Targeting the Ubiquitin Proteasome Cascade (UPS) in Multiple Myeloma

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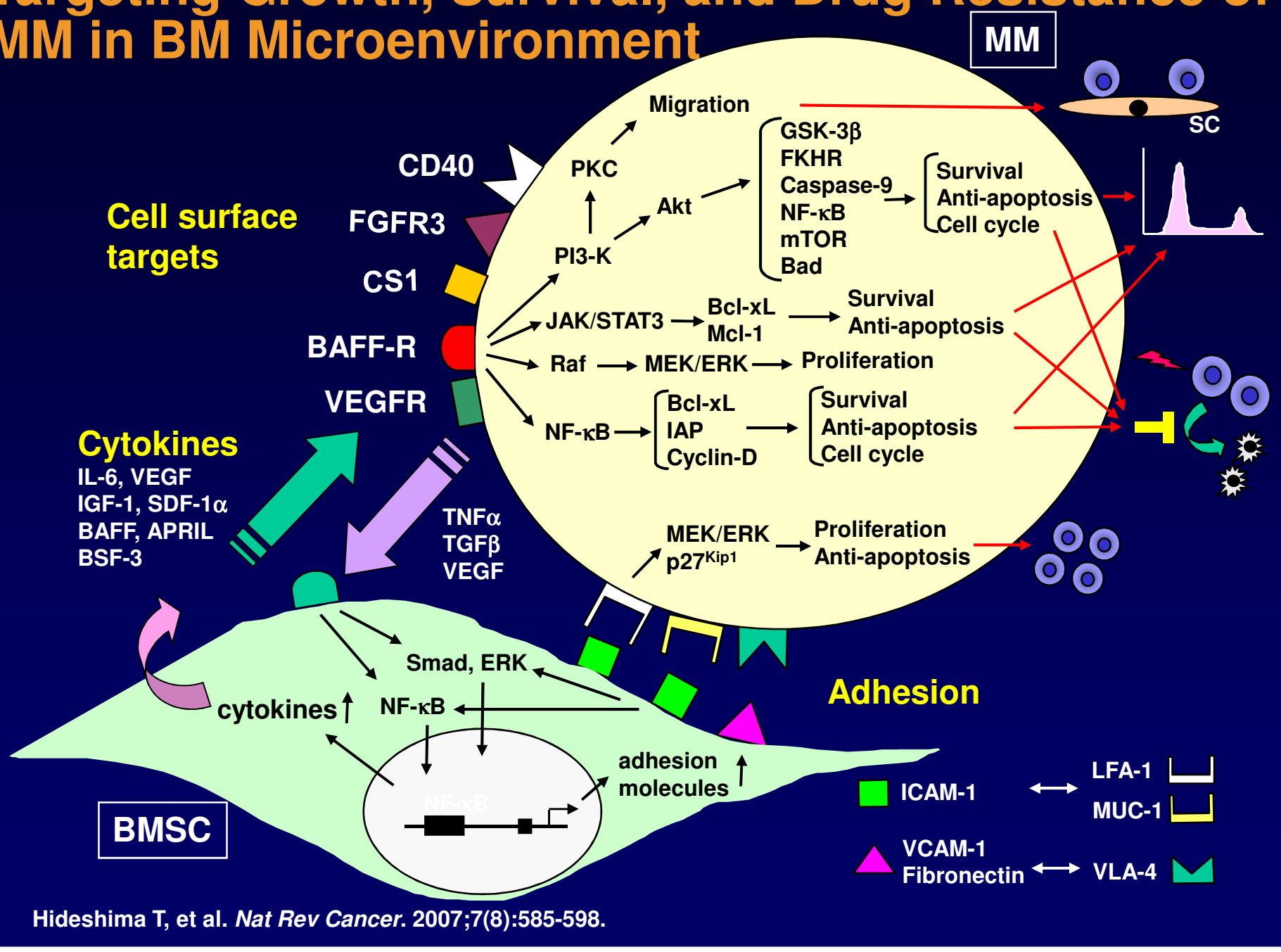
Conflict of Interest: Kenneth C. Anderson, M.D.

Consultancy: Celgene, Onyx, Sanofi Aventis, and Gilead

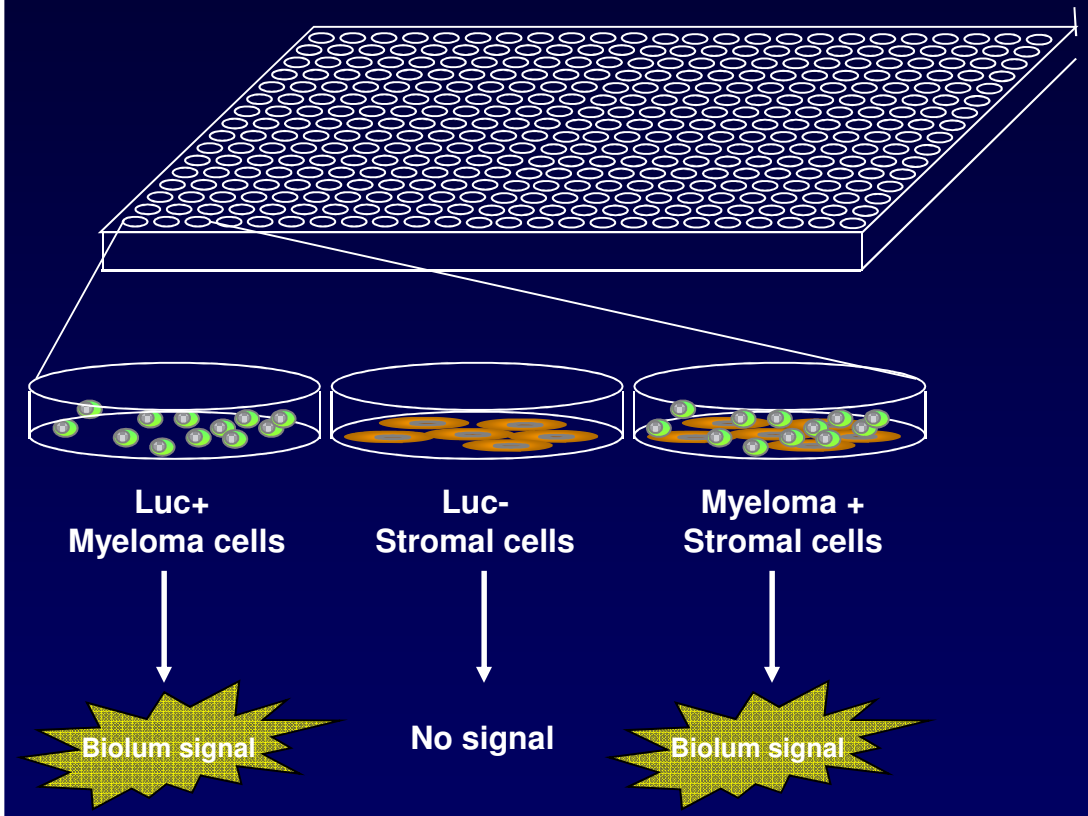
Scientific Founder: Acetylon, Oncopep

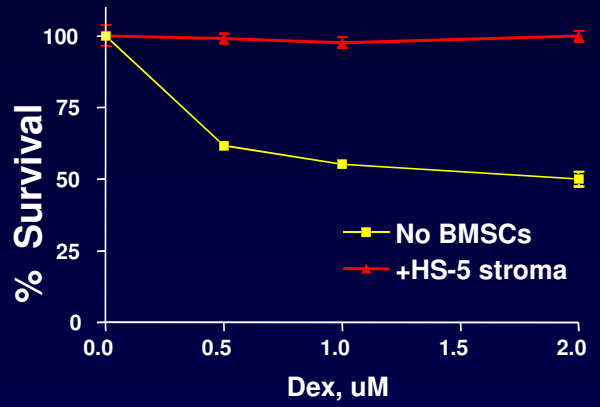
Targeting Growth, Survival, and Drug Resistance of MM in BM Microenvironment



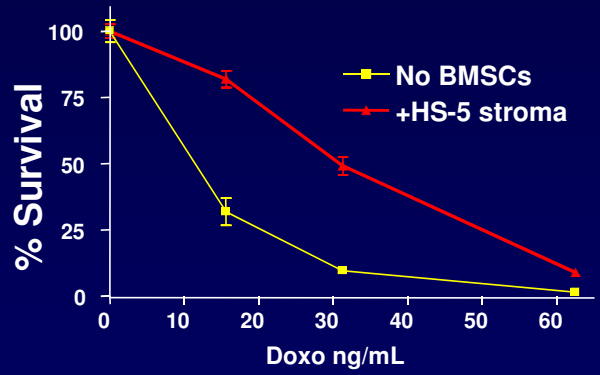
Hideshima T, et al. *Nat Rev Cancer*. 2007;7(8):585-598.

High-Throughput Screening of MM With BMSCs to Define Optimal Single Agents/Combinations

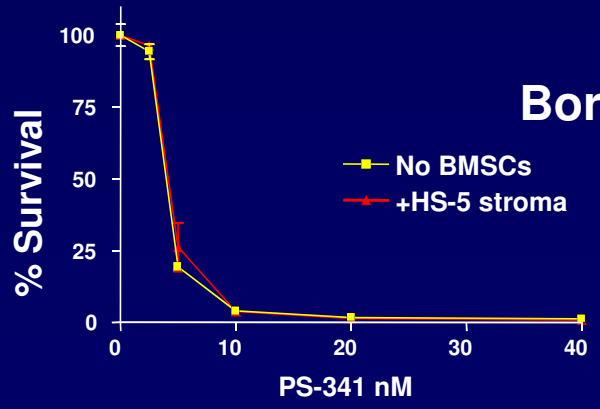




Dex



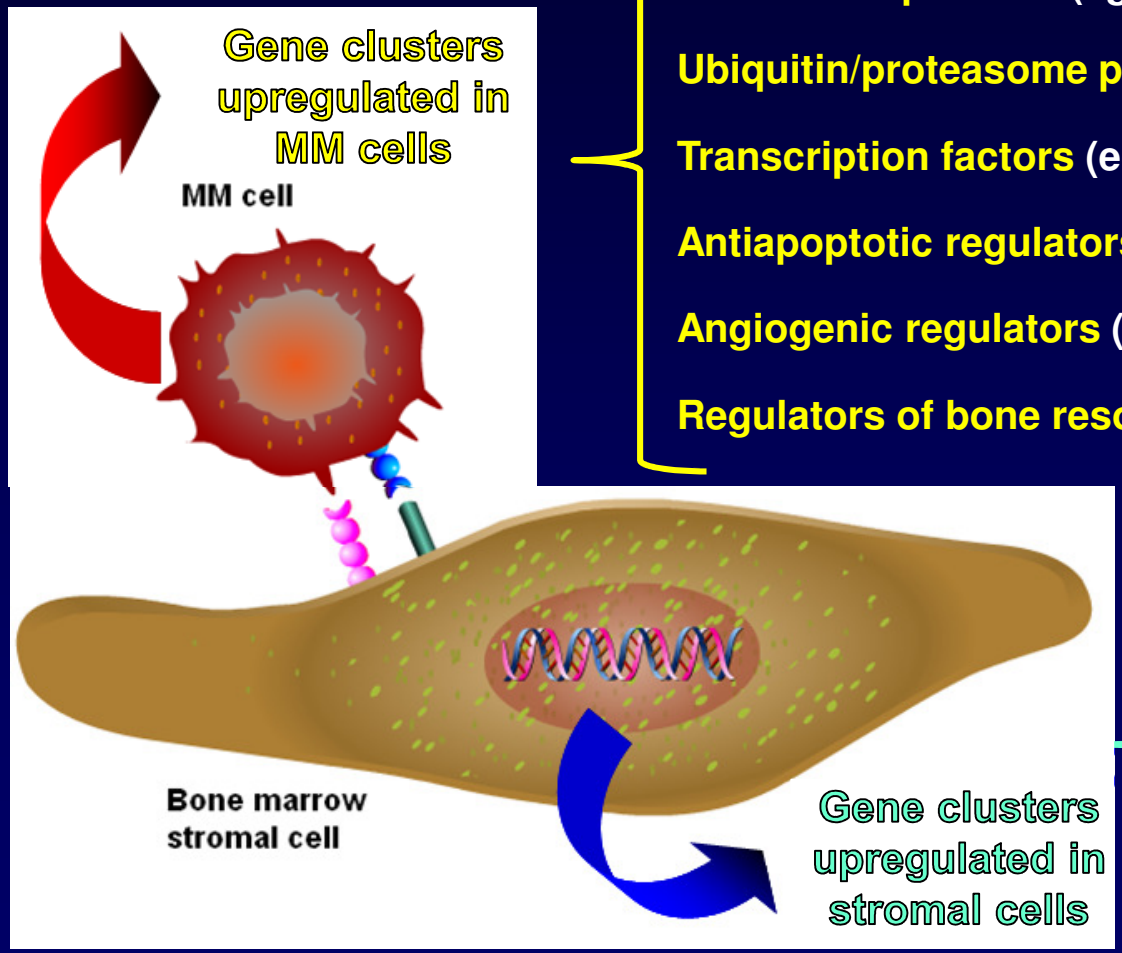
Doxo



Bortezomib

McMillin DW, et al. *Nat Med*. 2010;16(4):483-489..

Gene Clusters Modulated by MM-BMSC Interactions

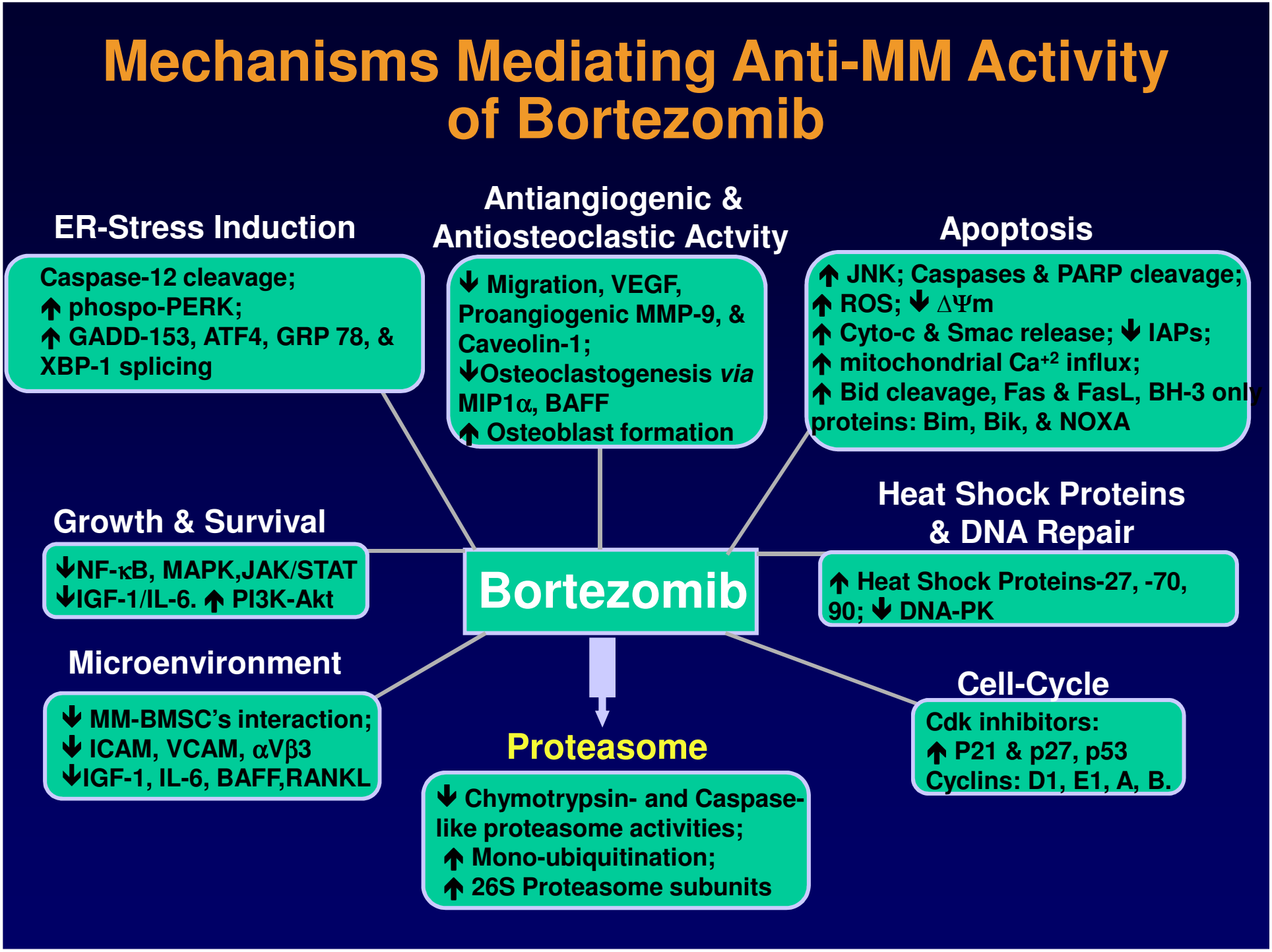


- **Cytokine/growth factors** (eg, IL-6)
- **Heat shock proteins** (eg, hdp90, hsp70, hsp27)
- **Ubiquitin/proteasome pathway members**
- **Transcription factors** (eg, NF-κB)
- **Antiapoptotic regulators** (eg, FLIP, cIAP-2, survivin, Mcl-1)
- **Angiogenic regulators** (eg, IL-8)
- **Regulators of bone resorption** (eg, IL-1β)

- **Proliferative cytokines** IL-6
- **Angiogenic growth factors** VEGF
- **Cell adhesion molecules** integrin-β5
- **ECM proteins**
- **Ubiquitin/proteasome pathway members**

McMillin DW, et al. *Nat Med*. 2010;16(4):483-489..

Mechanisms Mediating Anti-MM Activity of Bortezomib



Integration of Novel Therapy Into Myeloma Management

- Bortezomib, lenalidomide, thalidomide, doxorubicin, carfilzomib, pomalidomide
- Target MM in the bone marrow microenvironment to overcome conventional drug resistance *in vitro* and *in vivo*
- Effective in relapsed/refractory, relapsed, induction, consolidation, and maintenance therapy
- Eight FDA approvals and median survival prolonged from 3-4 to 6-7 years, with additional prolongation from maintenance
- New approaches needed to treat and ultimately prevent relapse

Chromosomes and Prognosis in Multiple Myeloma

For conventional low-dose and high-dose therapy:

Nonhyperdiploid worse prognosis than hyperdiploid
t(11;14), hyperdiploidy -standard risk
t(4;14), del(17p), del(13q14)-high risk

For novel treatments

Bortezomib, but not lenalidomide, can at least partially overcome t(4;14), del(13q14)-

del(17p) p53 remains high risk

Fonseca R, et al. *Leukemia*. 2009;23(12):2210-2221. Sagaster V, et al. *Leukemia*. 2007;21(1):164-168.

Continued Overall Survival Benefit After 5 Years' Follow-Up With Bortezomib-Melphalan-Prednisone (VMP) Versus Melphalan-Prednisone (MP) in Untreated Multiple Myeloma

- Persistent **significant OS benefit** with VMP vs MP; **13.3-month increase in median OS**
  - Seen across multiple pre-specified patient subgroups
  - Maintained after 5 years' follow-up and despite substantial use of novel agent–based salvage therapies
- OS subanalyses in patients receiving subsequent therapy demonstrate importance of providing **optimal first-line treatment incorporating bortezomib**
  - Rather than reserving bortezomib for salvage therapy and using conventional first-line treatment
- **No emerging safety signal for SPMs following VMP**
  - Thorough data collection; <5% of patients lost to follow-up

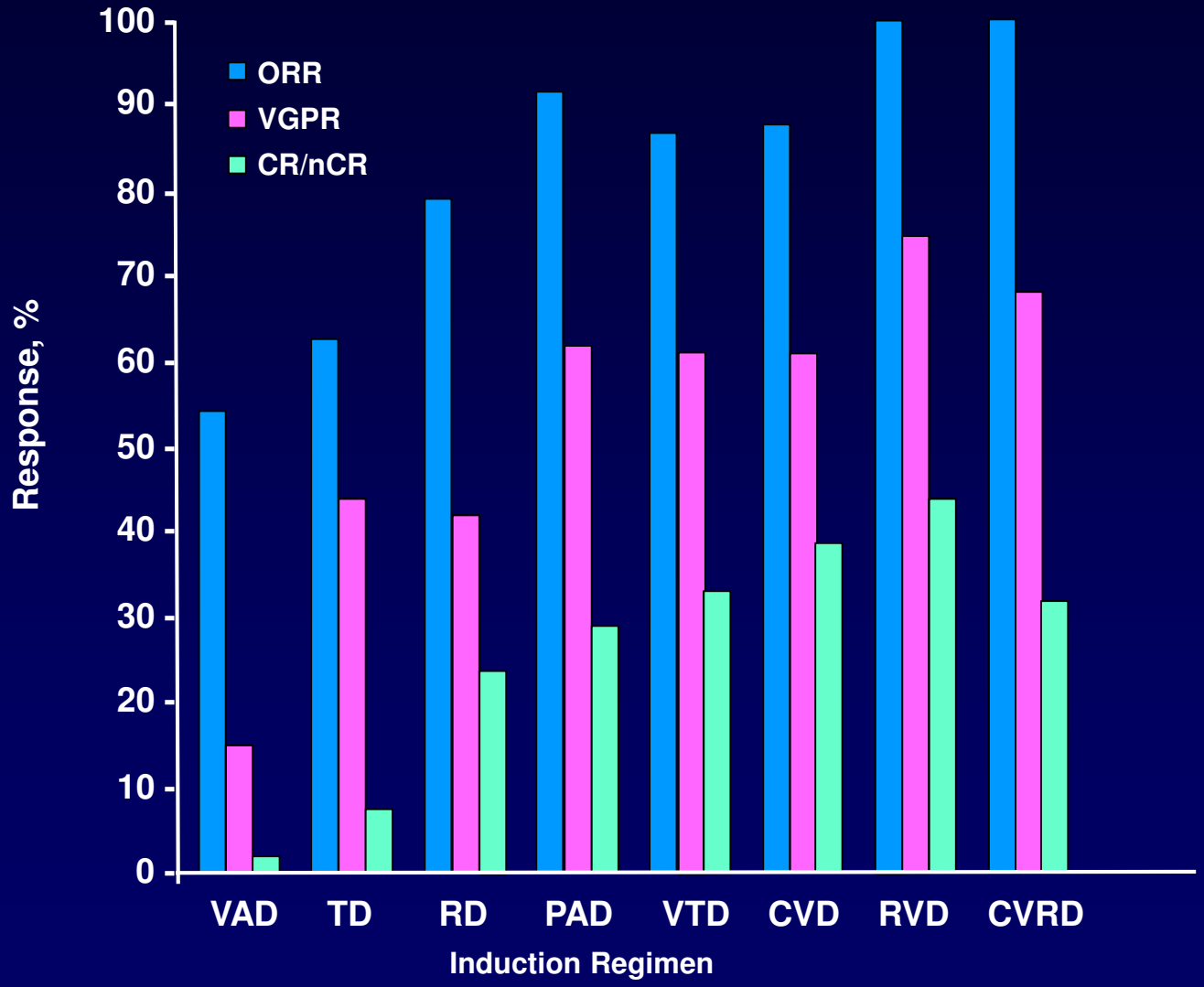
San Miguel JF, et al. *J Clin Oncol.* 2013;31(4):448-455.

Conclusion

	VMPT-VT	VMP	P value
5-year PFS	29%	13%	<0.0001
5-year TNT	41%	19%	<0.0001
5-year OS	61%	51%	0.01
3-year OS from relapse	47%	46%	0.63

Palumbo A, et al. *Blood*. 2012;120: Abstract 200.

Combinations in the Upfront Treatment of MM



Stewart AK, et al. *Blood.* 2009;114(27):5436-5443.

Lenalidomide and Bortezomib/Lenalidomide-based Consolidation

Study details

IFM 2005-02[1]
- Len consolidation (2 mos)
- Maintenance randomization: Len vs placebo

IFM 2008[2]
- VRD induction
- ASCT
- VRD consolidation (2 cycles)
- Len maintenance

Response data

n=572	Pre-consolidation	Post-consolidation	p
CR (IF$^-$)	14%	20%	<0.0001
≥ VGPR	58%	67%	<0.0001

n=31	Post-induction	Post-ASCT	Post-consolidation
sCR	13%	26%	38%
CR	10%	10%	10%
≥ VGPR	62%	68%	84%
≥PR	94%	91%	94%

[1]Attal M,et al. *Haematologica* 2011; 96 (s1): S23; oral presentation at IMW 2011
[2]Roussel M,et al. *Blood* 2010 ;116:(Abstract 624), oral presentation

HOVON 65 MM / GMMG-HD4 study: Bortezomib Induction and Maintenance Therapy

n=744, median age 57

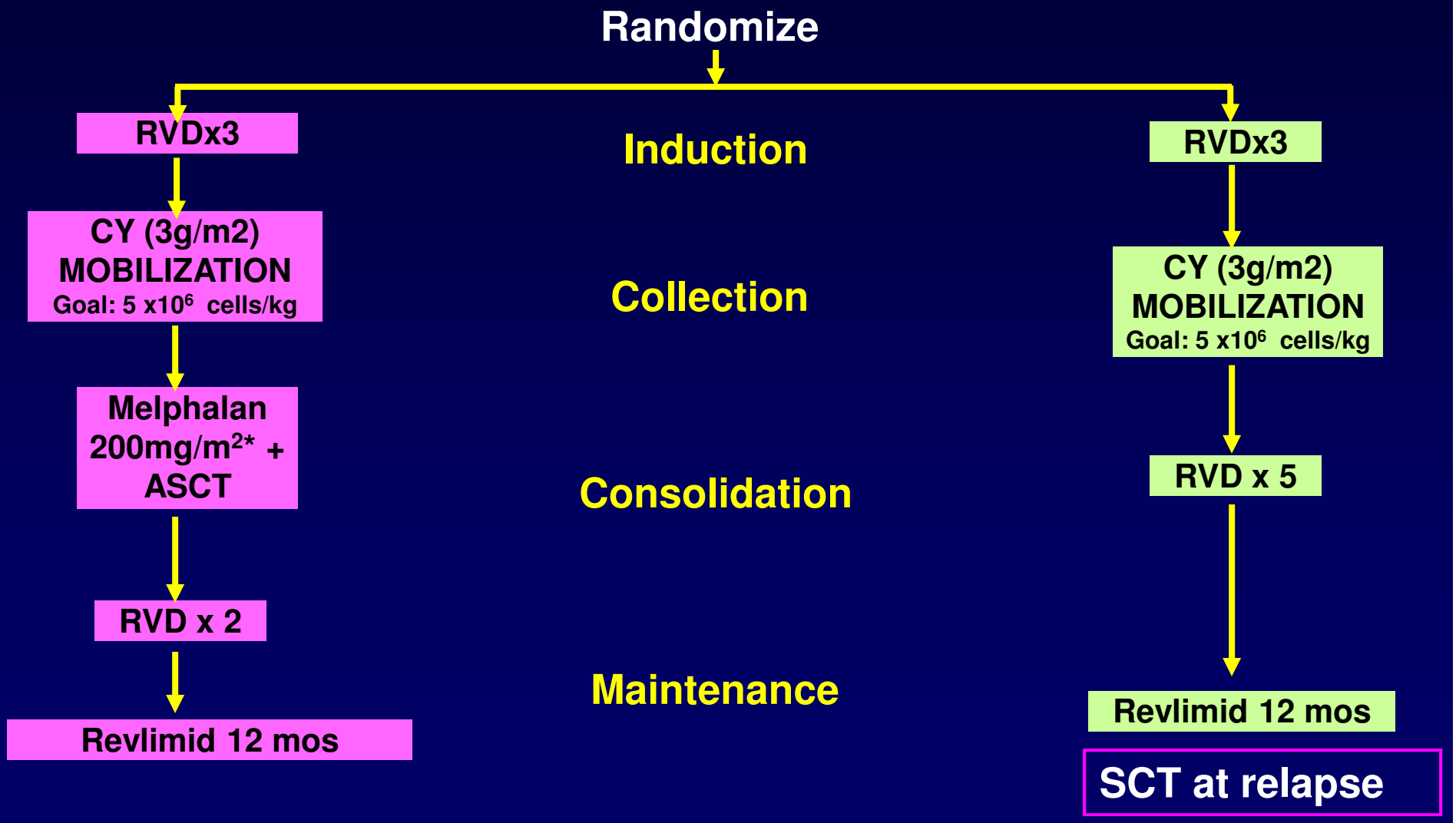
Study details	Median follow up	n	Results			
			≥nCR	≥VGPR	PFS	OS
PAD/HDM/ Bortezomib		205	49%*	76%*	36 m*	Median not reached
vs	39 m					HR=0.73 (0.56-0.96)
VAD/HDM/ Thalidomide		239	34%	55%	27m	p=0.02

*significant difference between arms

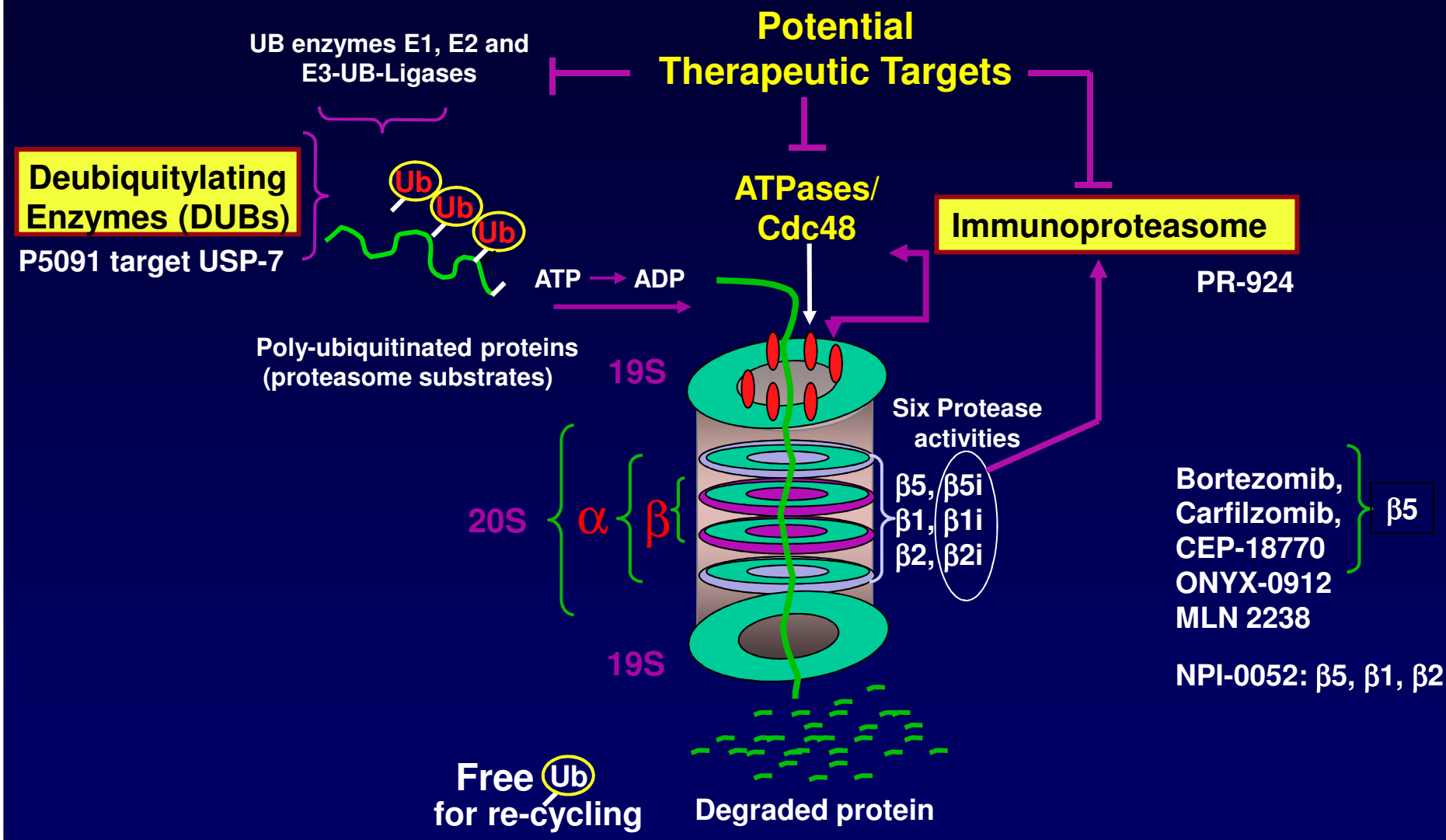
Sonneveld P,et al. *J Clin Oncol* 2012; 30(24):2946-2955.

IFM/DFCI Study in Newly Diagnosed MM Stem Cell Candidates

Genomic Profiling Over Time

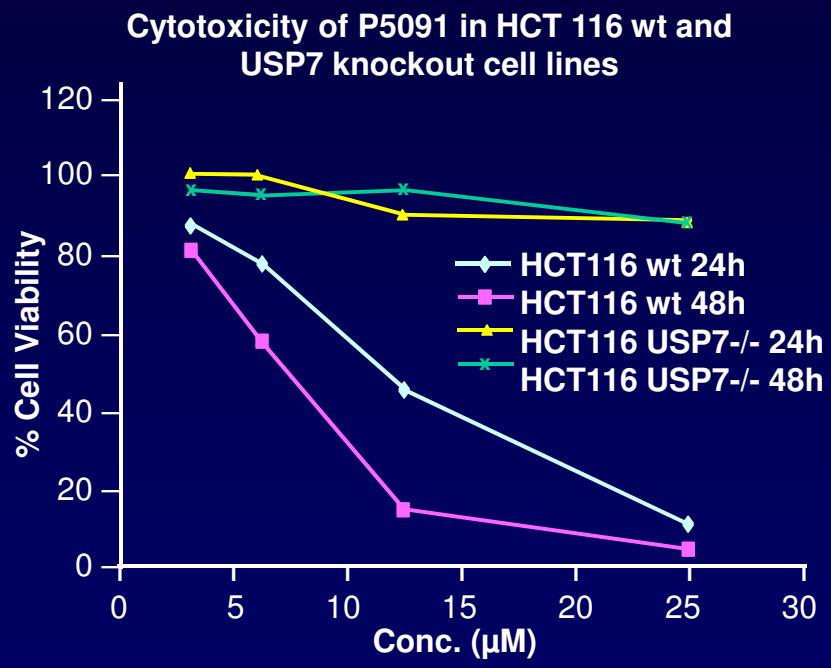


Proteasome: Present and Future Therapies

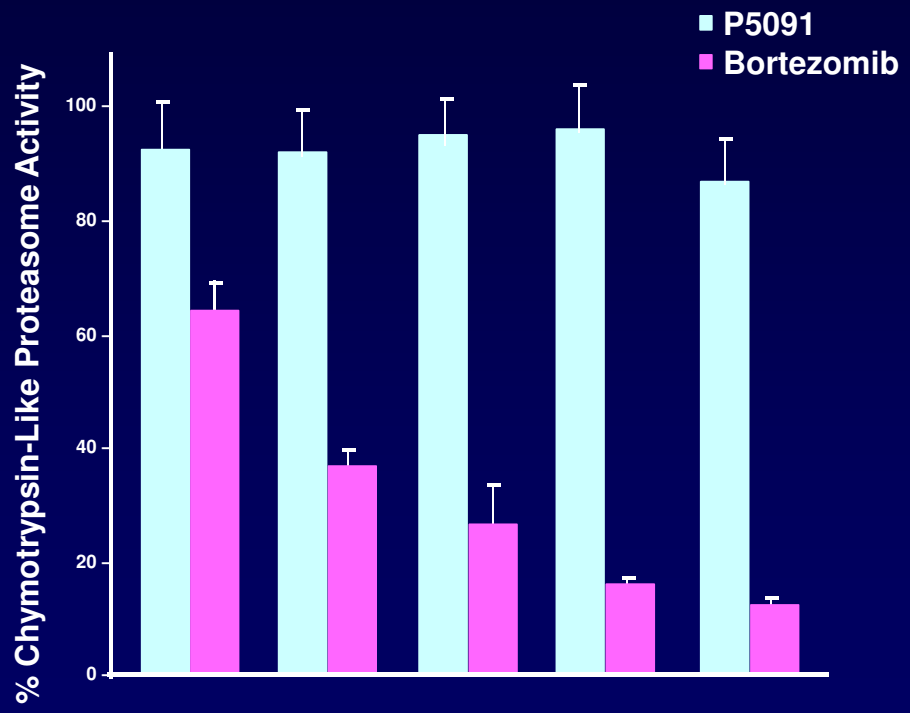


P5091 Specifically Targets USP-7 and Does Not Alter Proteasome Activity

USP-7 Knockout



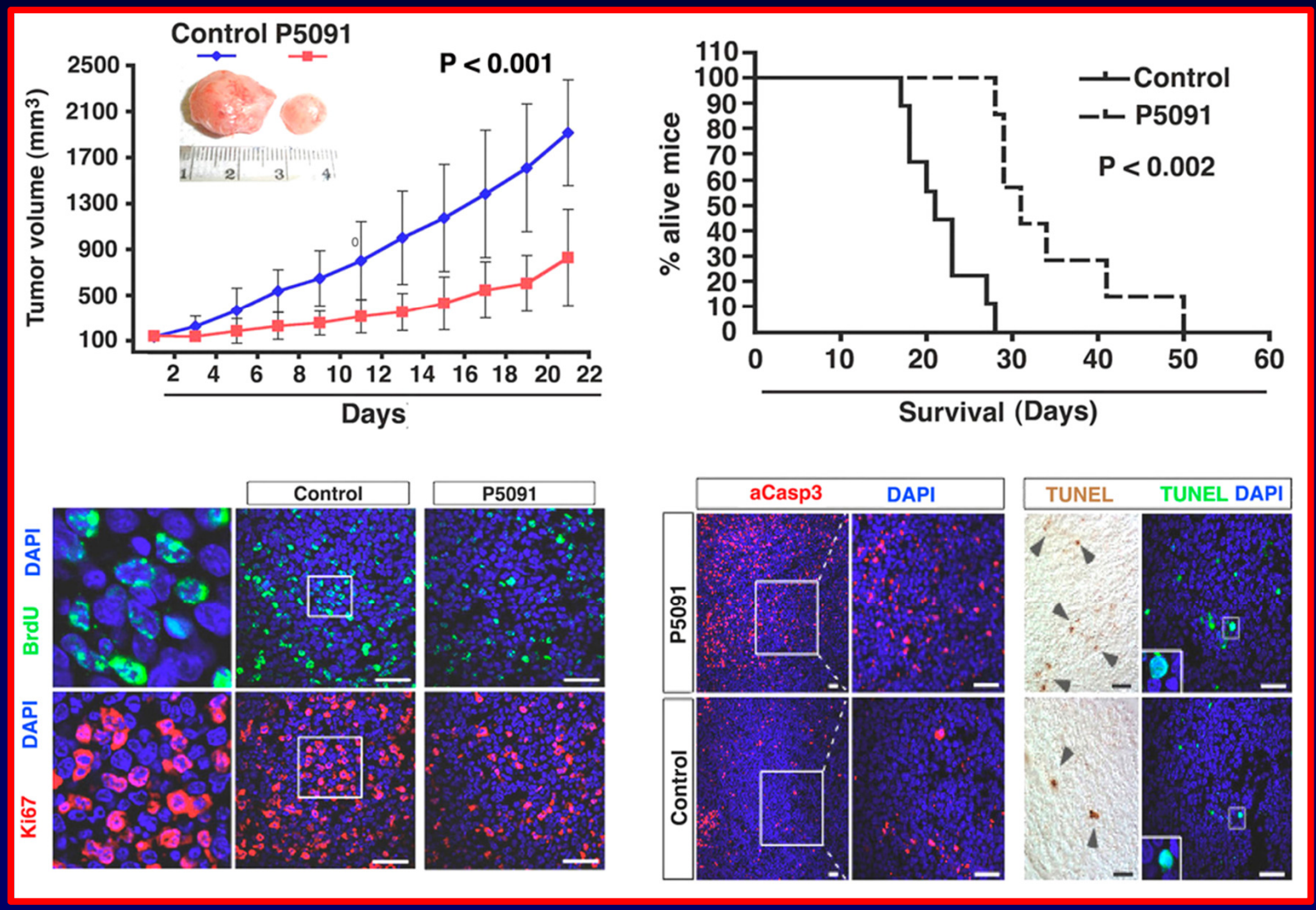
Proteasome Activity Assay



P5091 (μM)	2.5	5	7.5	10	12.5
Bortez (nM)	1	3	5	7	9

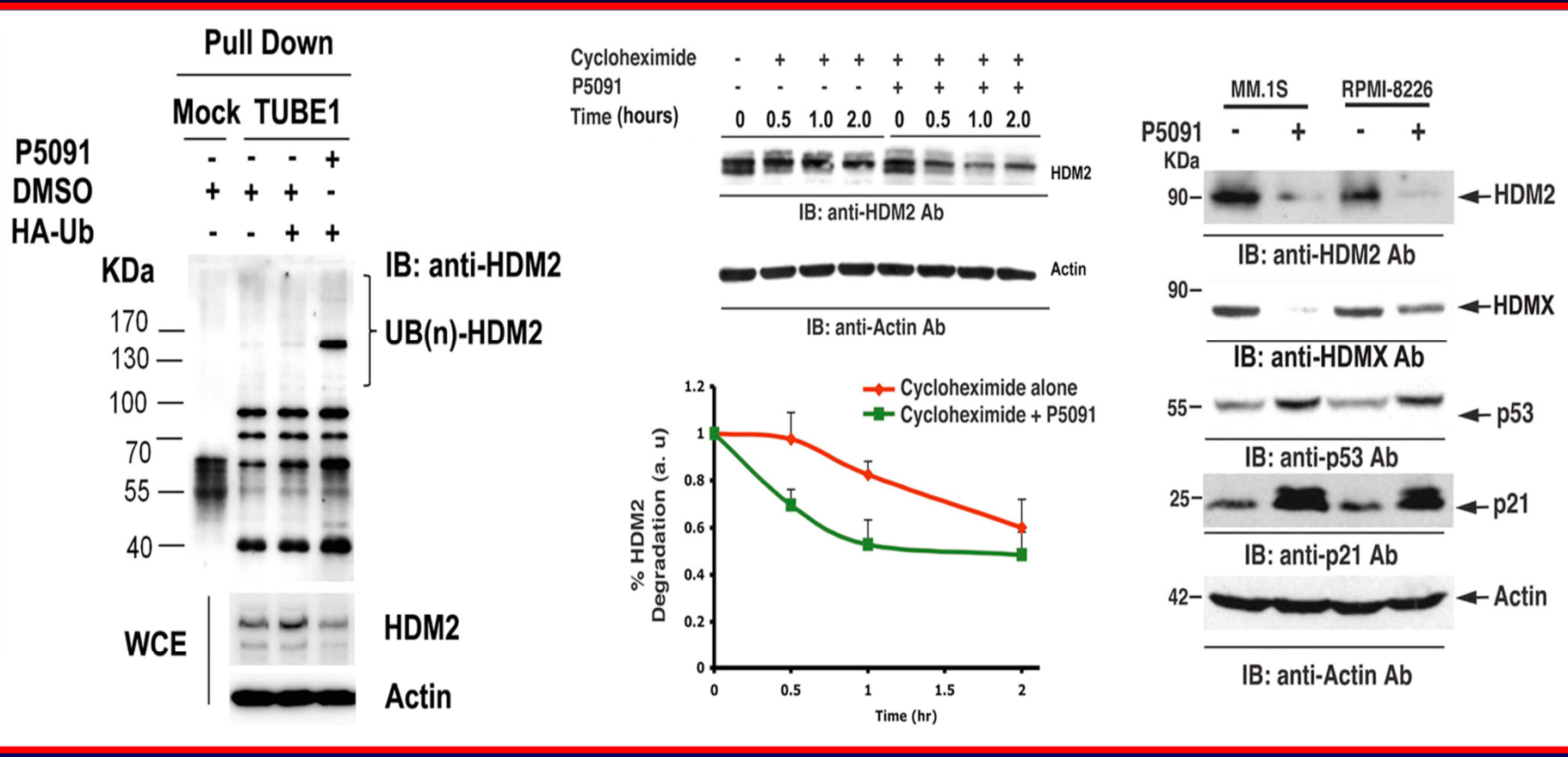
Chauhan D, et al. *Cancer Cell*. 2012;22(3):345-358.

P5091 Inhibits Tumor Growth and Prolongs Survival in Human Plasmacytoma Xenograft Model



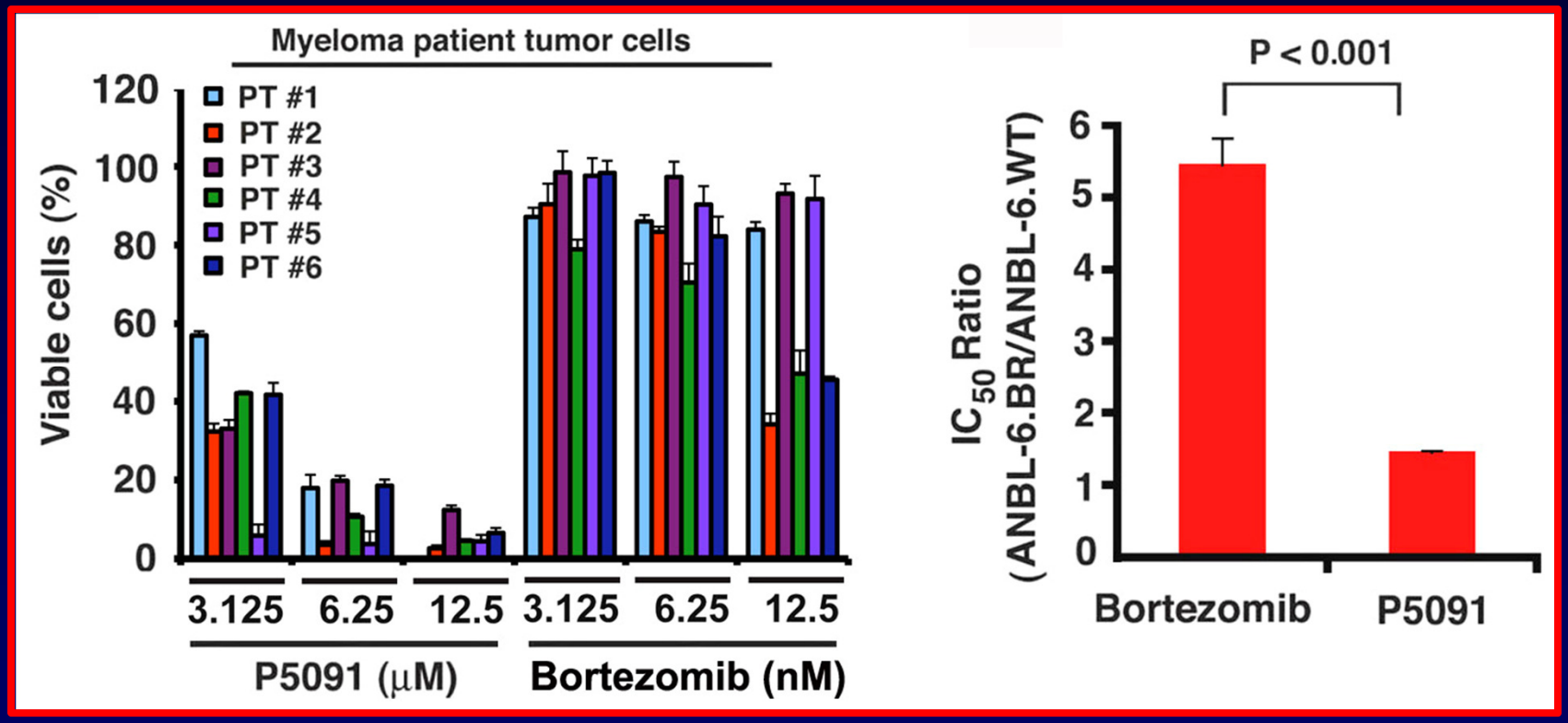
Chauhan D, et al. *Cancer Cell*. 2012;22(3):345-358.

P5091 Targets USP7 Substrate HDM2 and Activates HDM2-p53-p21 Signaling Pathways



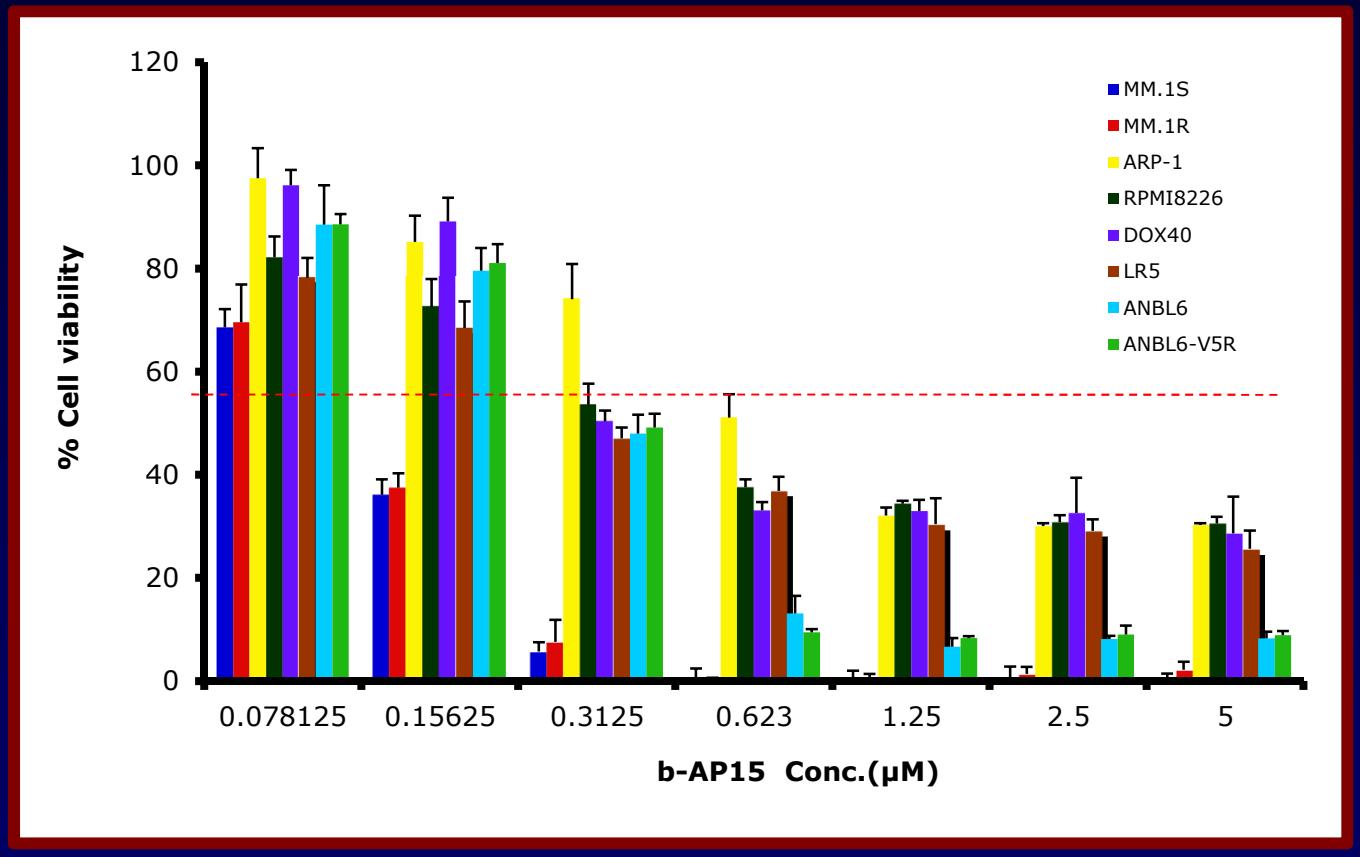
Chauhan D, et al. *Cancer Cell*. 2012;22(3):345-358.

P5091 Overcomes Bortezomib-Resistance in MM cells



Chauhan D, et al. *Cancer Cell*. 2012;22(3):345-358.

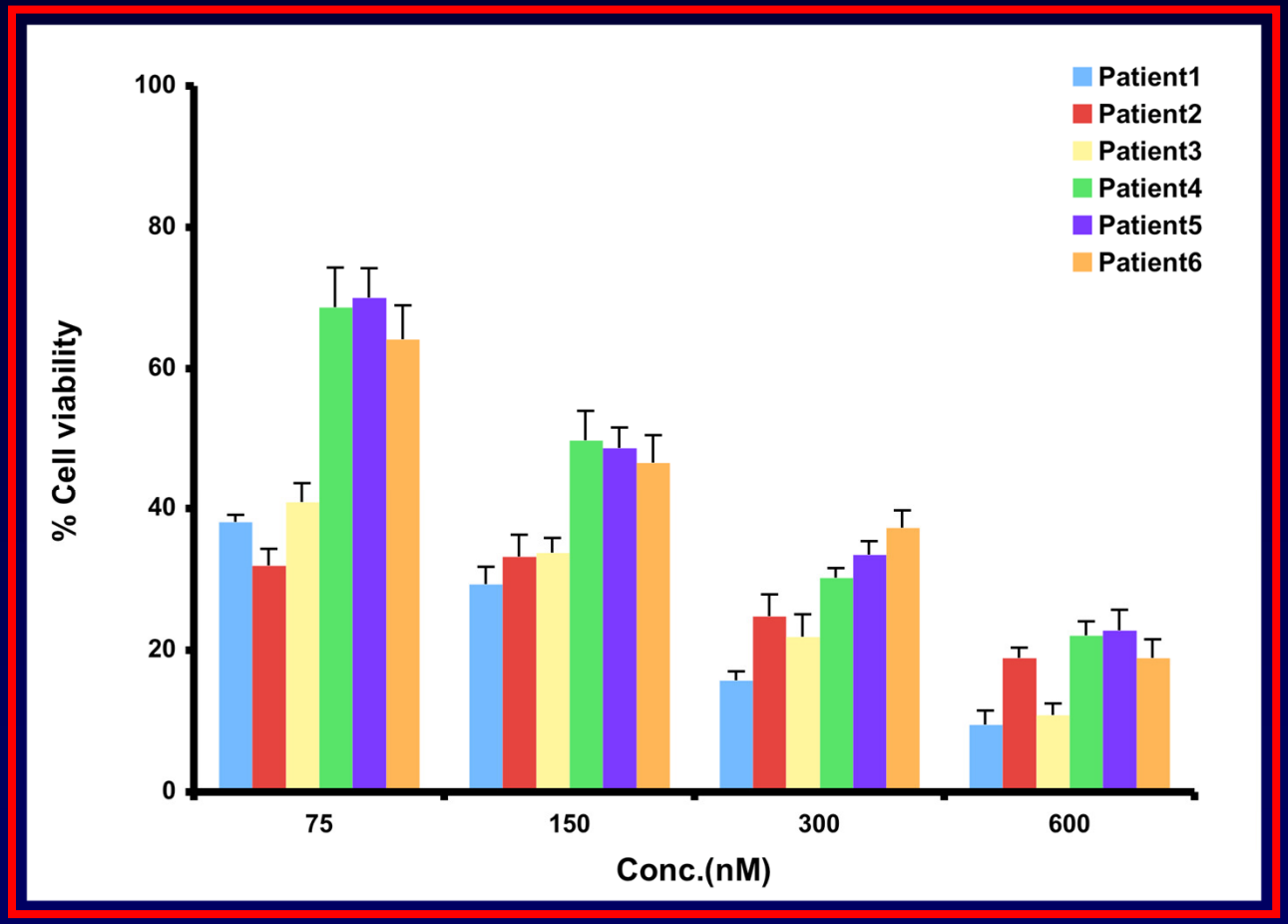
Anti-Myeloma Activity of a Novel USP14/UCHL5 DUB Inhibitor b-AP15



Myeloma cell lines were treated with indicated concentrations of b-AP15 for 48 hours, harvested, and then analyzed for viability using MTT assays.

Chauhan,Tian, et al. 2013.

Cytotoxicity of b-AP15 Against MM Patient Tumor Cells

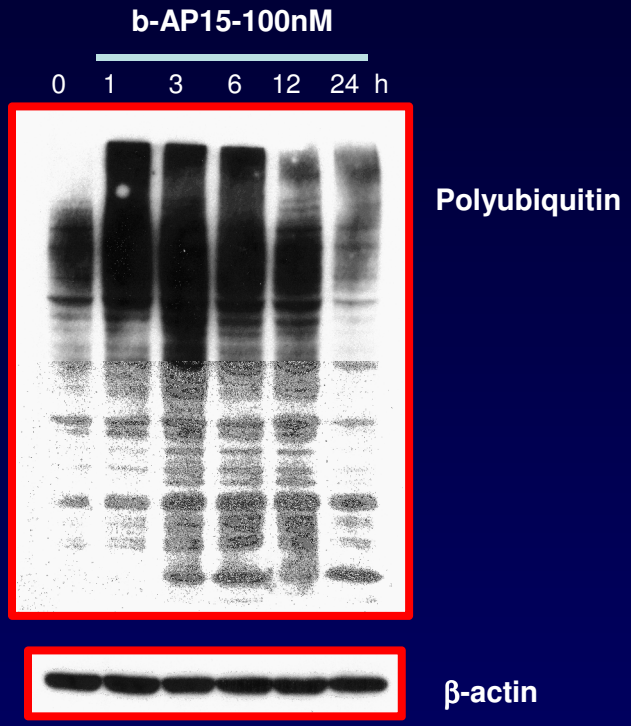


MM (CD138-positive) cells from 6 patients were treated with indicated concentrations of b-AP15 for 24 hours, harvested, and then analyzed for viability using MTT assays.

Treatment for 24h

Chauhan,Tian, et al. 2013.

b-AP15 Induces Polyubiquitination

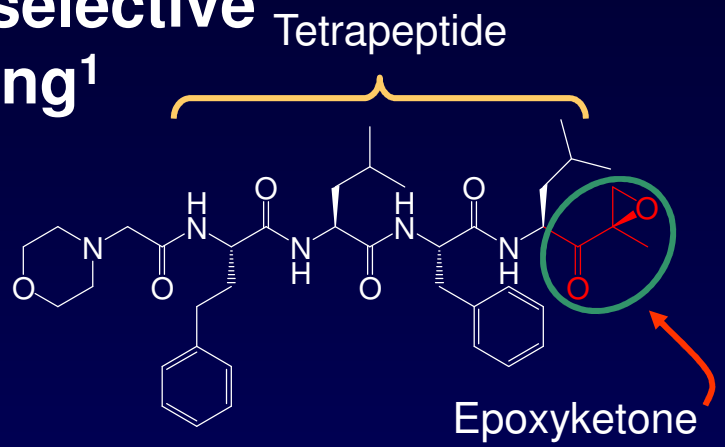


MM.1S cells were treated with indicated concentrations of b-AP15 for 0, 1, 3, 6, 12, or 24 hours, harvested, and total protein lysates were then analyzed for polyubiquitination by western blot analyses.

Chauhan,Tian, et al. 2013.

Carfilzomib: A Novel Proteasome (Chymotryptic) Inhibitor

- Novel chemical class with highly selective and irreversible proteasome binding[1]
- Improved antitumor activity with consecutive-day dosing[2]
- No neurotoxicity in animals[2]
- Durable responses in relapsed and relapsed/ refractory MM w/o neuropathy[3]
- Carfilzomib, lenalidomide, Dex versus lenalidomide, Dex phase III trial for new drug approval[4]



1.Demo SD, et al. *Cancer Res.* 2007;67(13):6383-6391. 2.Kirk CJ, et al. *Blood.* 2008;112: Abstract 2765. 3.Siegel DS, et al. *Blood.* 2012;120(14):2817-2825. 4.National Institutes of Health. Available at: http://clinicaltrials.gov/show/NCT01080391. Accessed: March 21, 2013.

Benefit of Carfilzomib in Relapsed/Refractory MM: Meaningful ORR, DOR, and OS

Response Category	Total N = 266, n (%)	
CR	1 (0.4)	ORR = 22.9% (95% CI: 18.0, 28.5)
VGPR	13 (4.9)	
PR	47 (17.7)	
MR	34 (12.8)	CBR = 35.7% (95% CI: 30.0, 41.8)
SD	81 (30.5)	
PD	69 (25.9)	
Not evaluable	21 (7.9)	

Duration of response

Median DOR = 7.8 months (95% CI: 5.6, 9.2)

Overall Survival

Median OS = 15.4 months (95% CI: 12.5, 19.0)

Siegel DS, et al. *Blood.* 2012;120(14):2817-2825.

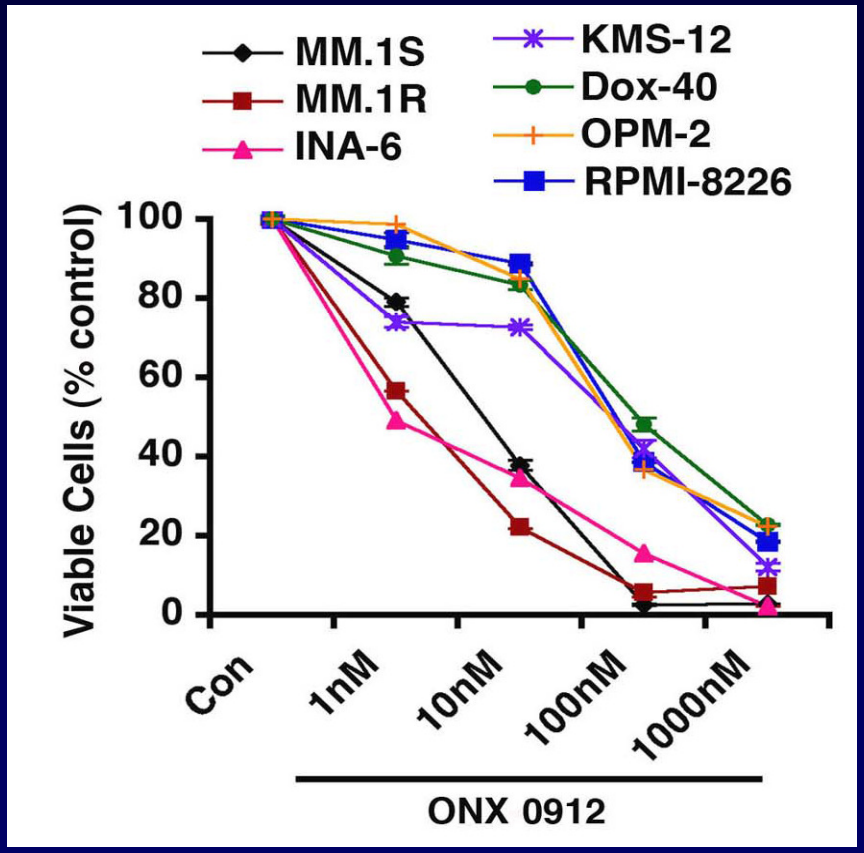
CRd in Relapsed and Upfront MM

- Response to CRd therapy was high, with an ORR of 78%
  - 41% VGPR or better[1]
- CRd well-tolerated with durable responses[1]
- ASPIRE phase III open-label, international, multicenter trial comparing CRd to Rd in R/R MM fully enrolled.[2]
- Remarkable extent and frequency of response to CRd upfront (100% ORR, 80% CR, nCR after 12 cycles)[2]

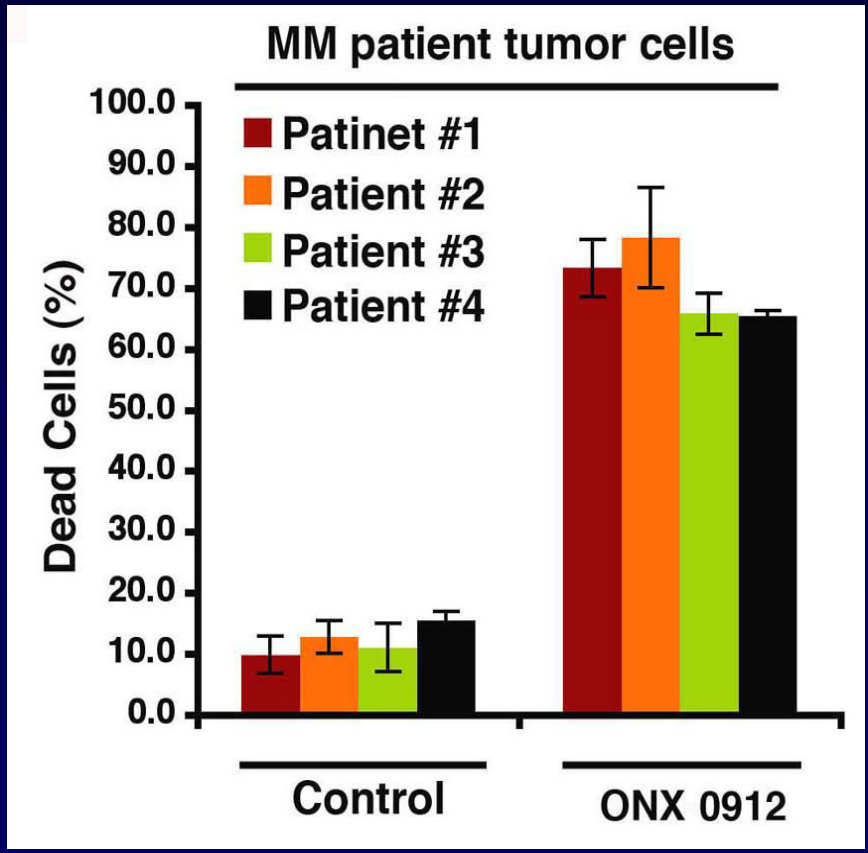
1.Wang M, et al. *J Clin Oncol*. 2011;29(suppl): Abstract 8025. 2. Jakubowiak AJ, et al. 2013. *Blood.* In press.

In Vitro Anti-MM Activity of Oral Inhibitor ONX 0912 (Opromazib)

Myeloma Cell Lines



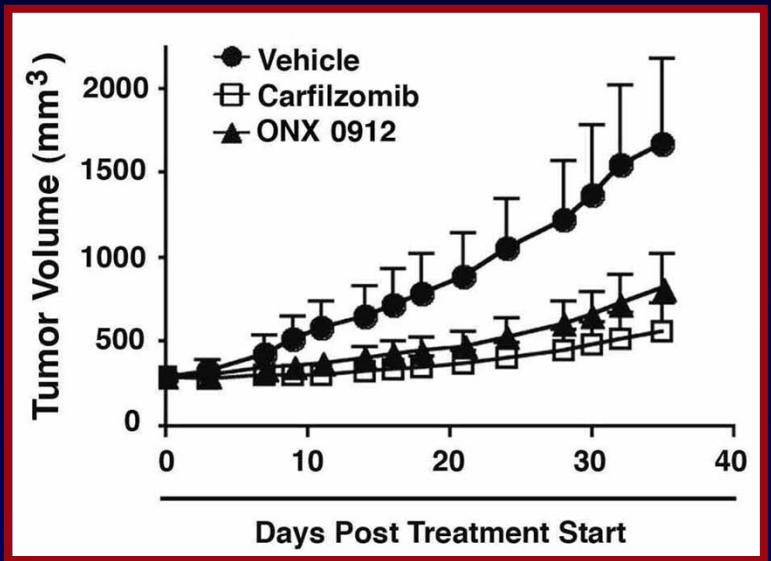
Patient Tumor Cells

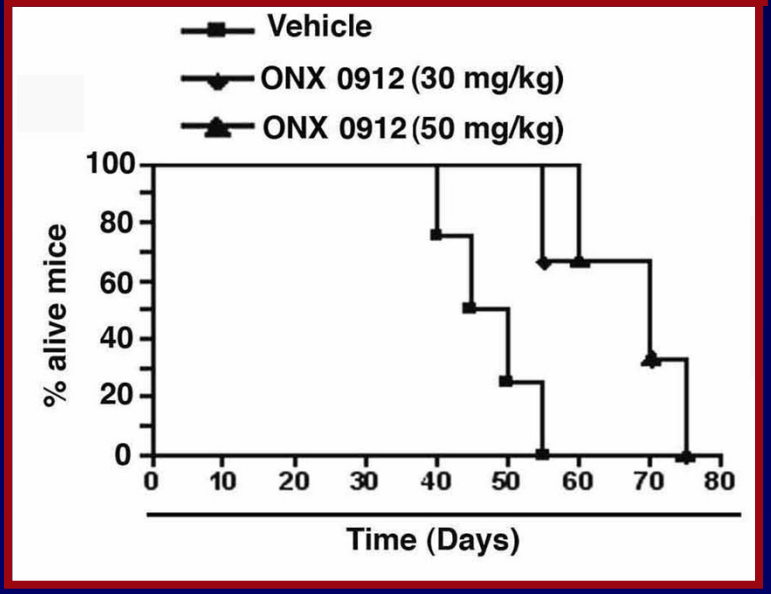


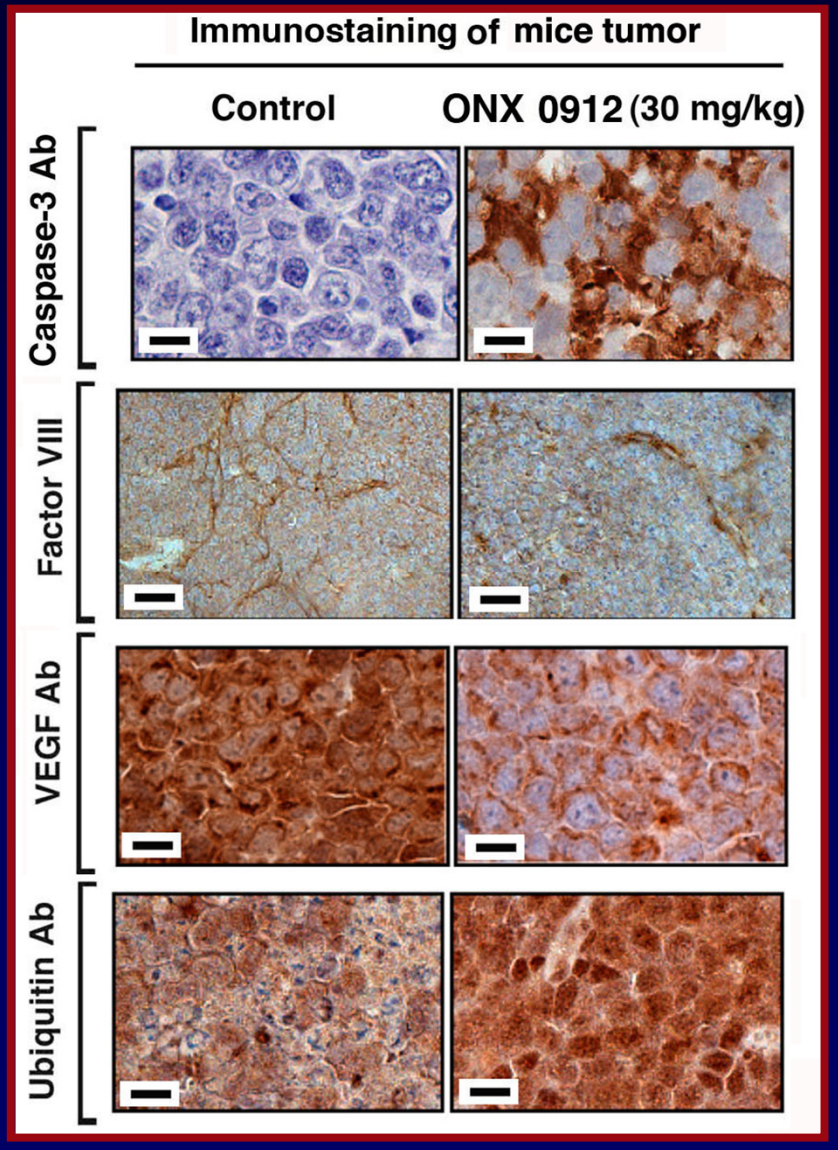
- Phase I clinical trial opromazib ongoing

Chauhan D, et al. *Blood*. 2010;116(23):4906-4915..

Anti-Myeloma Activity of ONX 0912 *In Vivo*

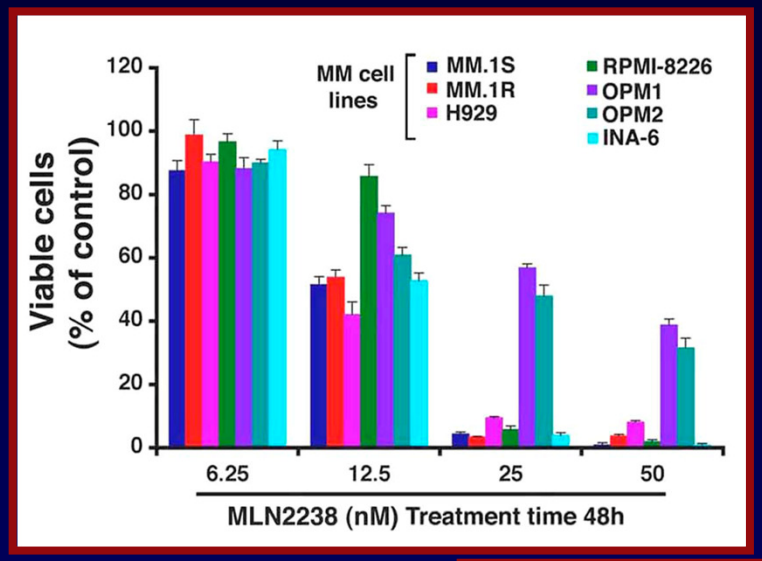


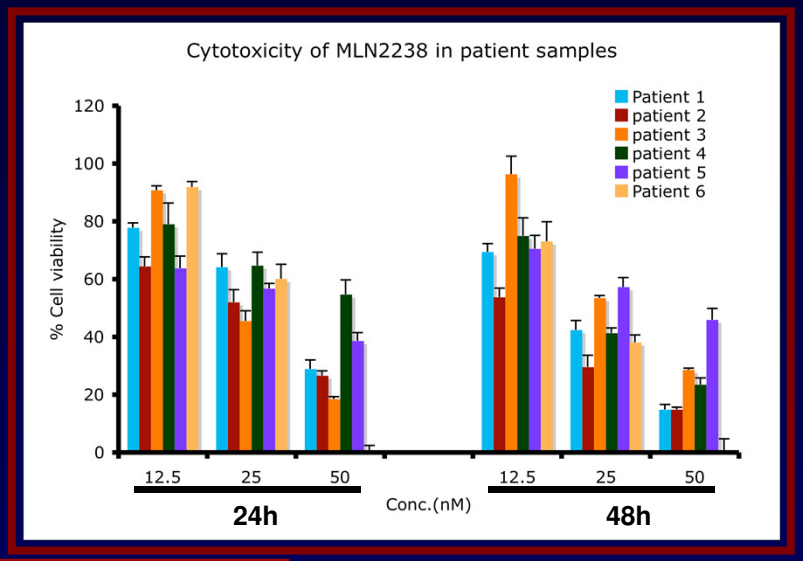


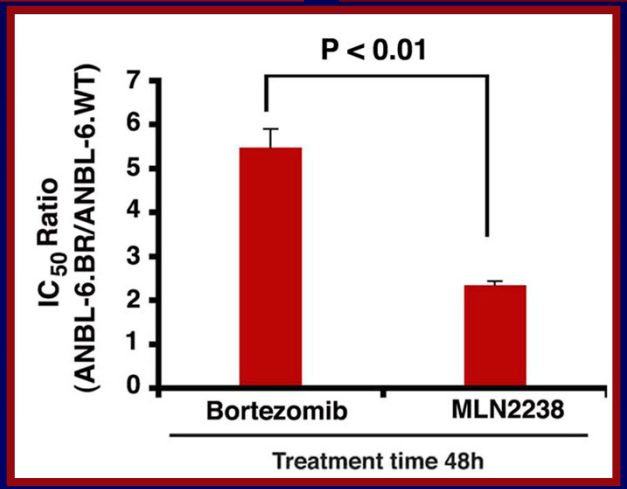


Chauhan D, et al. *Blood*. 2010;116(23):4906-4915..

MLN2238/9708 Decreases Cell Viability in MM Cells and Overcomes Bortezomib-Resistance

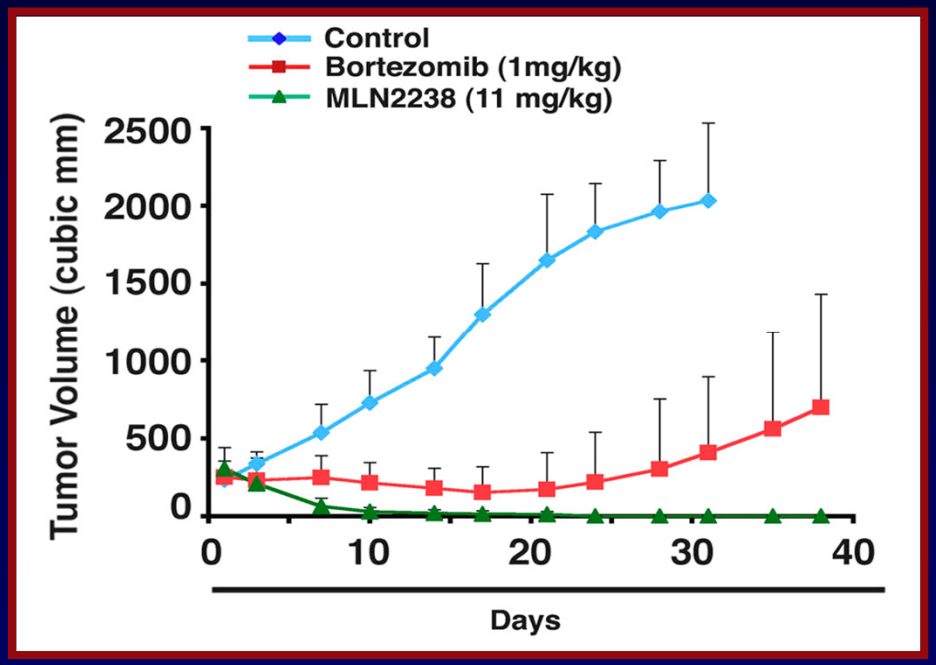


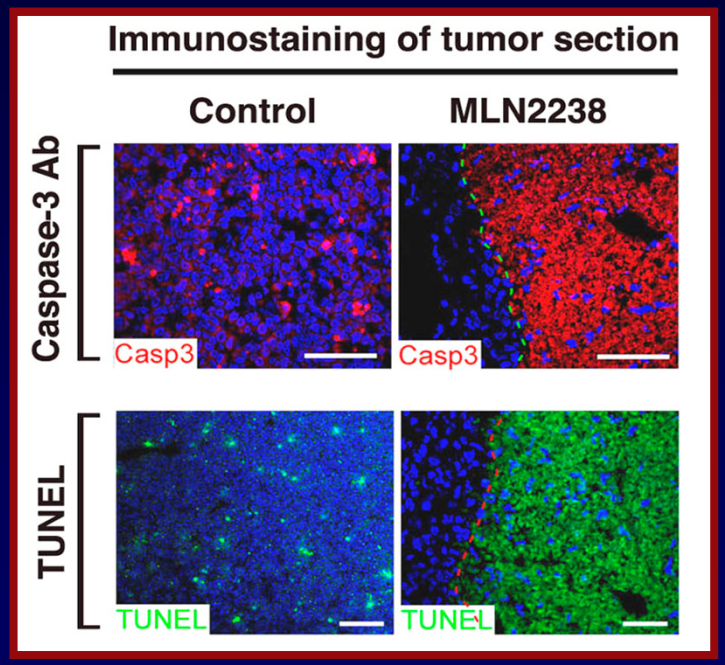




Chauhan D, et al. *Clin Cancer Res.* 2011;17(16):5311-5321.

MLN2238/9708 Oral Chymotryptic Inhibitor More Potently Blocks MM Cell Growth *In Vivo* than Bortezomib

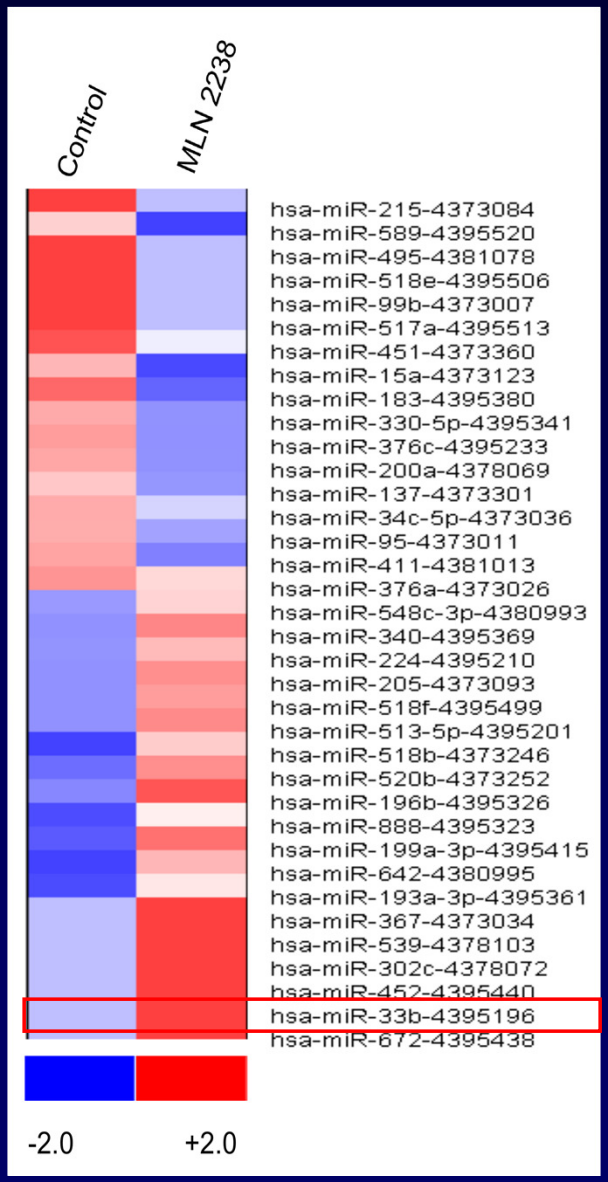




Clinical trials ongoing in relapsed/refractory MM and with lenalidomide-Dex as initial therapy

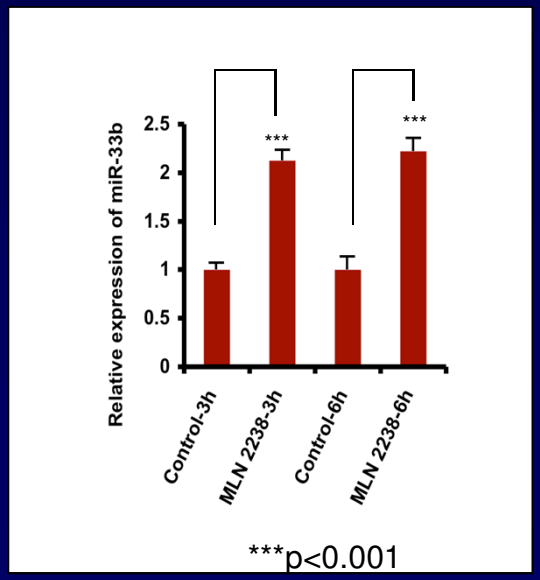
Chauhan D, et al. *Clin Cancer Res.* 2011;17(16):5311-5321.

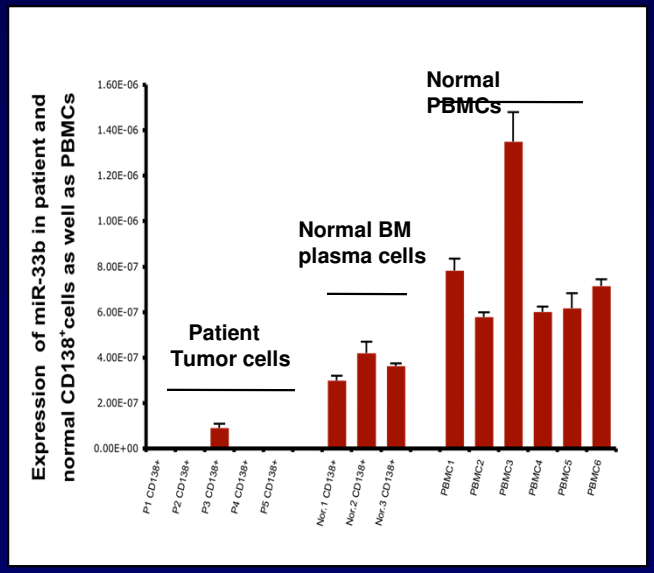
MLN2238/9708-Triggered miRNA Alterations in MM Cells



ΔΔCT>1.5 or ΔΔCT <-1.5	36 miRNAs
ΔΔCT>1.5 (upregulation)	19 miRNAs
ΔΔCT <-1.5 (downregulation)	17 miRNAs

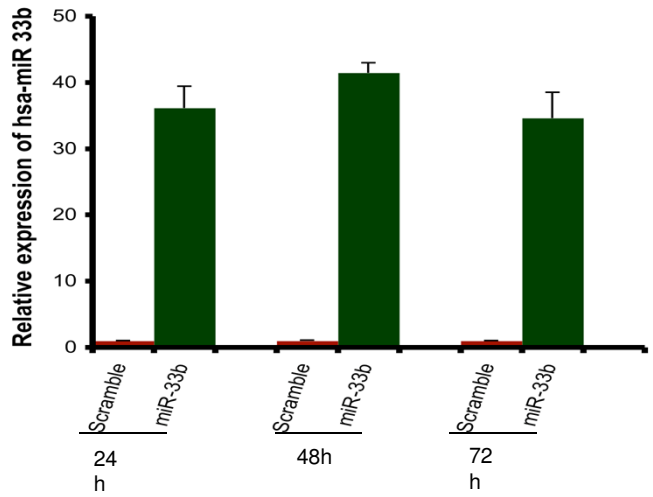
qPCR Validation miR-33B expression

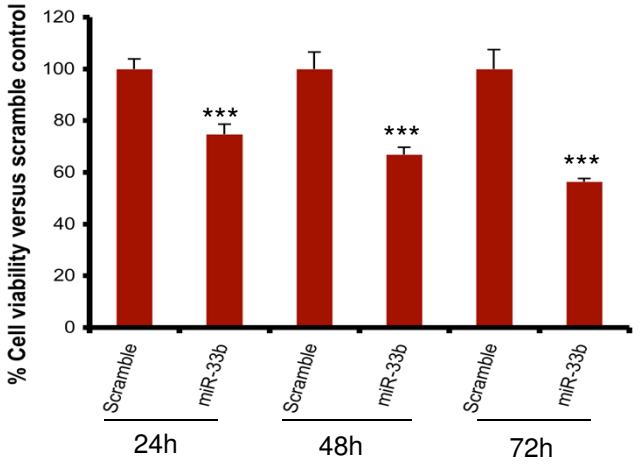


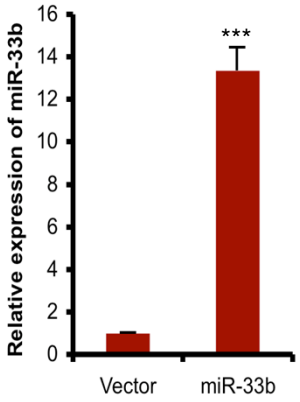


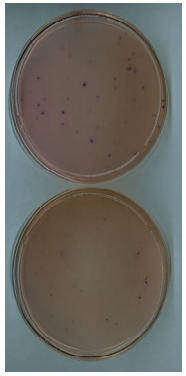
Tian Z, et al. *Blood*. 2012;120(19):3958-3967.

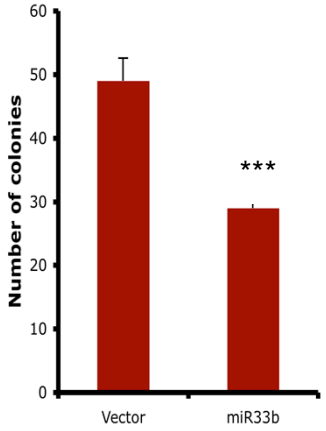
Overexpression of miR-33b Decreases Viability, Colony Formation, and Enhances Anti-MM Activity of MLN2238/9708 in MM Cells

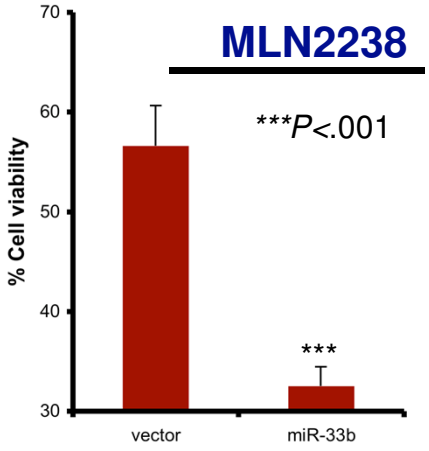












***p<0.001

Tian Z, et al. *Blood*. 2012;120(19):3958-3967.

MLN9708 in Relapsed and/or Refractory MM: Expansion Cohorts of a Phase I Dose-Escalation study

- **46 pts evaluable for response**
  - 21 in dose-escalation cohorts
  - 30 in expansion cohorts (including 6 from dose-escalation cohorts)
- **6 pts have achieved ≥PR**
  - 1 CR, confirmed by bone marrow (PI-naïve expansion cohort)
  - 5 PRs (1 each at 1.2 and 2.23 mg/m^2 in dose-escalation cohorts; 1 in RRMM and 2 in bortezomib-relapsed expansion cohorts)
- **1 pt achieved MR** (bortezomib-relapsed expansion cohort; 40% M-protein reduction)
- All 7 pts remain in response, with duration of disease control of up to 15.9 months
- **28 pts have achieved SD**
  - 14 in dose-escalation cohorts
  - 9, 5, and 2 in RRMM, bortezomib-relapsed, and PI-naïve expansion cohorts
  - Durable, with disease stabilization for up to 12.9 months

Richardson PG, et al. *Blood.* 2011;118: Abstract 301.

Phase I/II Study of MLN9708, Lenalidomide, and Dex in Patients With Previously Untreated MM

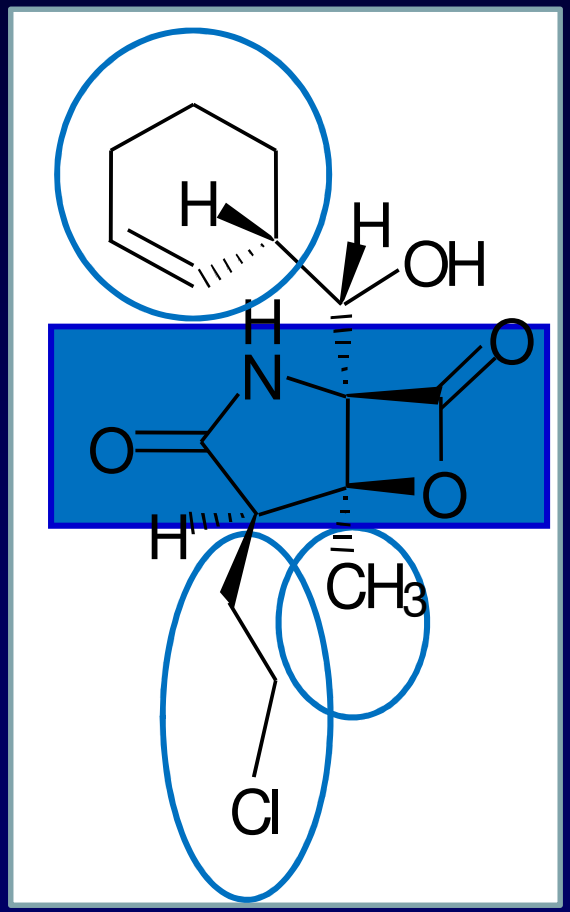
- Oral weekly MLN9708, lenalidomide, and dexamethasone is well tolerated
  - incidence of PN has been limited

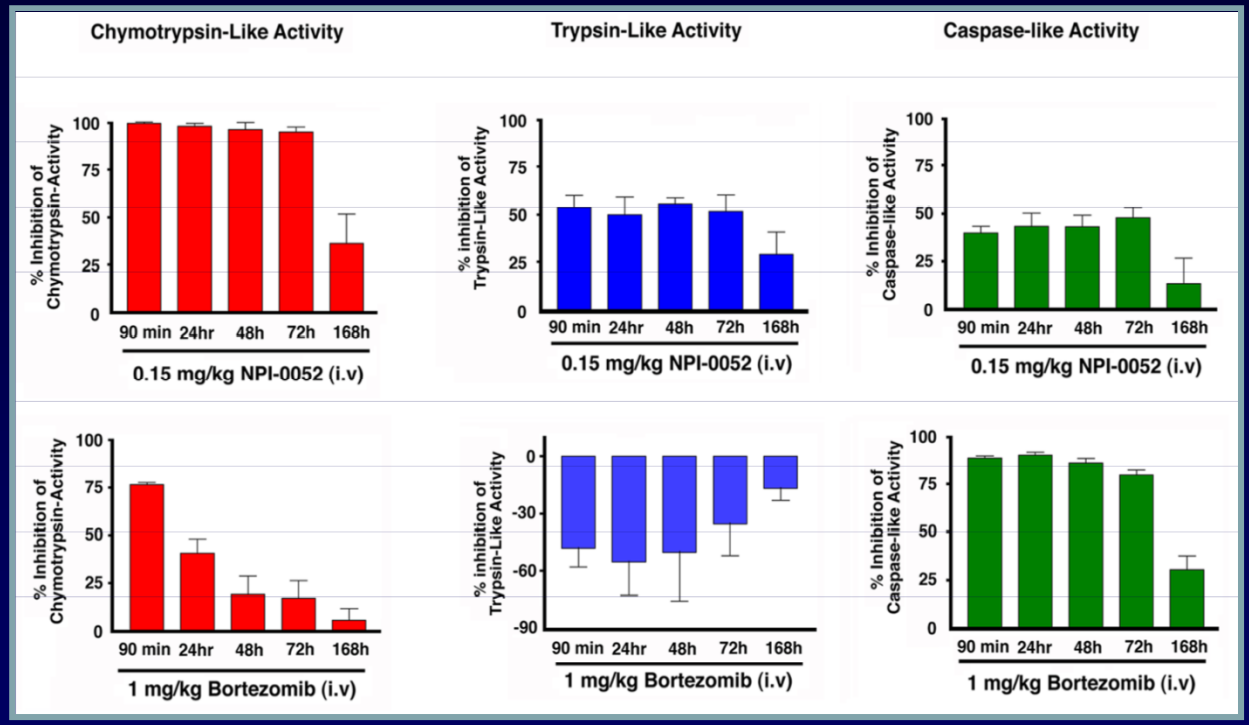
  At median drug exposure of 6 months, 92% PR or better, including ≥VGPR 55% and CR 23%
  - Responses increased with number of cycles and deepened over time
  - 88% of patients achieving CR who were evaluable for MRD status were confirmed as MRD-negative
- A phase III trial of MLN9708 plus lenalidomide–dexamethasone versus placebo plus lenalidomide–dexamethasone in patients with relapsed and/or refractory MM is currently enrolling (NCT01564537) for new drug approval

Kumar SK, et al. *Blood*. 2012;120: Abstract 332. National Institutes of Health. Available at: http://clinicaltrials.gov/show/NCT01564537. Accessed: March 22, 2013.

Marizomib: A Non-Peptide Proteasome Inhibitor Induces Rapid, Broad, and Prolonged Inhibition

Marizomib (NPI-0052)





- Exhibits high levels of proteasome inhibition without toxicities associated with bortezomib
- Active in bortezomib and IMiD resistant myeloma preclinically

Chauhan D, et al. *Cancer Cell*. 2005;8(5):407-419.

NPI-0052 Marizomib : Novel Proteasome Inhibitor

- Phase I, open-label, dose-escalation study in patients with relapsed and relapsed/refractory MM (N=32)
  - Best response (paraprotein; EBMT criteria): SD in 18 (58%)
    - SD >6 mos: 9 (28%)
  - Recommended phase II dose: 0.7 mg/m^2
  - Generally well-tolerated
    - Common AEs: fatigue, nausea/vomiting, dizziness, and headache
    - No neuropathy, neutropenia, or thrombocytopenia
    - NB: Ongoing study of twice weekly 0.5mg dosing achieving responses and well tolerated in R/R MM

Richardson P et al. *Blood*. 2011;118(21):Abstract 302.

Responses to Marizomib +/- Dexamethasone in Evaluable Pts at Full Dose [≥0.4 mg/m^2]* Twice Weekly (n = 21)**

All Pts		
EBMT		
≥ SD	11/20	55%
MR + PR	3/20	15%
Uniform Criteria		
≥ SD	12/21	57%
PR + VGPR	4/21	19%

Pts Refractory to Bortezomib		
EBMT		
≥ SD	8/12	67%
MR + PR	2/12	17%
Uniform Criteria		
≥ SD	8/12	67%
PR + VGPR	2/12	17%

Median Duration of Response (All Pts) = 133 days (~5 mos)

Pts Exposed to Bortezomib		
EBMT		
≥ SD	11/19	58%
MR + PR	3/19	16%
Uniform Criteria		
≥ SD	11/19	58%
PR + VGPR	3/19	16%

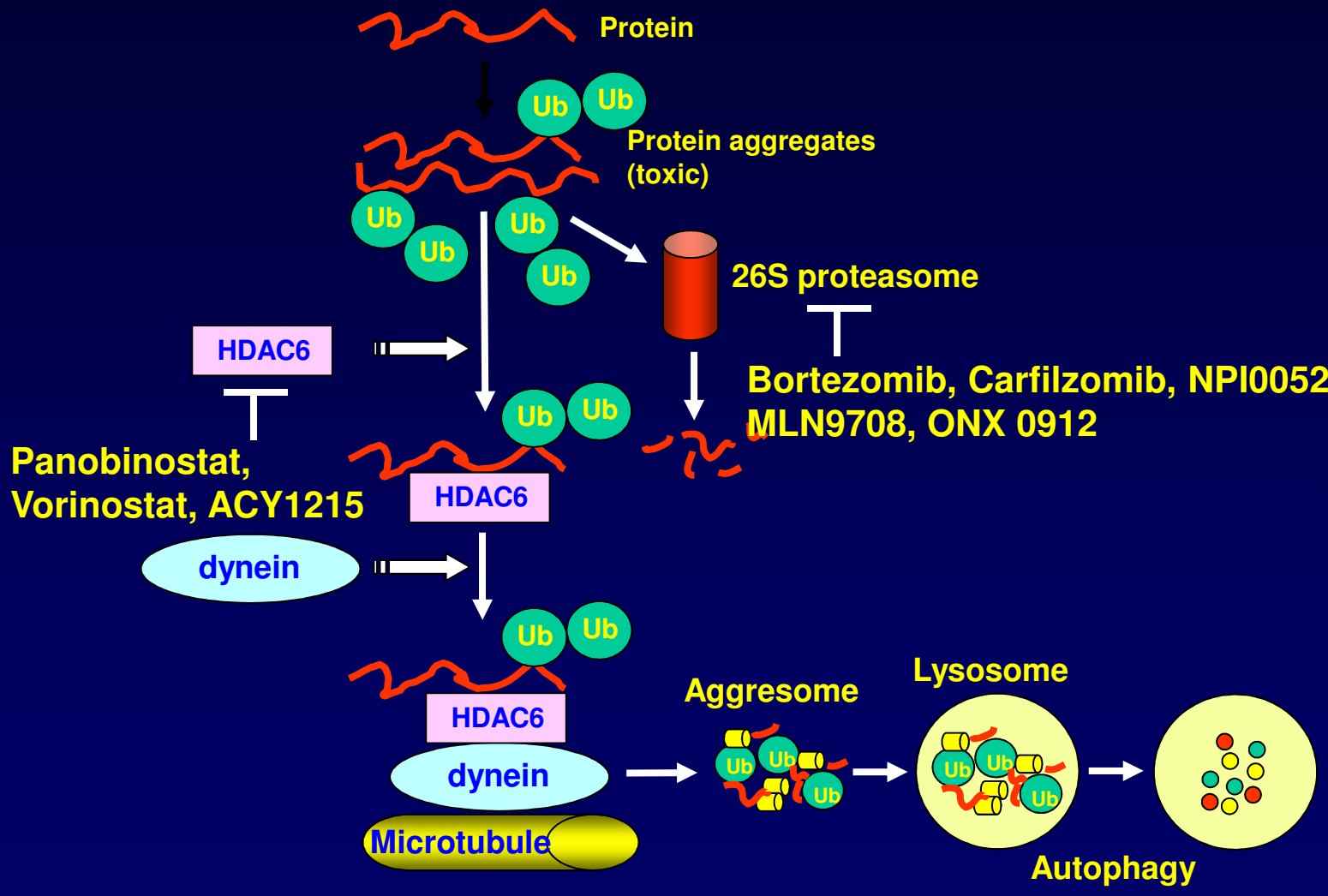
Pts Refractory to Lenalidomide		
EBMT		
≥ SD	8/13	62%
MR + PR	3/13	23%
Uniform Criteria		
≥ SD	9/14	64%
PR + VGPR	4/14	29%

- **Response criteria defined with baseline SPEP ≥0.5 g/dL or UPEP ≥200 mg/24h with at least 2 assessments after treatment day 1 for EBMT; also by Freelite® for uniform criteria.
- Refractory defined as having PD during or within 60 days of last regimen.

*As of 05 Dec 11

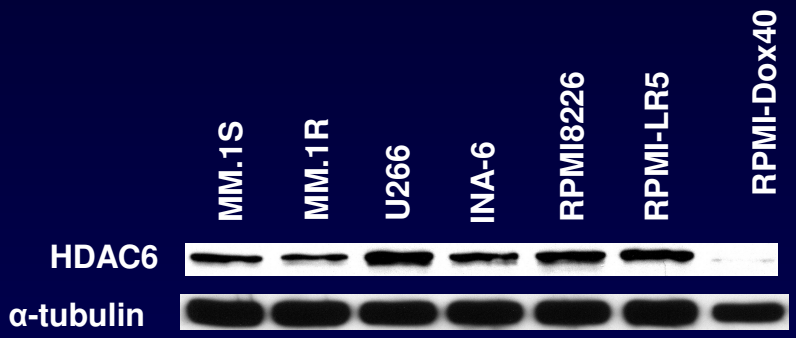
Richardson PG, et al. *Blood*. 2011;118: Abstract 302.

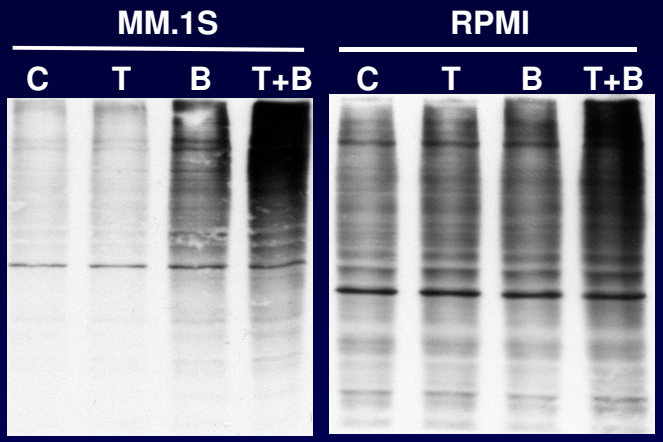
Development of Rationally-Based Combination Therapies (HDAC and Proteasome Inhibitors)

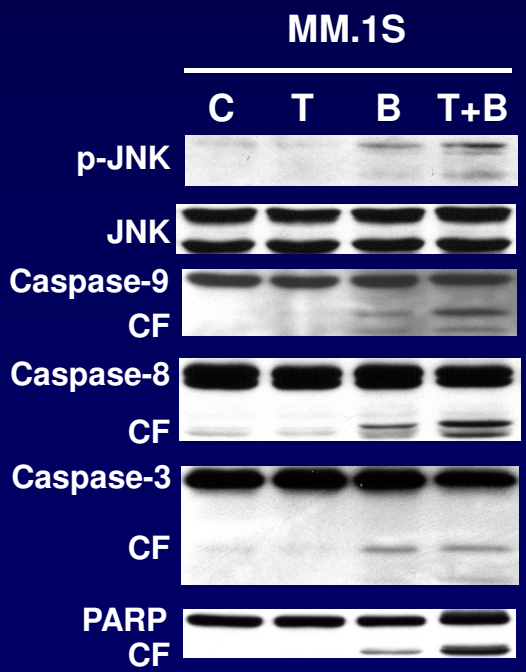


Hideshima T, et al. *Clin Cancer Res*. 2005;11(24 Pt 1):8530-8533. Catley L, et al. *Blood*. 2006;108(10):3441-3449.

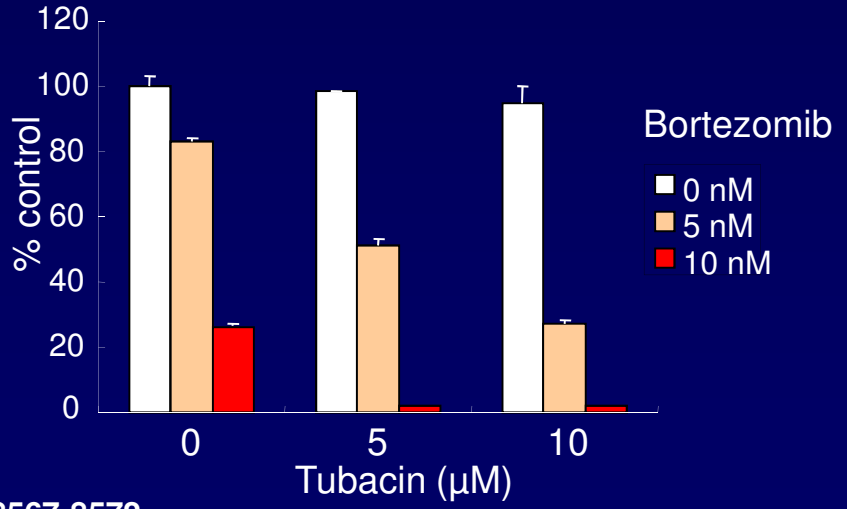
Targeting Proteasome and Aggresome Triggers Synergistic MM Cytotoxicity







C: control T: tubacin (5μM)
B: bortezomib (5 nM)



Hideshima T, et al. *Proc Natl Acad Sci U S A*. 2005;102(24):8567-8572.

VANTAGE 088: An International, Multicenter, Randomized, Double-Blind Study of Vorinostat or Placebo with Bortezomib in Relapsed MM

- The combination of vorinostat + bortezomib is active in patients with relapsed and refractory MM
  - Significant improvement in response rate
  - ORR 54% vs 41% (*P*<.0001); CBR 71% vs 53% (*P*<.0001)

- PFS and TTP were prolonged in the combination arm compared with bortezomib alone
  - PFS hazard ratio reduction of 23% (*P* = .01); 7.63 months (6.9–8.4) versus 6.83 months (5.7–7.7)

- The combination was associated with side effects of thrombocytopenia, diarrhea, and fatigue limiting prolonged therapy

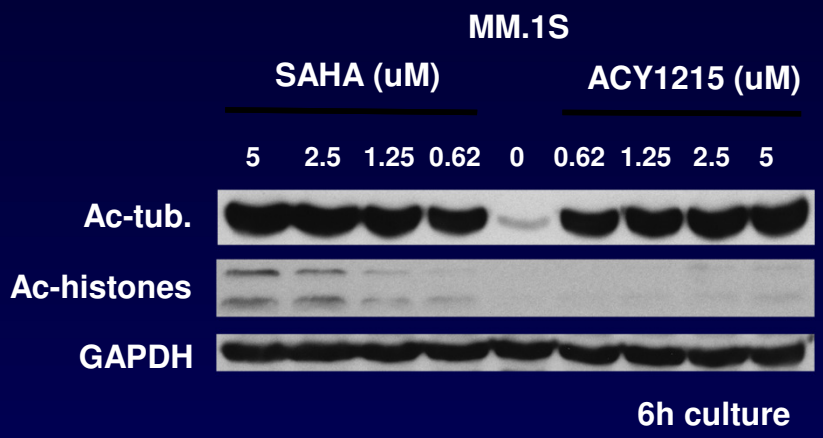
Dimopoulos MA, et al. *Blood*. 2011;118: Abstract 811.

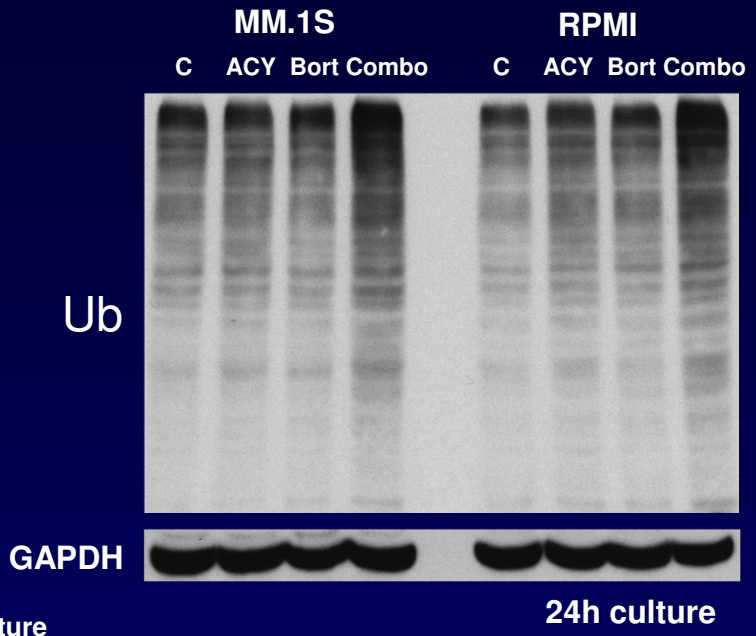
Bench to Bedside Translation of HDAC 6 Selective Inhibitor ACY 1215 Rocilinostat

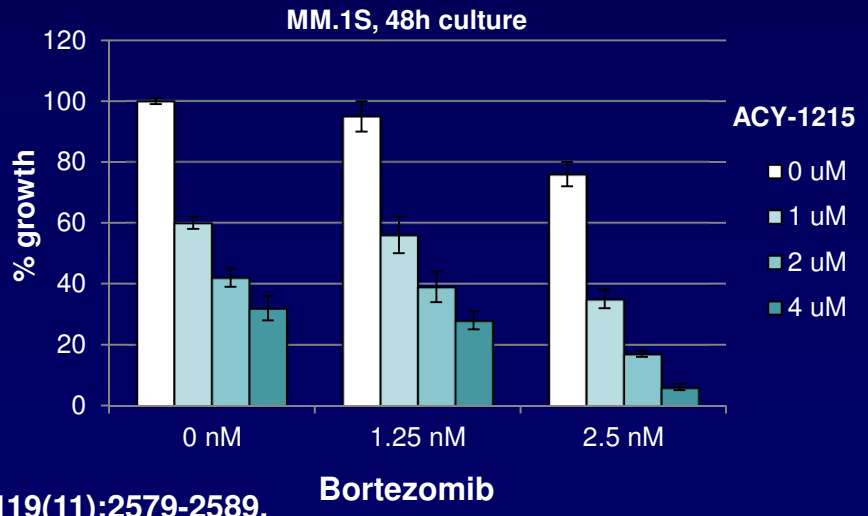
- Orally bioavailable, highly potent, selective inhibitor of HDAC 6 synthesized in fall 2009
- Synergistic MM cytotoxicity with bortezomib *in vitro* and *in vivo*
- Favorable PK/PD, toxicity profile
- Phase Ia/Ib/II clinical trial of ACY1215, alone and with bortezomib and with lenalidomide/dexamethasone, ongoing

Santo L, et al. *Blood*. 2012;119(11):2579-2589.

HDAC6 Selective Inhibitor ACY-1215 Enhances Bortezomib-Induced Cytotoxicity Clinical Trials Ongoing

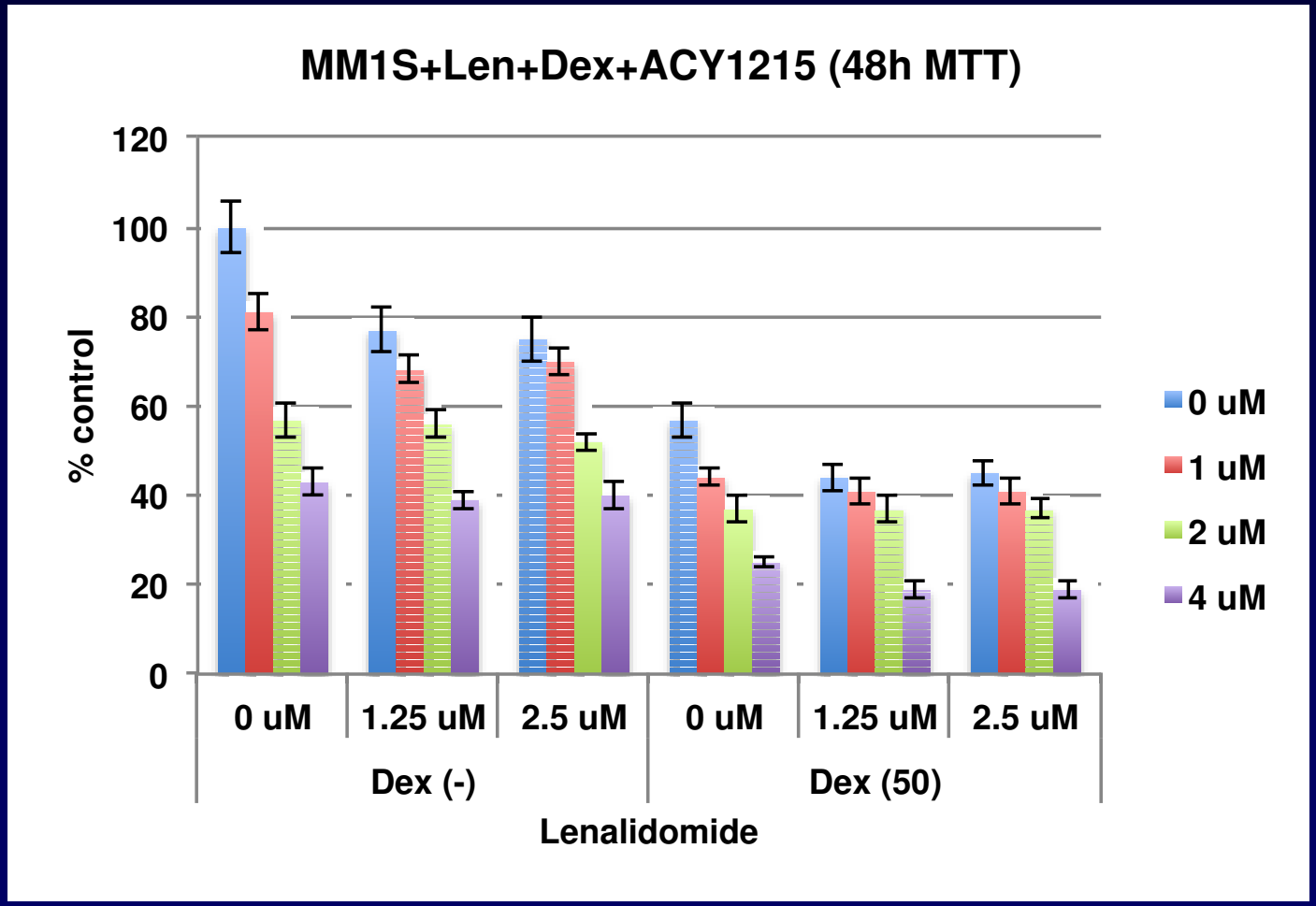






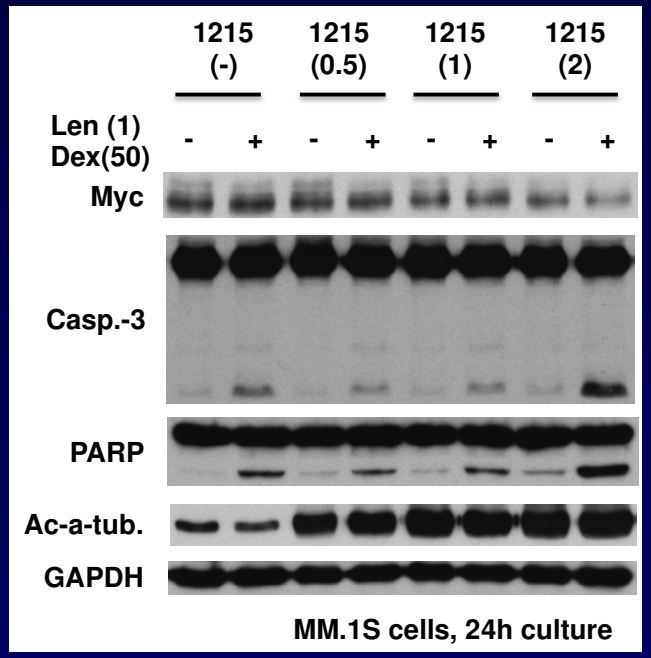
Santo L, et al. *Blood*. 2012;119(11):2579-2589.

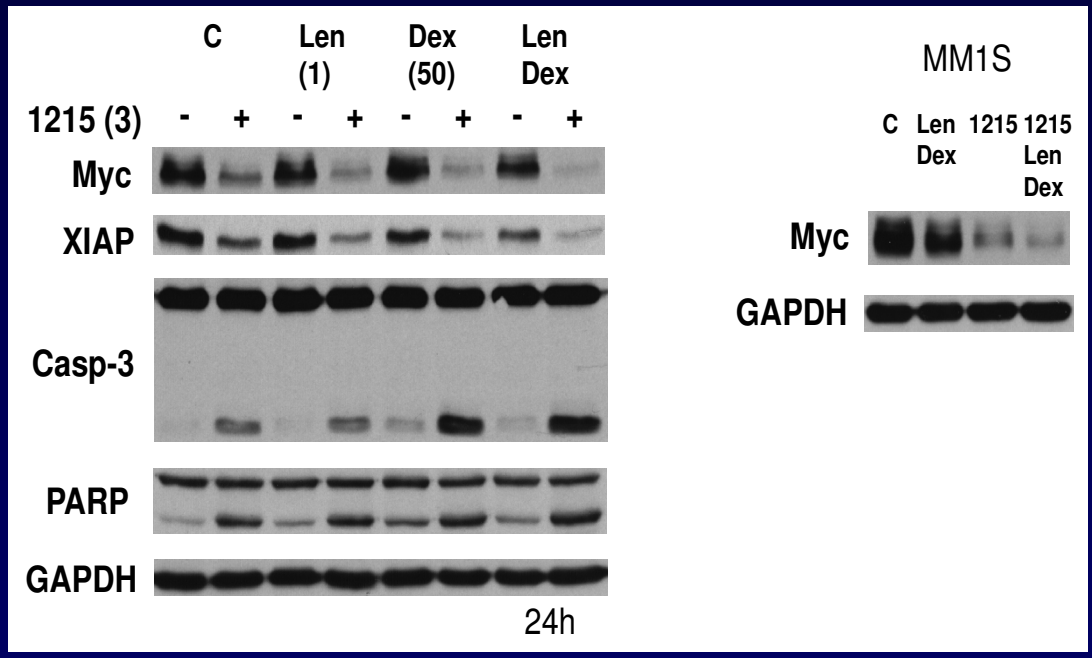
Synergistic Cytotoxicity of Lenalidomide and Dexamethasone is Augmented by ACY-1215



Hideshima et al. 2013.

ACY-1215, Lenalidomide and Dexamethasone Inhibits c-Myc Expression





Hideshima et al. 2013.

Predicting Proteasome Inhibitor Response

MM Relatively Resistant to PI

- **High proteasome capacity**
- **Low proteasome load**

→ Low level proteasome stress

MM Highly Sensitive to PI

- **Low proteasome capacity**
- **High proteasome load**

→ High level proteasome stress

Bianchi G, et al. *Blood.* 2009;113(13):3040-3049.

Mutations in Myeloma

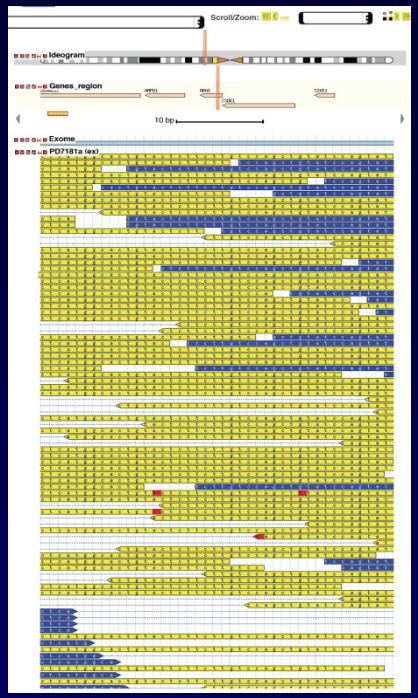
19 patients each with newly diagnosed and relapsed MM

- **Protein homeostasis:** 42% including FAM46C, RPL10, RPS6KA1, EIF3B, XBP1, LRRK2[1]
- **NF-kB signaling:** 10 point mutations, 4 additional structural re-arrangements affecting coding
  - NB confers bortezomib sensitivity[1]
- **Histone-methylating enzymes:** WHSC1, UTX, MLL[1]
- **BRAF:** 4% activating[1]

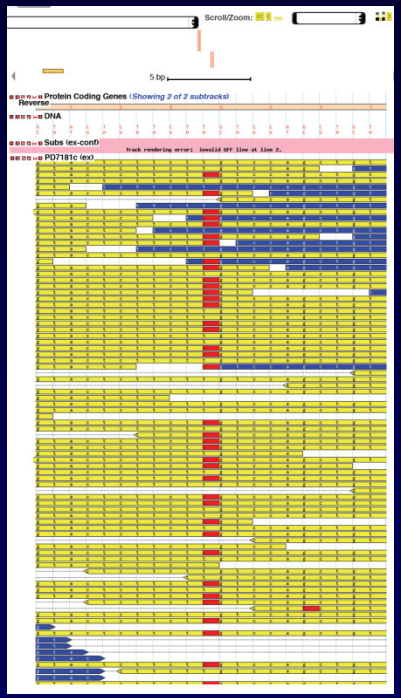
NB **PSMB5 b5** proteasome subunit mutation confers proteasome inhibitor resistance in laboratory, not identified in clinic[2]

1. Chapman MA, et al. *Nature*. 2011;471(7339):467-472. 2. Lichter DI, et al. *Blood*. 2012:120(23):4513-4516.

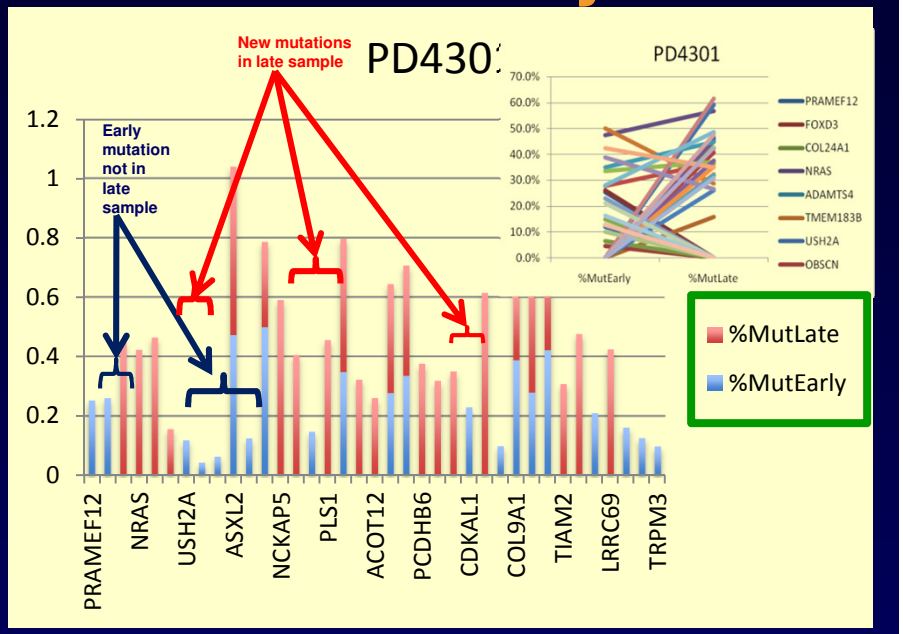
Whole Genome Sequencing Identifies Acquisition of New Changes in MM: 71 Patient Study

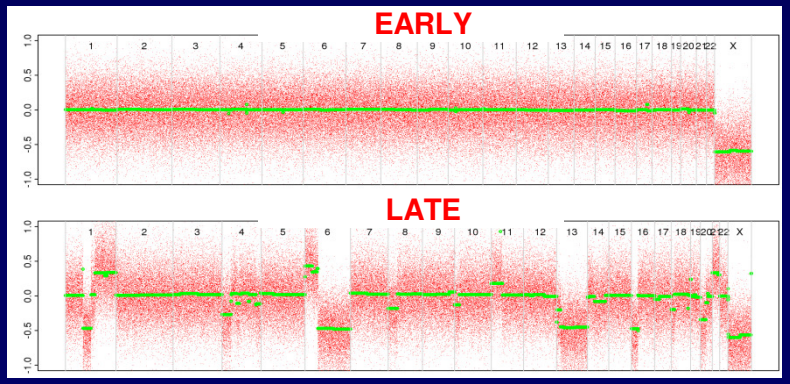


Early Tumor

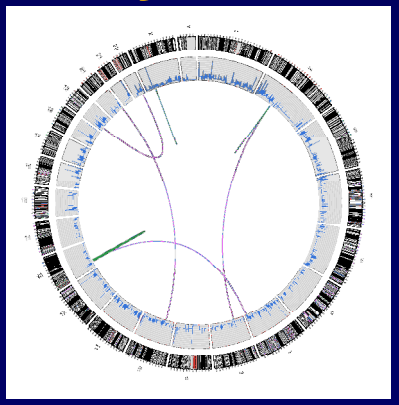


Late Tumor

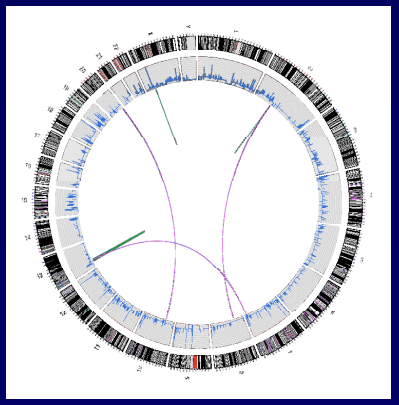




Early Tumor



Late Tumor



Munshi NC, et al. *Blood.* 2011;118: Abstract 297.

Current and Future Directions Targeting UPS

1. Incorporation of proteasome inhibitors into induction, consolidation and maintenance therapy.

2. Development of next generation agents targeting protein homeostasis

3. Development of rationally-based combination therapies

4. Utilization of proteasome stress and genomics profiling for improved classification and personalized therapy

Myeloma will be a chronic illness, with sustained CR in a significant fraction of patients.

United Nations Against Myeloma:

Bench to Bedside Research Team

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Deborah Doss
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Karin Anderson
Shannon Viera
Katherine Redman
Amber Walsh
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Holly Bartel
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Jeffrey Sorrell

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Italy

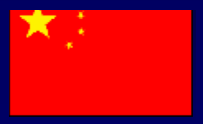
Israel

Japan

Canada

Germany

Austria



China

Teru Hideshima
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