Effect of new anti-myeloma drugs on bone microenvironment cells

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Discosures: Research funding: Celgene, Novartis

New anti-myeloma drugs

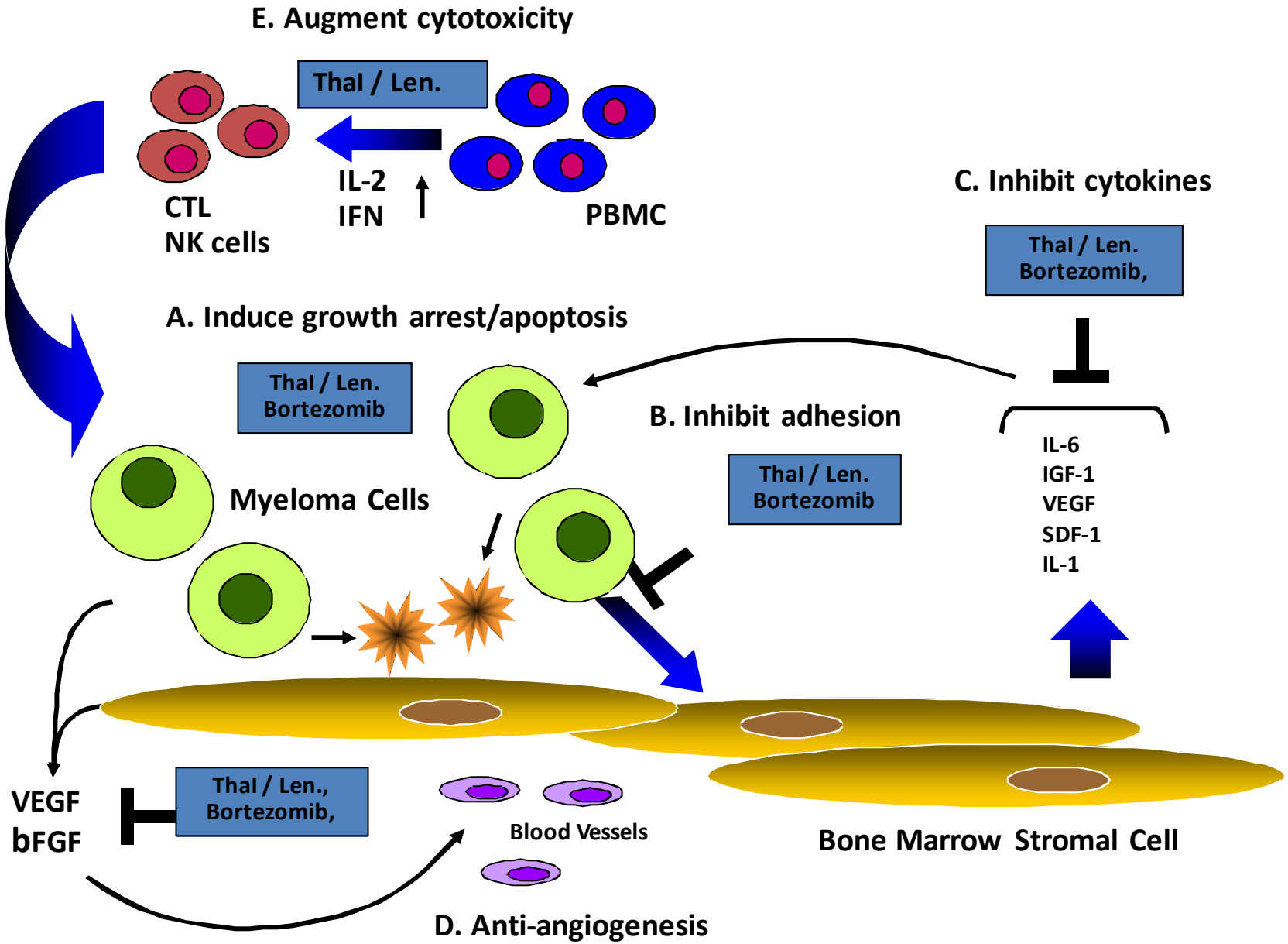
- **Targeting growth factors / receptors**
  - Anti-IL-6 antibody
    - Sant7
  - VEGF receptor inhibitor
    - PTK787/ZK
    - GW654652
  - IGF-1R inhibitor
    - AVE1642
    - GSK1838705A-GSK1904529A
  - FGFR tyrosine kinase inhibitor
    - CHIR258
  - Anti CD40 inhibitor
    - SNG40
  - CS1
    - Elotuzumab

- **Targeting directly tumor cells**
  - HSP-90 inhibitors
    - Geldanamicyne
    - Tanespimycin
  - **Histone deacetylase inhibitor**
    - SAHA (vorinostat)
    - LBH589 (panobinostat)

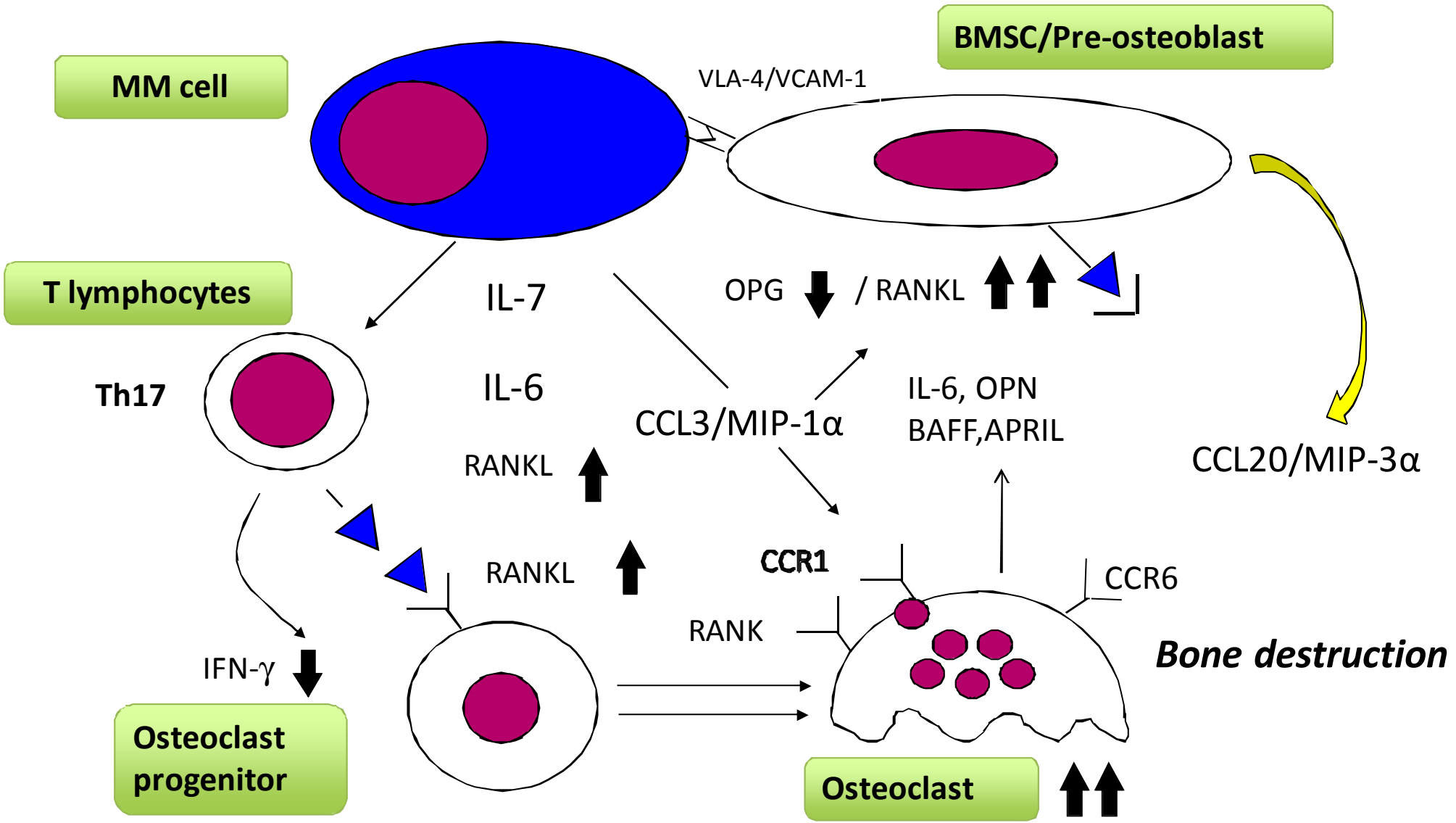
- **Targeting cell signaling pathway**
  - –Farnesyl transferase inhibitor (Ras inhibitor)
    - •Tipifarnib or Zarnestra
  - –mTOR inhibitor
    - •Rapamycin
    - •CCI-779
  - –Akt inhibitor
    - •Perifosine
  - –PKC inhibitor
    - •Enzastaurin
  - –TRAIL receptor activator
    - •HGS-ETR
  - –MAPK inhibitor
    - •SCIO-469

- **Multi-target drugs**
  - **– Proteasome inhibitors: Bortezomib, Carfilzomib, NPI-0052**
  - **– Thalidomide**
  - **– IMiDS: Lenalidomide, Pomalidomide**
  - – Arsenic trioxide

New drugs targeting MM cells and the microenvironment

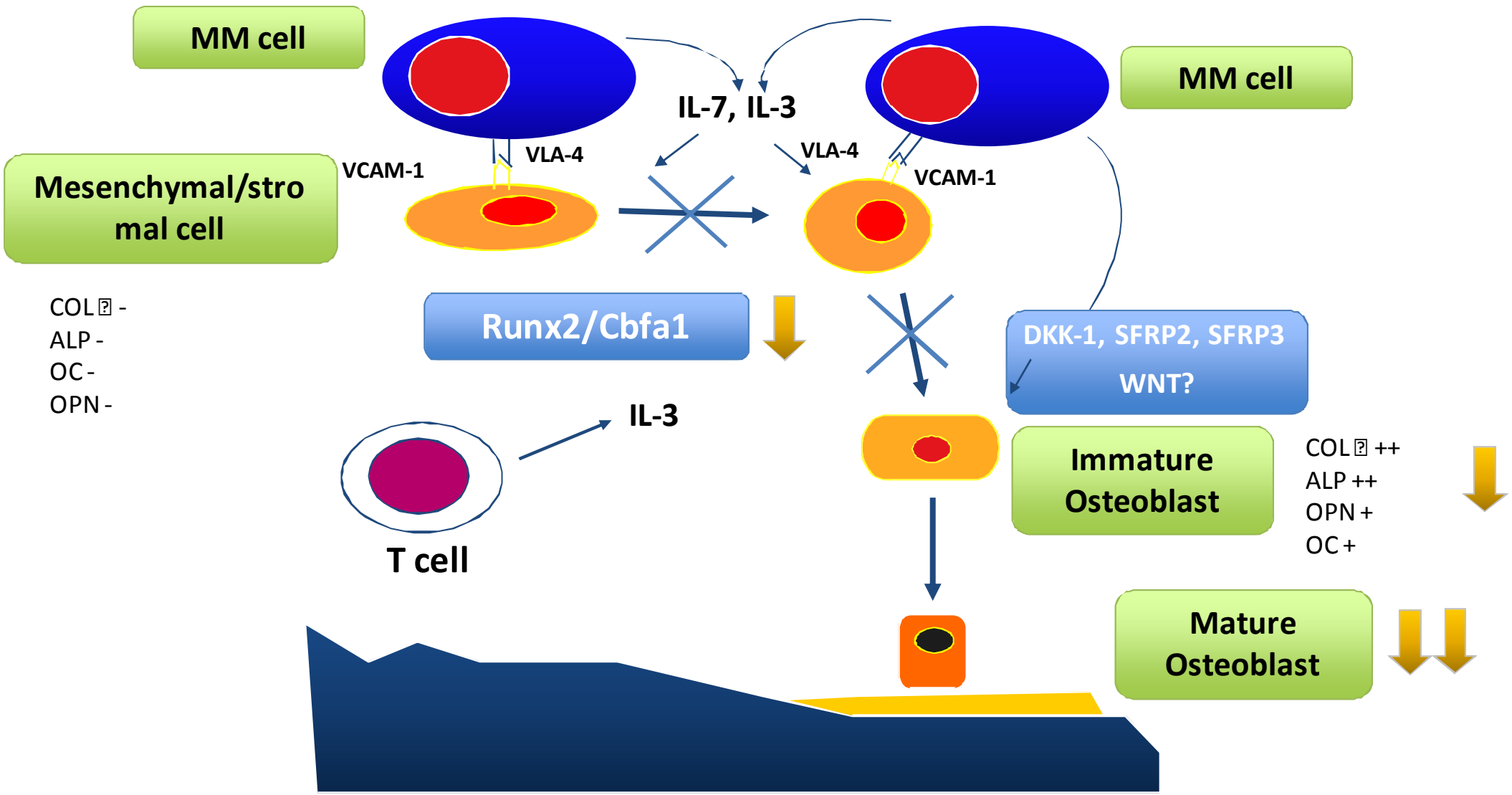


Pathophysiology of osteoclast activation in multiple myeloma (MM)



Giuliani N et al Blood 2001 and Blood 2002; Giuliani N et al. Cancer Res 2008; Choi SJ et al. J Clin Invest. 2001; Abe M et al. Leukemia 2006; Noonan K et al. Blood 2010

Pathophysiology of Osteoblast inhibition in MM



Tian E et al. *NEJM, 2003*
Giuliani N et al. *Cancer Res,* 2007

Ehrlich LA et al *Blood,* 2005
Giuliani N et al *Blood,* 2005

Key question:

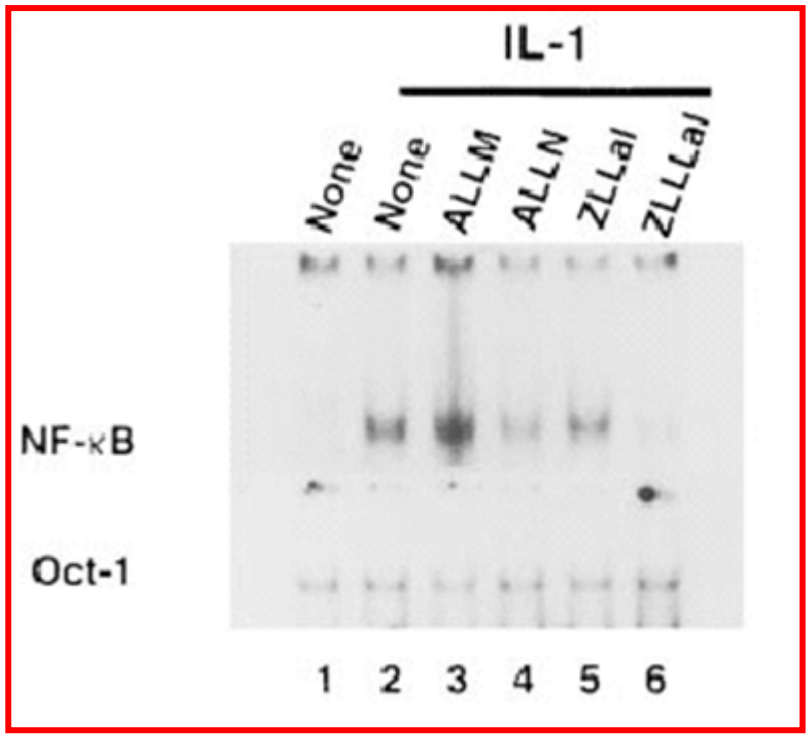
Which are the effects of the new drugs on bone microenvironment cells?

Proteasome inhibitors

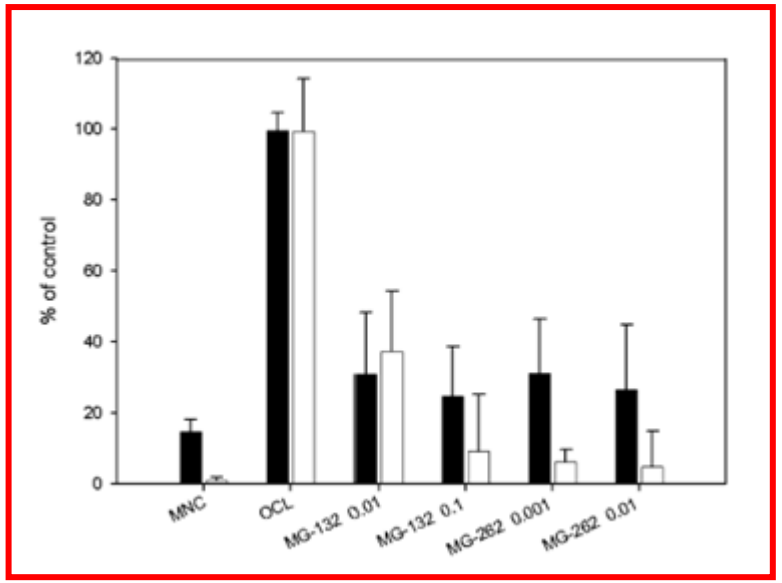
IMiDs

HDAC inhibitors

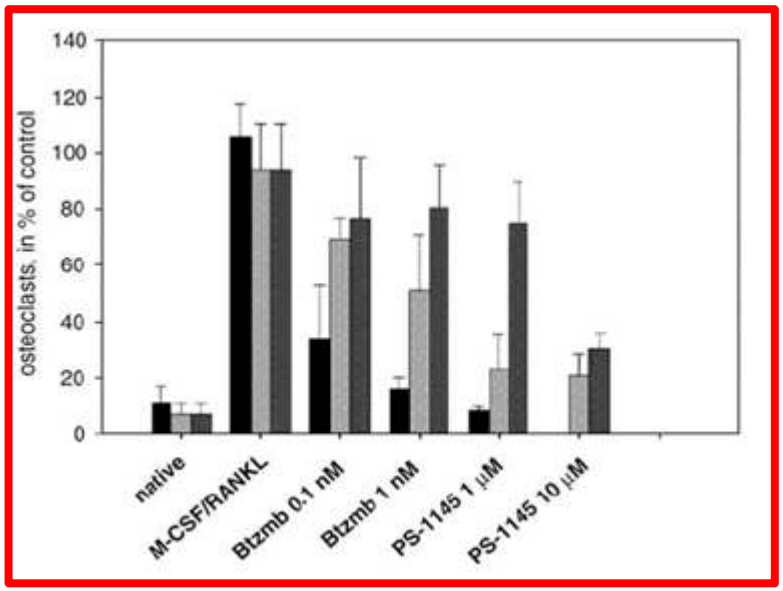
Proteasome inhibitors block osteoclast formation



Jimi E et al. J Biol Chem, 1998

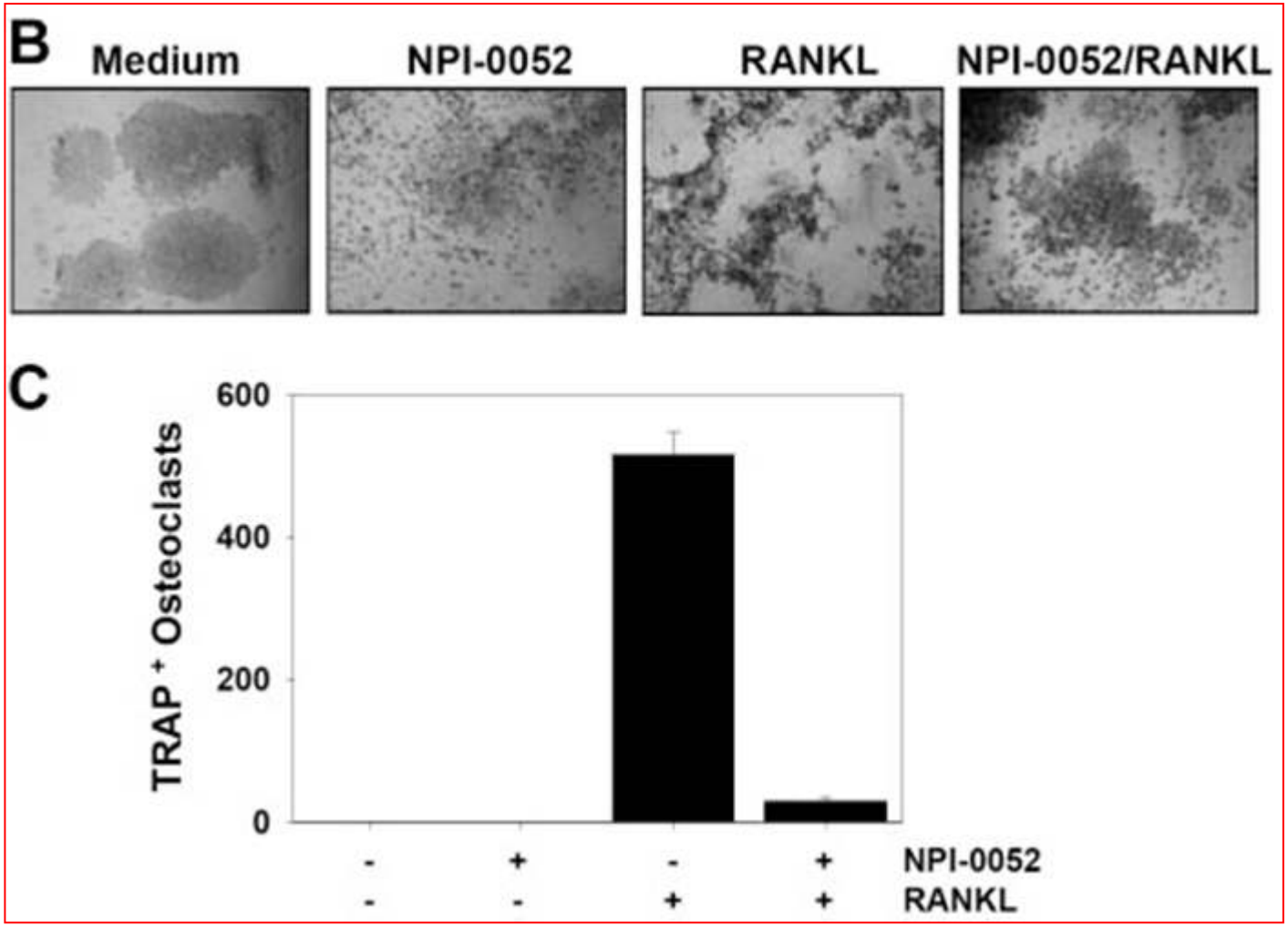


Zavrinski I et al. Biochem Biophys Res Commun 2005



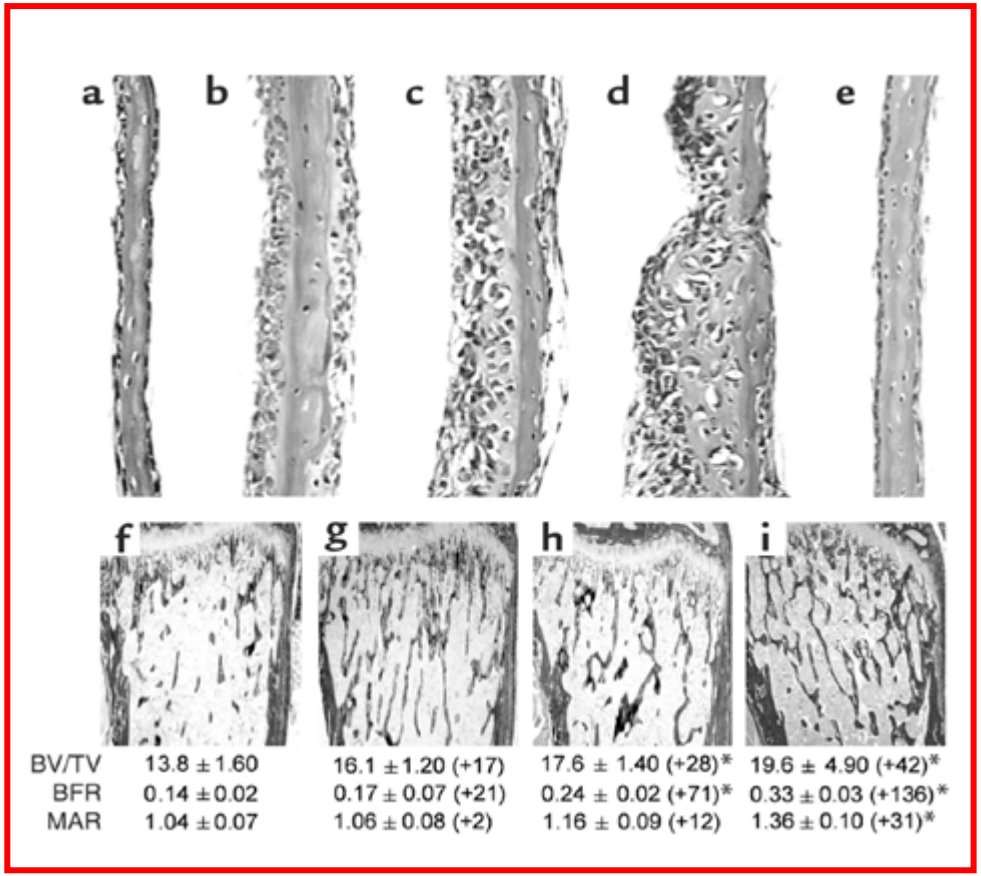
Von Metzler et al. Leukemia 2007

The new proteasome inhibitor NPI-0052 (Salinosporamide A) blocks RANKL-induced osteoclast formation

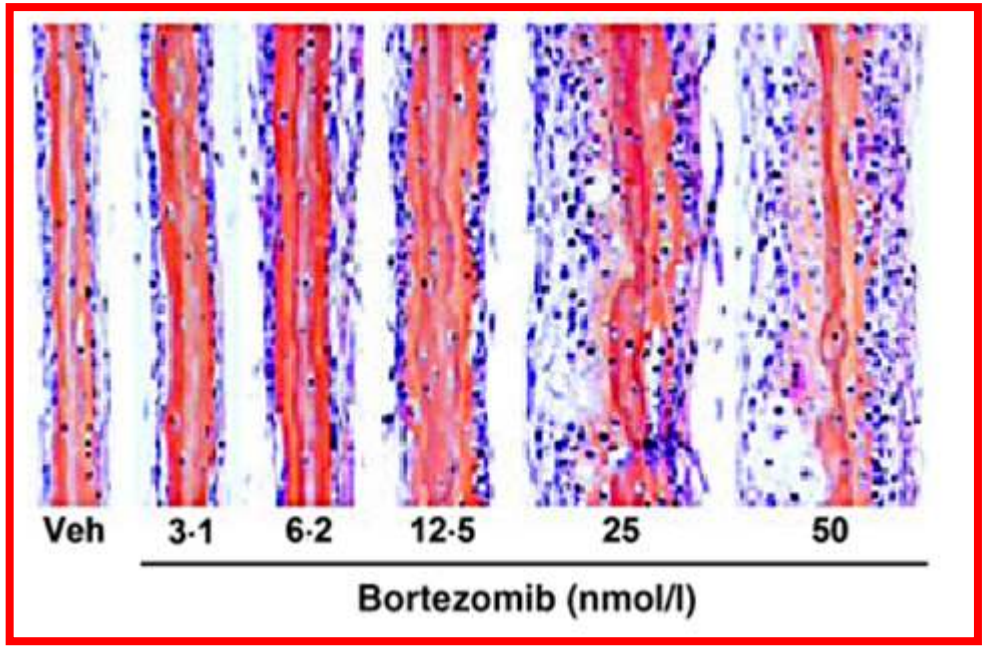


Ahn KS et al. Blood, 2007

Proteasome inhibitors stimulate bone formation in mice

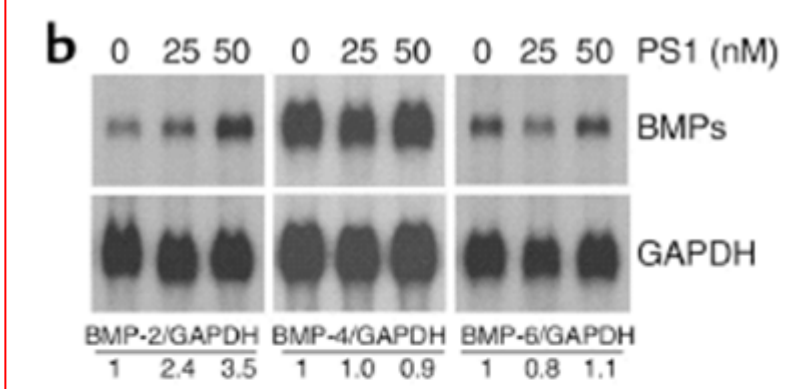


Garrett IR et al. J Clin Invest, 2003



Oyajobi et al. Br J Haematol, 2007

BMPs stimulation by proteasome inhibitors



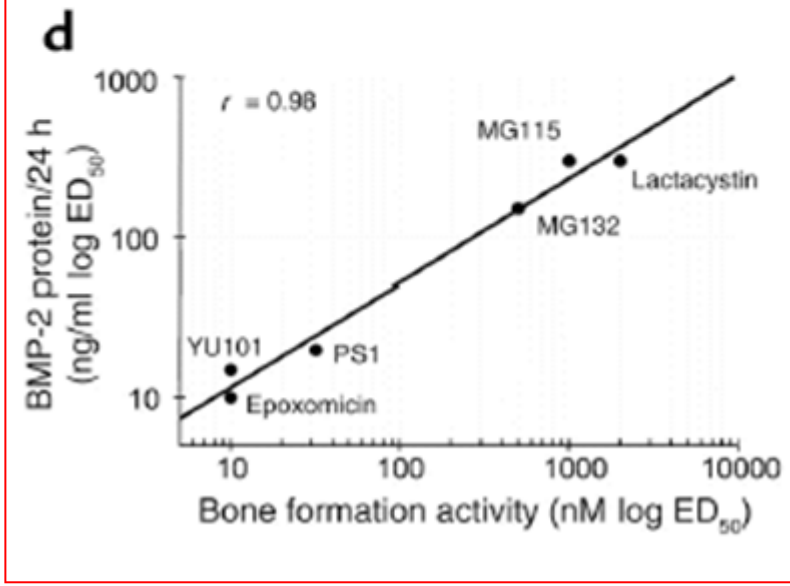


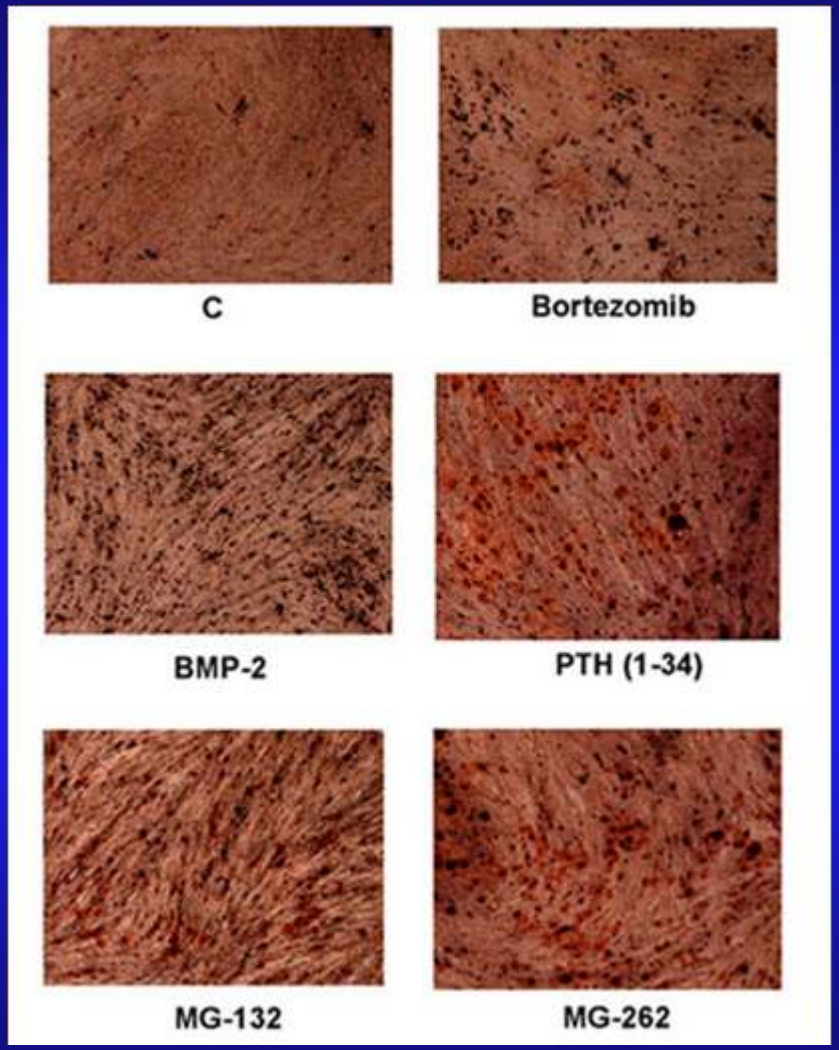
Table 4
Effects of different proteasome inhibitors at multiple concentrations over 24 hours on the production of BMP-2 protein into HU09 cell-conditioned medium

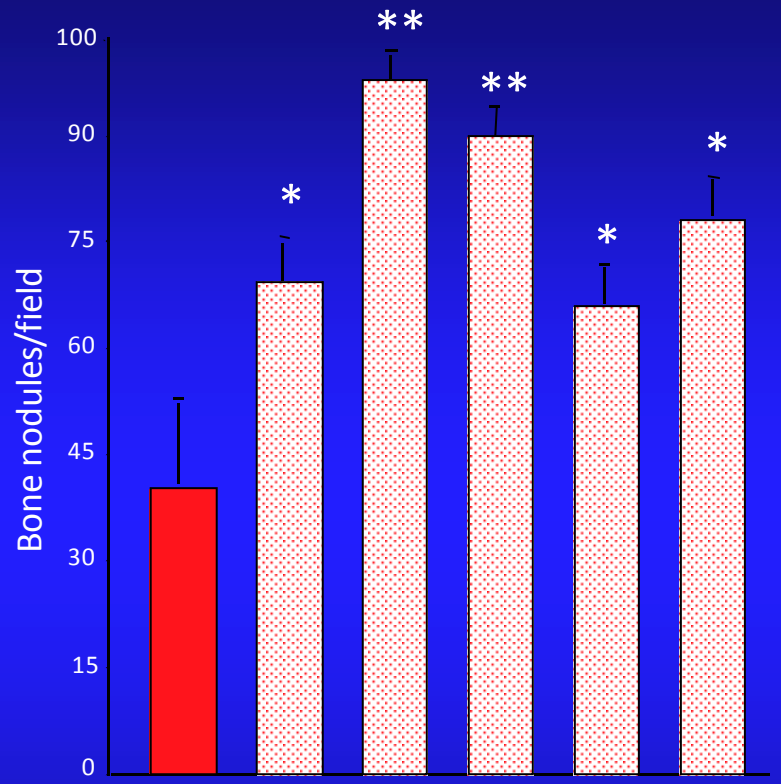
Treatment	Concentration (μM)	BMP-2 protein (pg/ml)
YU101	Vehicle alone	37 ± 5
	0.0025	56 ± 15
	0.005	59 ± 20
	0.010	119 ± 22[A]
	0.020	163 ± 12[A]
	0.040	212 ± 27[A]
PS1	Vehicle alone	67.2 ± 5
	0.0062	98 ± 12[A]
	0.0125	139 ± 15[A]
	0.025	158 ± 18[A]
	0.050	152 ± 22[A]
	0.100	156 ± 16[A]
Epoxomicin	Vehicle alone	36 ± 6
	0.01	50 ± 8
	0.02	108 ± 14[A]
	0.04	172 ± 9[A]
	0.08	75 ± 11[A]
MG132	Vehicle alone	71 ± 13
	0.035	65 ± 4
	0.075	84 ± 13
	0.100	107 ± 11[A]
	0.300	140 ± 14[A]
	0.600	145 ± 6[A]
MG115	Vehicle alone	71 ± 12
	0.300	71 ± 11
	0.600	149 ± 15[A]
	0.1200	74 ± 3
Lactacystin	Vehicle alone	36 ± 6
	125	37 ± 8
	250	83 ± 12[A]
	500	117 ± 15[A]

Data are expressed as means ± SEM. [A]$P < 0.05$ versus vehicle alone.

Garrett IR et al. J Clin Invest, 2003

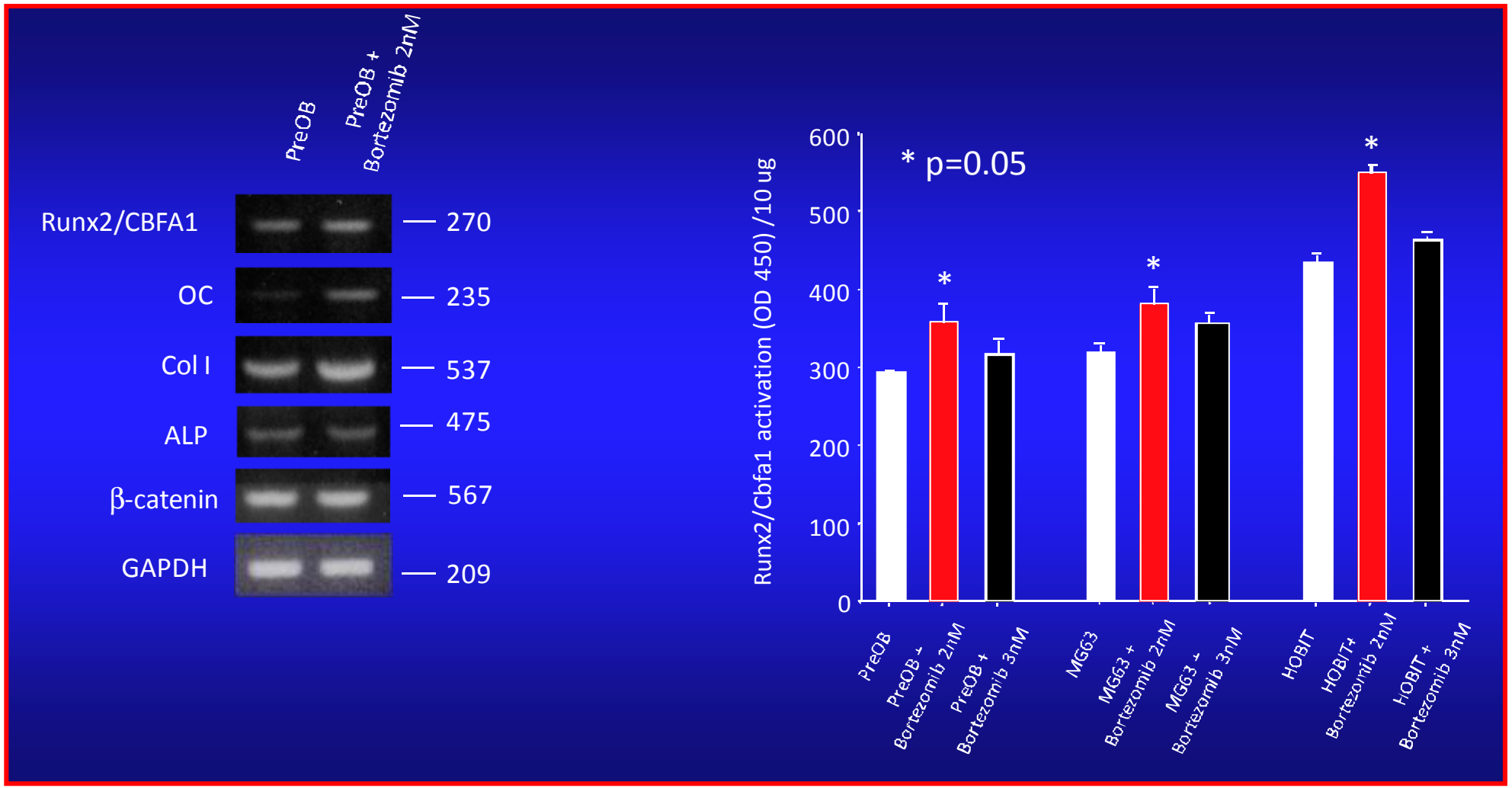
Bortezomib increases osteogenic differentiation of hMSC





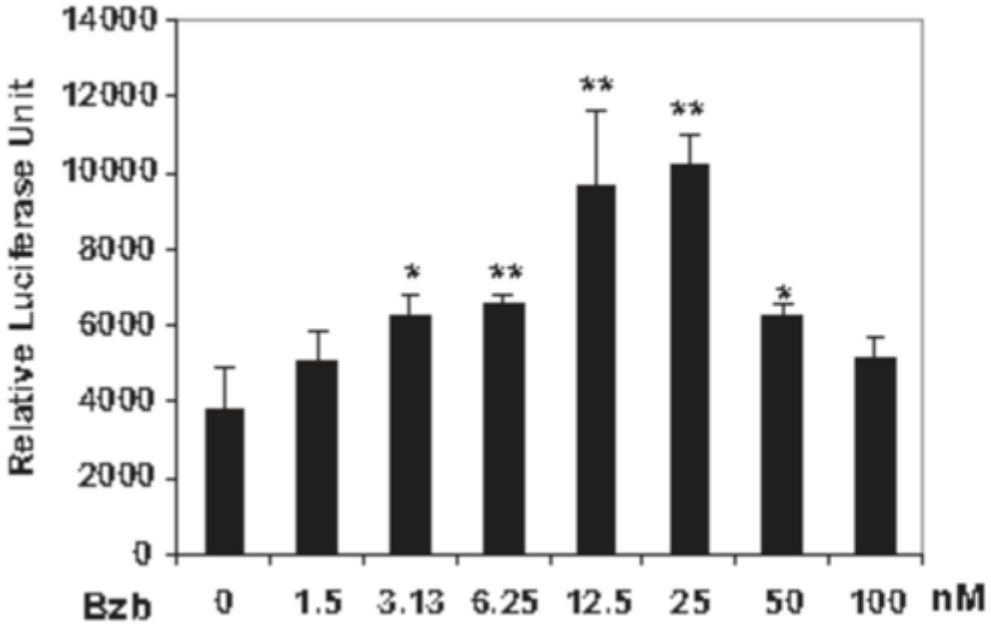
Giuliani et al. Blood 2007

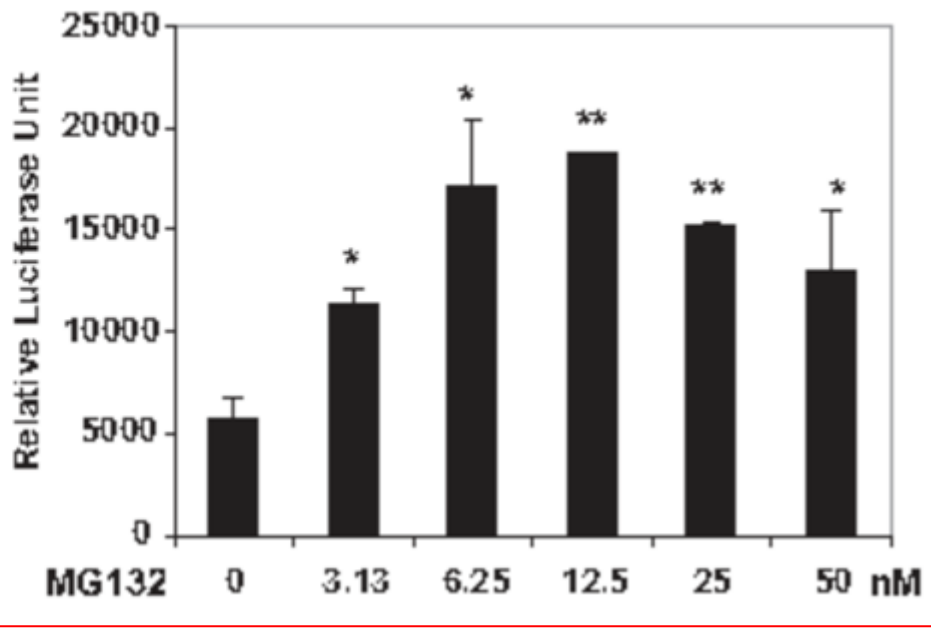
Bortezomib increases the expression and activity of Runx2 and the osteogenic related markers



Giuliani et al. Blood 2007

Proteasome inhibitors activates TCF trascriptional activity in osteoblasts and MSC

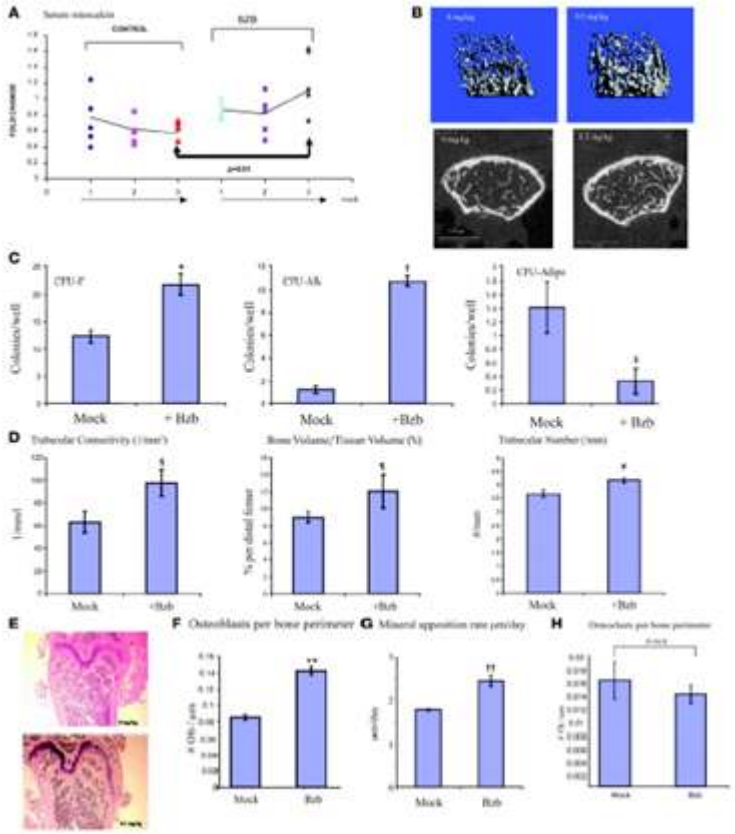


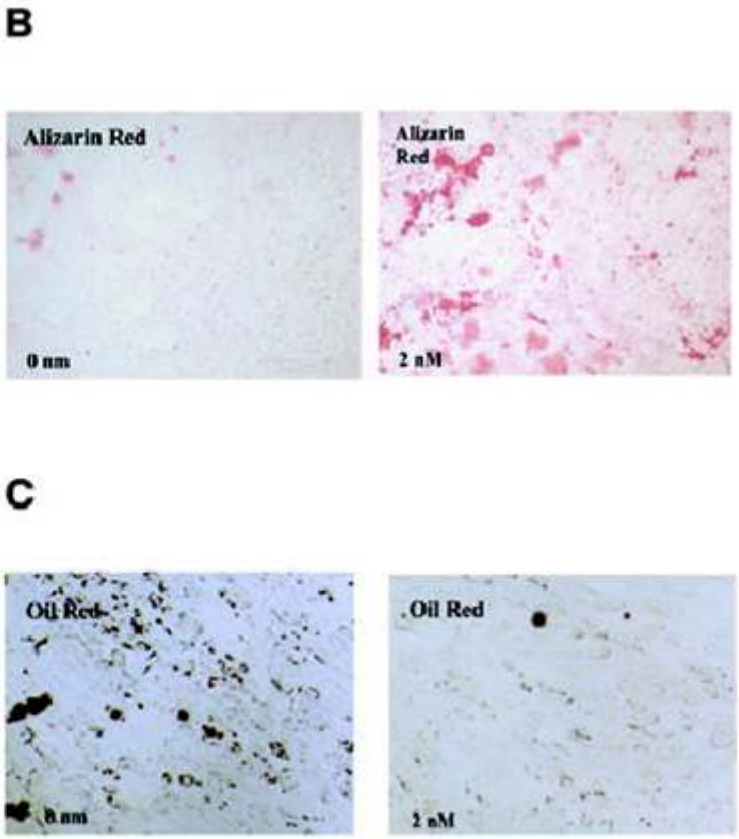


Quiang YW et al. Blood 2009

Pharmacologic targeting of a stem/progenitor population in vivo is associated with enhanced bone regeneration in mice

Siddhartha Mukherjee,[1,2,3,4] Noopur Raje,[3,5] Jesse A. Schoonmaker,[1,4] Julie C. Liu,[6] Teru Hideshima,[5] Marc N. Wein,[7] Dallas C. Jones,[7] Sonia Vallet,[5] Mary L. Bouxsein,[8] Samantha Pozzi,[5] Shweta Chhetri,[5] Y. David Seo,[1,4] Joshua P. Aronson,[1,4] Chirayu Patel,[4] Mariateresa Fulciniti,[5] Louise E. Purton,[1,4] Laurie H. Glimcher,[7] Jane B. Lian,[6] Gary Stein,[6] Kenneth C. Anderson,[5] and David T. Scadden[1,2,3,4]

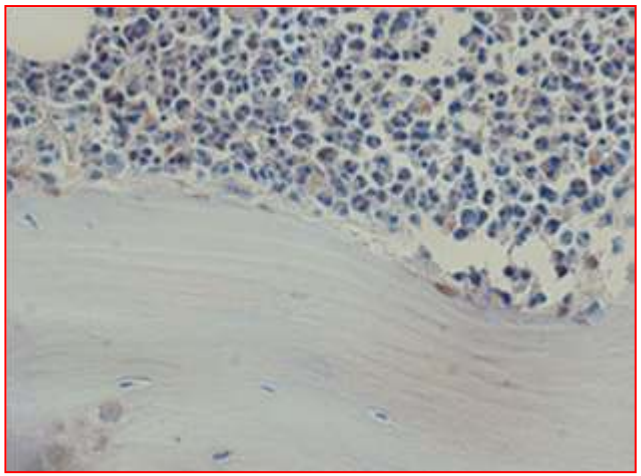


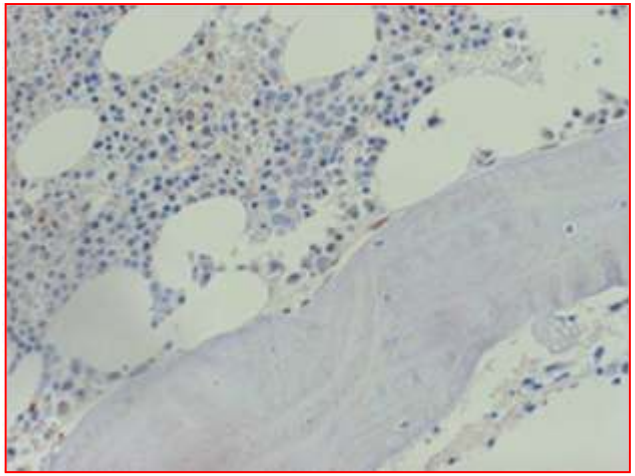


The Journal of Clinical Investigation http://www.jci.org Volume 118 Number 2 February 2008

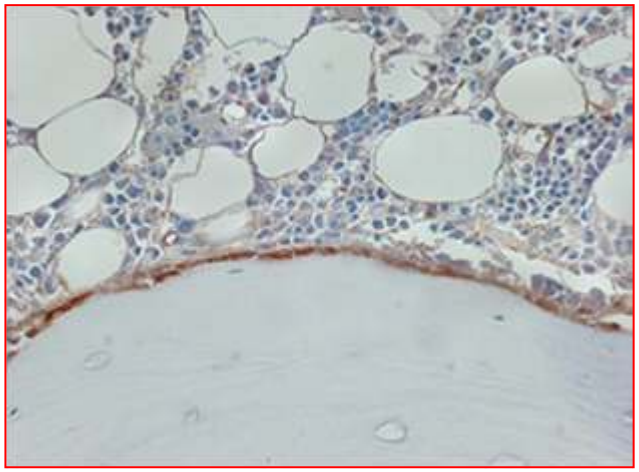
Bortezomib activates osteoblasts in vivo

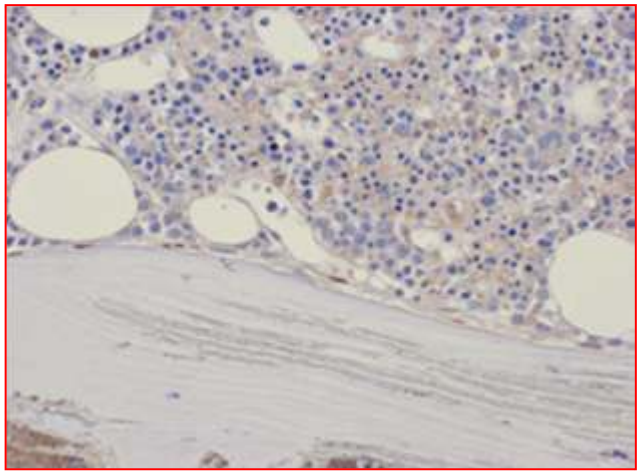
Pre Bortezomib





Post Bortezomib



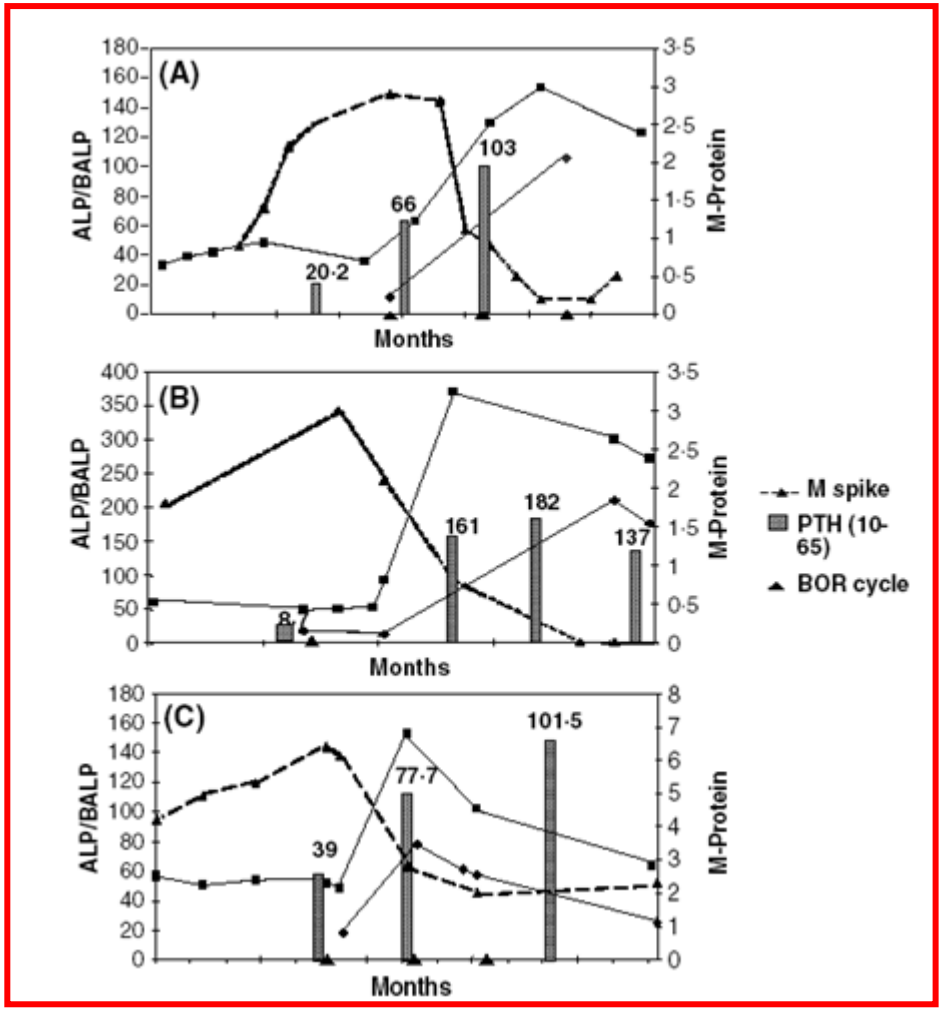


MM “responder”

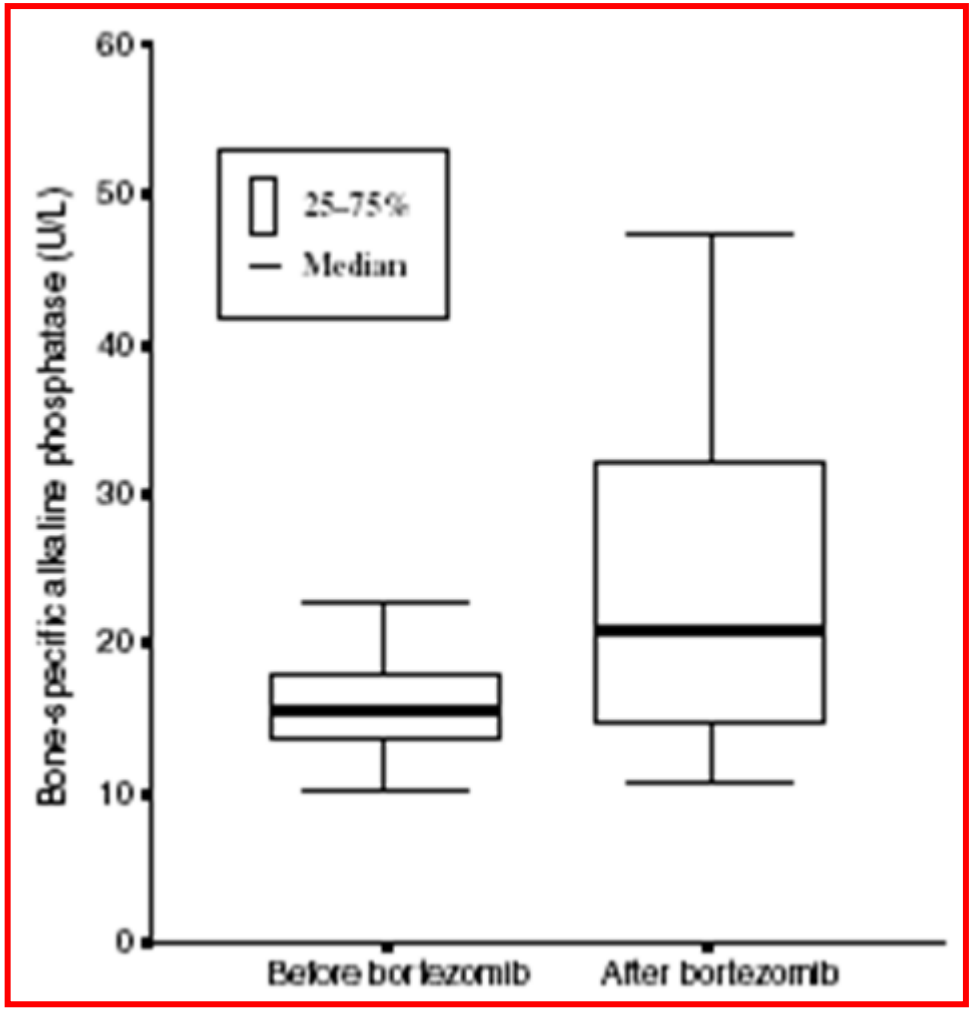
MM “non responder”

Giuliani N et al. Blood 2007

Bortezomib stimulates bone alkaline phosphatase level in MM patients



Zangari M et al. *Br J Haematol*, 2005



Heider U et al. *Eur J Hematol*, 2006

Key question:

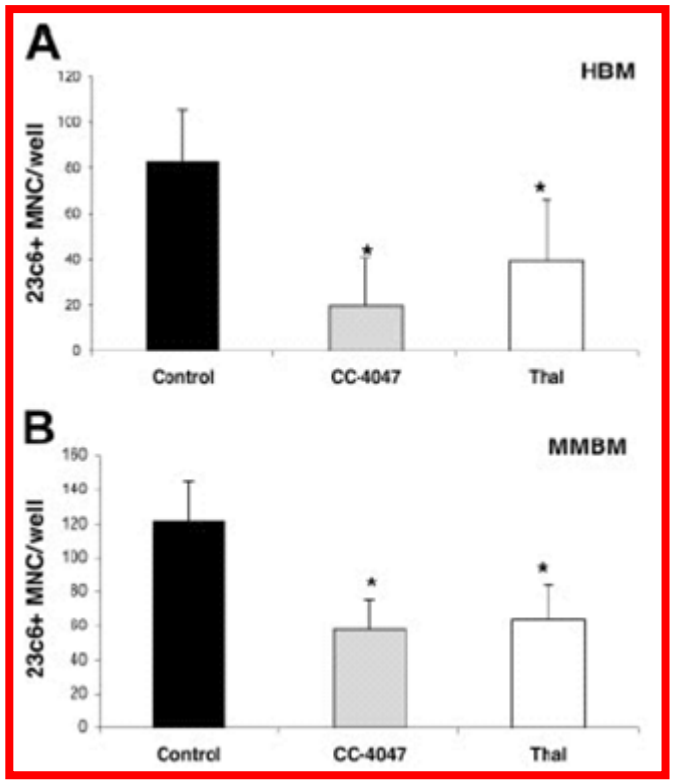
Which are the effects of the new drugs on bone microenvironment cells?

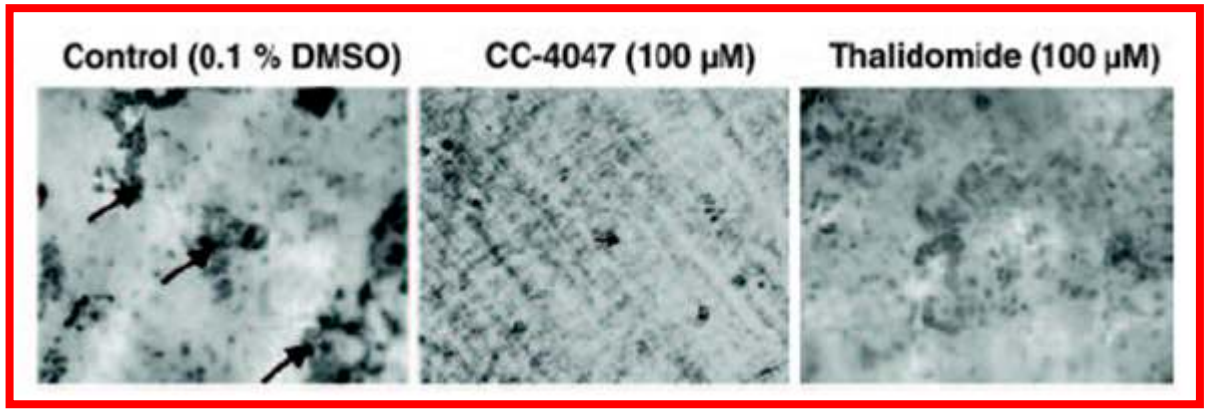
Proteasome inhibitors

IMiDs

HDAC inhibitors

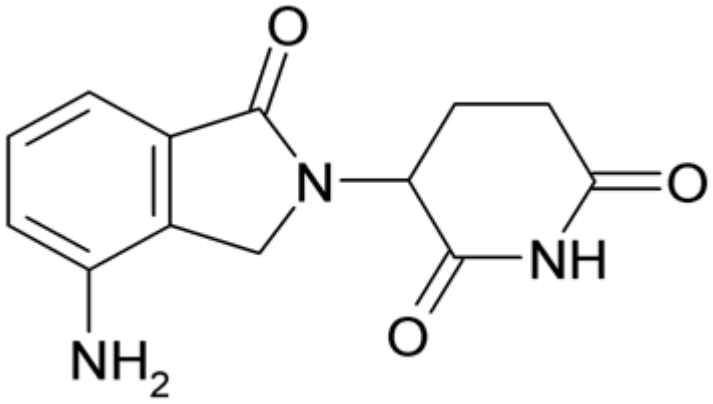
Thalidomide and IMiDs inhibit osteoclast formation and activity



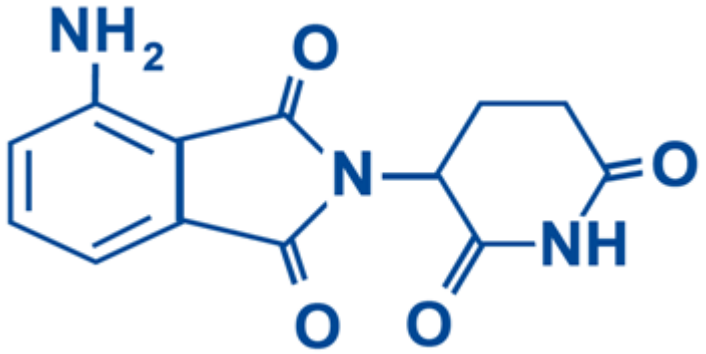


Anderson G. et al. Blood 2006

- Immunomodulatory drugs (IMIDs®), such as Lenalidomide (LEN) and the new more potent Pomalidomide (POM), inhibit osteoclast formation directly through the block of osteoclast maturation.[1-3]



Lenalidomide

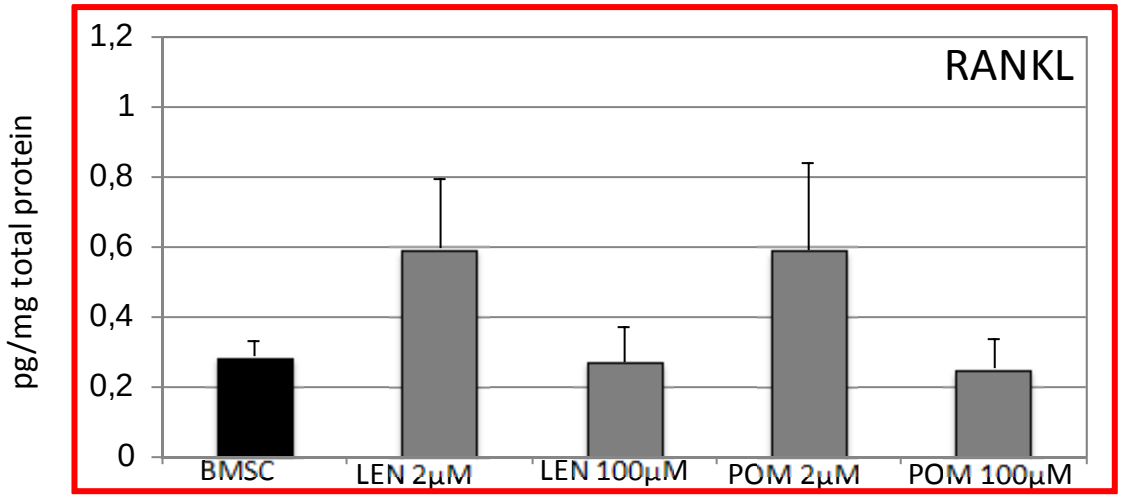


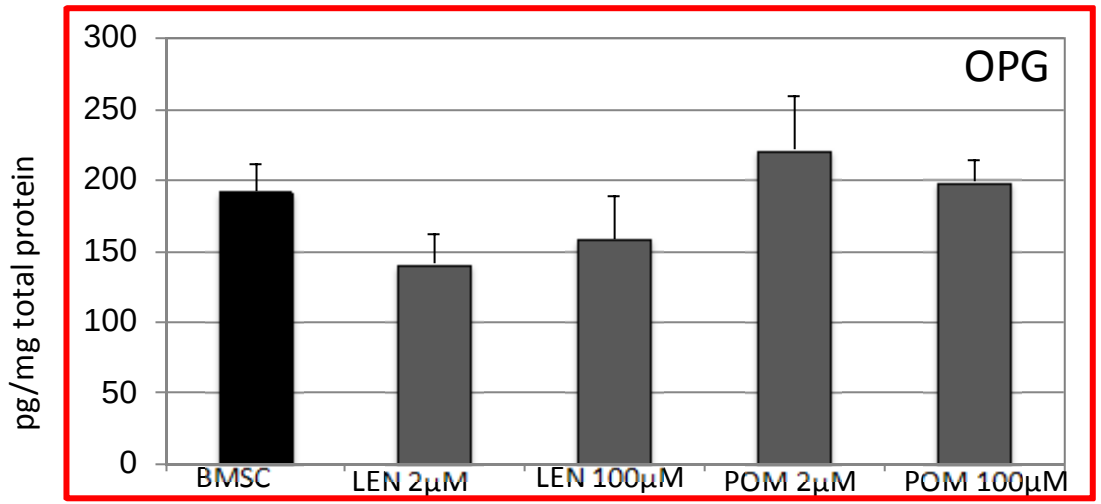
Pomalidomide

- Which is the potential effects of the IMiD® LEN and POM on the mechanisms involved in MM-induced osteoclast formation ?

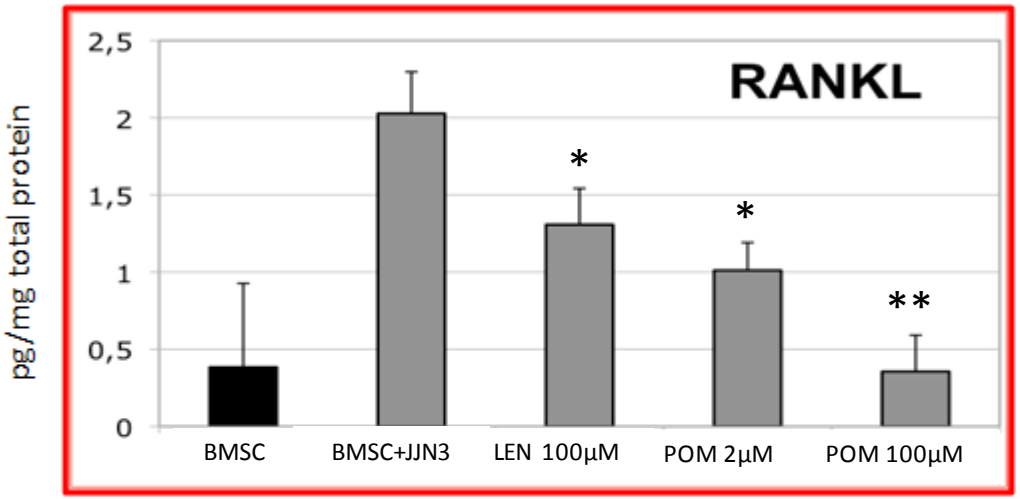
[1] Breitkreutz I et. al. *Leukemia*. 2008; [2]Anderson G et al. *Blood*. 2006; [3]Lacy MQ, et al. *Am J Hematol*. 2010

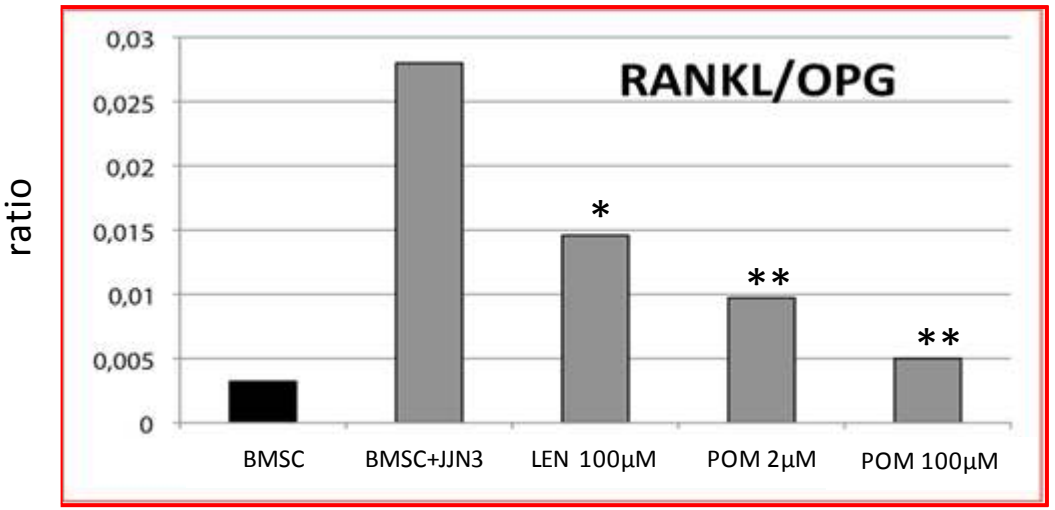
IMiD® compounds effect on RANKL and OPG production by BMSC/osteoprogenitor cells

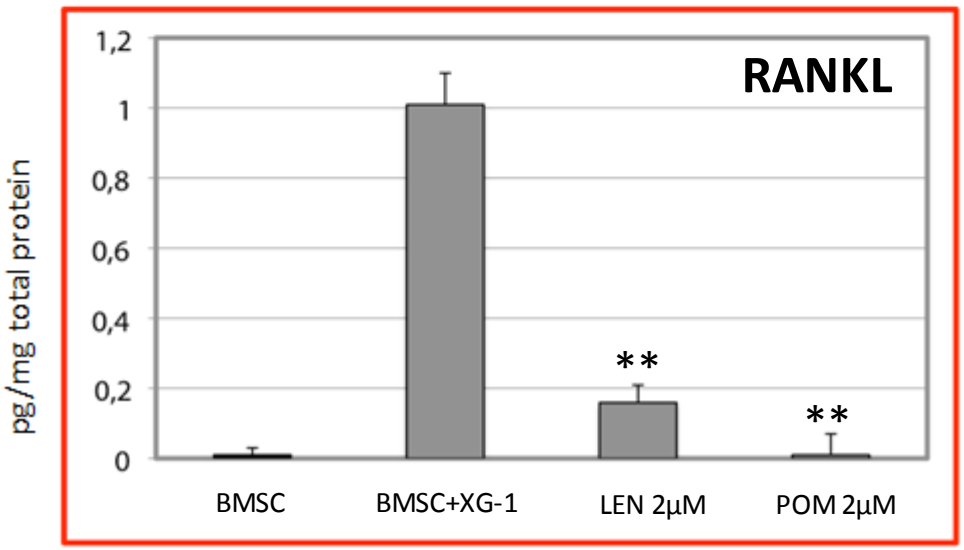


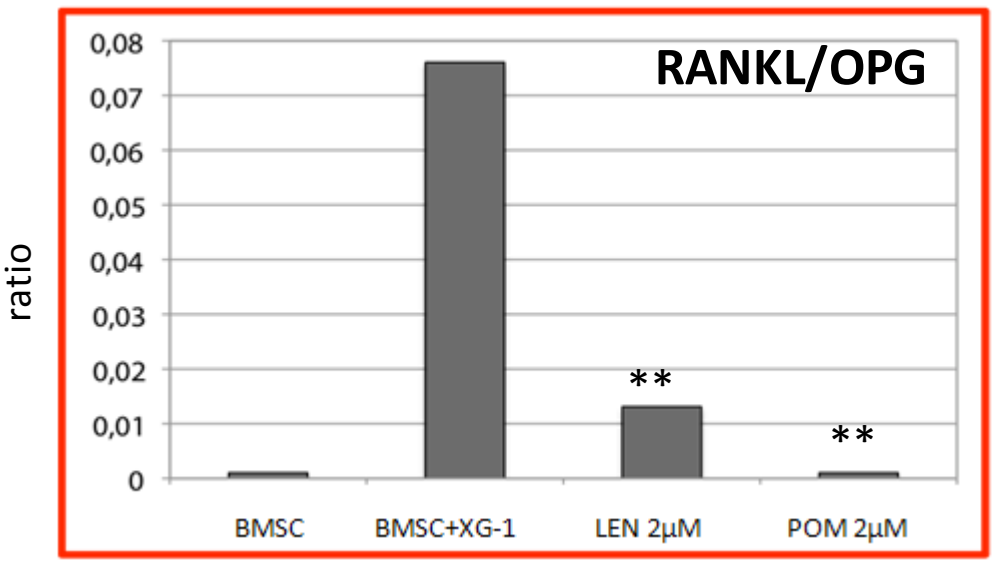


IMiD® compounds blunt RANKL up-regulation in co-culture

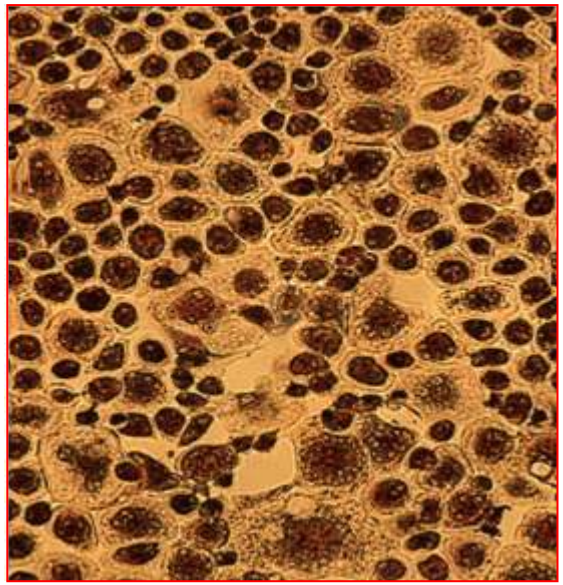




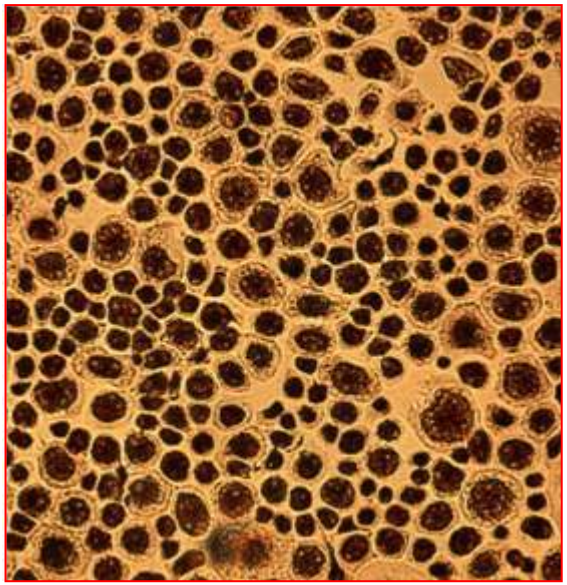




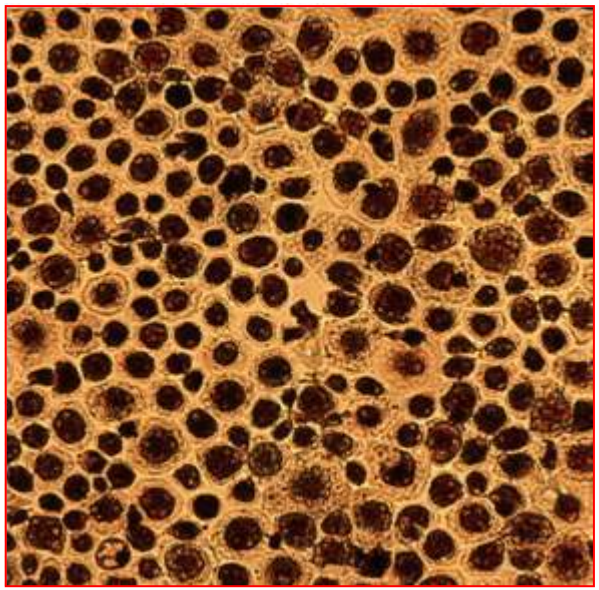
The pro-osteoclastogenic property of the CM of BMSC/MM cells was reduced in the presence of IMiDs®



Vehicle

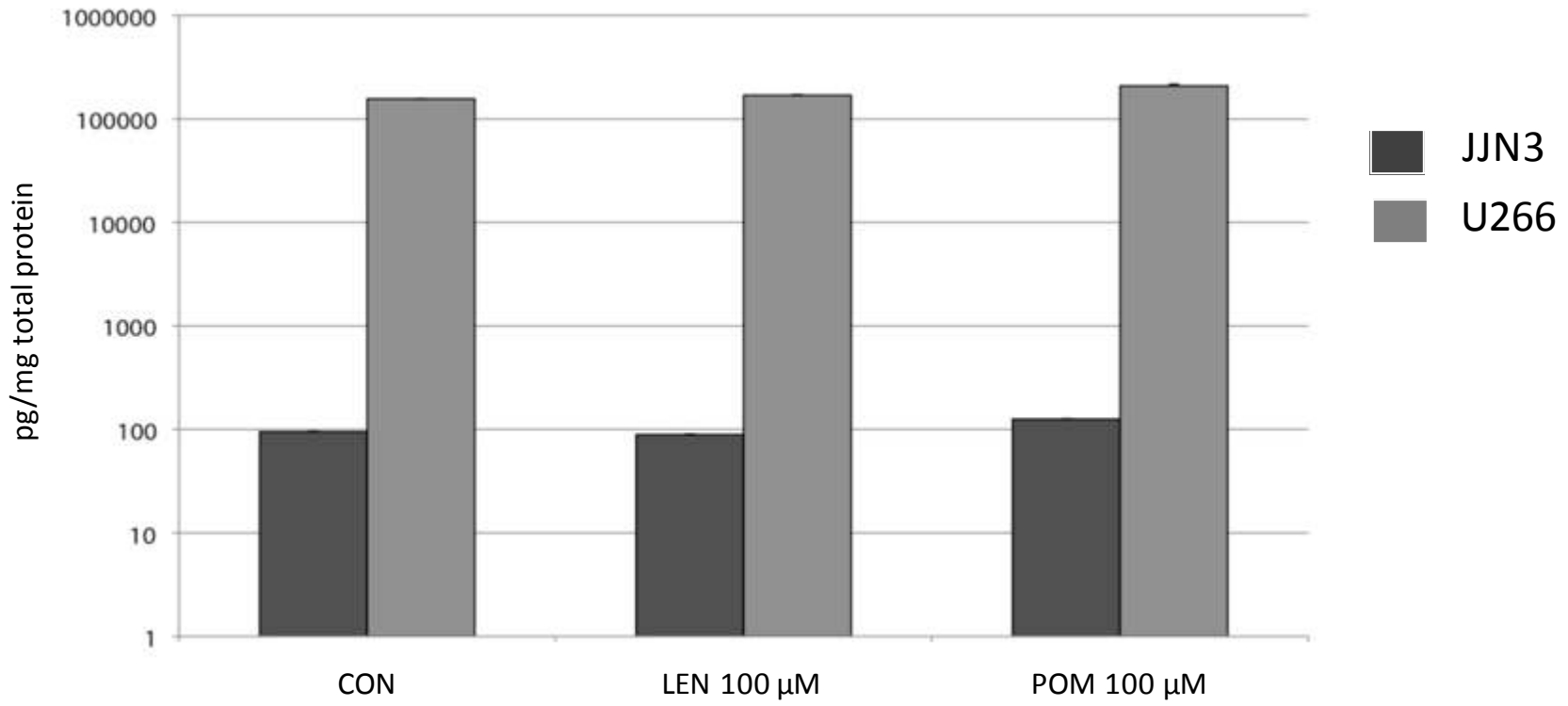


LEN 2μM

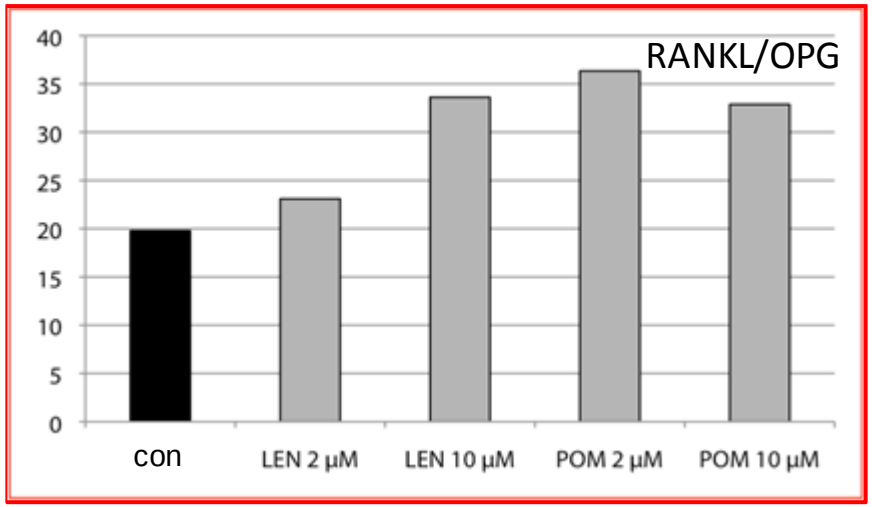


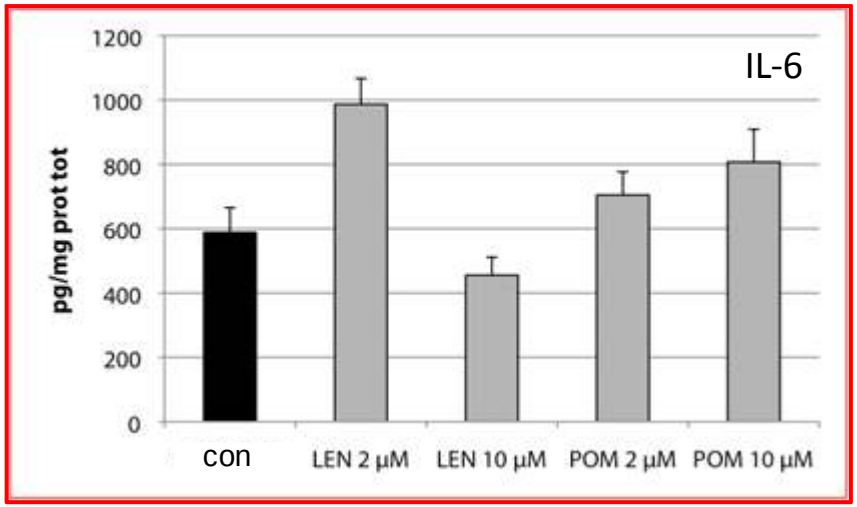
POM 2μM

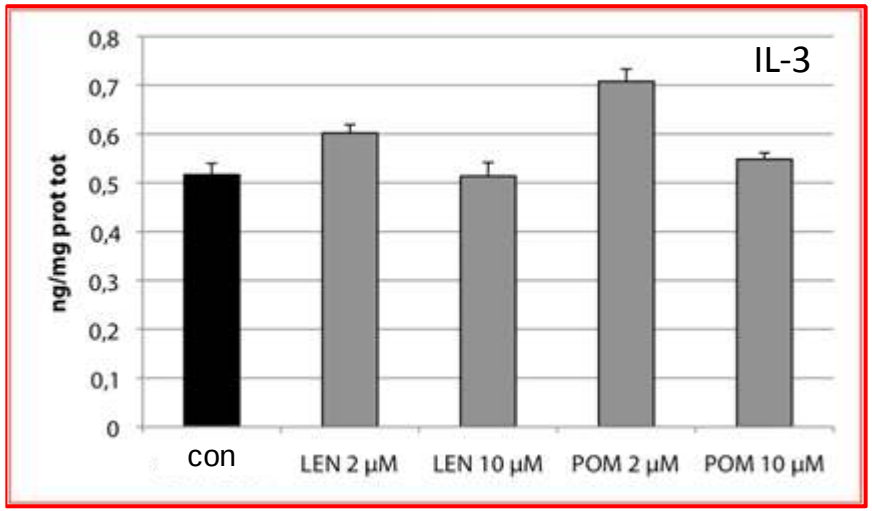
Effect of IMiD® compounds on the secretion of the pro-osteoclastogenic cytokines by MM cells: CCL3/MIP-1α

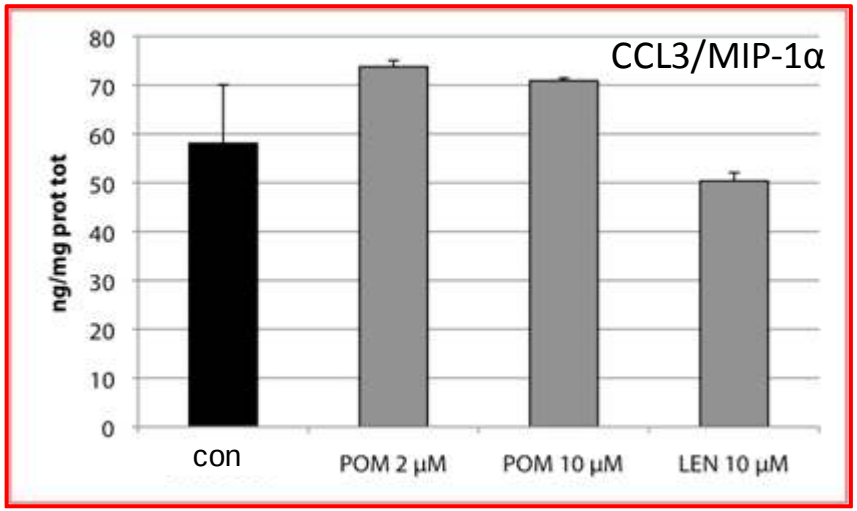


Effect of IMiD® compounds on osteoclastogenic cytokine secretion by activated peripheral blood mononuclear cells (PBMCs)









Adhesion molecules were significantly modulated at gene level by IMiD® compounds in MM cells

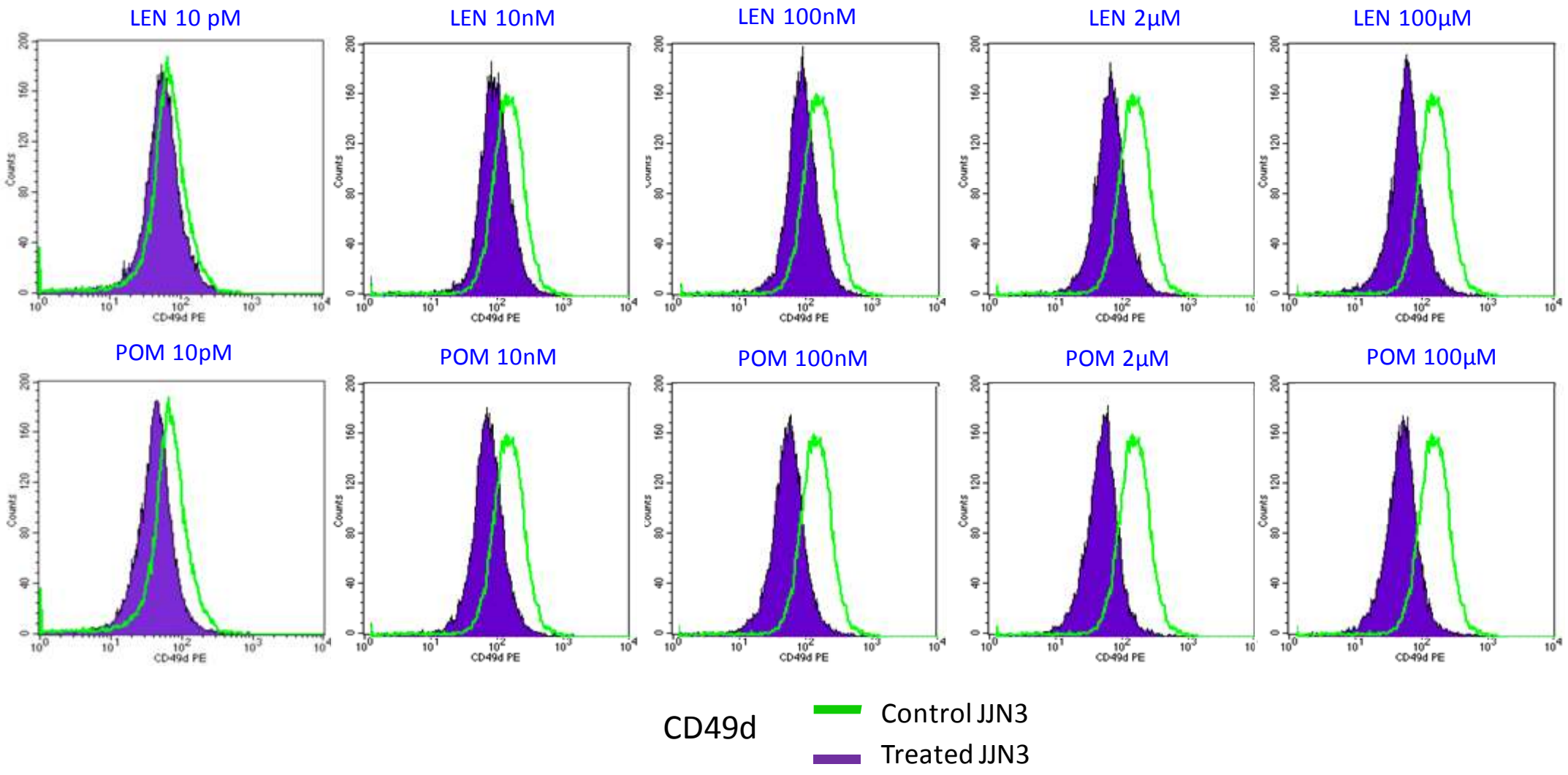
By microarray analysis (Affymetrix ® Genechips U133 Plus 2.0) we found:

- 71 and 214 genes were significantly modulated in hBMSC by LEN and POM, respectively, including those belonging to *focal adhesion, cell cycle, BMP2, TGF-β* and *IL-6 signaling.*
- 40 and 83 genes were significantly modulated by LEN and POM, respectively, in HMCLs (JJN3).

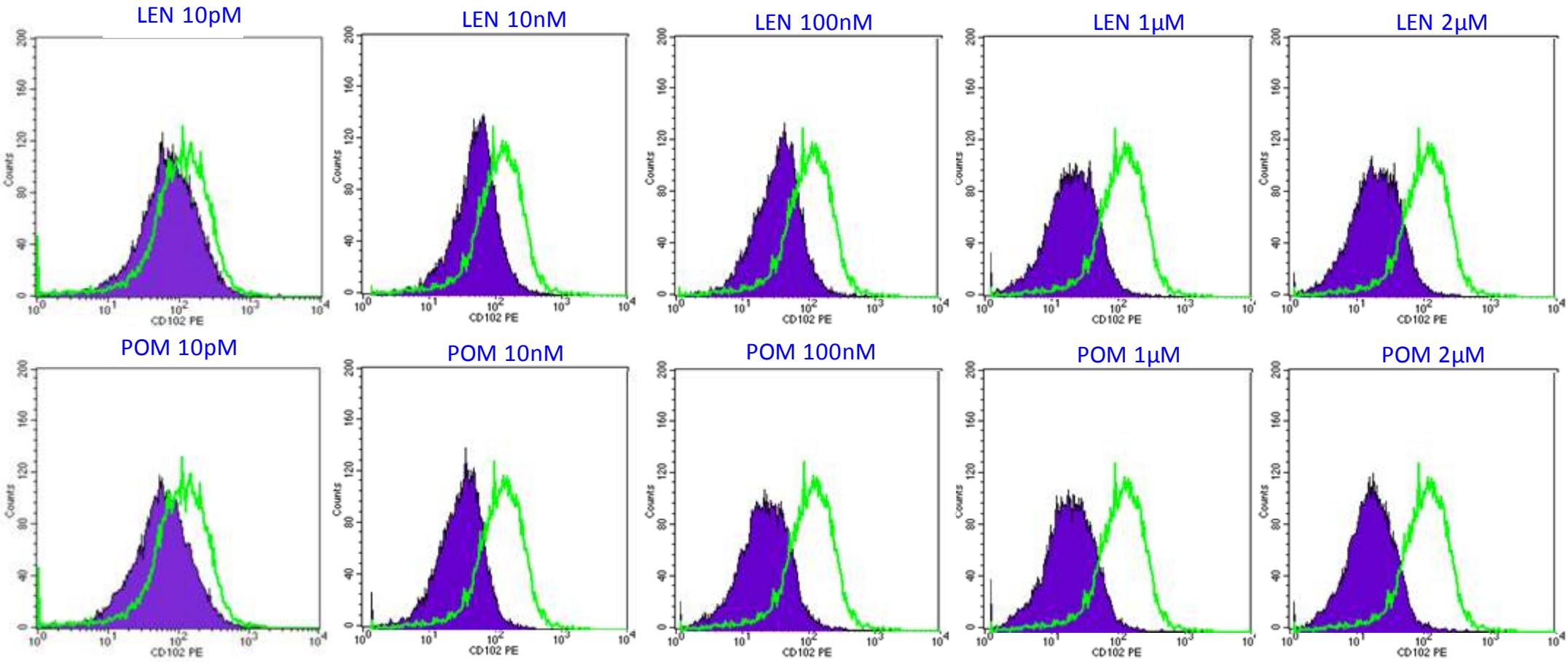
Downregulation of *ITGA4* (CD49d), *ITGA8* and *ICAM2* (CD102)

Probe Set	Gene Title	Gene symbol	Fold change LEN vs CON	Fold change POM vs CON
213416_at	integrin, alpha 4	*ITGA4*	*0.43*	*0.49*
214265_s_at	Integrin, alpha 8	***ITGA8***	***0.33***	***0.23***
213620_s_at	Intercellular adhesion molecule 2	***ICAM2***	***0.42***	***0.48***
205692_s_at	CD38 molecule	*CD38*	*1.59*	*2.36*

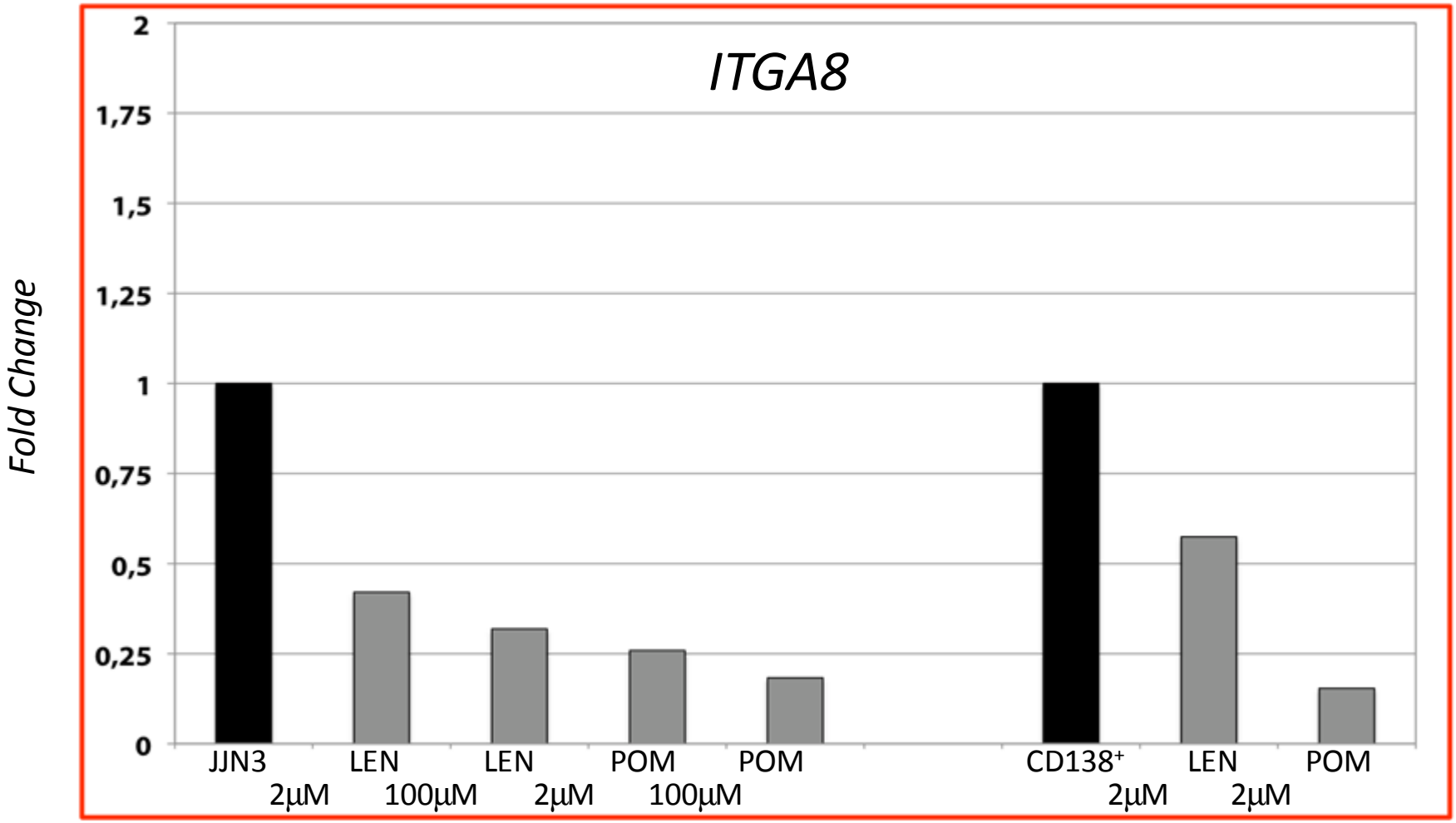
IMiD® compounds reduce VLA-4 (CD49d) expression by MM cells at a wide range of concentrations



IMiD® compounds reduce CD102 expression by MM cells



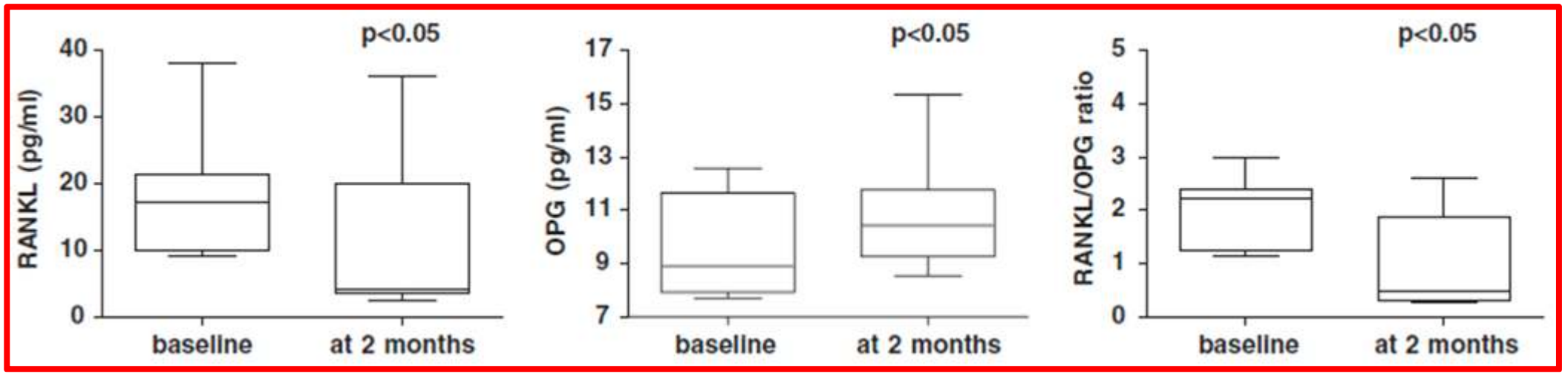
ITGA8 is a target of IMiD® compounds in cells



SUMMARY

- IMiD® compounds inhibit MM-induced osteoclast formation either directly or through the inhibition of RANKL/OPG ratio targeting the expression of adhesion molecules by MM cells such as CD49d or ICAM-2 (CD102) and ITAG8.

Lenalidomide treatment reduces RANKL/OPG ratio serum level in MM patients



Breitkeutz et al. Leukemia 2008

Key question:

Which are the effects of the new drugs on bone microenvironment cells?

Proteasome inhibitors

IMiDs

HDAC inhibitors

In vitro and *in vivo* effects of HDAC inhibitors on bone cells

	Cell/animal model	Effect
In vitro	Osteoclasts	• Promote apoptosis (Yi et al., 2007) • Suppress differentiation/maturation (Rahman et al., 2003; Nakamura et al., 2005)
	Osteoblasts	• Promote differentiation (Haberland et al., 2010) • Increase alkaline phosphatase production (Iwami and Moriyama, 1993; de Boer et al., 2006) • Increase osteoblastic gene expression (Sakata et al., 2004; Schroeder and Westendorf, 2005; Haberland et al., 2010) • Increase mineralized matrix production (de Boer et al., 2006; Haberland et al., 2010) • Increase Runx2 transcriptional activity (Schroeder and Westendorf, 2005)
	Mesenchymal stem cells (MSC)	• Promote apoptosis (Di Bernardo et al., 2009) • Promote cell cycle arrest (Di Bernardo et al., 2009) • Decrease proliferation (Di Bernardo et al., 2009; Lee et al., 2009) • Decrease pluripotency (Di Bernardo et al., 2009; Lee et al., 2009) • Increase osteoblastic lineage differentiation (Cho et al., 2005; de Boer et al., 2006; Di Bernardo et al., 2009; Lee et al., 2009) • Decrease adipocytic lineage differentiation (Lee et al., 2009; Haberland et al., 2010) • Decrease neural lineage differentiation (Lee et al., 2009) • Decrease chondrocytic lineage differentiation (Lee et al., 2009)
In vivo	Humans	• Decrease bone mineral density (Sheth et al., 1995; Boluk et al., 2004; Elliott et al., 2007) • Increase fracture risk (Vestergaard et al., 2004) • Cause teratogenic craniofacial defects (Vajda et al., 2004)
	Rats	• Decrease bone mineral content (Nissen-Meyer et al., 2007)
	Mice	• Decrease trabecular bone mass (strain-dependent) (Pratap et al., 2010; McGee-Lawrence et al., 2010; Senn et al., 2010)

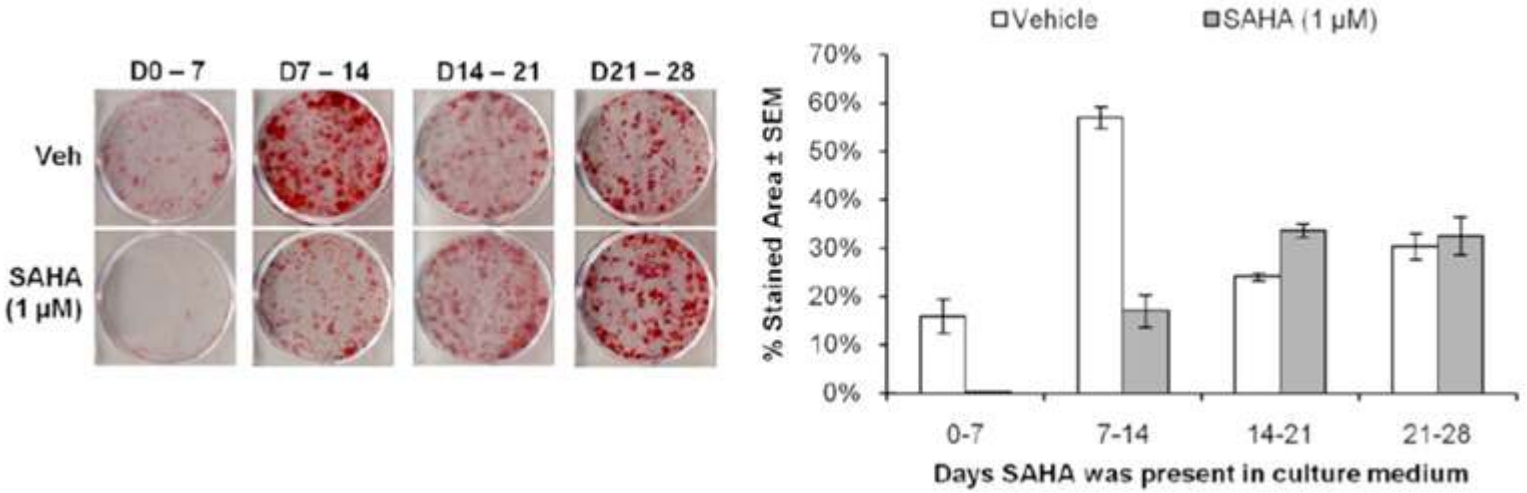
McGee-Lawrence ME et al. Gene 2011

HDAC inhibitor SAHA (Vorinostat) causes bone loss by inhibiting immature osteoblasts

Static and dynamic histomorphometry in the distal femoral metaphysis.

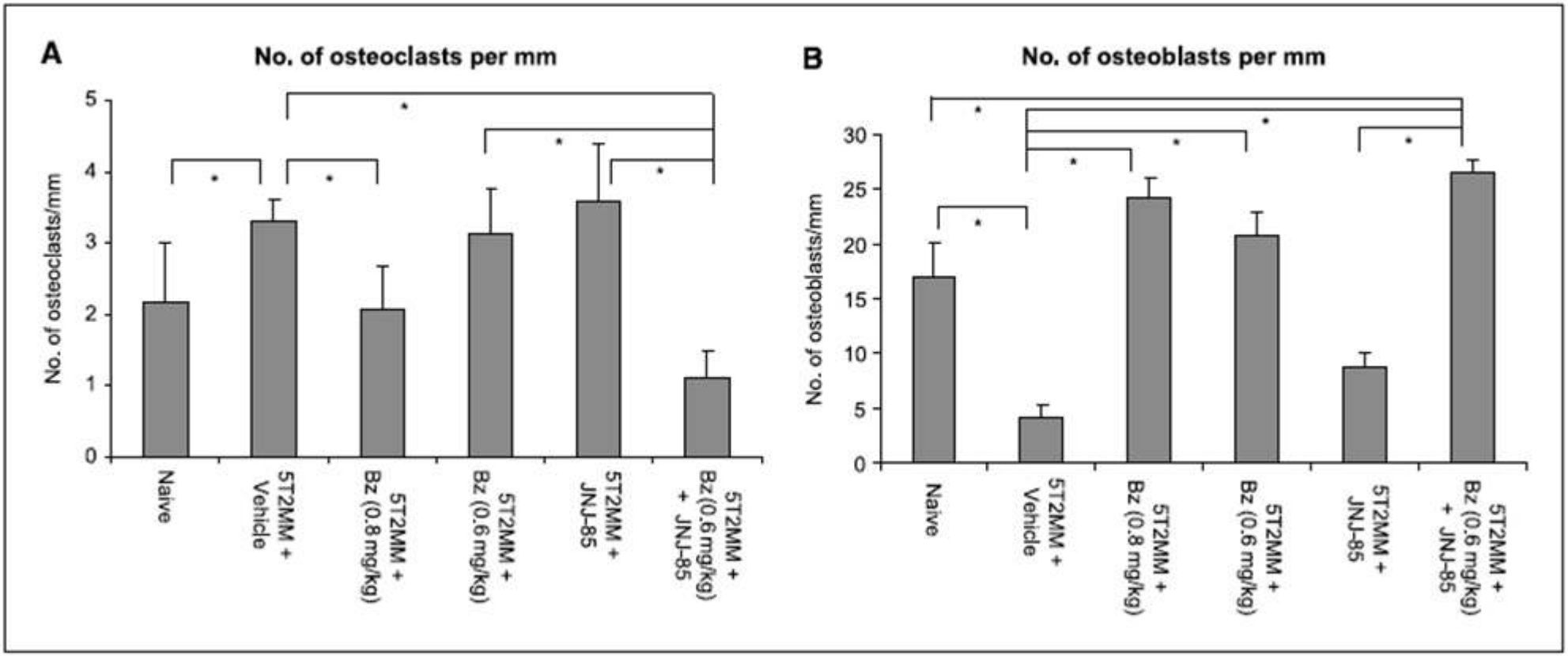
	Oc.S/BS, %	N.Oc/B.Pm, #/mm	Ob.S/BS, %	N.Ob/B.Pm, #/mm	MS/BS, %	MAR, μm/day	BFR/BV, %/day
Vehicle	6.01 (4.41)	4.64 (3.10)	10.71 (3.02)	8.81 (2.68)	35.7 (4.5)	1.46 (0.14)	3.52 (0.63)
SAHA	3.66 (2.21)	2.75 (1.54)	6.96 (4.28)	5.47 (2.93)	36.0 (4.3)	1.73 (0.15)	4.18 (0.60)
p-value	0.149	0.101	**0.036**	**0.016**	0.867	**0.0008**	**0.034**

Means of 10 samples (standard deviations) are shown.



McGee-Lawrence ME et al. Bone 2011

HDAC inhibitors in combination with Bortezomib show a positive effect on MM bone disease in mice



Deleu S et al. Cancer Res 2009

CONCLUSIONS

- The new class of anti–myeloma drugs such as proteasome inhibitors, IMiDs and HDAC inhibitors show a significant effects on both osteoclasts and osteoblasts with potential impact on bone remodeling process and MM bone disease in MM patients

- Osteoclasts and osteoblasts are targets of the new anti-myeloma drugs whose effect may be due in part to their action on the bone microenvironment cells.

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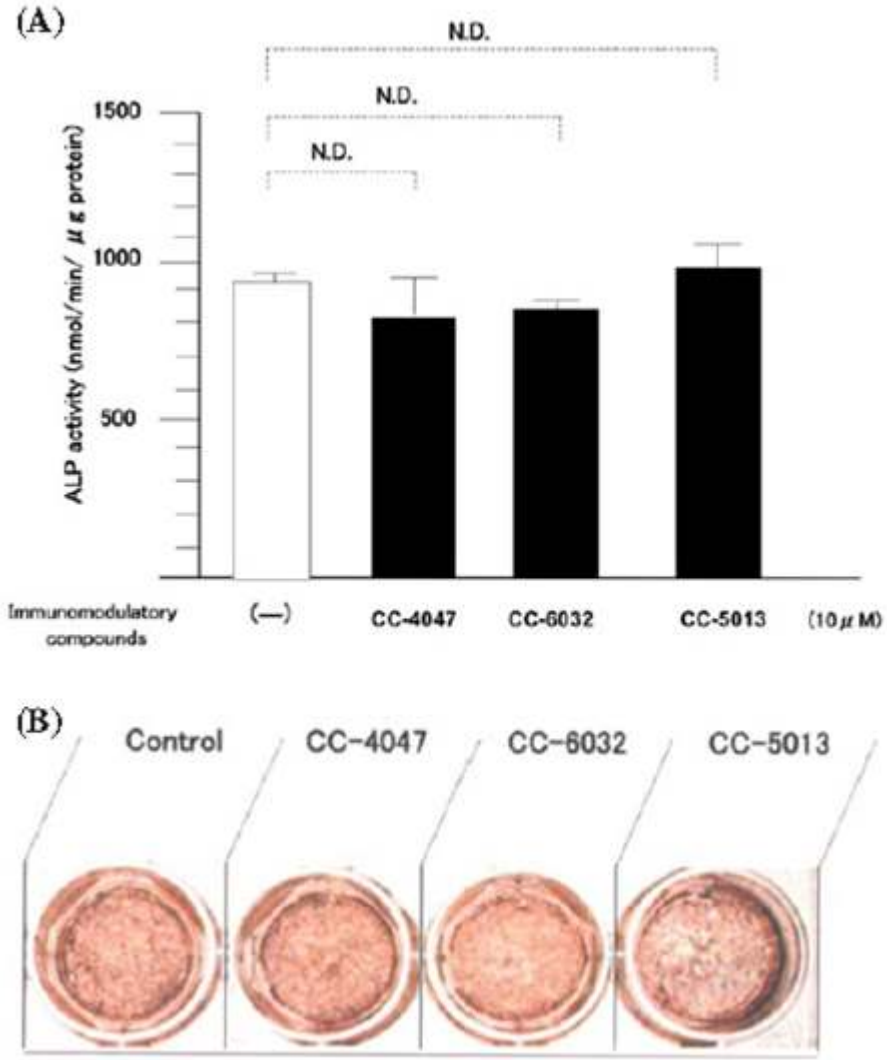
INTERNATIONAL MYELOMA FOUNDATION

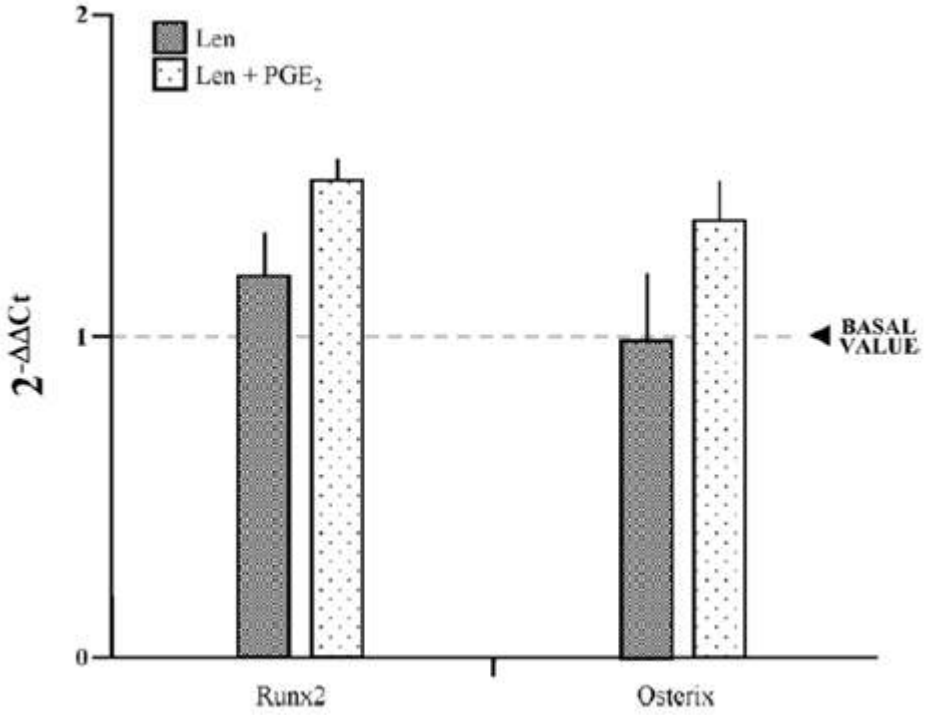
Improving the quality of life of myeloma patients while working toward prevention and a cure

AIRC

IMiD® compounds have not effect on osteogenic differentation



Munemasa S et al. In J Oncol 2008



De Matteo M et al. Leuk Res 2010