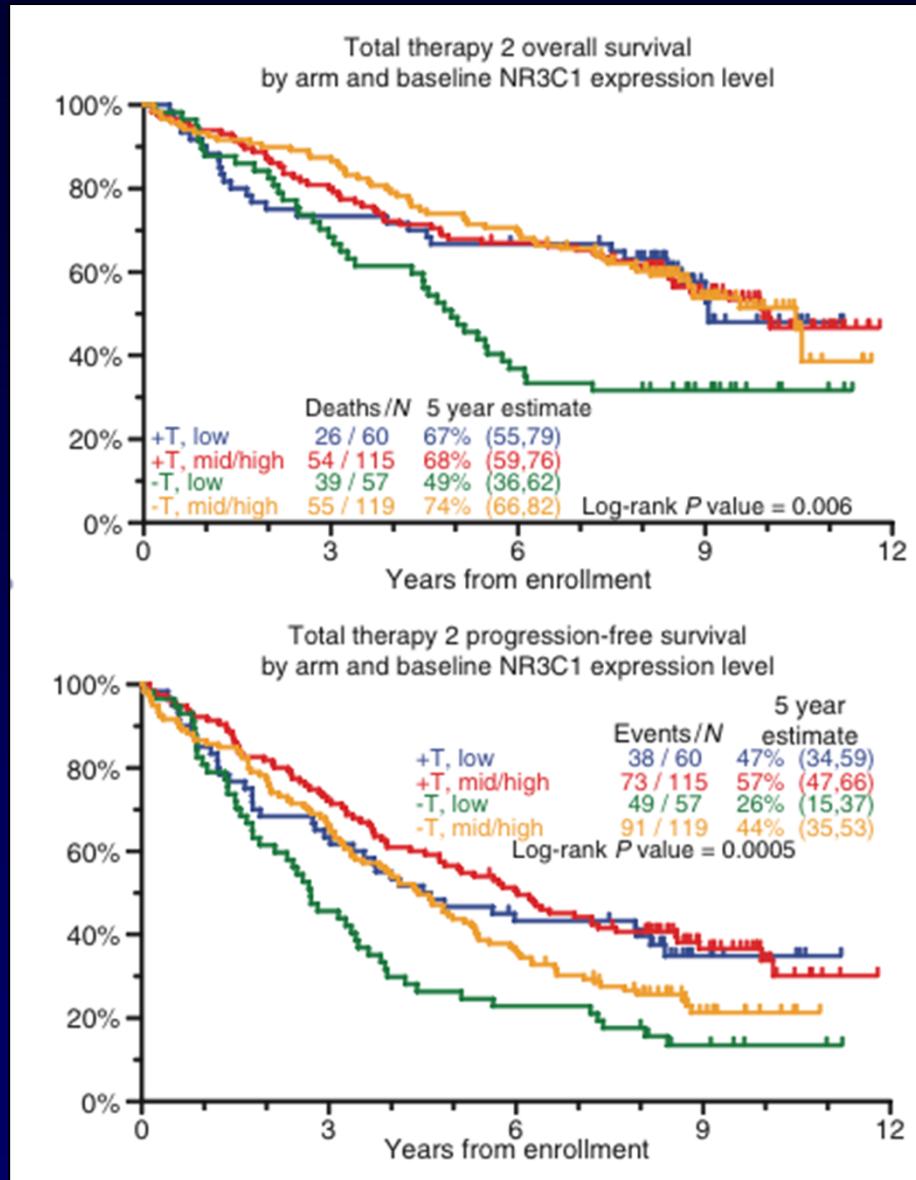
Mechanisms

1. Receptor (NR3C1): Low level of expression¹
2. Mutation in NR3C1, whole sequencing²

Glucocorticoid receptor signaling

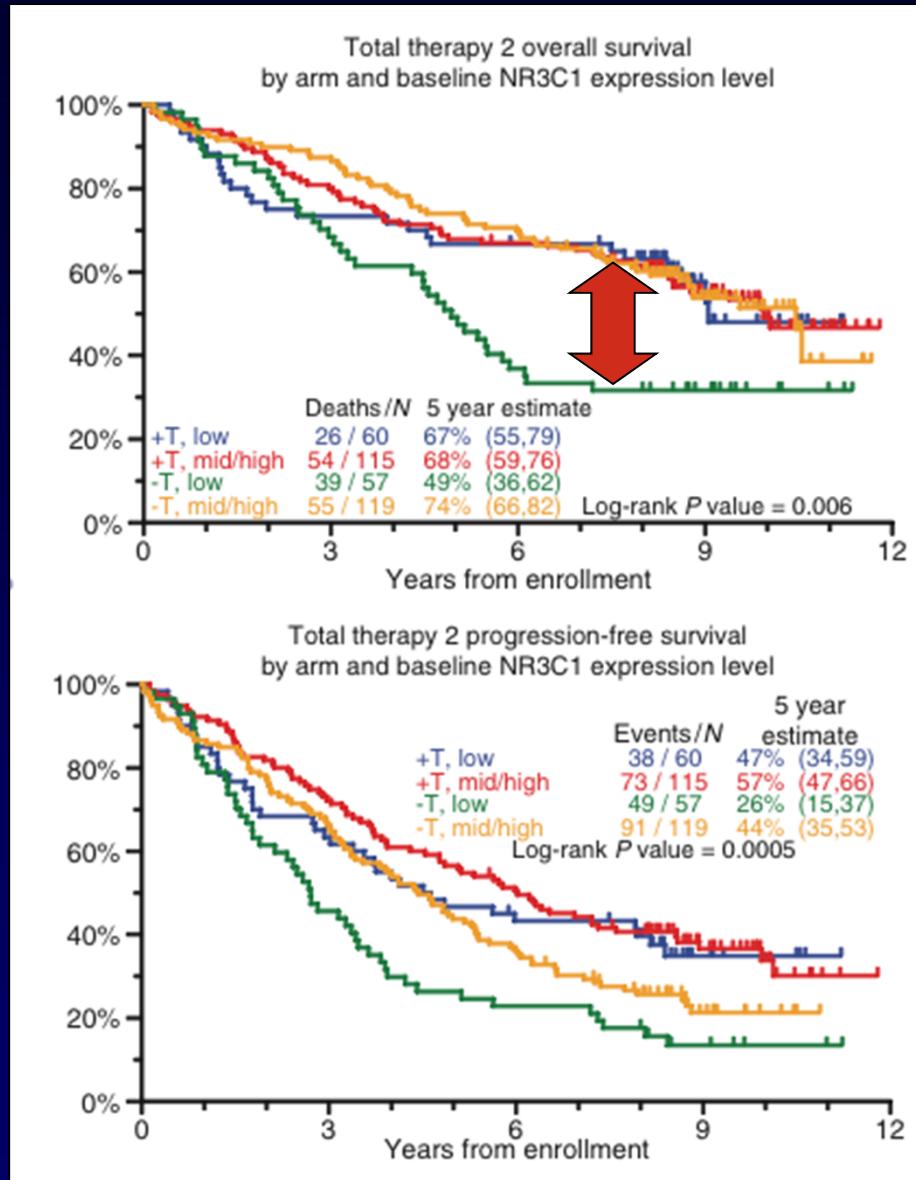
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1. Heuck CJ, et al. *Clin Cancer Res*. 2012;18(19):5499-5506. 2. Egan JB, et al. *Br J Haematol*. 2013;161(5): 748-751.



- Baseline NR3C1 gene expression
- Purified plasmocytes
- Affymetrix

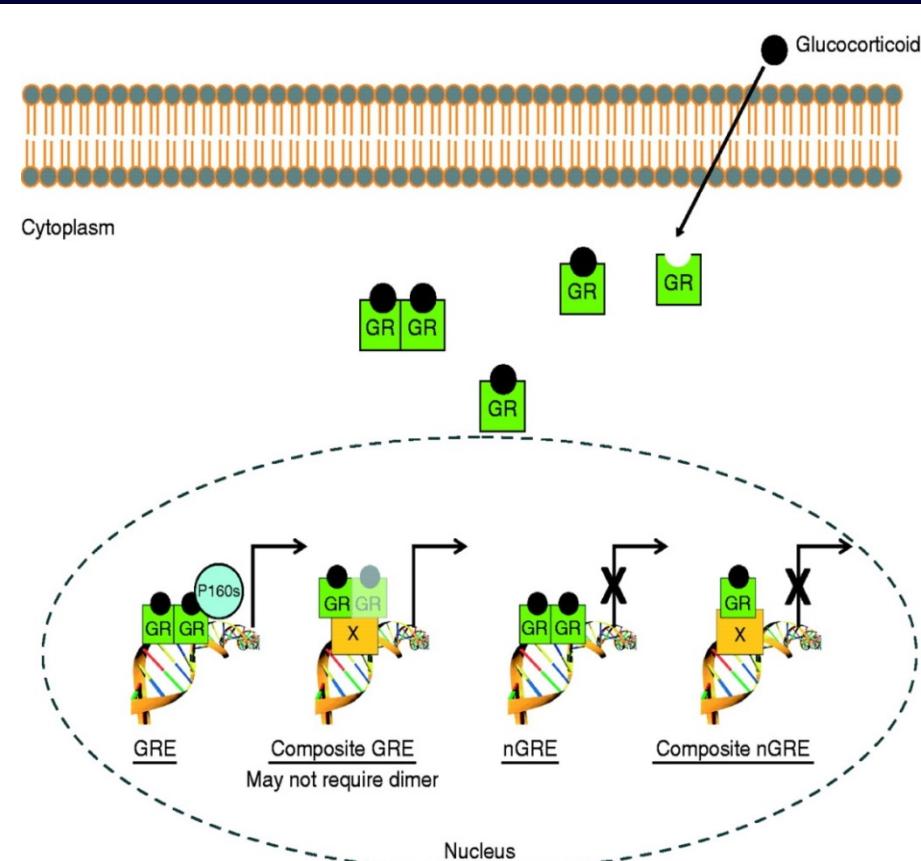
Low vs high +/- thalidomide



- Baseline NR3C1 gene expression
- Purified plasmocytes
- Affymetrix

Low vs high +/- thalidomide

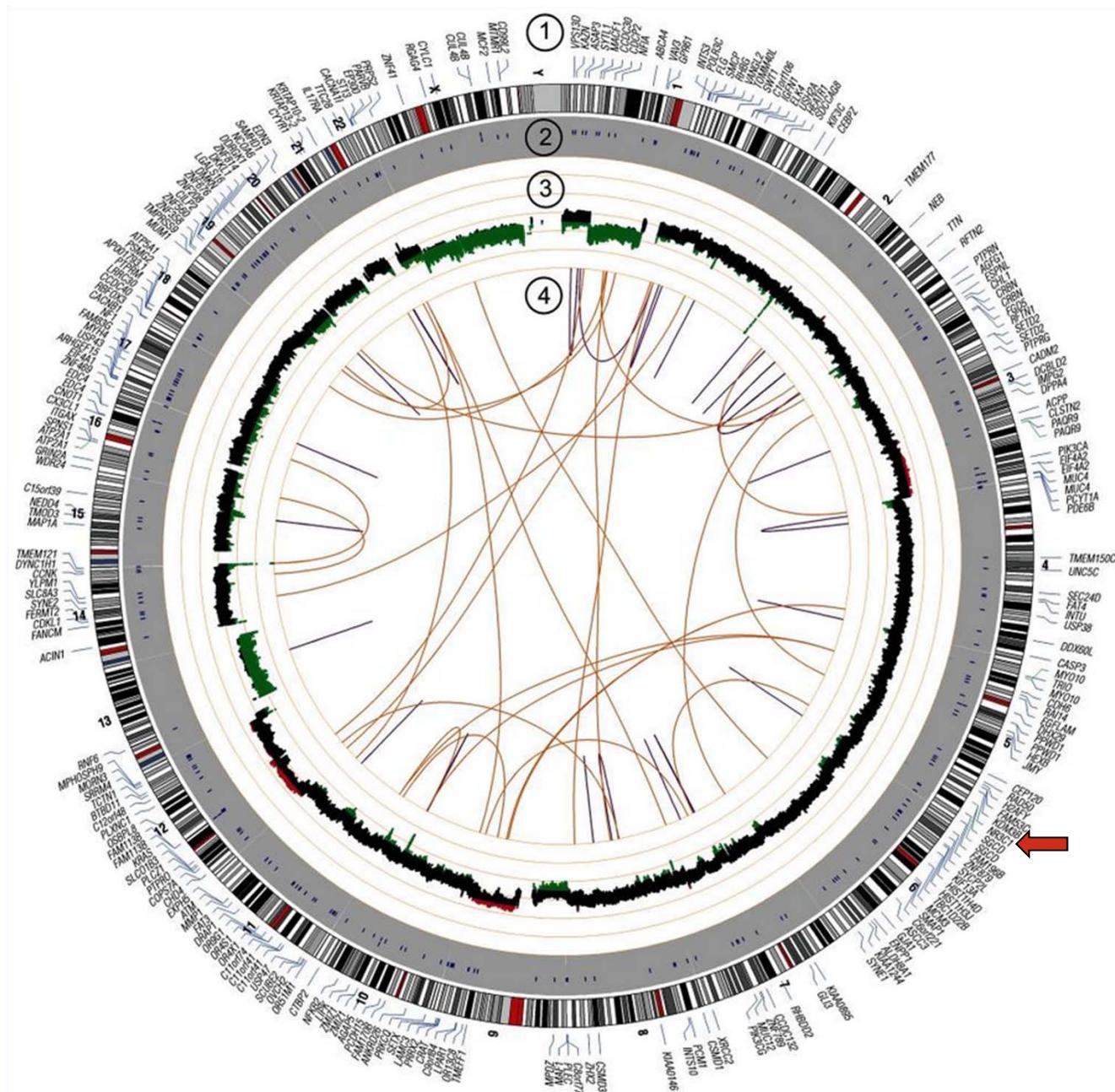
Resistance to Dexamethasone



Mechanisms

1. Receptor: Low level of expression¹
2. Mutation in NR3C1, whole genome sequencing²

1. Heuck CJ, et al. *Clin Cancer Res*. 2012;18(19):5499-5506. 2. Egan JB, et al. *Br J Haematol*. 2013;161(5): 748-751.



Circos plot depicting genome wide somatic variants, rearrangements, and copy number changes derived from next generation sequencing

NR3C1 on chromosome 5

Summary of Clinically Relevant Single Nucleotide Variations

| Chr | hg19 position | SNV | Gene | SIFT | Polyphen2 | Effect | Amino acid | dbSNP |
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Chr, chromosome; SNV, single nucleotide variation; SIFT, Sorting Intolerant

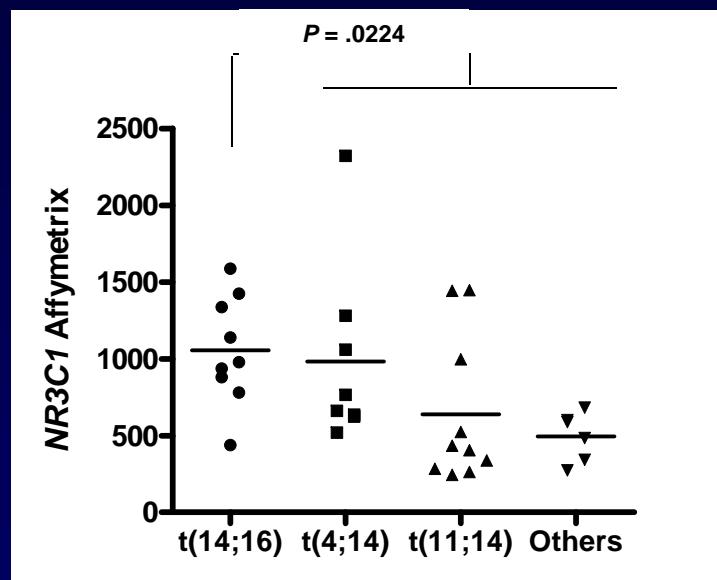
From Tolerant program; Polyphen2, Polymorphism Phenotyping v2 tool; dbSNP,

Single Nucleotide Polymorphism Database

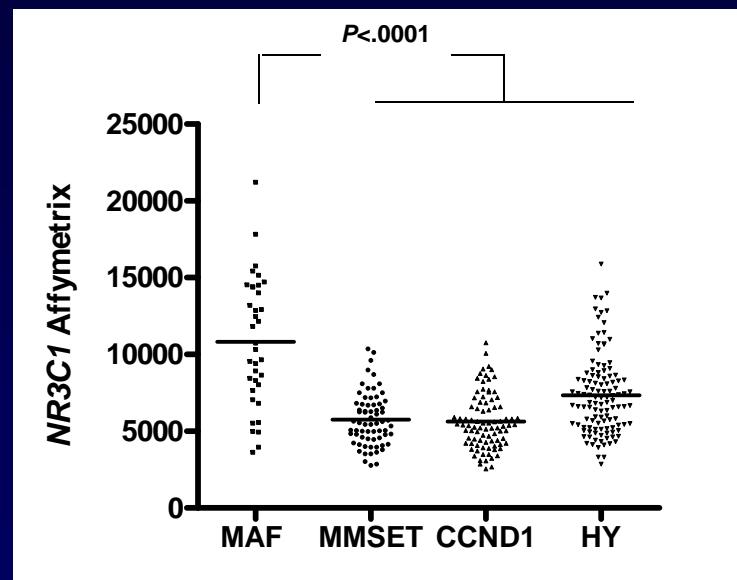
**Extramedullary myeloma whole genome sequencing reveals novel mutation
in the glucocorticoid receptor in multi drug resistant disease**

(Paradoxically) MAF Subgroup Significantly Expressed Higher Levels of GR

Human myeloma cell lines (HMCLs)



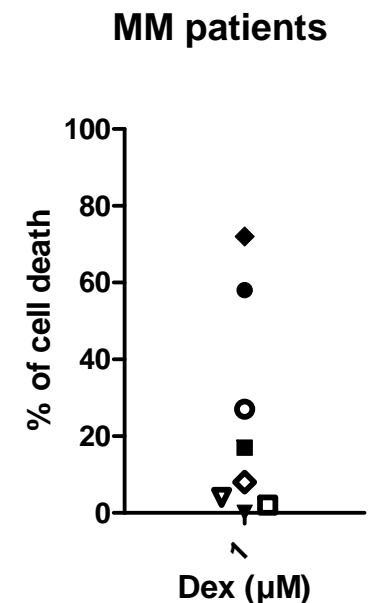
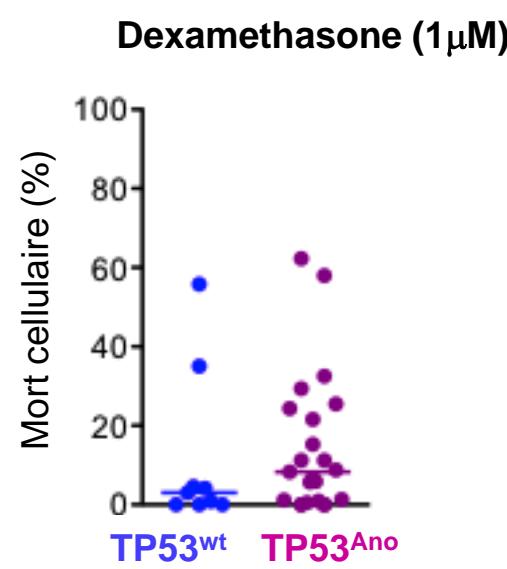
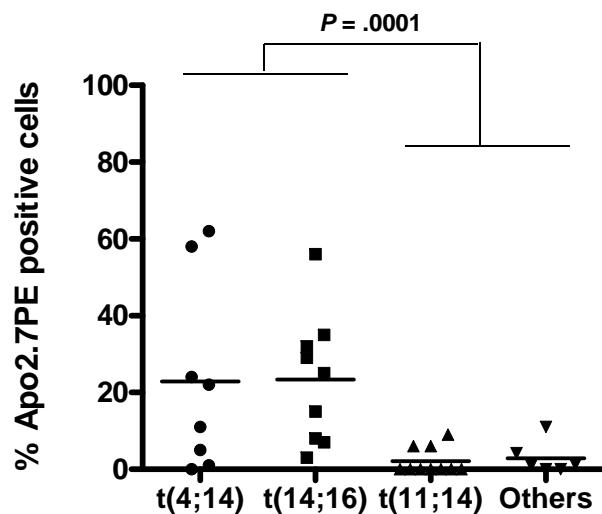
Newly diagnosed MM patients



Ch5?

- GR expression is highest in HMCLs and group of patients over-expressing MAF

Apoptosis Induced by Dexamethasone Single-Agent



Variable

- Dexamethasone sensitivity is superior in t(4;14) and t(14;16) HMCL subgroups
- P53 pathway is not involved in resistance to steroids

Resistance to Melphalan

Resistance to Melphalan (First Synthesized 1953)

Melphalan:

- Alkylating agent actively transported into cells
- It exerts its cytotoxic effect through the formation of **DNA cross-links / damages**
- Melphalan's cytotoxic effects are related to its concentration and the duration of exposure to melphalan of the cell
- Induction of cell death when DNA repair pathways are overflowed

Resistance to Melphalan (First Synthesized 1953)

Mechanisms of resistance:

- Multidrug resistance (MDR) (P-glycoprotein [PgP])
- Resistance to apoptosis: p53
- DNA repair (various genes, including *FANC* and *MMSET*)

Resistance to Melphalan (First Synthesized 1953)

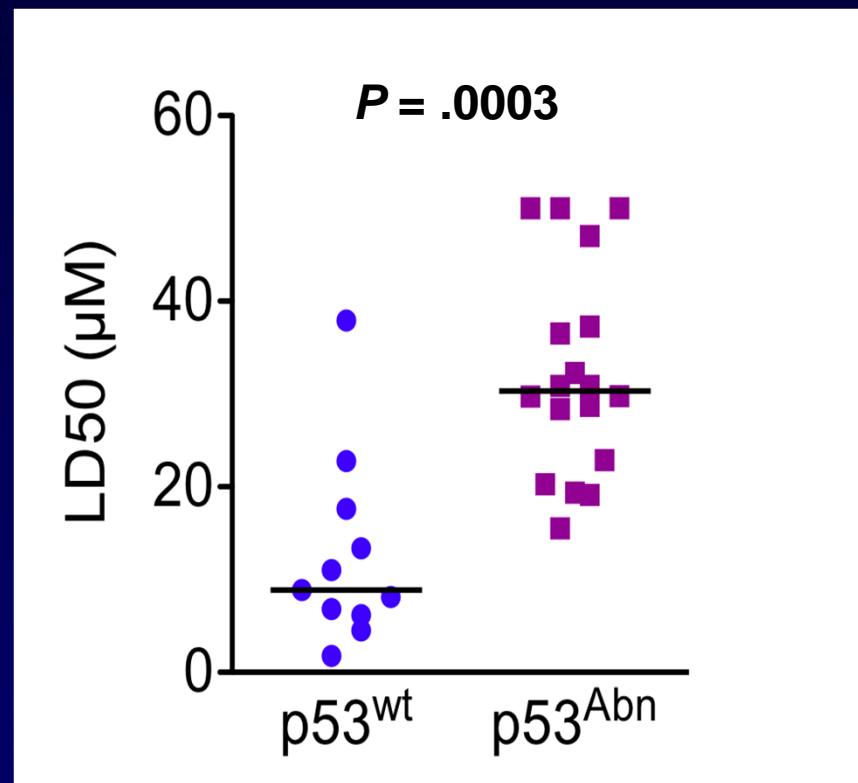
Mechanisms of resistance:

- MDR (pgp)
- Resistance to apoptosis: p53
- DNA repair (various genes, including *FANC* and *MMSET*)

Therefore t(4;14) (*MMSET*) and 17p deletions (p53) are associated with resistance to melphalan

Resistance to Melphalan, *In Vitro*, Human Myeloma Cell Lines

Melphalan



p53

Surget S, et al; *Leuk Lymphoma*. 2014;55(9):2165-2173. UMR 892 Inserm, Nantes

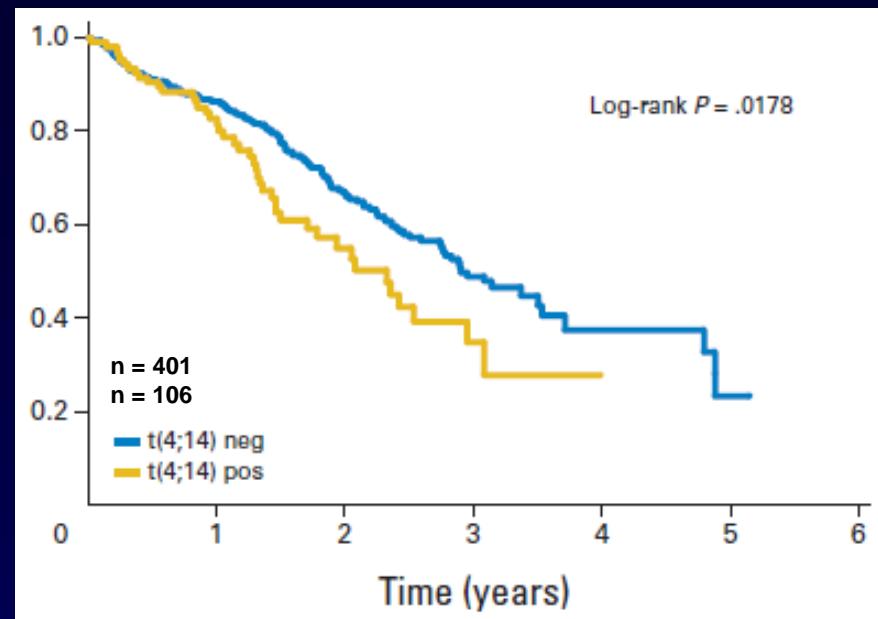




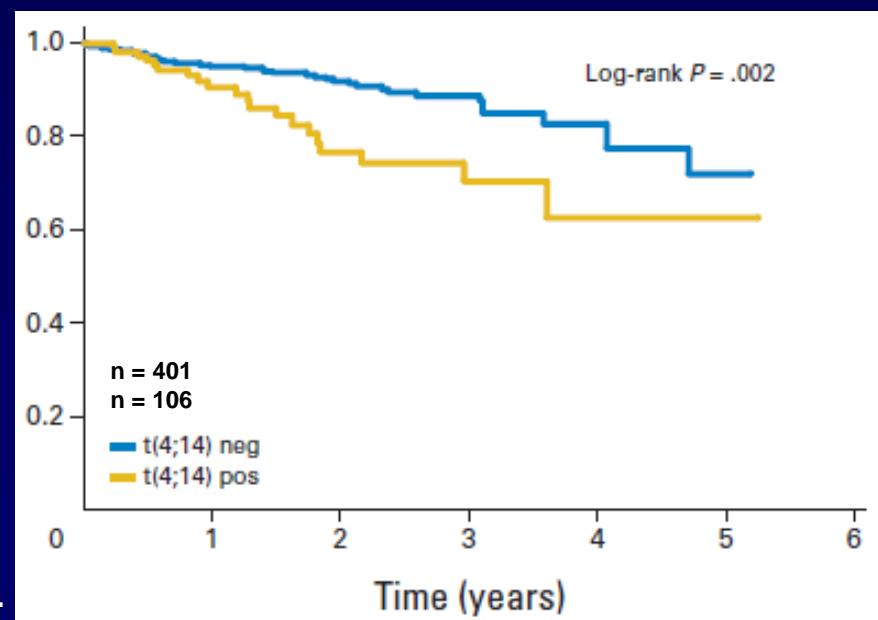
**Bortezomib plus dexamethasone
induction
Melphalan 200 and ASCT**

17p and resistance to melphalan

Event-Free Survival (EFS)



Overall Survival



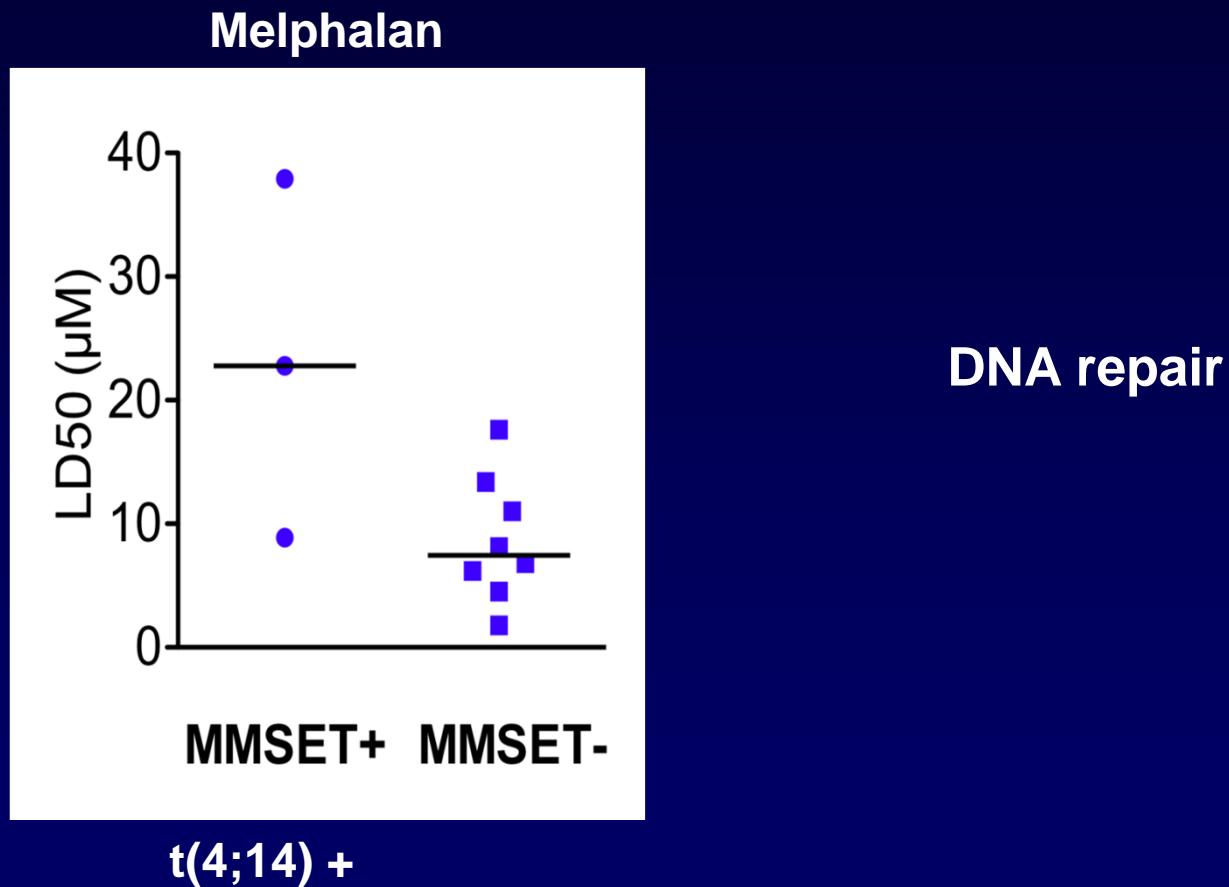
Resistance to Melphalan

***MMSET*: enzyme, histone methyltransferase**

- Methylation of histone H4
- Recruitment protein 53BP1
- Initiation of DNA repair

t(4;14): Overexpression of *MMSET*; DNA repair is enhanced/increased; resistance to DNA damages induced by melphalan

Resistance to Melphalan, *In Vitro*, Human Myeloma Cell Lines

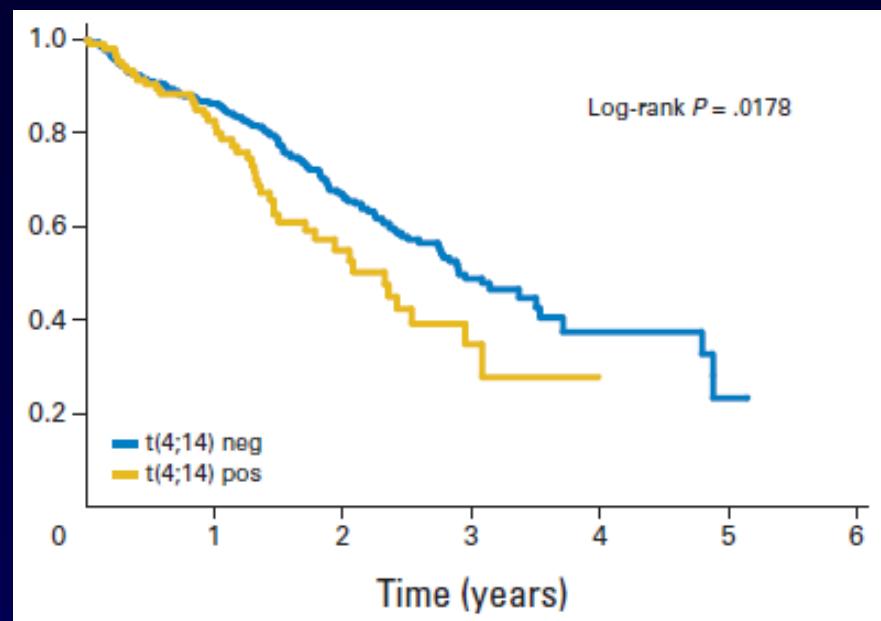




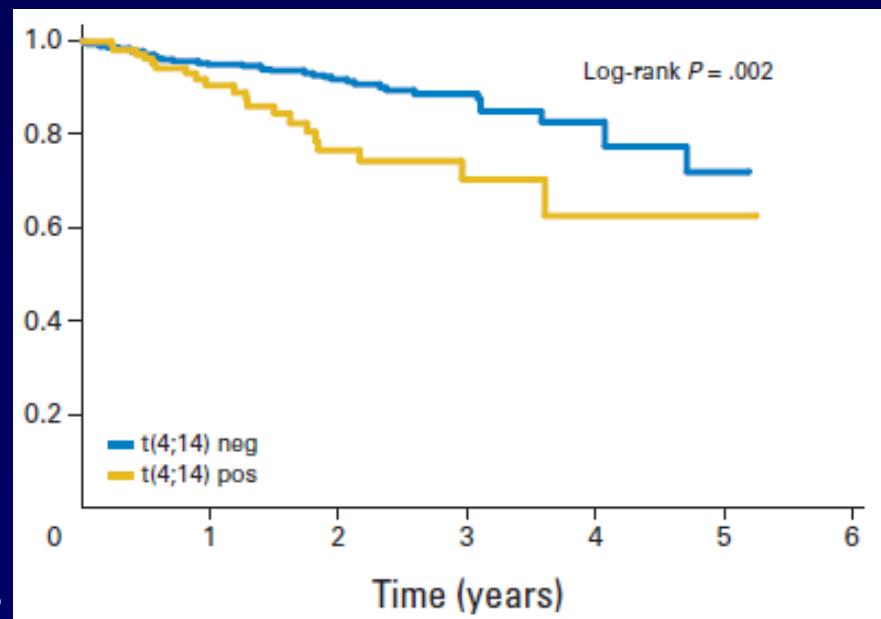
Bortezomib plus dexamethasone induction Melphalan 200 and ASCT

T(4;14) and resistance to melphalan

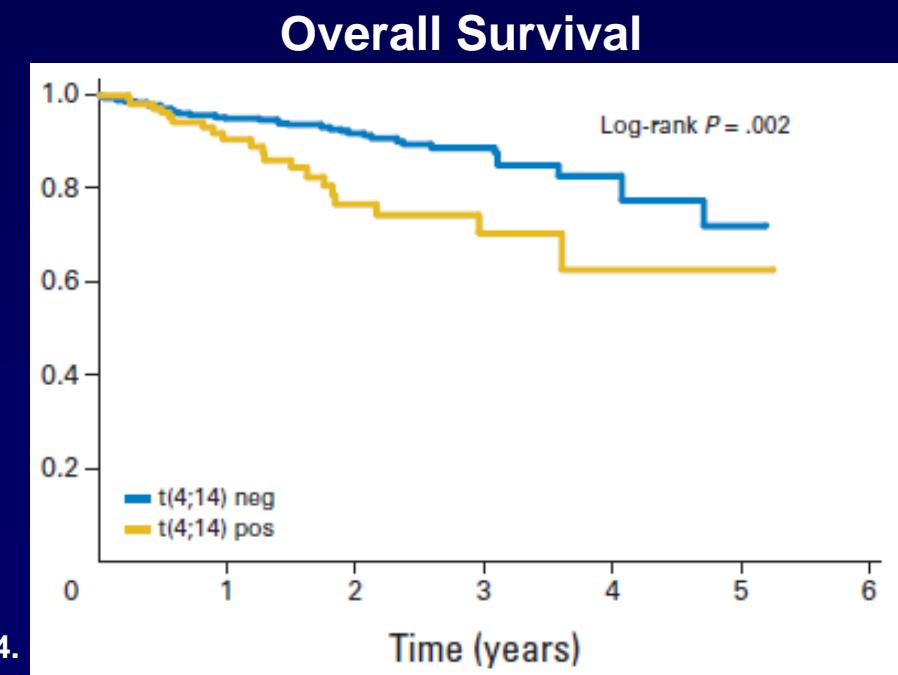
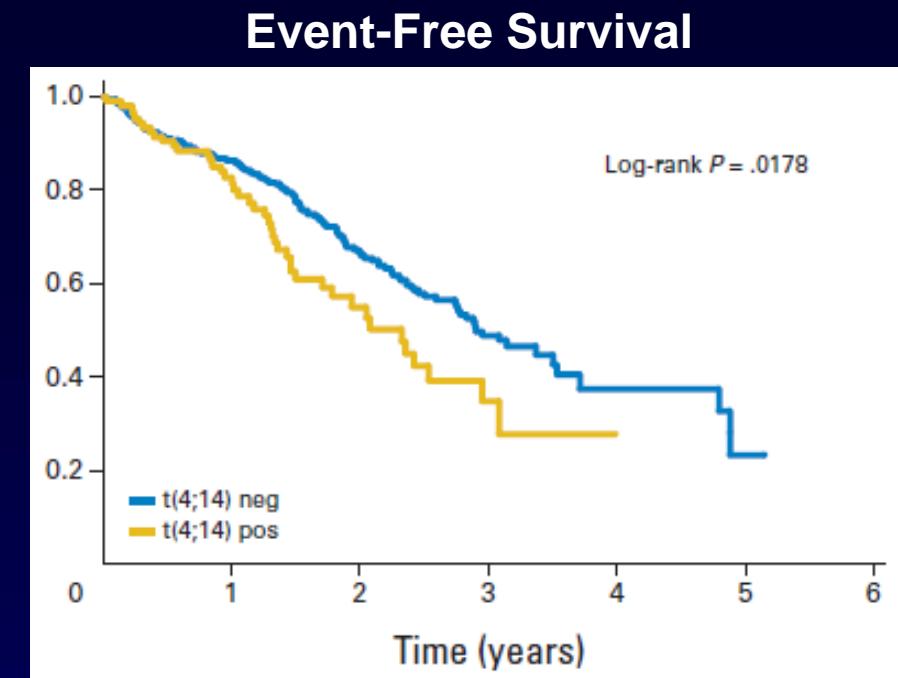
Event-Free Survival



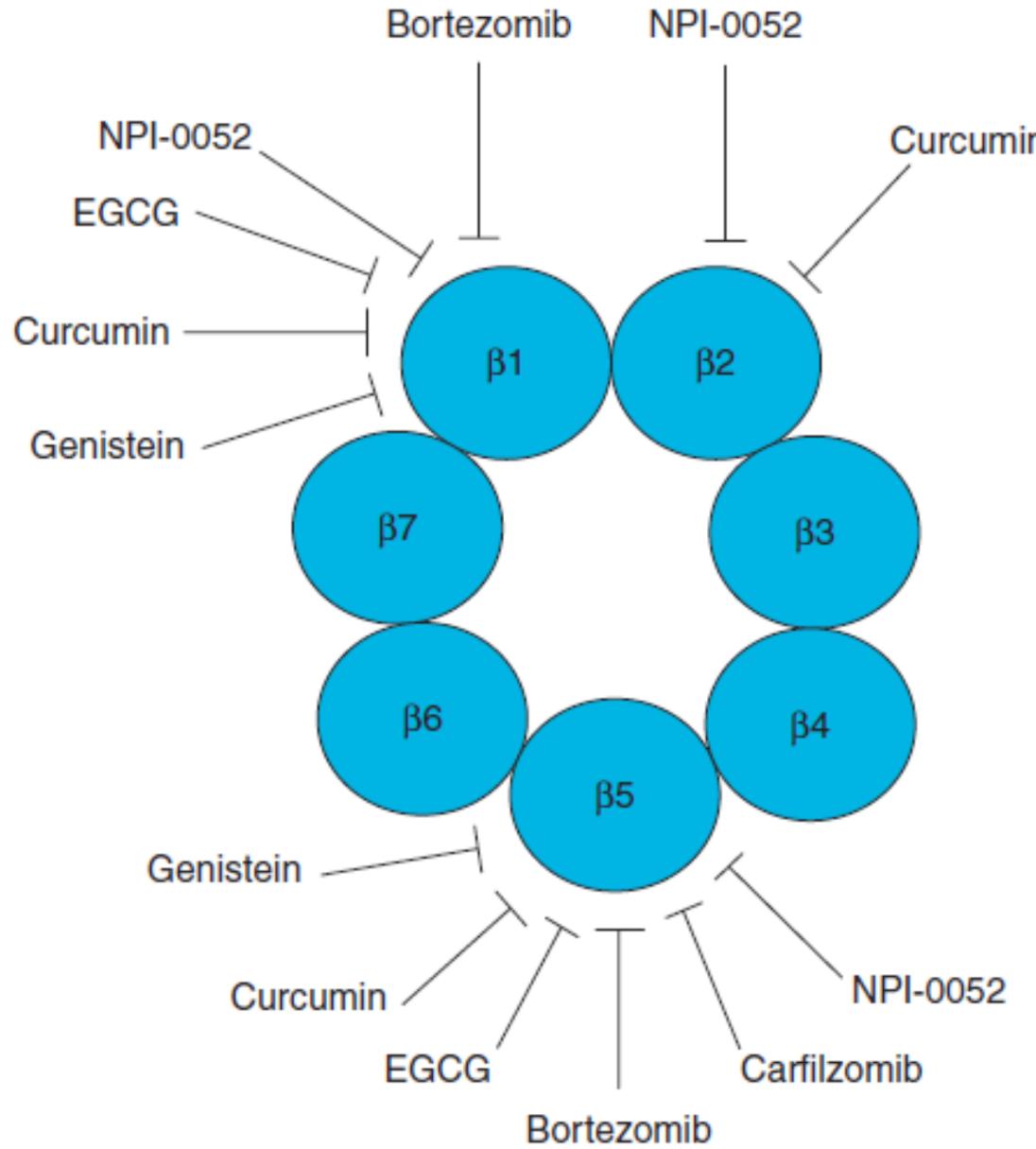
Overall Survival

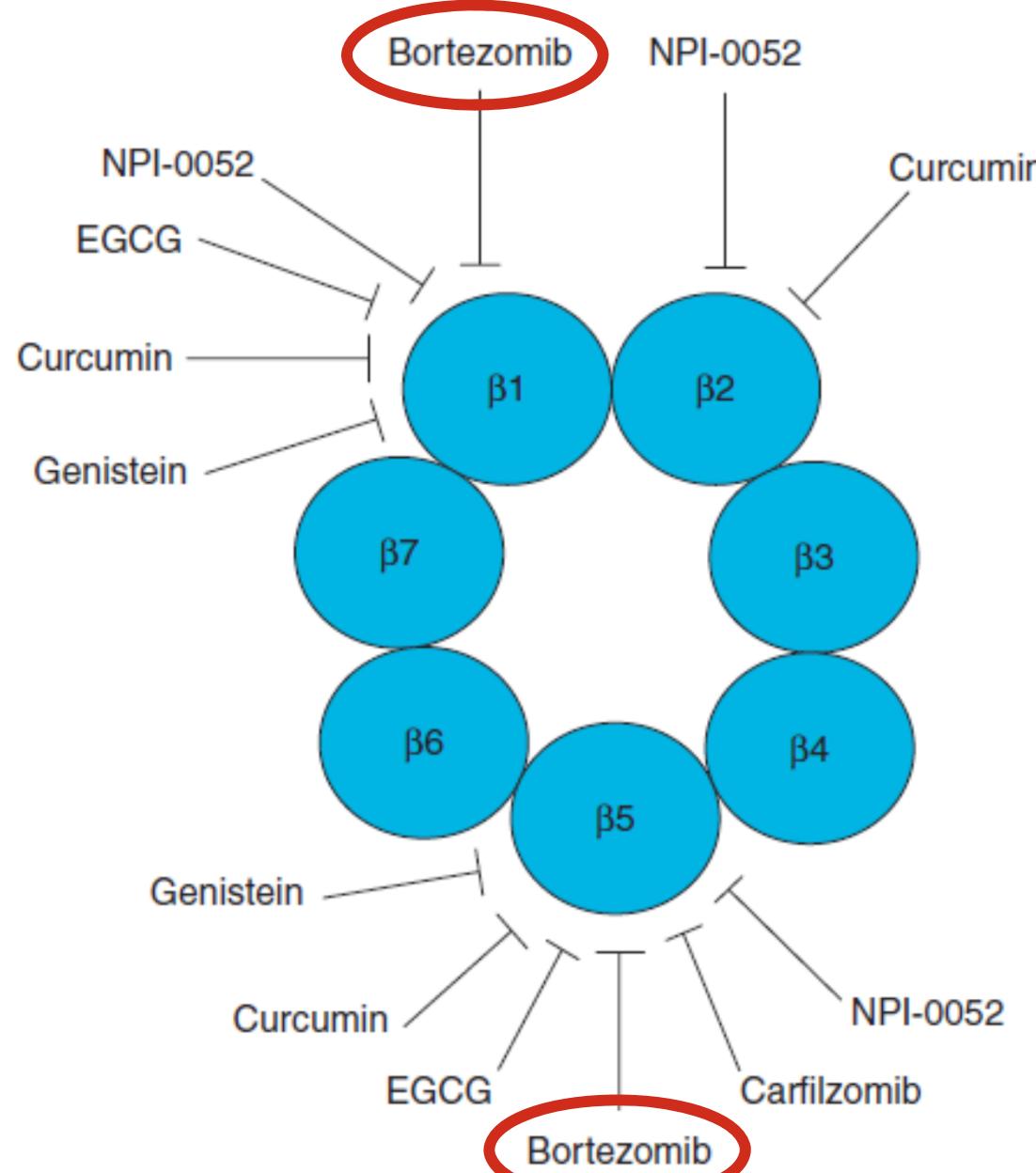


T(4;14)
VAD or bortezomib plus
dexamethasone induction



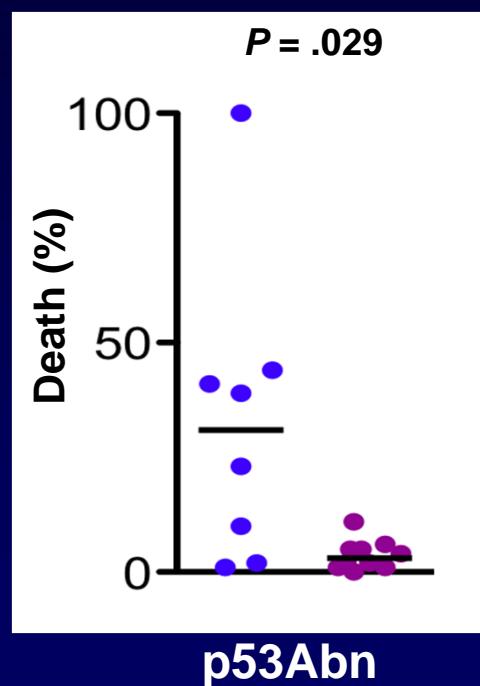
Resistance to Proteasome Inhibitors



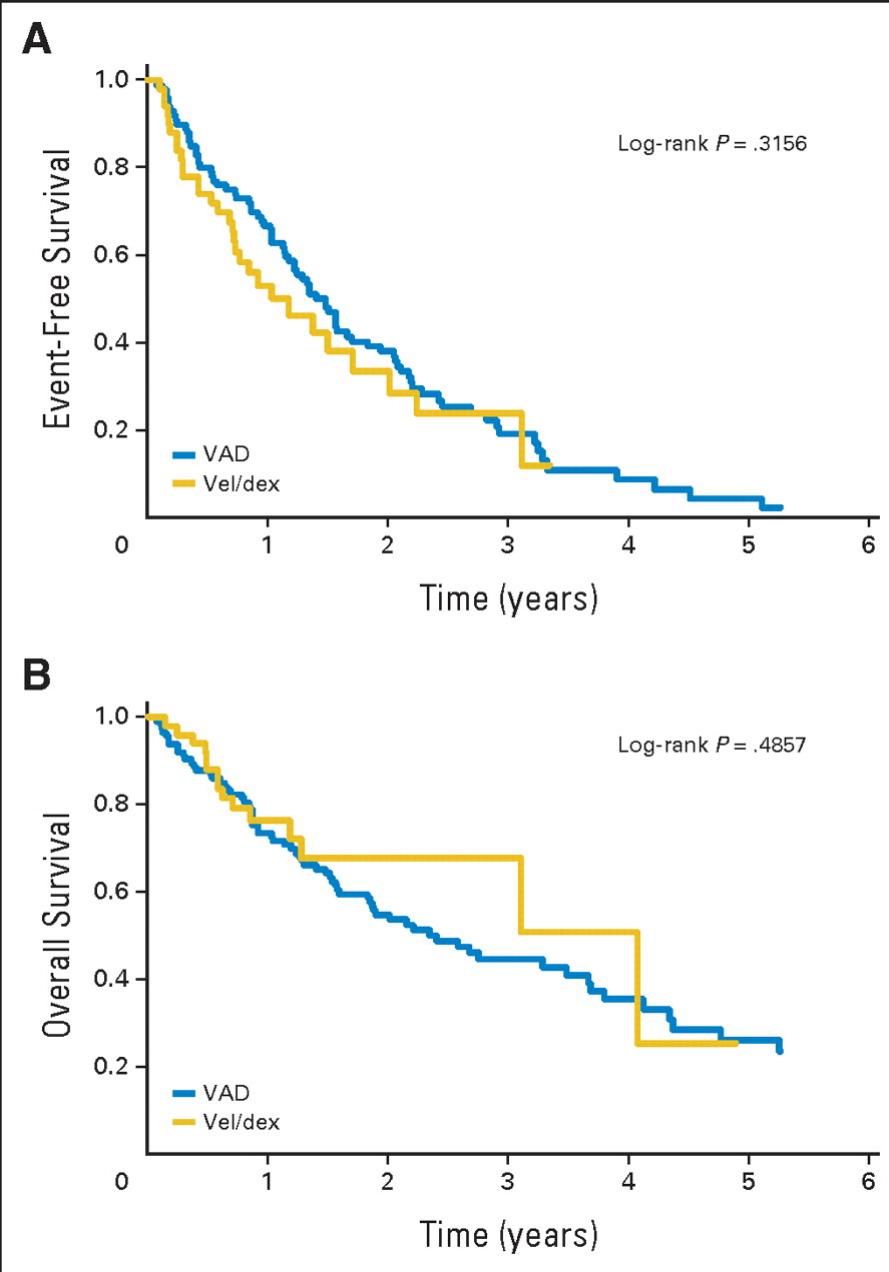


Resistance to Bortezomib, *In Vitro*, HMCL

Bortezomib

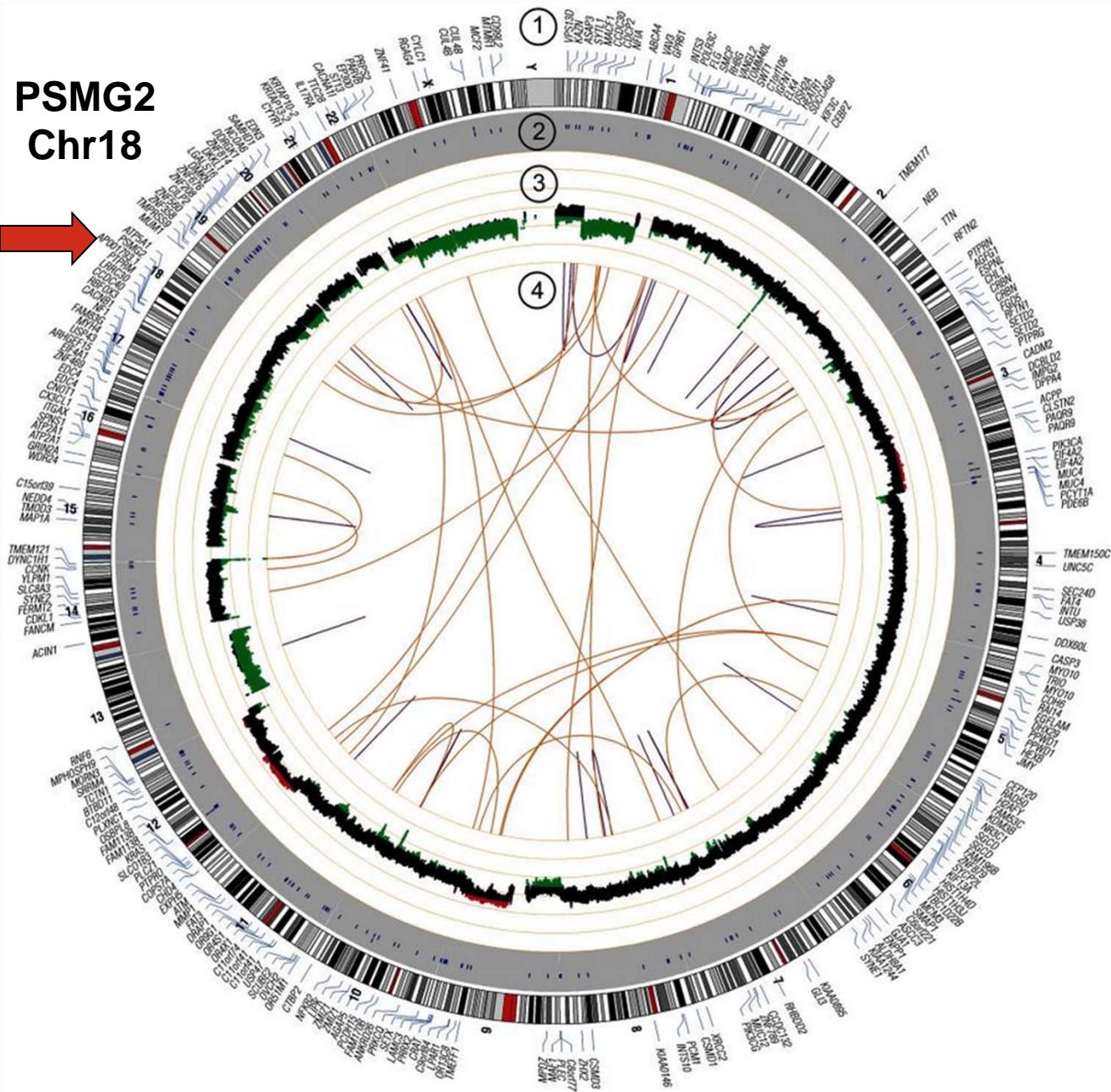


p53



17p VAD or bortezomib plus dexamethasone induction

A) EFS (B) OS in patients with del(17p) treated with bortezomib-dexamethasone induction ($n = 54$) or with VAD induction ($n = 119$; EFS and OS in years; $P = .32$ for EFS; $P = .49$ for OS)



Circos plot depicting genome wide somatic variants, rearrangements, and copy number changes derived from next generation sequencing

Mutation

Whole genome sequencing

Summary of Clinically Relevant Single Nucleotide Variations

| Chr | hg19 position | SNV | Gene | SIFT | Polyphen2 | Effect | Amino acid | dbSNP |
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Chr, chromosome; SNV, single nucleotide variation; SIFT, Sorting Intolerant

From Tolerant program; Polyphen2, Polymorphism Phenotyping v2 tool; dbSNP,

Single Nucleotide Polymorphism Database

Extramedullary myeloma whole genome sequencing reveals mutation on PSMG2, proteasome assembly chaperone 2, involved in 20S proteasome maturation

(Mutations in proteasome assembly components contribute to proteasome inhibitor resistance)

Egan JB, et al. *Br J Haematol.* 2013;161(5):748-751.

Overview of Some of the Molecular Effects of PIs That Contribute to Their Antimyeloma Activity

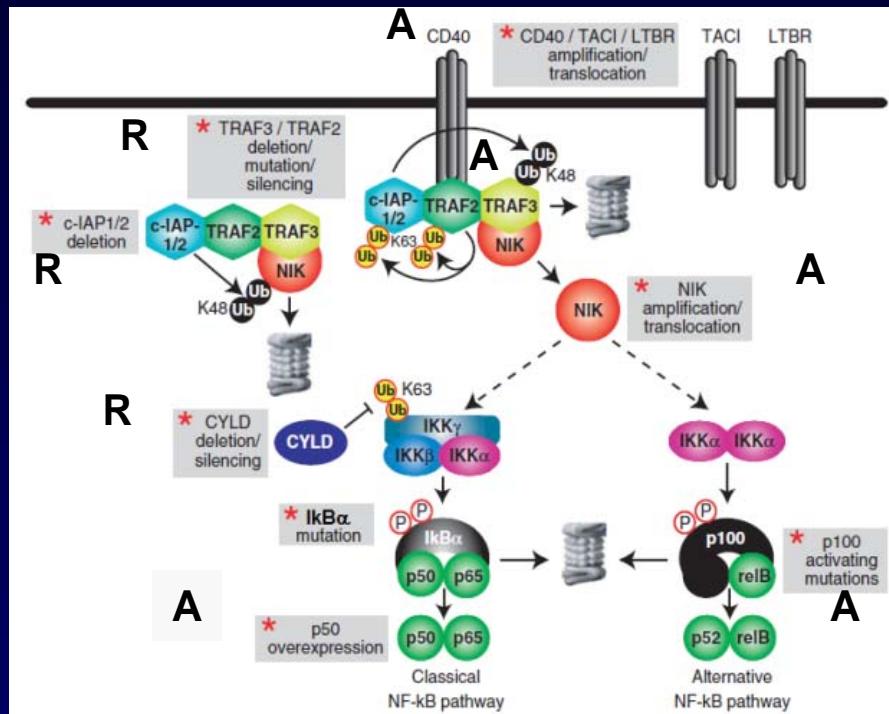
| Target | Mechanism | Consequence |
|---|--|---|
| α_4 -Integrin BH3 proteins Calcium | Downregulate expression of VLA-4 Stabilize BIK, NOXA and BIM Disrupt mitochondrial calcium uniporter | Overcome adhesion-mediated drug resistance Contribute to activation of Bax and Bak Dysregulate intracellular calcium storage; induce caspase activation |
| Caveolin 1 | Inhibit VEGF-triggered caveolin phosphorylation; decrease caveolin expression | Reduce myeloma cell migration and survival |
| Cdkis HIF-1 α | Stabilize Cdkis such as p21 and p27 Stimulate FIH | Arrest the cell cycle Inhibit tumor angiogenesis and tumor adaptation to hypoxia |
| HLA | Downregulate surface expression of class I molecules | Enhance natural killer cell-mediated lysis of myeloma cells |
| HSP-90 | Induce HSP-90 expression and cell-surface exposure | Enhance dendritic cell-mediated induction of immunity |
| IL-6 | Reduce stromal cell production of IL-6 through NF- κ B; also down-regulate gp130 through a caspase-mediated process | Suppress IL-6-mediated growth and survival signals |
| IGF-1 | Downregulate IGF-1 and IGF-1R expression | Suppress IGF-1-mediated growth and survival signals |
| JNK | Activate JNK | Upregulate Fas and activate caspase-8 and caspase-3 |
| Mcl-1 | Induce Mcl-1 cleavage | Reduce antiapoptotic Mcl-1; produce proapoptotic Mcl-1 fragments |
| MKP-1 | Induce MKP-1 expression | Inhibit p44/42 MAPK-mediated growth and survival signals |
| NF- κ B | Stabilize I κ B | Multiple mechanisms (please see text for more details) |
| p53 | Cause accumulation and phosphorylation of p53 | Induce downstream targets such as p21, NOXA and Bax |
| ROS | Induce reactive oxygen species production | Promote mitochondrial injury with release of pro-apoptotic factors |
| UPR | Induce proapoptotic UPR genes; suppress antiapoptotic UPR responses | Activate caspase-mediated apoptosis |
| VEGF | Suppress stromal cell production of VEGF | Reduce myeloma cell migration and marrow angiogenesis |

Overview of Additional Mechanisms of Action of PIs That Add to the Antimyeloma Activity of Other Chemotherapeutics

| Target | Mechanism | Consequence |
|------------------|---|--|
| Bcl-2 | Induce Bcl-2 phosphorylation and cleavage | Sensitize to multiple cytotoxic agents |
| DNA-PK | Suppress expression, and induce cleavage, of DNA PK and other DNA damage repair enzymes | Sensitize to DNA damaging agents such as alkylators and anthracyclines |
| NF-κB | Stabilize IκB | Sensitize to multiple cytotoxic agents |
| PgP | Inhibit normal maturation of precursor forms of PgP | Reduce multi-drug resistance to chemotherapeutics subject to PgP |
| Survivin | Reduce survivin levels in combination with cytotoxics | Sensitize to DNA damaging drugs |
| Topo-I | Prevent tumor-induced degradation of Topo-I | Sensitize to agents that inhibit Topo-I |
| Topo-II α | Stabilize Topo-II α | Sensitize to agents that inhibit Topo-II α |

Signaling Pathways: Few Recurrent Mutation but Many Different Ones

- NF-κB pathway
 - Negative regulators inactivation
 - Positive regulators activation
 - Driver genes



- Combined genetic alterations ~ 20 % of the patients have constitutively activated NF-κB pathway

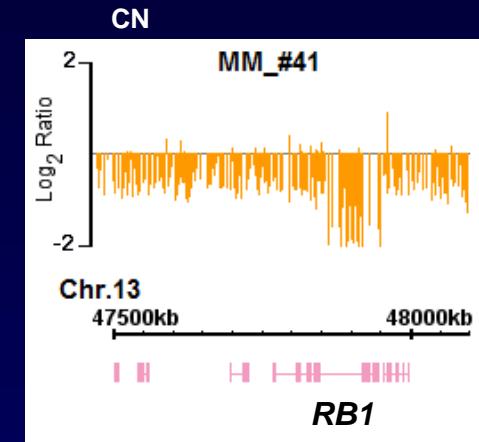
Multimodal Genomic Analysis of MM: Focal Vision, Localized Analysis

- Focal abnormalities (targeting suppressor genes or oncogenes)

- Homozygous deletion:

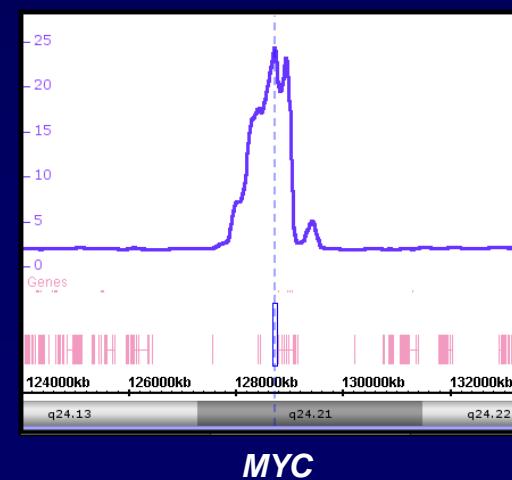
CDKN2C, CDKN2A/CDKN2B, RB1, TP53,

TRAF2, WWOX, FAM46C...



- Amplification (>3 copies): infrequent!

MYC, CD40, NFKB1...

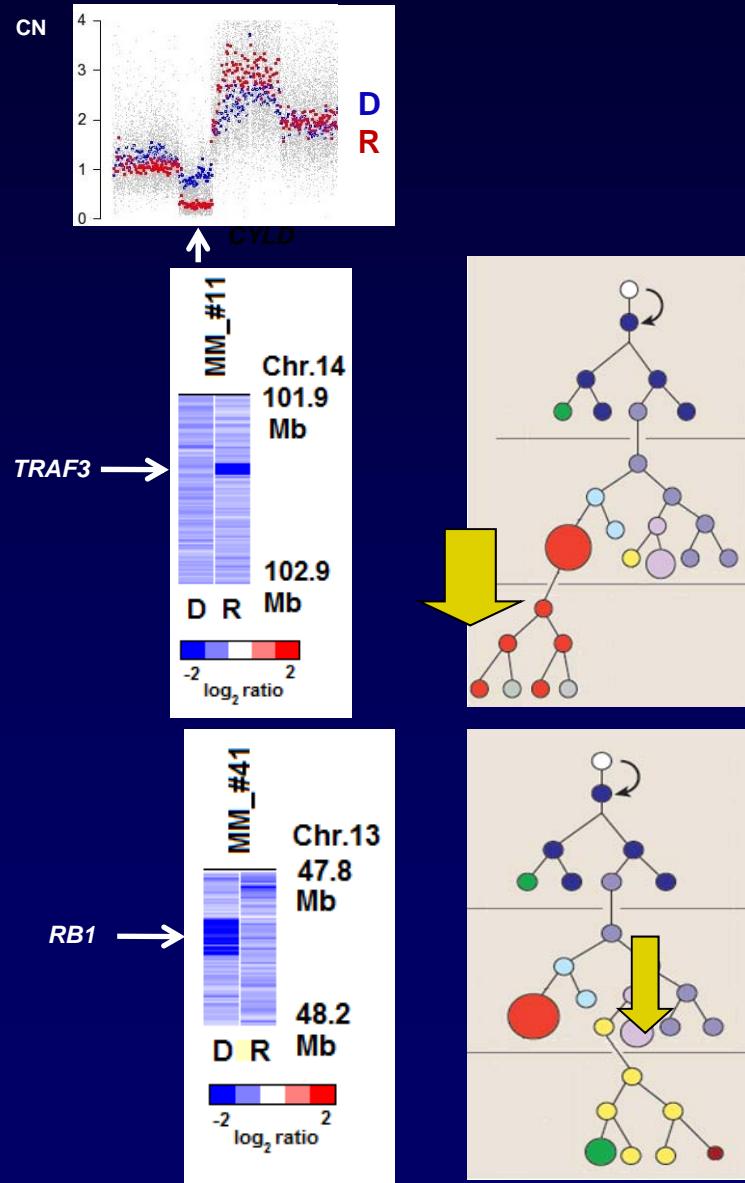


Drug Resistance Mechanisms: Different Time Points in the Course of the Disease

- Genomic analysis of 24 paired samples: Diagnosis / relapse (early stages)
- Genomic analysis of 24 paired samples: Relapse 1 / relapse 2 (late stages)

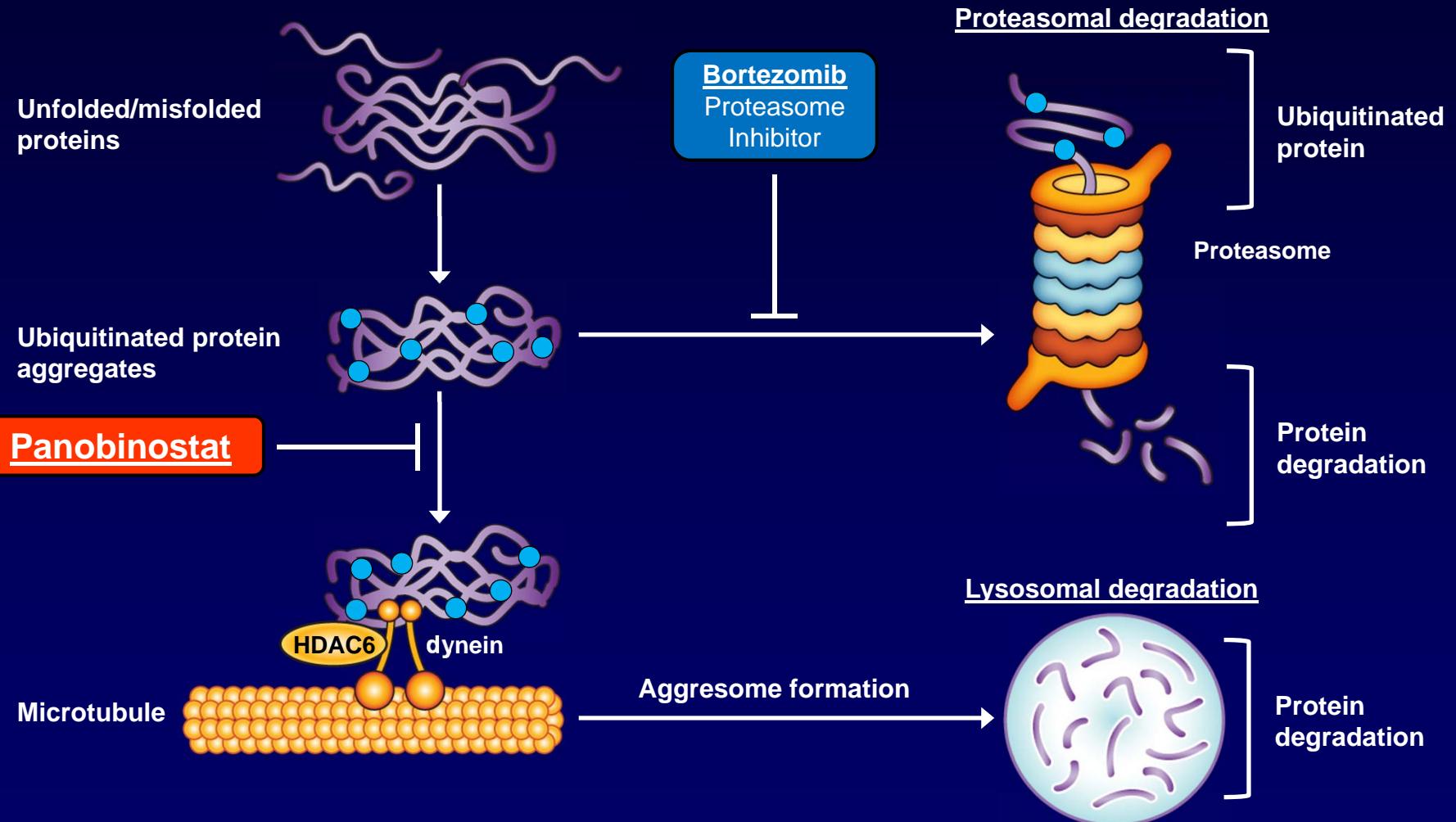
Three Evolutionary Trajectories (Paired Samples Diagnosis/Relapse) Role of Therapy, Treatment Pressure?

1. No copy number alterations (CNAs) change: With or without relative proportion of subclonal populations changes; no new acquisition
2. Linear evolution: With CNAs acquisition over time; *traf3* (nfkb inhibitor), bortezomib effective
3. Nonlinear/branching evolution: loss of CNAs over time. Bortezomib induction. 2 copies → 1 copy, ancestral clone (yellow)



Panobinostat + Bortezomib

Dual Inhibition of Protein Degradation Pathways



Hideshima T, et al. *Mol Cancer Ther.* 2011;10(11):2034-2042.

[Comment on Richardson et al, page 2331](#)

Panobinostat to revert bortezomib resistance

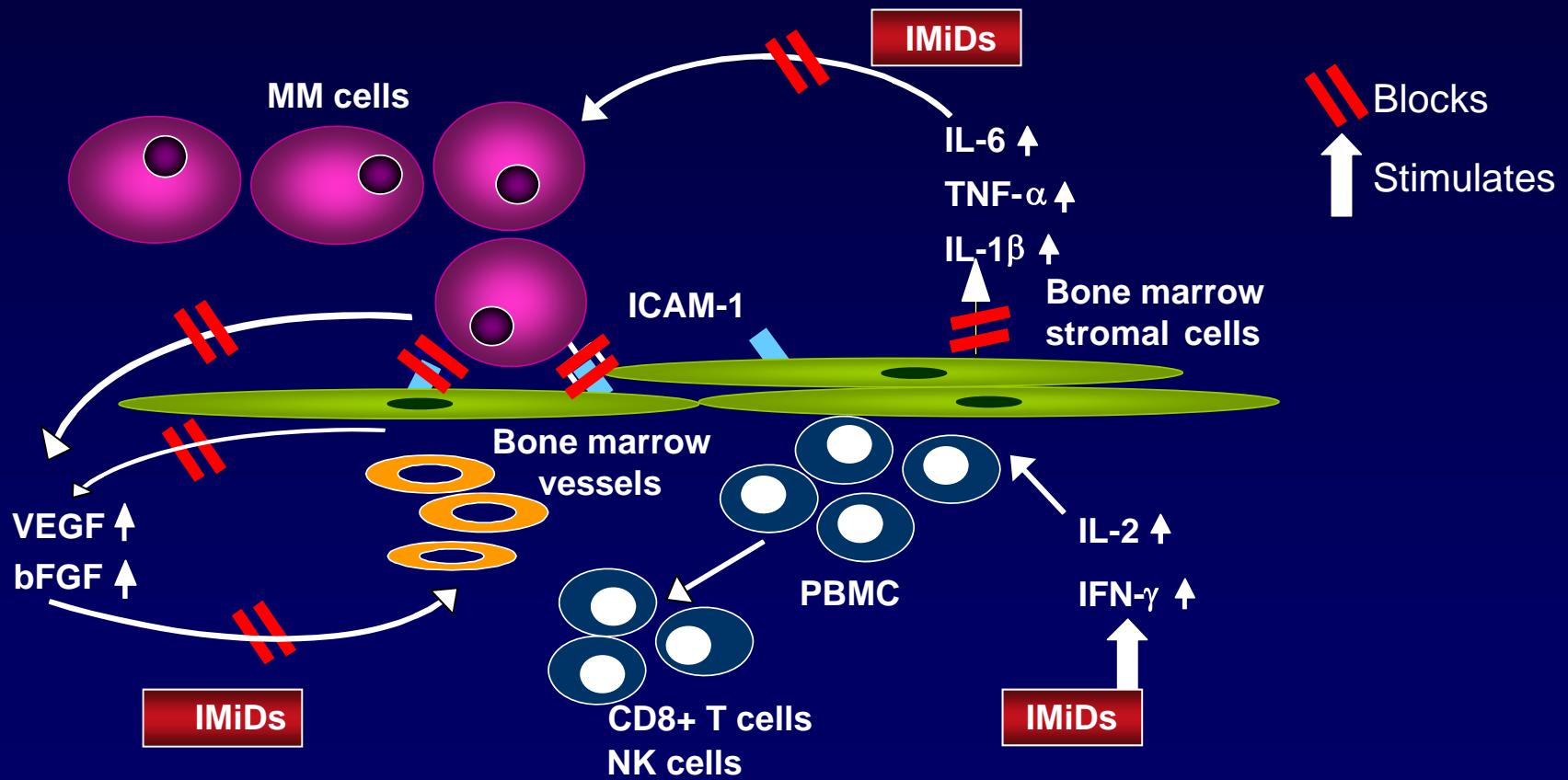
Philippe Moreau¹ ¹UNIVERSITY HOSPITAL NANTES

In this issue of *Blood*, Richardson et al present convincing results demonstrating that panobinostat, an oral pan-deacetylase inhibitor, in combination with bortezomib and dexamethasone, can recapture responses in bortezomib-refractory multiple myeloma (MM).¹

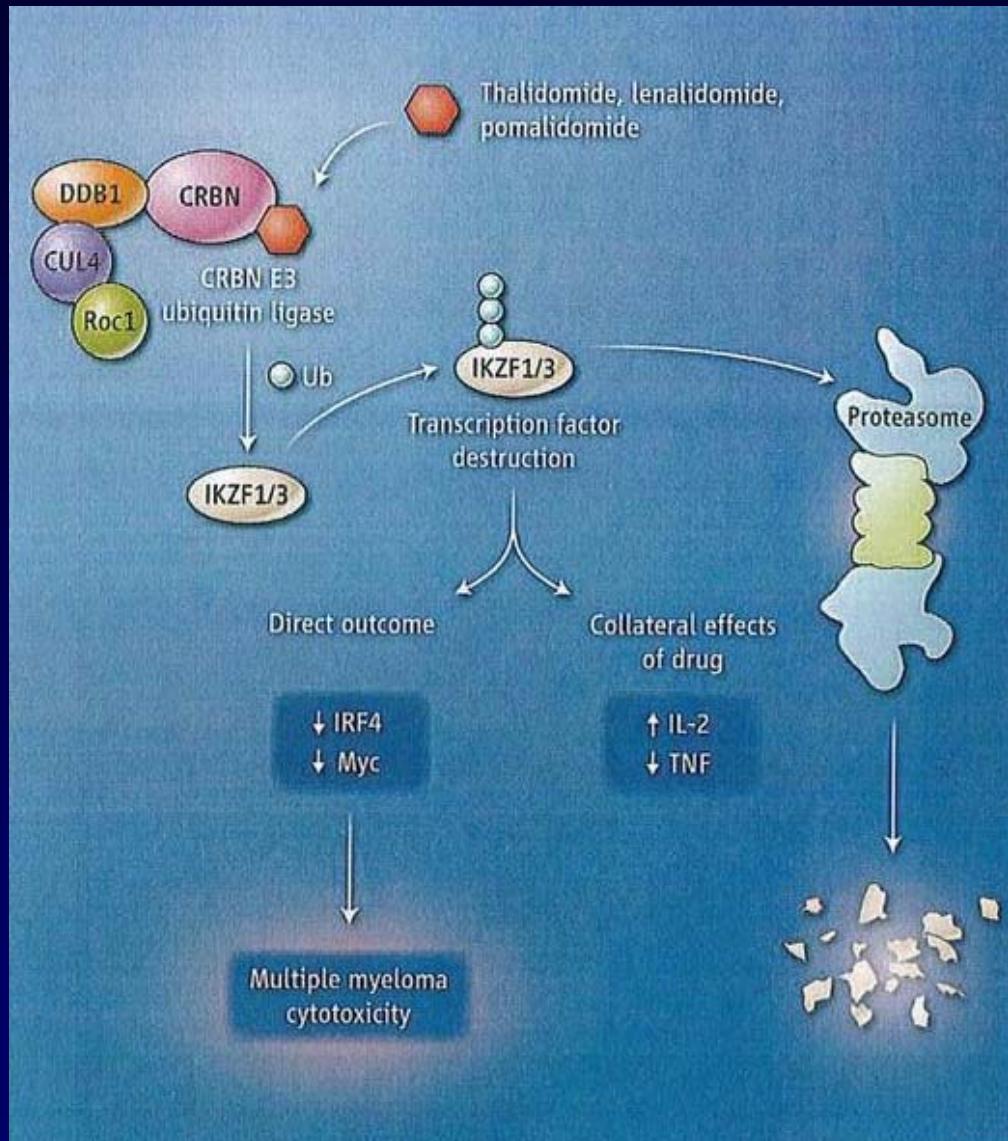
Resistance to IMiDs

Modes of Action of IMiDs in MM

IMiDs target MM cells and / in the bone marrow microenvironment



Hideshima T, et al. *Blood*. 2000;96(9):2943-2950. Richardson PG, et al. *Blood*. 2002;100(9):3063-3067.



IMiDs bind to the protein cereblon (CRBN), which activates the enzymatic activity of the CRBN E3 ubiquitin ligase complex.

The transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) are modified with ubiquitin (Ub) molecules, targeting them for proteolysis.

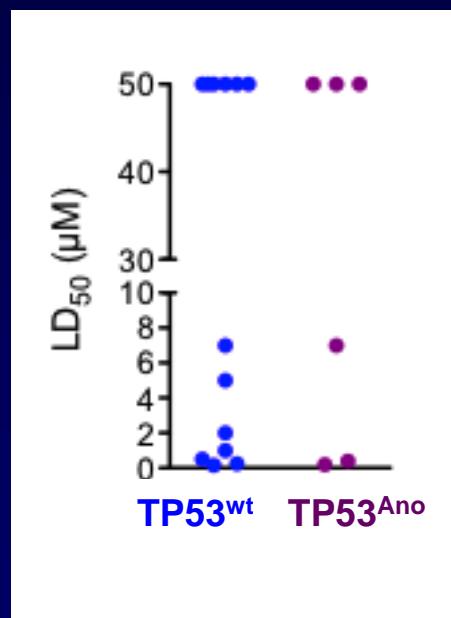
This alters the function of T cells and B cells, with a toxic outcome for multiple myeloma cells.

Resistance to IMiDs, HMCL, *In Vitro*

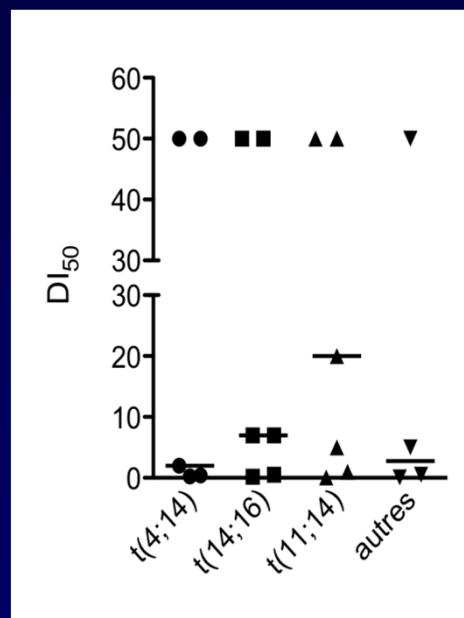
Cell death: IMiDs + dexamethasone¹

IMiDs alone: Inhibition of proliferation (maintenance story)

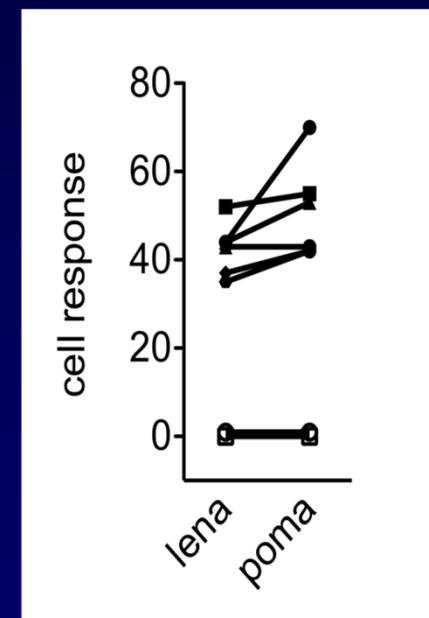
Lenalidomide



Lenalidomide



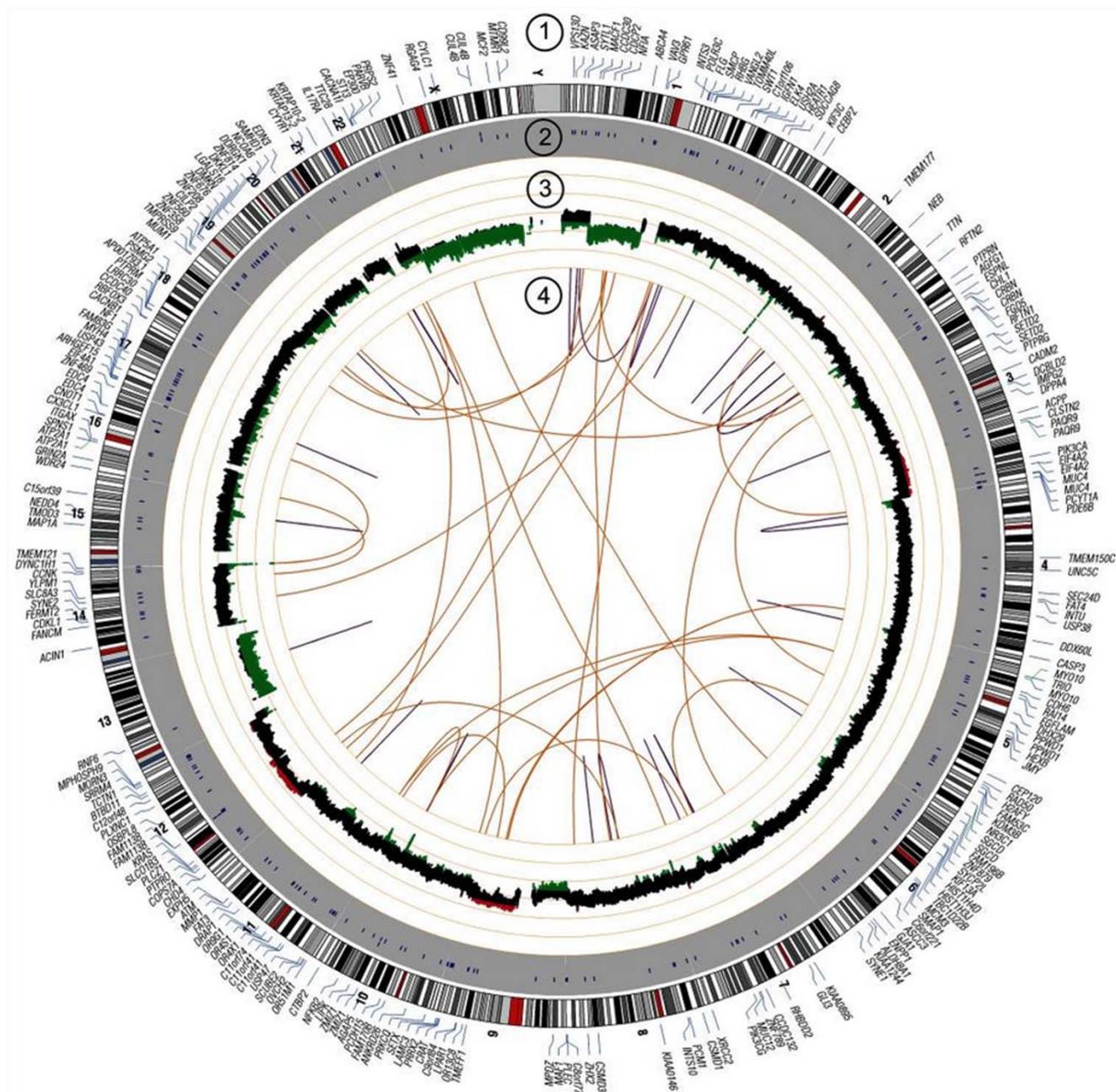
Lenalidomide/
Pomalidomide



No impact of p53

No impact of translocations

10 μM 1 μM



Circos plot depicting genome wide somatic variants, rearrangements, and copy number changes derived from next generation sequencing

Mutation

Whole genome sequencing

Summary of Clinically Relevant Single Nucleotide Variations

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Chr, chromosome; SNV, single nucleotide variation; SIFT, Sorting Intolerant

From Tolerant program; Polyphen2, Polymorphism Phenotyping v2 tool; dbSNP,
Single Nucleotide Polymorphism Database

Extramedullary myeloma whole genome sequencing reveals Q99* truncating mutation as well as a R283K point mutation were observed in CRBN, that was demonstrated as essential for the anti-MM action of IMiDs

Conclusions

- **New tools to evaluate drug resistance**
- **Samples: Diagnosis / relapse**
- **So many pathways, several mechanisms**
- **Dexamethasone is not a PI, is not an alkylator, is not an IMiD**
- **New classes of agents: Monoclonal antibodies, histone deacetylase inhibitors ...**
- **Combinations? Risk-adapted strategy**

...A very long way to go