Dal laboratorio al
nuove terapie ne
mieloma multipl

Nicola Giuliani MD, PhD

Dept. of Medicine and Surgery
University of Parma

U.O. Di Ematologia e CTMO, AOU di Parma

From the bench to the bedside: new treatment in multiple myeloma (MM)

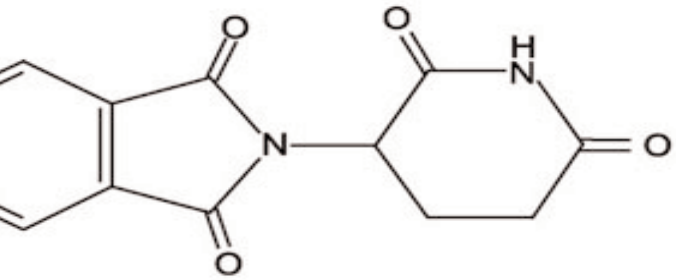
ew evidences on the mechanism of action of the anti-MM dru

Mechanisms of drug resistance and how overcome them.

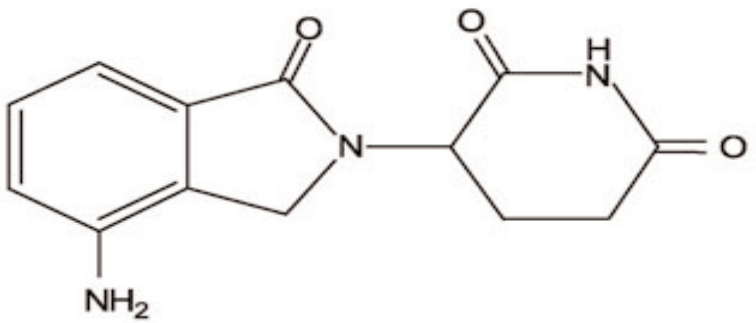
D-38 and CS-1: target for monoclonal antibodies.

heck-points inhibitors.

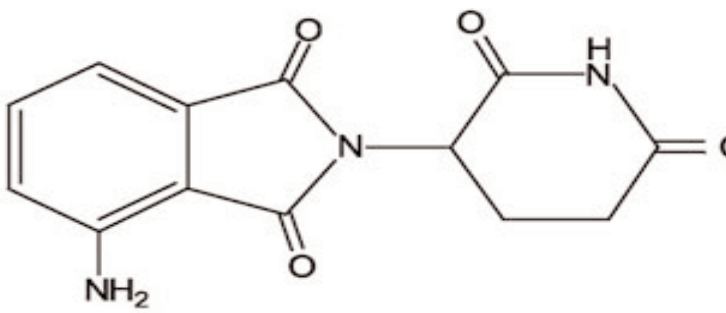
IMiDs®



THALIDOMIDE



LENALIDOMIDE



POMALIDOMIDE

- Immunomodulatory Drugs → Thalidomide derivatives
- Pleiotropic properties (direct anti-tumor effects; microenvironment effects, anti-angiogenic activity, anti-inflammatory properties and immunomodulatory effects)

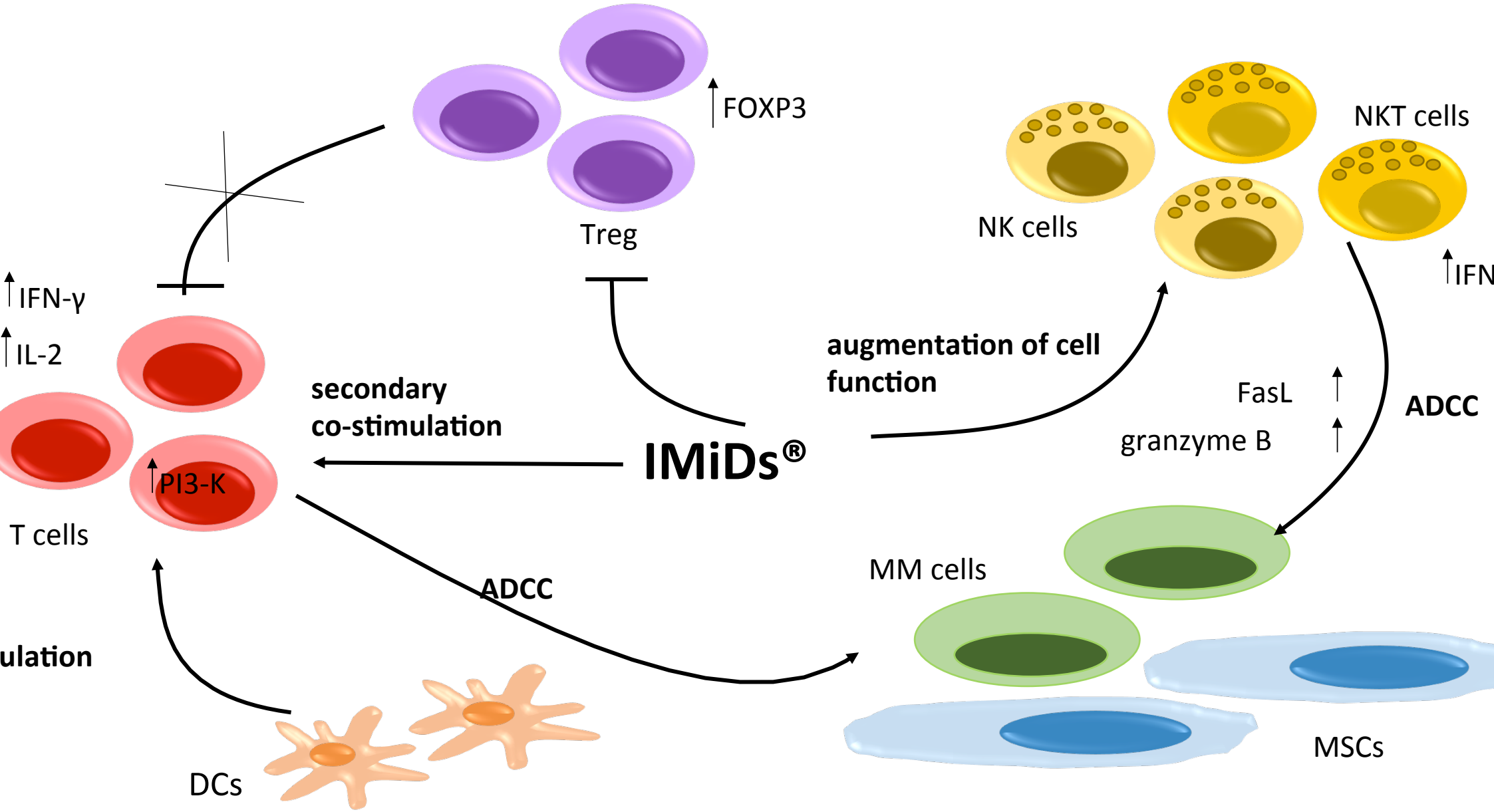
a L . et al, Leuk Res 2012

IMiDs® mechanisms of action (I)

Effect	Relative potency += potency factor 10		
	Thalidomide	Lenalidomide	Pomalidomide
Interference with tumor micro-environment interaction			
Anti-angiogenesis	++++	+++	+++
Anti-inflammatory properties	+	++++	+++++
Downregulation of adhesion molecules	+	++++	+++++
Anti-osteoclastogenic properties	+	++++	+++++
Direct anti-tumor effects			
Anti-proliferative activity	+	+++	+++
Immune modulation			
CD4+ and CD8+ T cell co-stimulation	+	++++	+++++
Tregs suppression	-	+	+
Th1 cytokine production	+	++++	+++++
NK and NKT cell activation	+	++++	+++++
Antibody-dependent cellular cytotoxicity (ADCC)	-	++++	++++

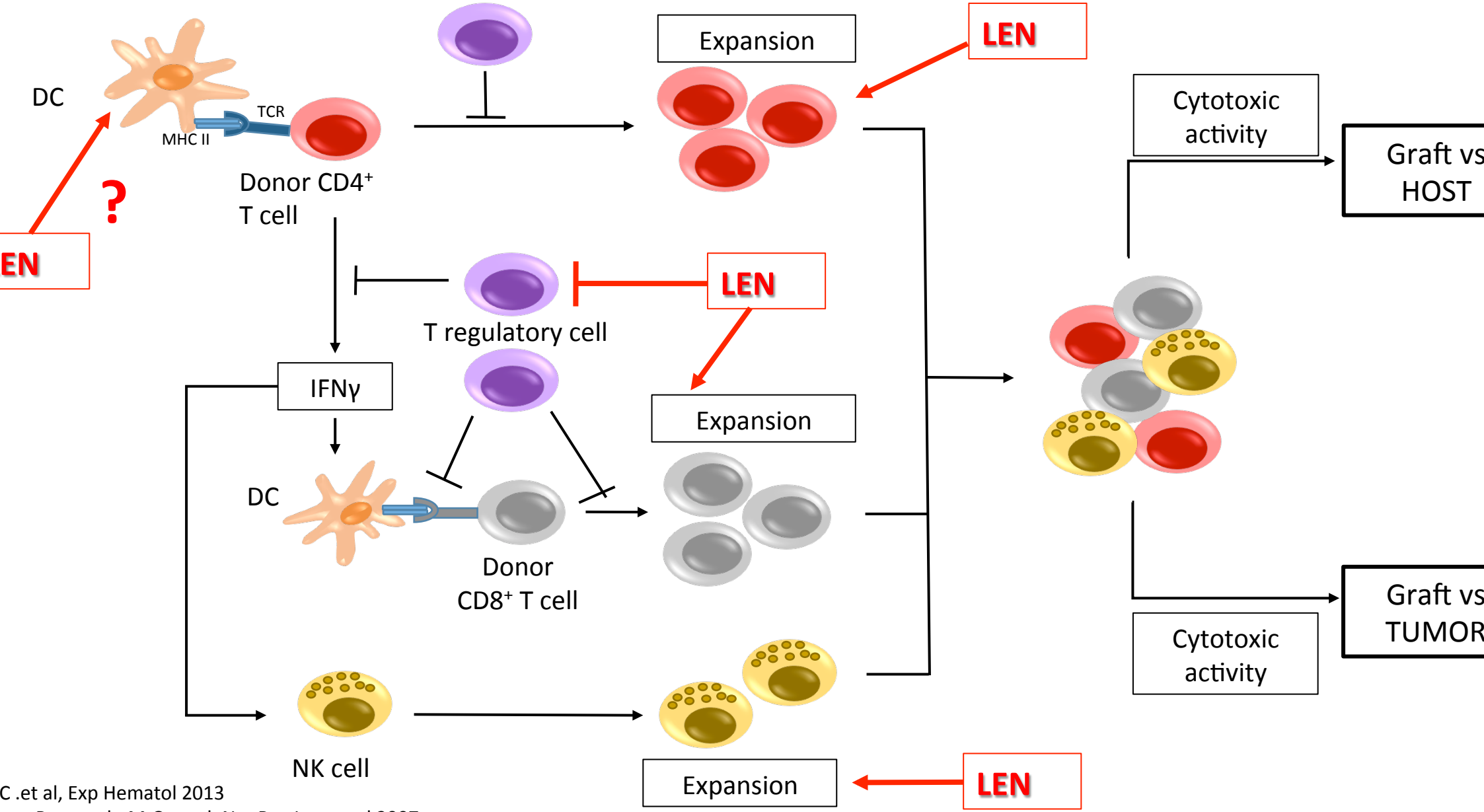
rom Sedlarikova L et al., Leuk Res 2012

IMiDs® mechanisms of action (II)



rom Sedlarikova L et al., Leuk Res 2012

Immunological *in vivo* effects of LEN



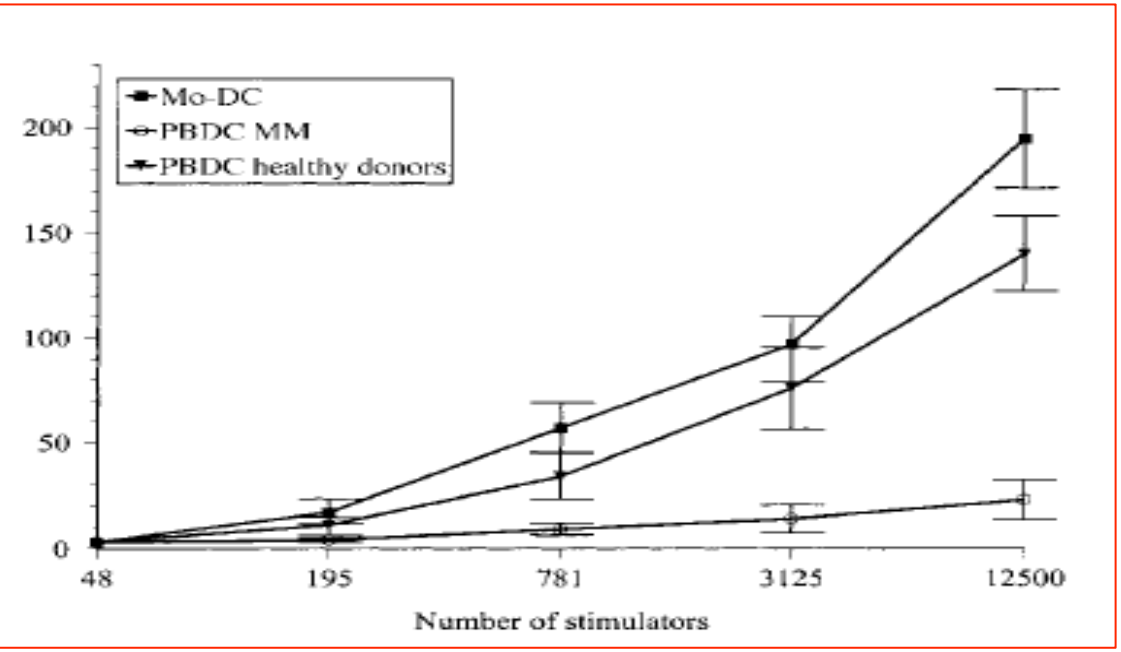
C .et al, Exp Hematol 2013

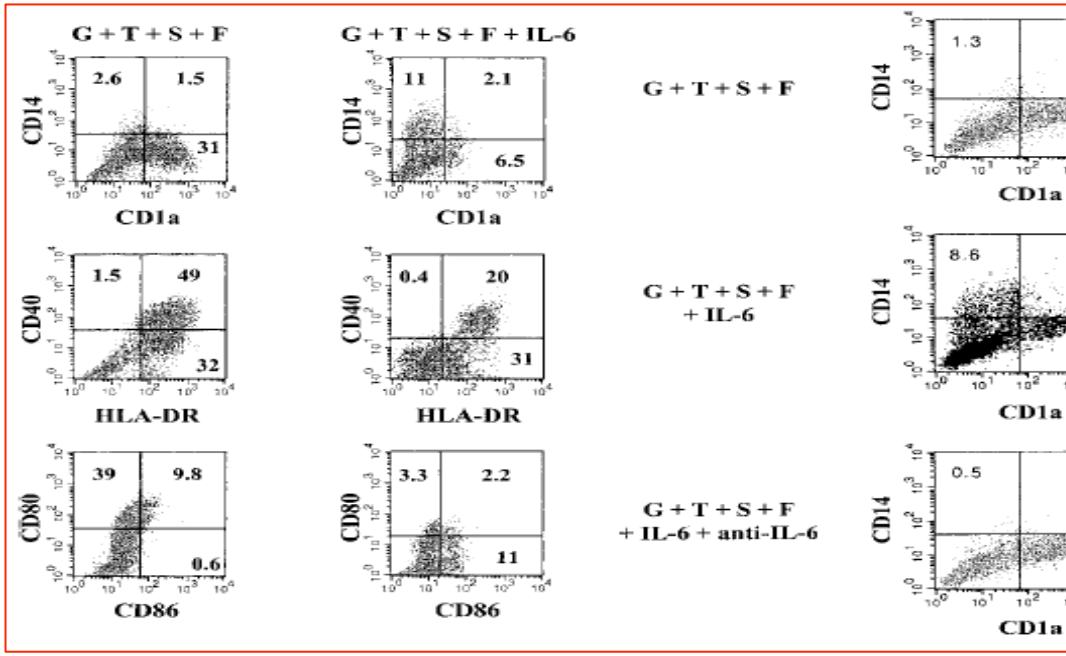
rom Roncarolo M.G .et al, Nat Rev Immunol 2007

DCs are defective in MM patients

↓ DC precursors in MM patients vs healthy donors (HD)s

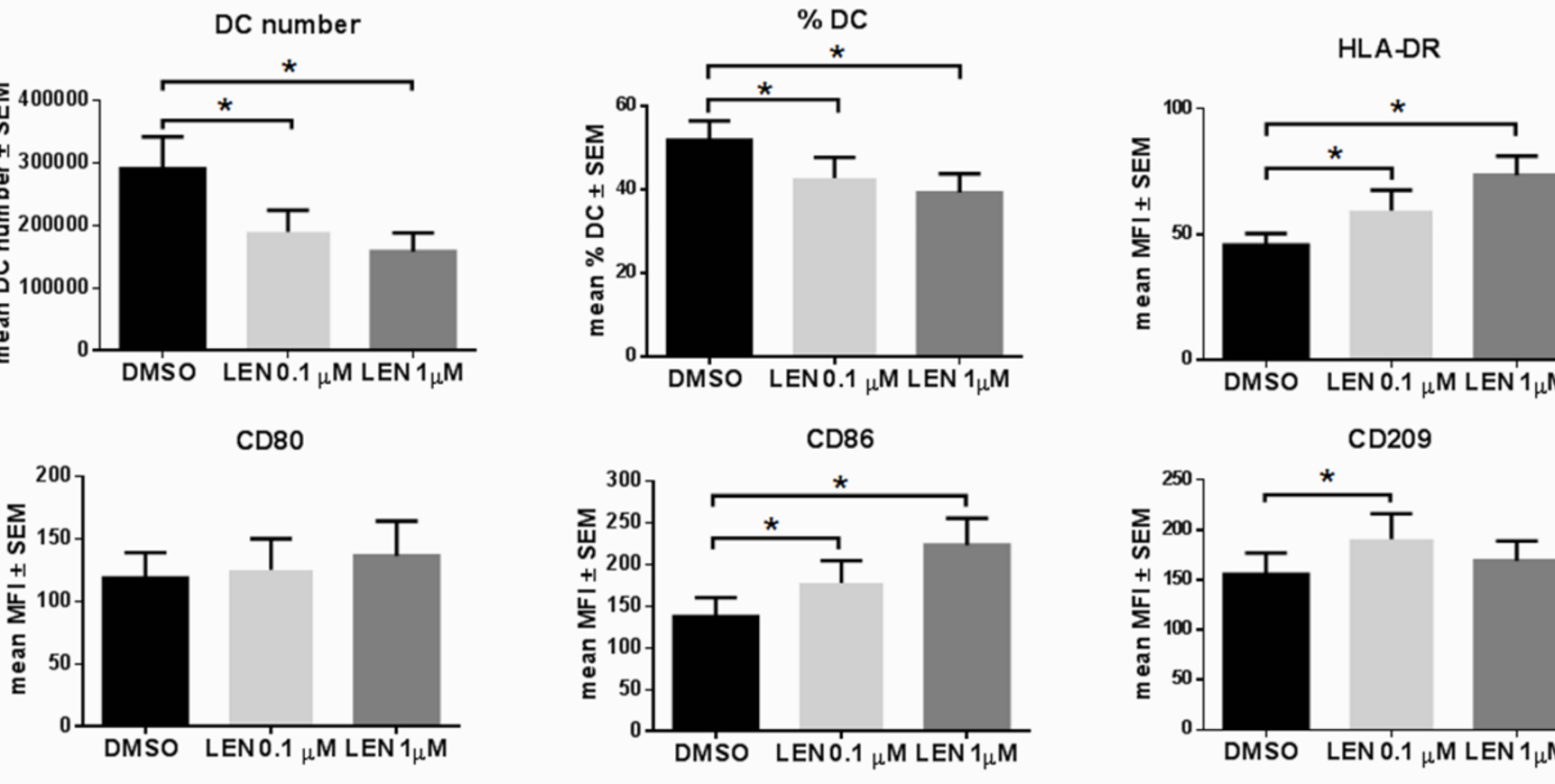
↓ HLA-DR, CD40, and CD80 on peripheral blood (PB) DCs of MM patients vs HDs





Ratta M, Fagnoni F et al., *Blood* 2007

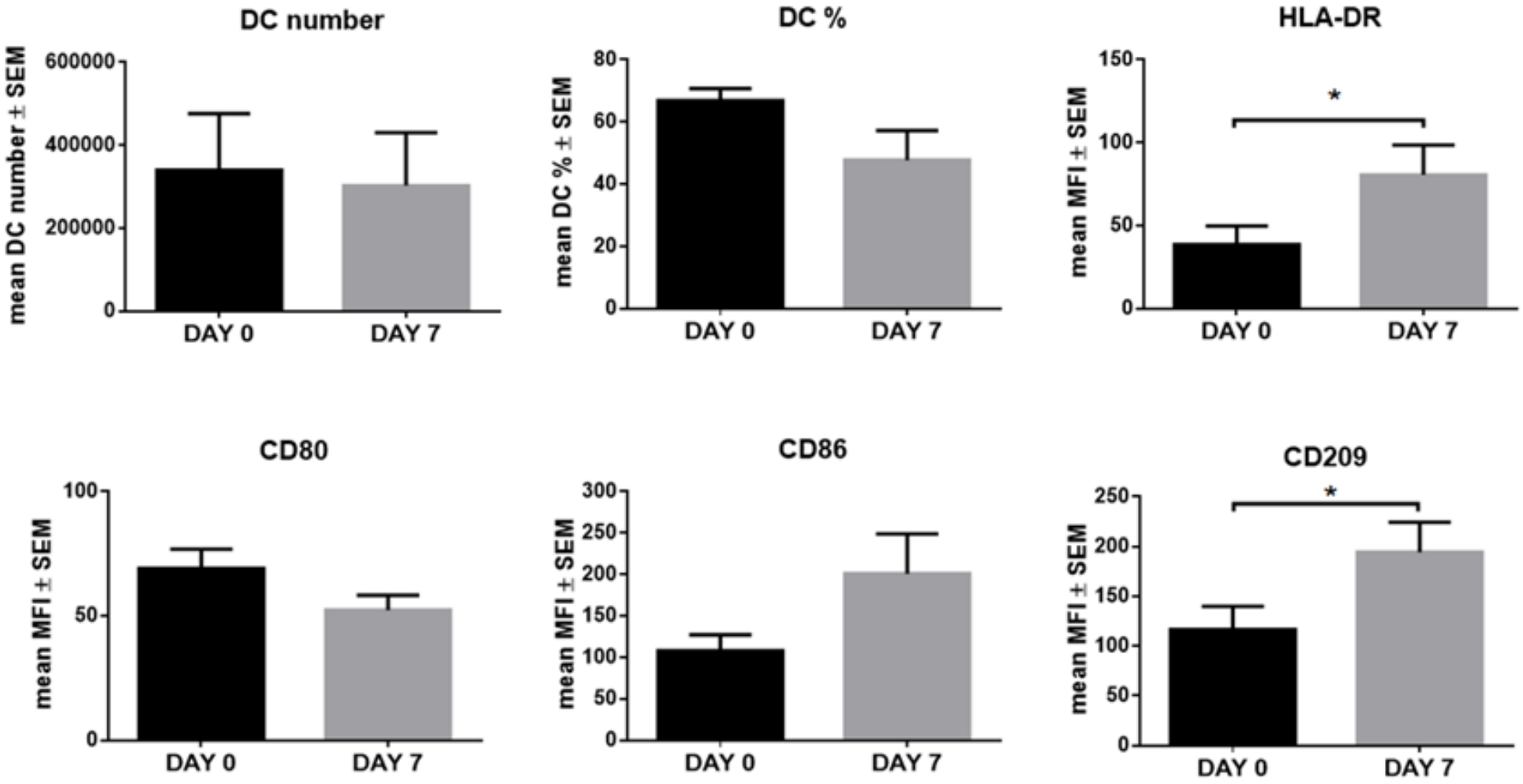
EN enhanced *in vitro* DC differentiation from BM an PB of MM patients



al, Oncotarget 2017, in press.

p calculated by paired Student's t test; *

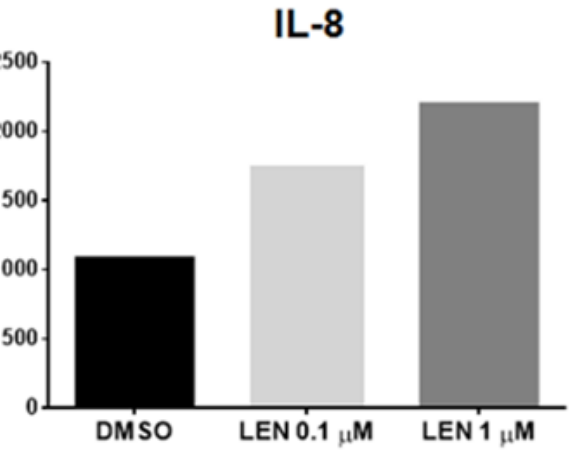
x vivo LEN treatment of MM patients increased *in vitro* D differentiation

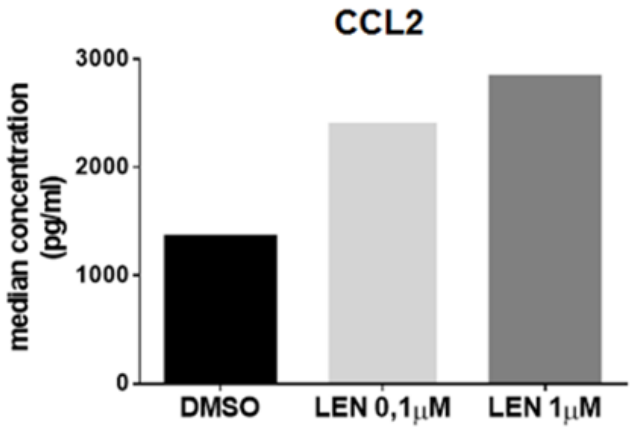


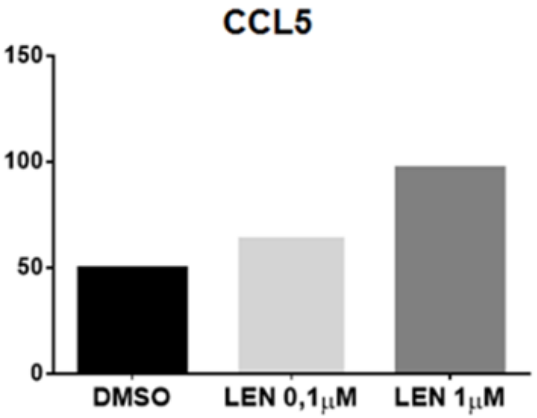
al, Oncotarget 2017, in press.

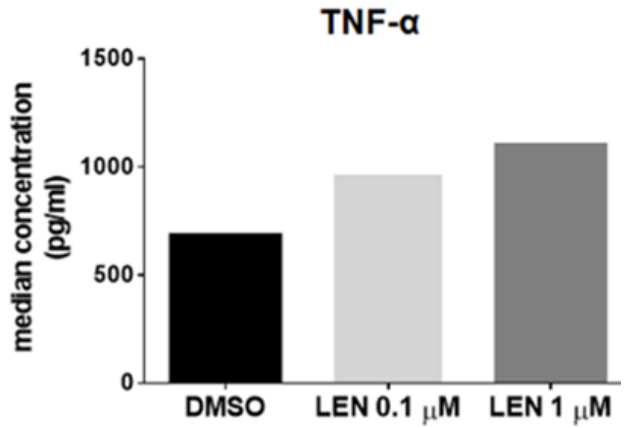
p calculated by paired Student's t test; *

EN increased chemokine/cytokine production and D ability to stimulate T cell proliferation

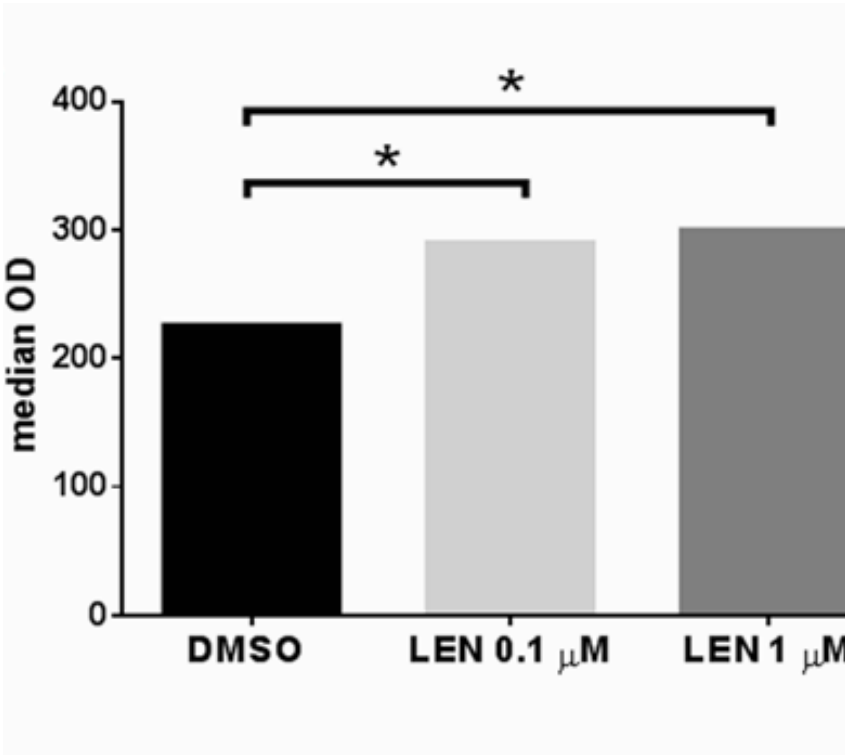








B



al, Oncotarget 2017, in press.

p calculated by Mann-Whitney test; *,

Effect of LEN on DCs: translational impact

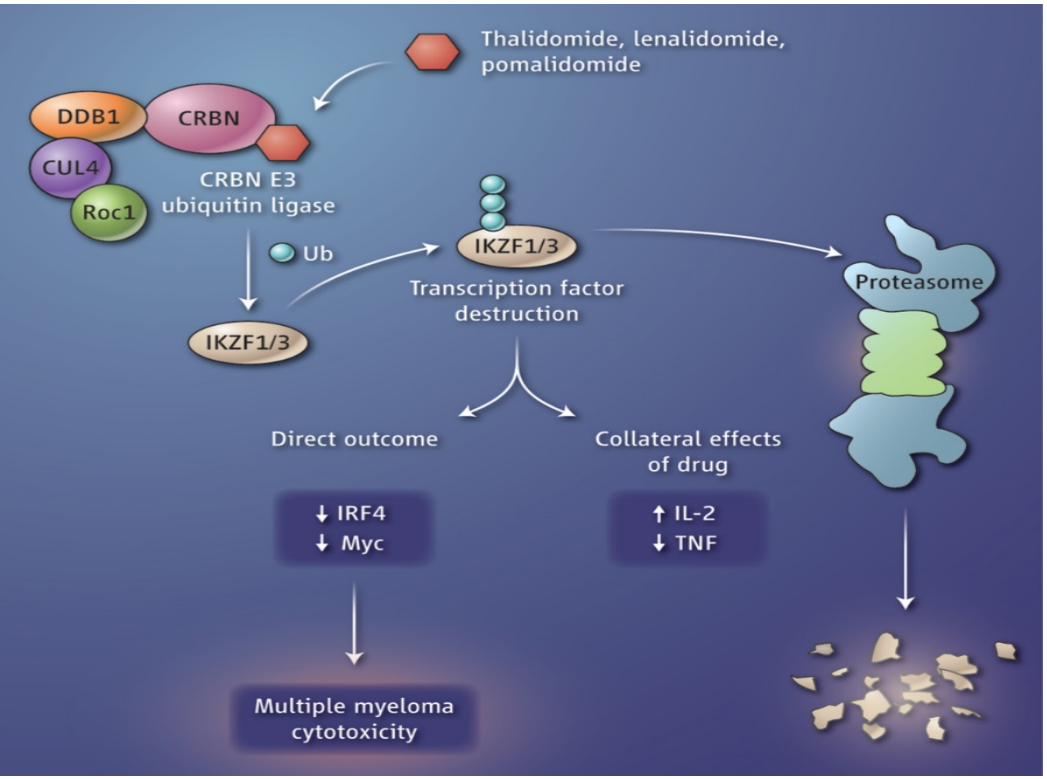
- LEN as maintenance therapy to restore immuno-dysfunction i MM patients.
- LEN to potentiate the graft-versus MM effect.
- LEN in the contest of a DC-based vaccination therapy.
- LEN in combination therapy to improve anti-MM immun response.

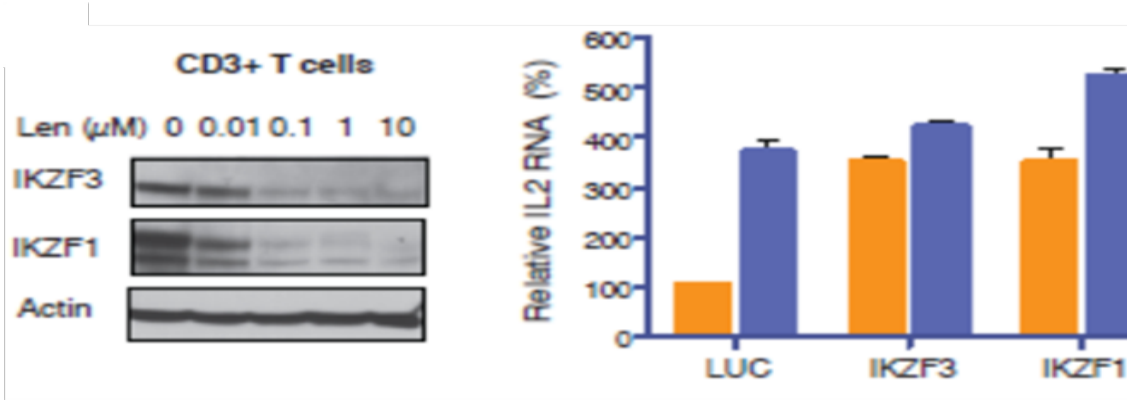
IMiDs®: molecular mechanism in MM and T cells

elective ubiquitination and degradation of two lymphoid transcription factors, IKZF1 and IKZF3, by the RBN-CRL4 ubiquitin ligase in MM cells and T cells

KZF1 (*ikaros*) and IKZF3 (*aiolos*) are essential transcription factors in MM

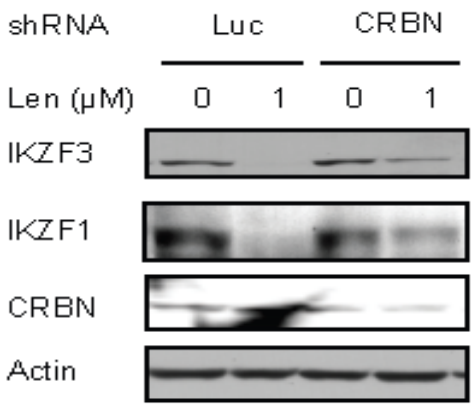
epletion of IKZF1 and IKZF3 by shRNA in T cells enhances IL-2 production

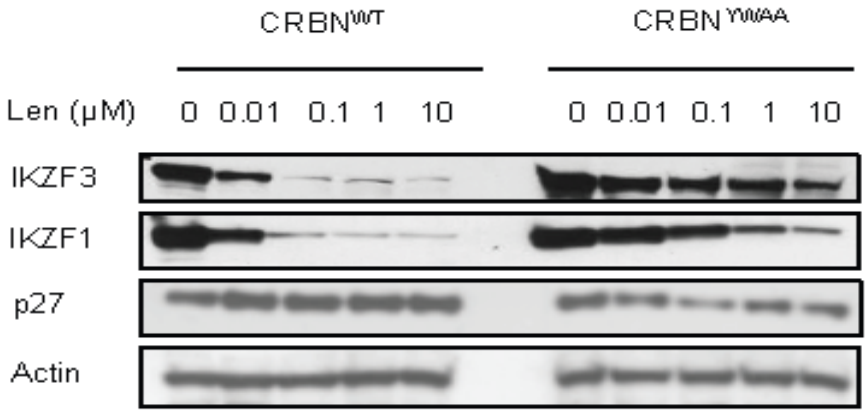


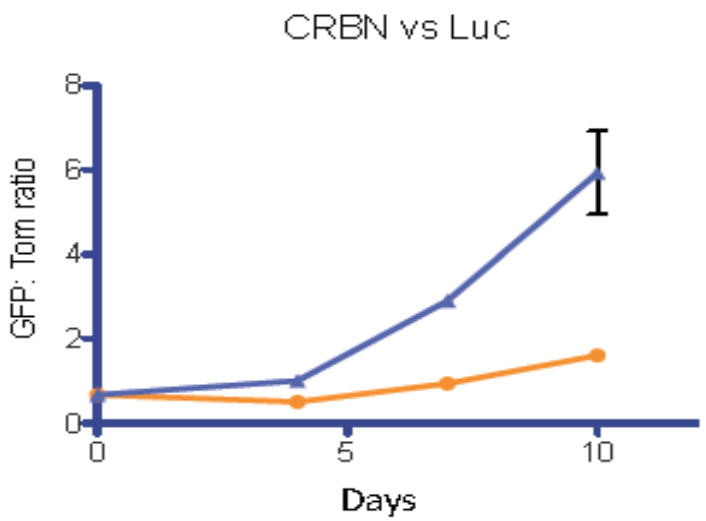


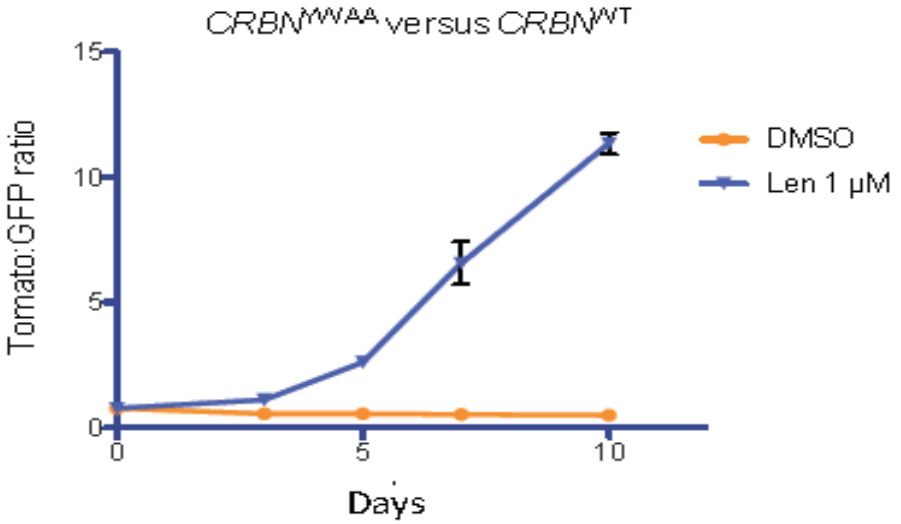
et al, Science 2014

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



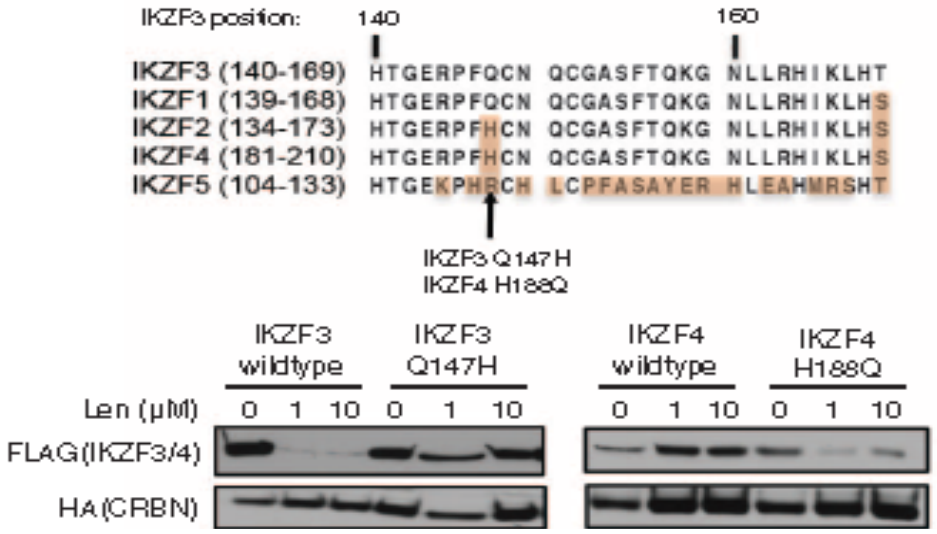


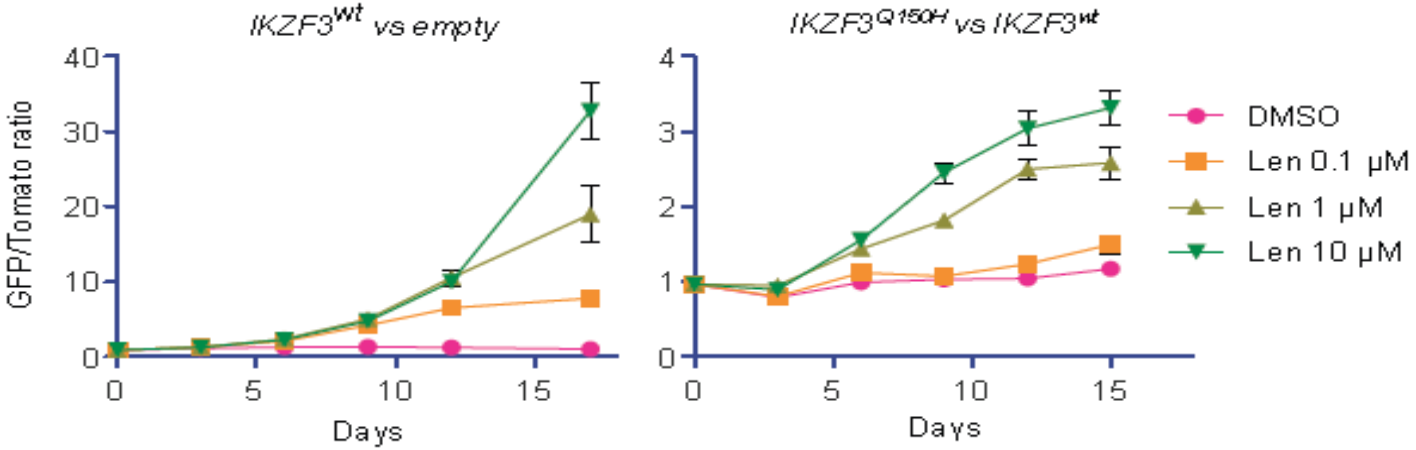


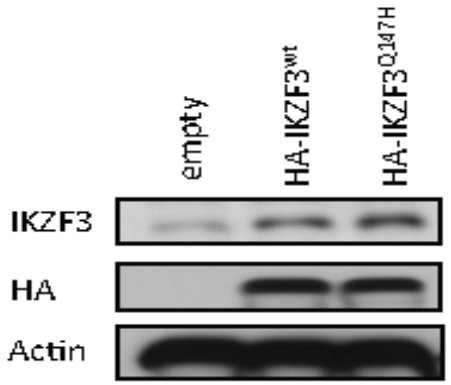


KrÖnke J et al, Scie

IKZF3 mutations or overexpression induce LEN resistance in MM cells

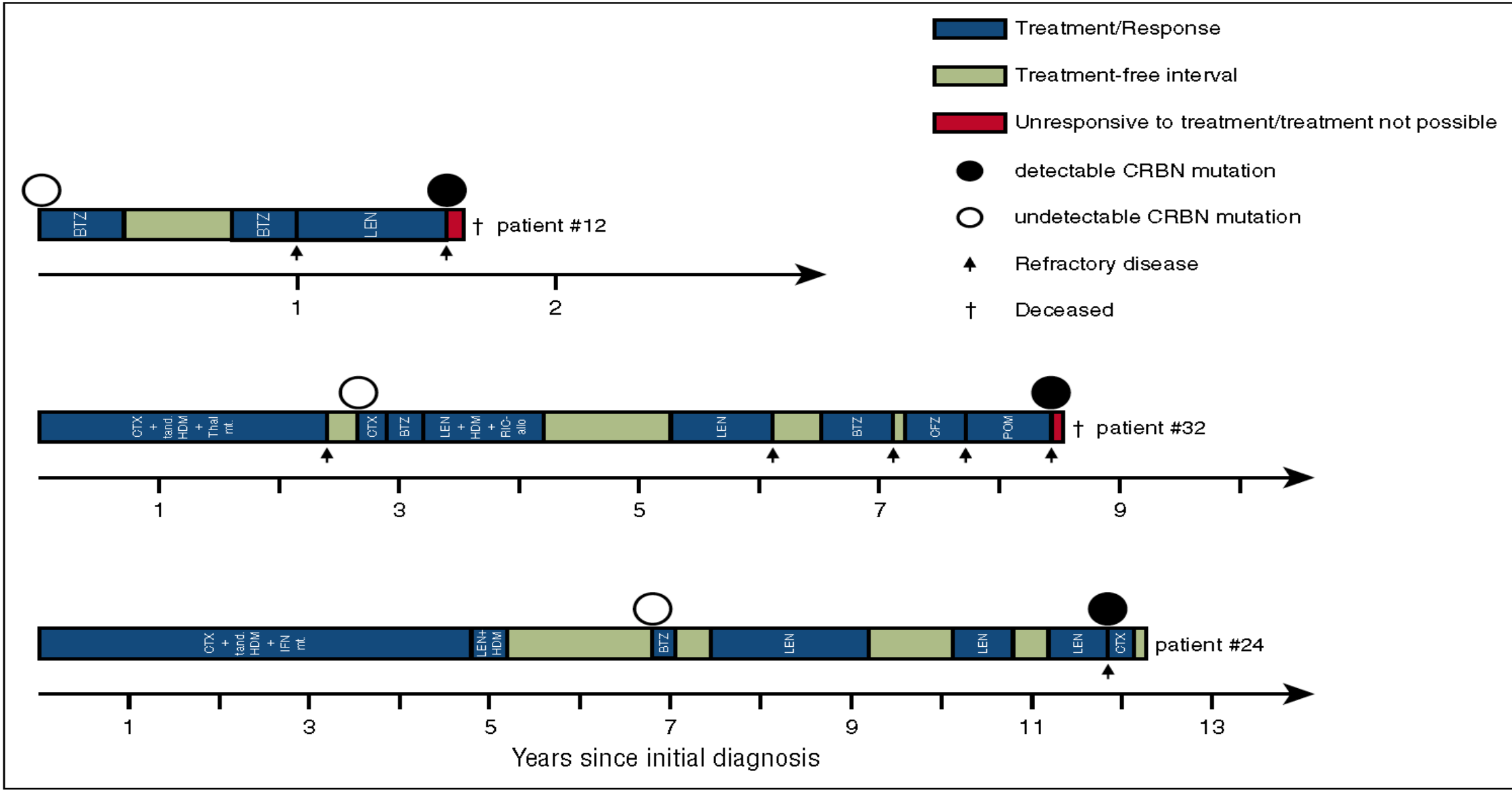






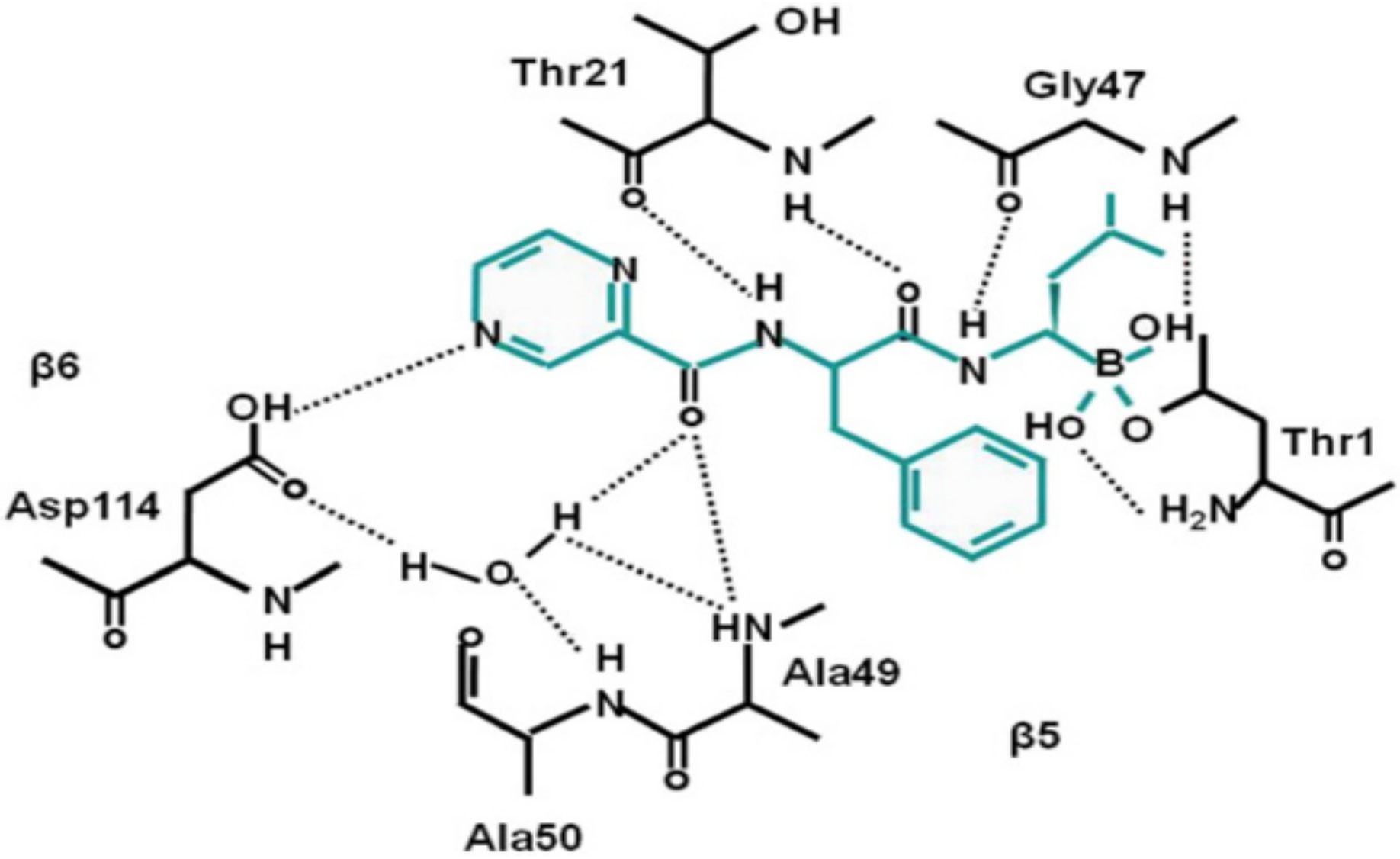
KrÖnke J et al, Scie

CRBN mutations and clinical course of MM patients



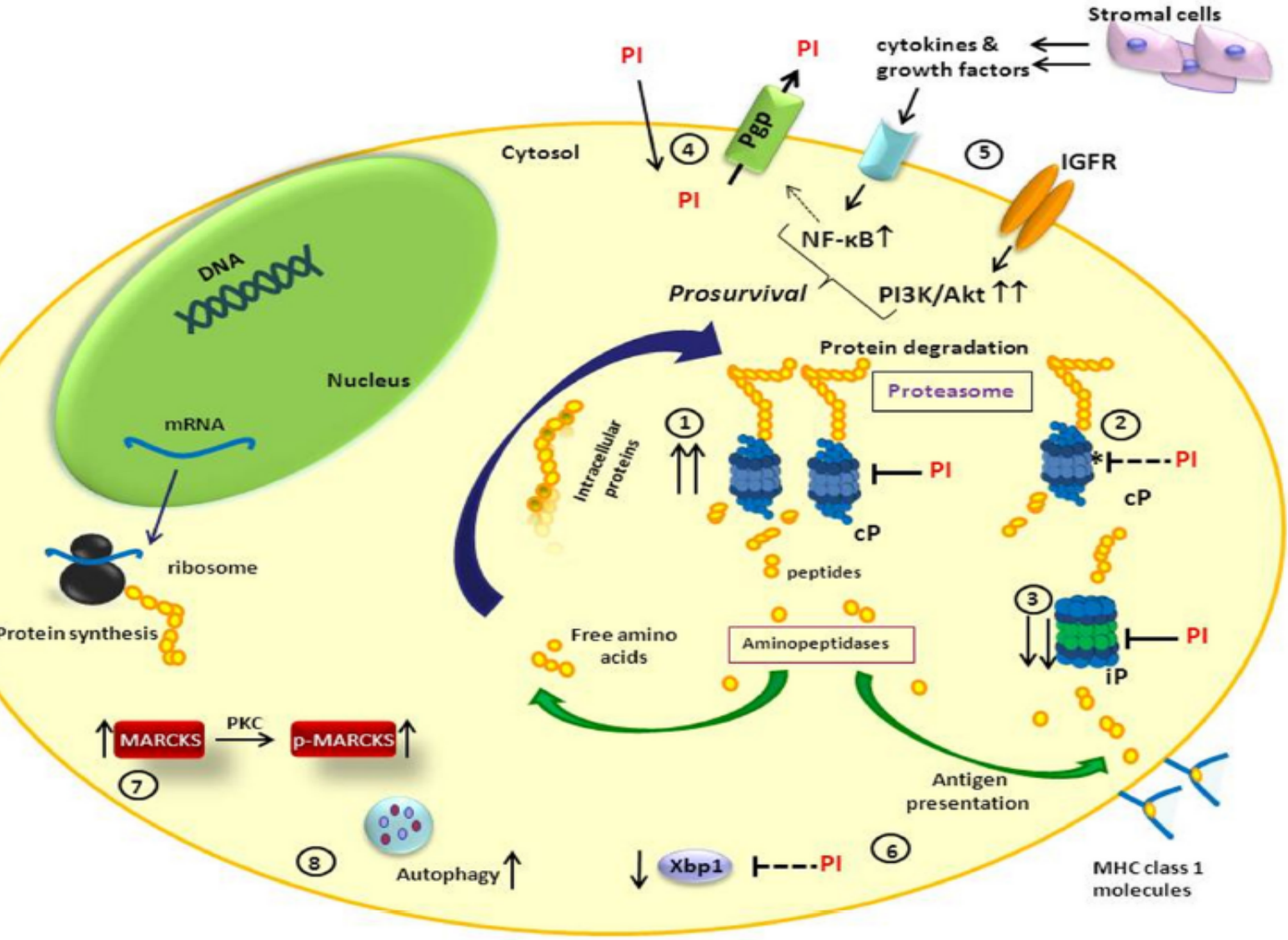
Kortum KM et al, Blo

Interaction of BOR and the proteasome subunit β5



Biomark Res 2013

Molecular mechanisms involved in BOR resistance



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

D. et al, Drug Resist Uptdat 2015

Leukemia (2015) **29,** 2184–2191

www.nature.com/leu

RIGINAL ARTICLE

Pharmacologic screens reveal metformin that suppresses GRP78-dependent autophagy to enhance the anti-myelom ffect of bortezomib

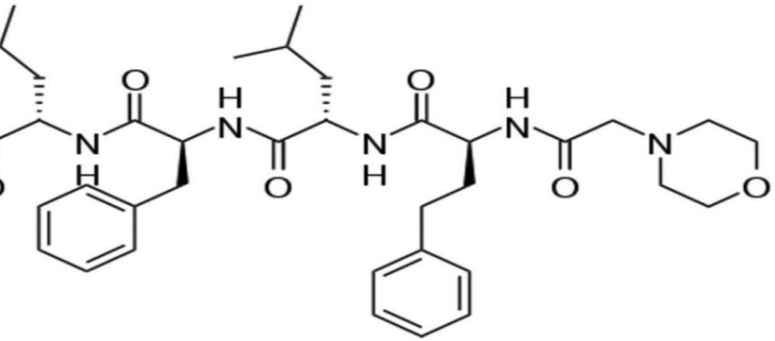
Jagannathan[1,2,7], MAY Abdel-Malek[1,2,7], E Malek[1,2], N Vad[1,2], T Latif[2,3], KC Anderson[4,5] and JJ Driscoll[1,2,3,6]

w.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 201

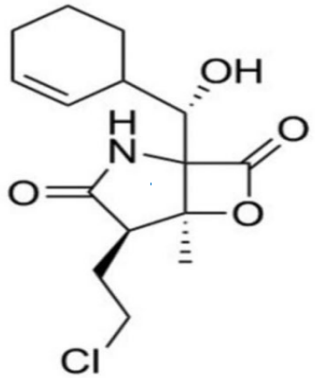
oncanonical SQSTM1/p62-Nrf2 pathway activation mediates roteasome inhibitor resistance in multiple myeloma cells via edox, metabolic and translational reprogramming

ene Riz[1], Teresa S. Hawley[2,3], Jeffrey W. Marsal[1] and Robert G. Hawley[1]

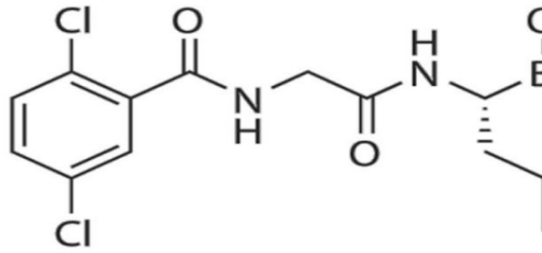
New Proteasome inhibitors (PI)s



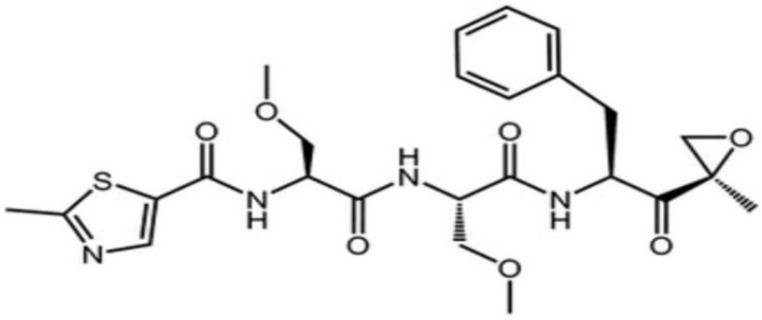
Carfilzomib



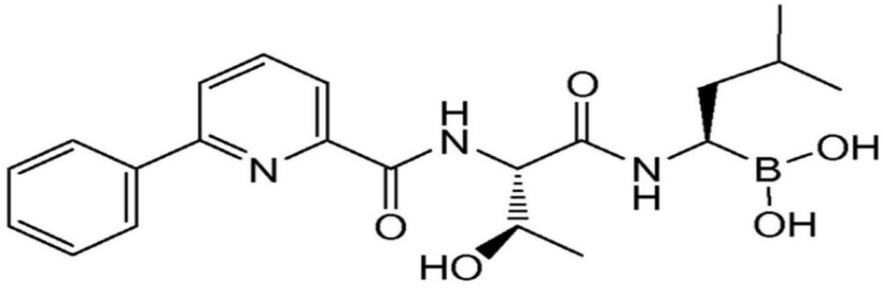
Marizomib



Ixazomib

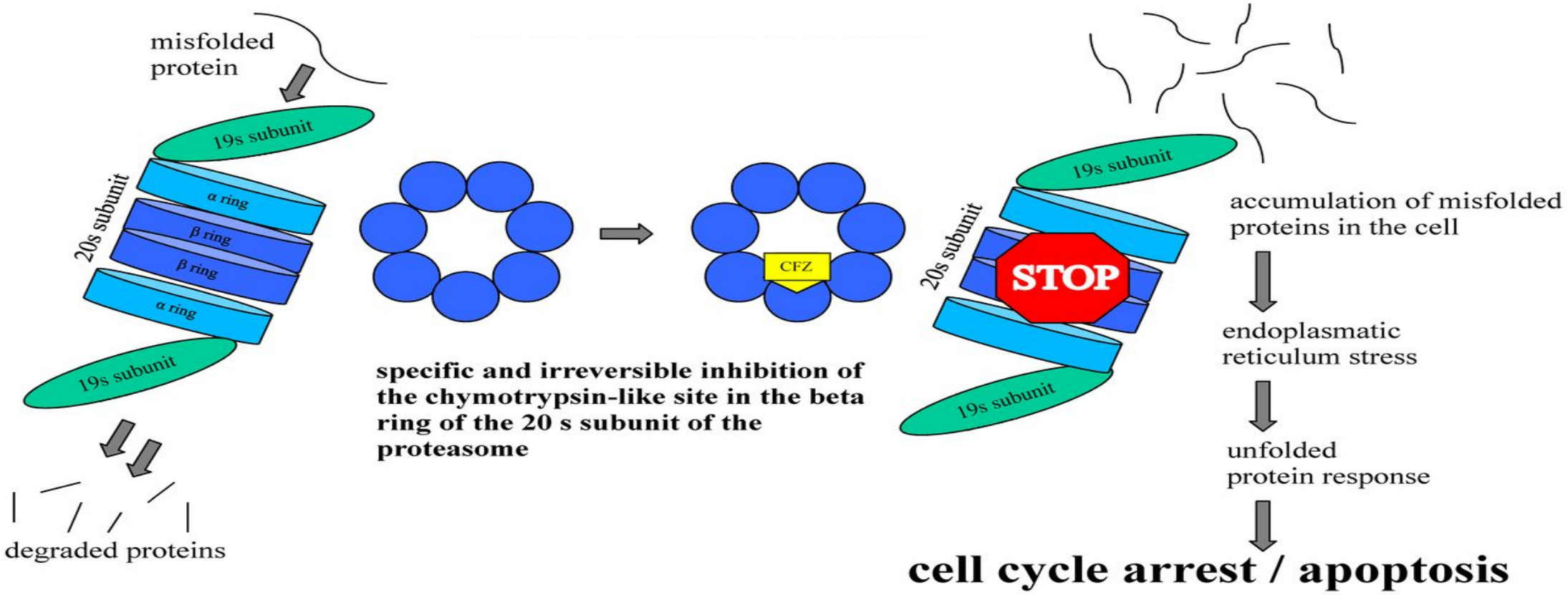


Oprozomib



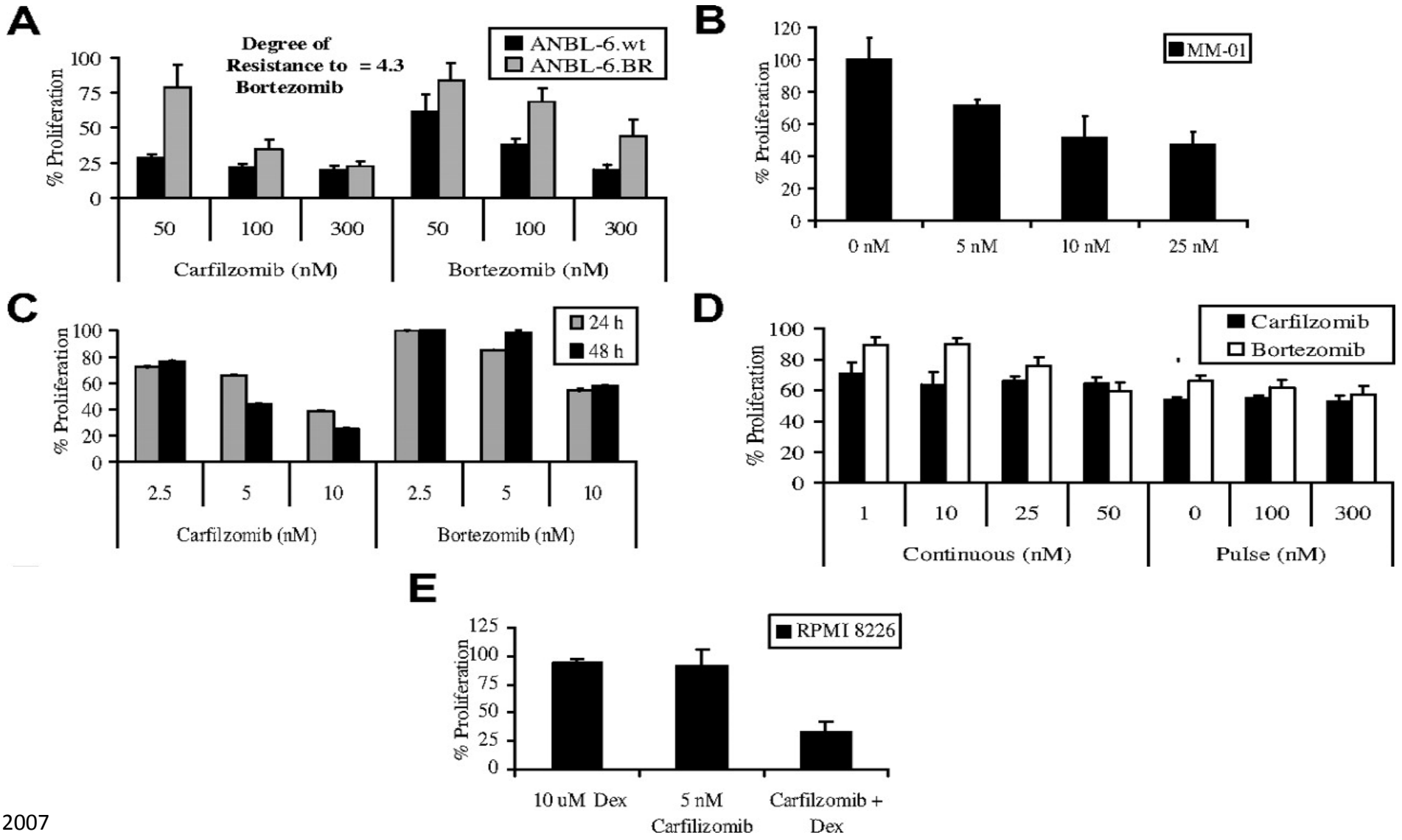
Delanzomib

Carfilzomib



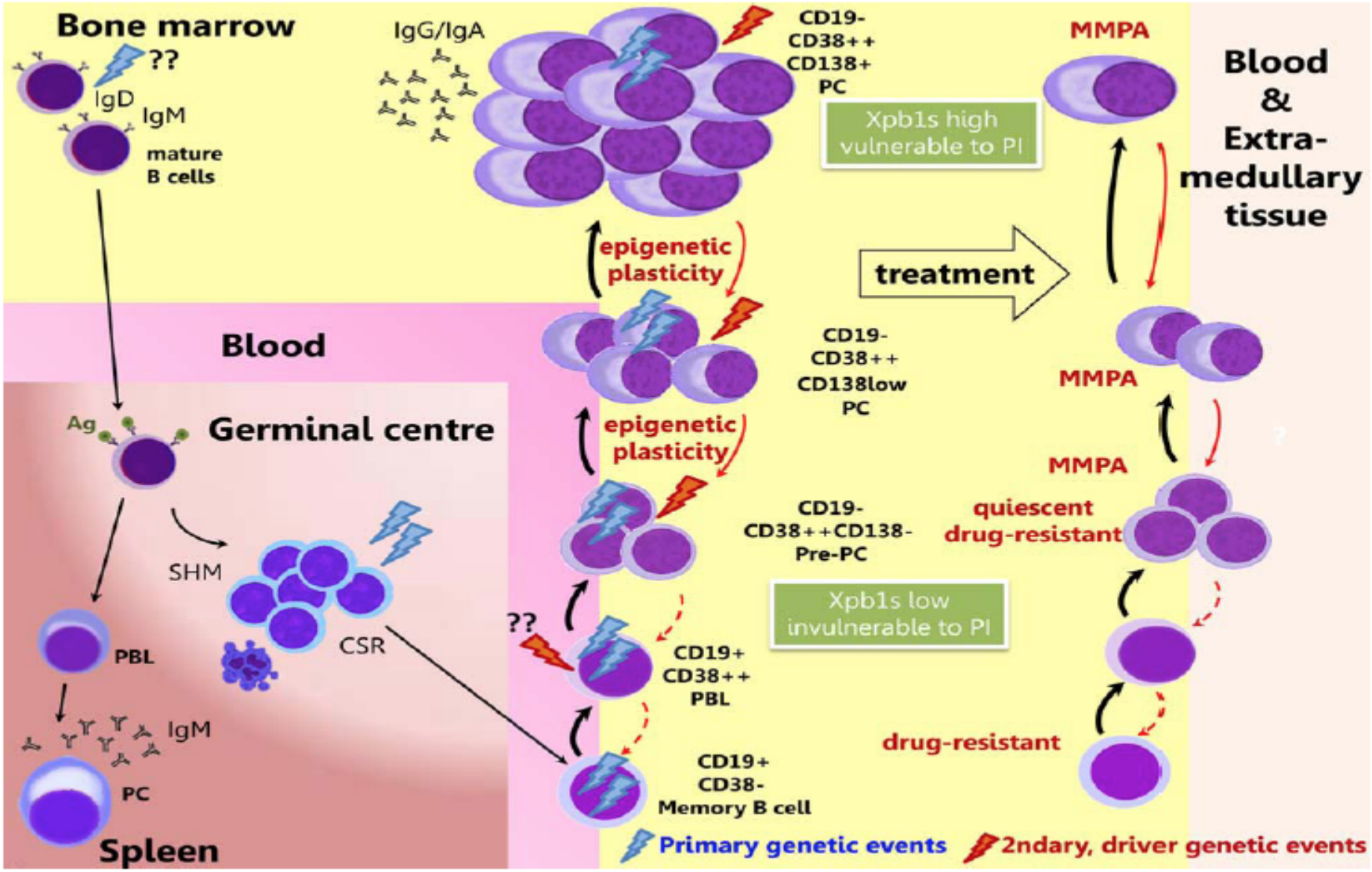
M. et al, Blood 2013

Carfilzomib an irreversible inhibitor of the ubiquitin-proteasome pathway against pre-clinical models of M



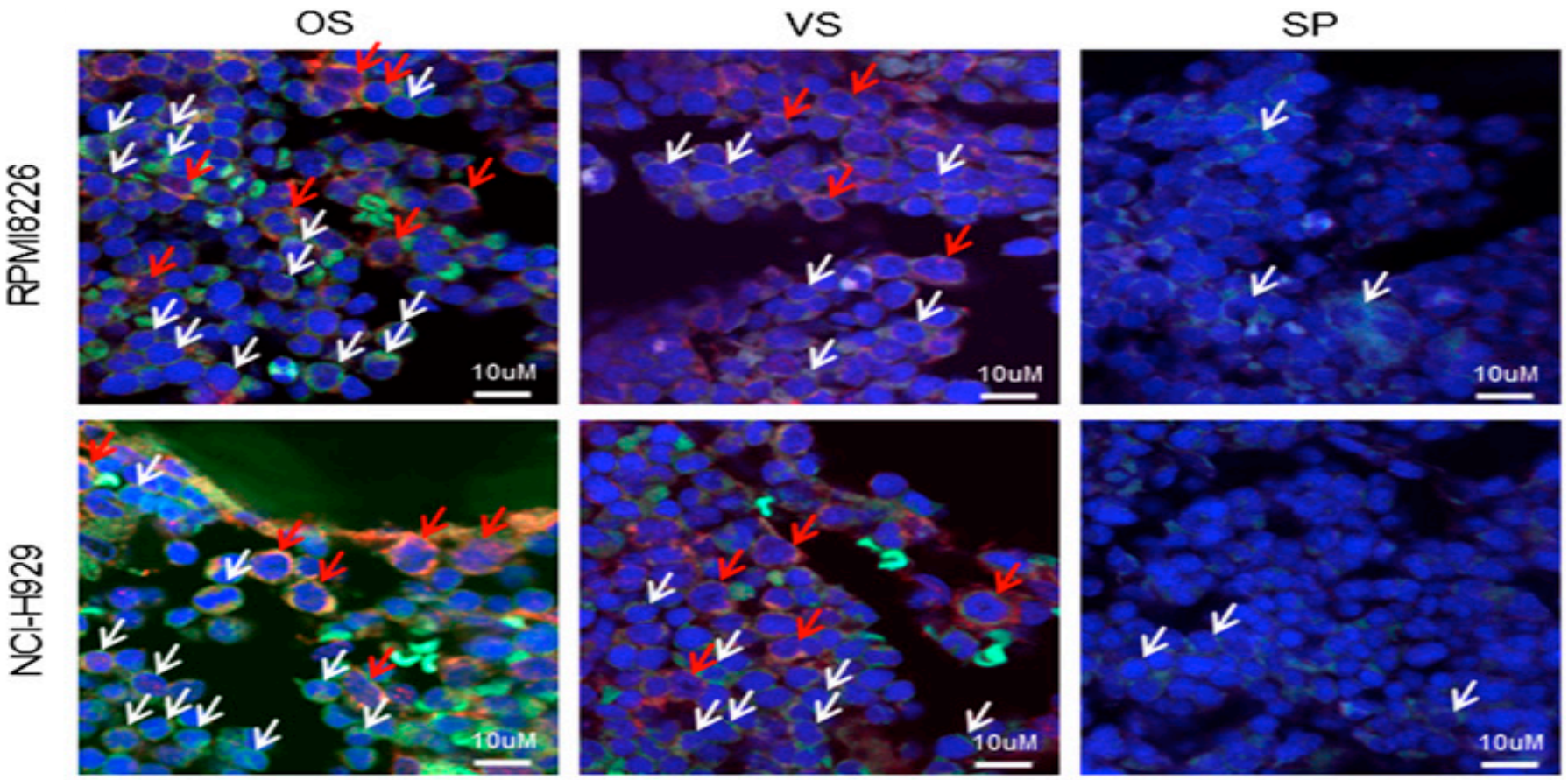
et al Blood 2007

nerging MM cancer stem cells as mechanisms of dr resistance



Karadimitris A et al, Stem C

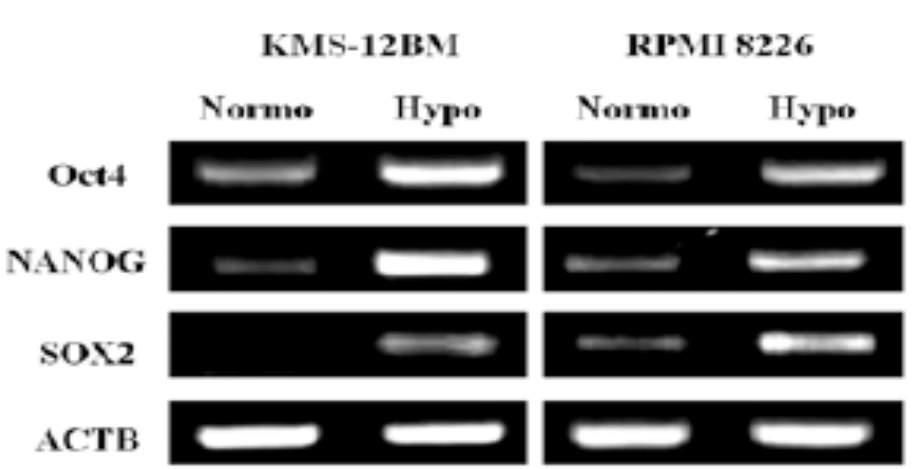
Quiescent MM cells preferably reside within the osteoblastic niche

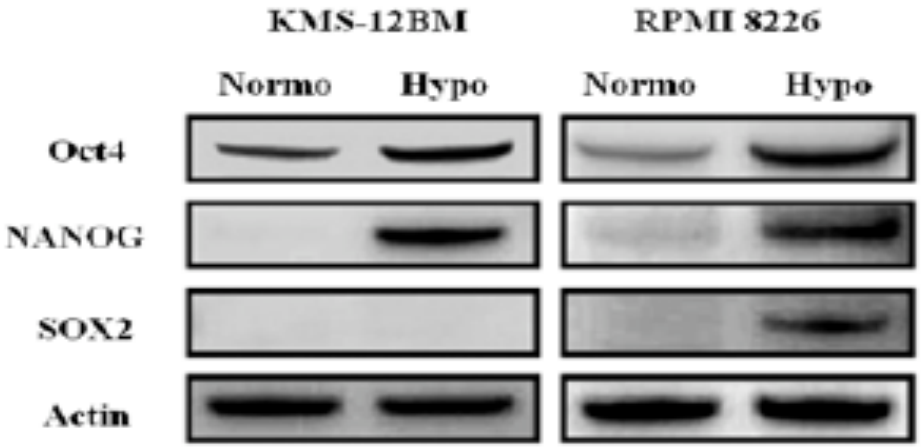


white arrow indicates PKH+CD138- cells (PKH: green)
red arrow indicates PKH+CD138+cells (PKH: green; CD138: red)

Chen Z et al, Blood

ypoxia induce stem cell-like transcriptional program MM cells

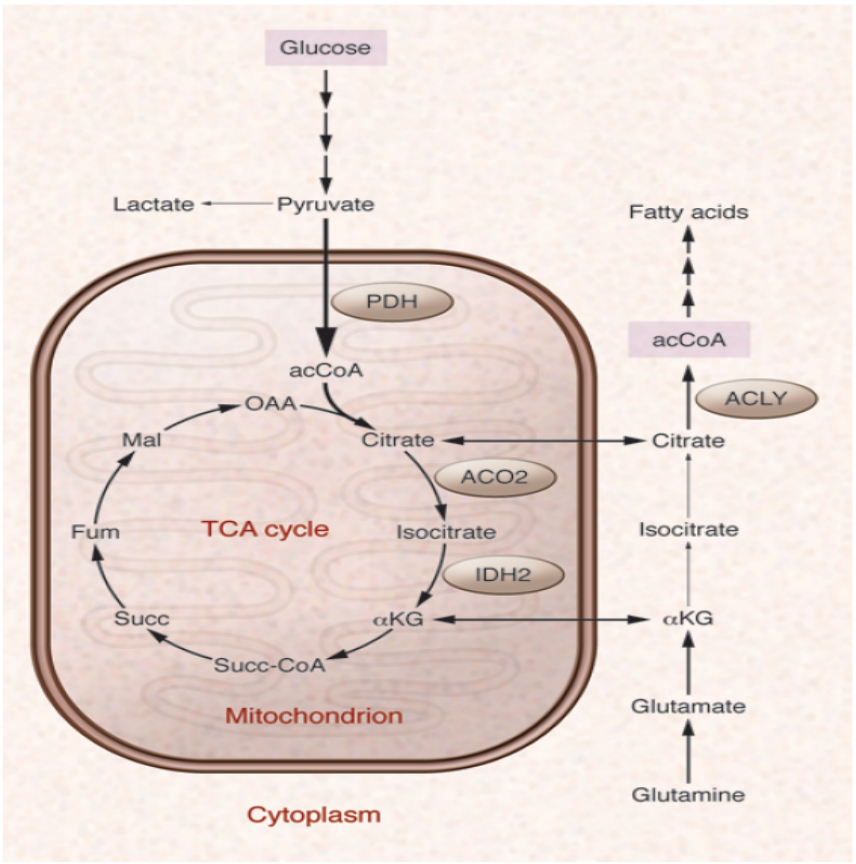




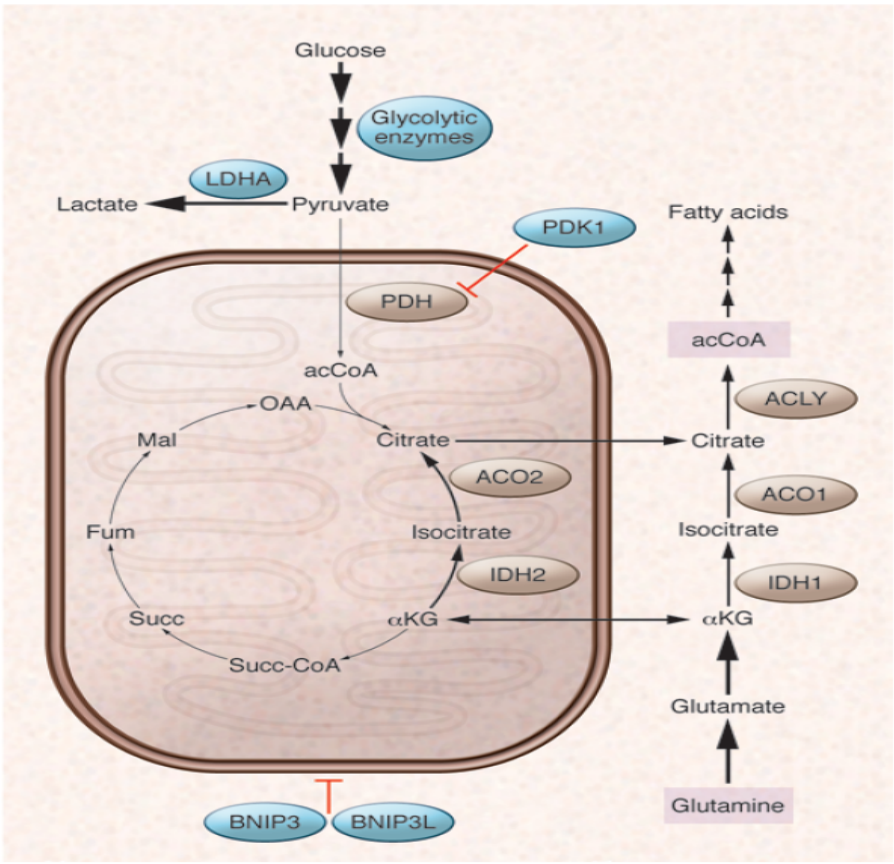
Kawano Y et al, Int J On

HIF-1 mediates metabolic responses to intratumoral hypoxia

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



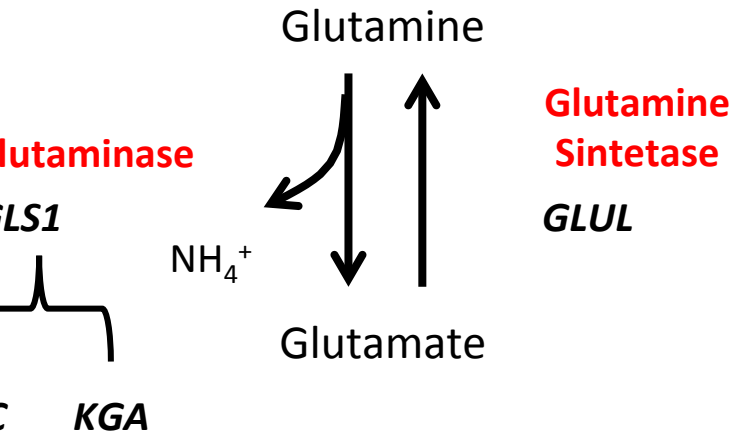
Well-oxygenated cells

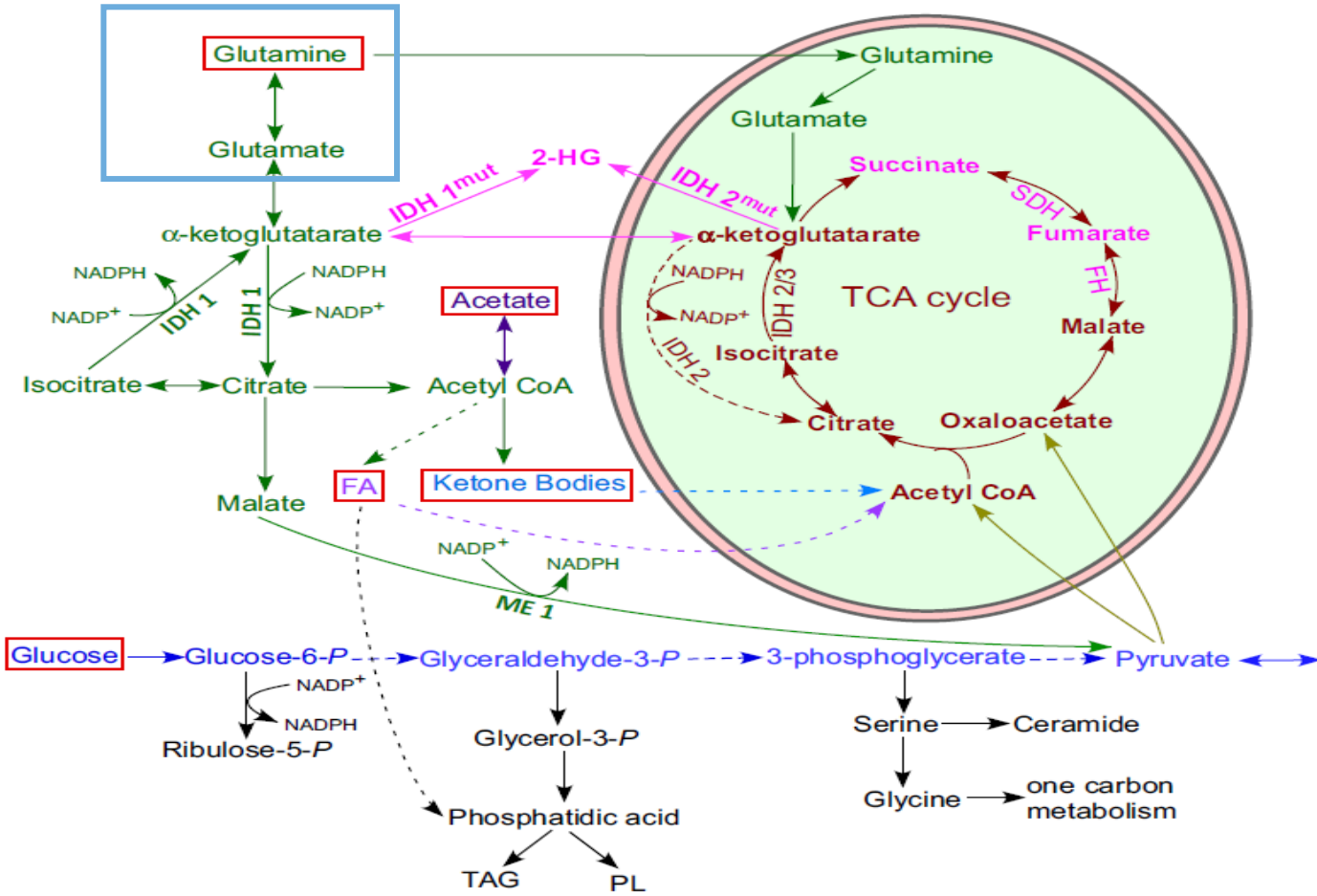


Hypoxic cells

holistic view of cancer bioenergetics: tochondrial function and respiration ay fundamental roles in the development d progression of diverse tumors

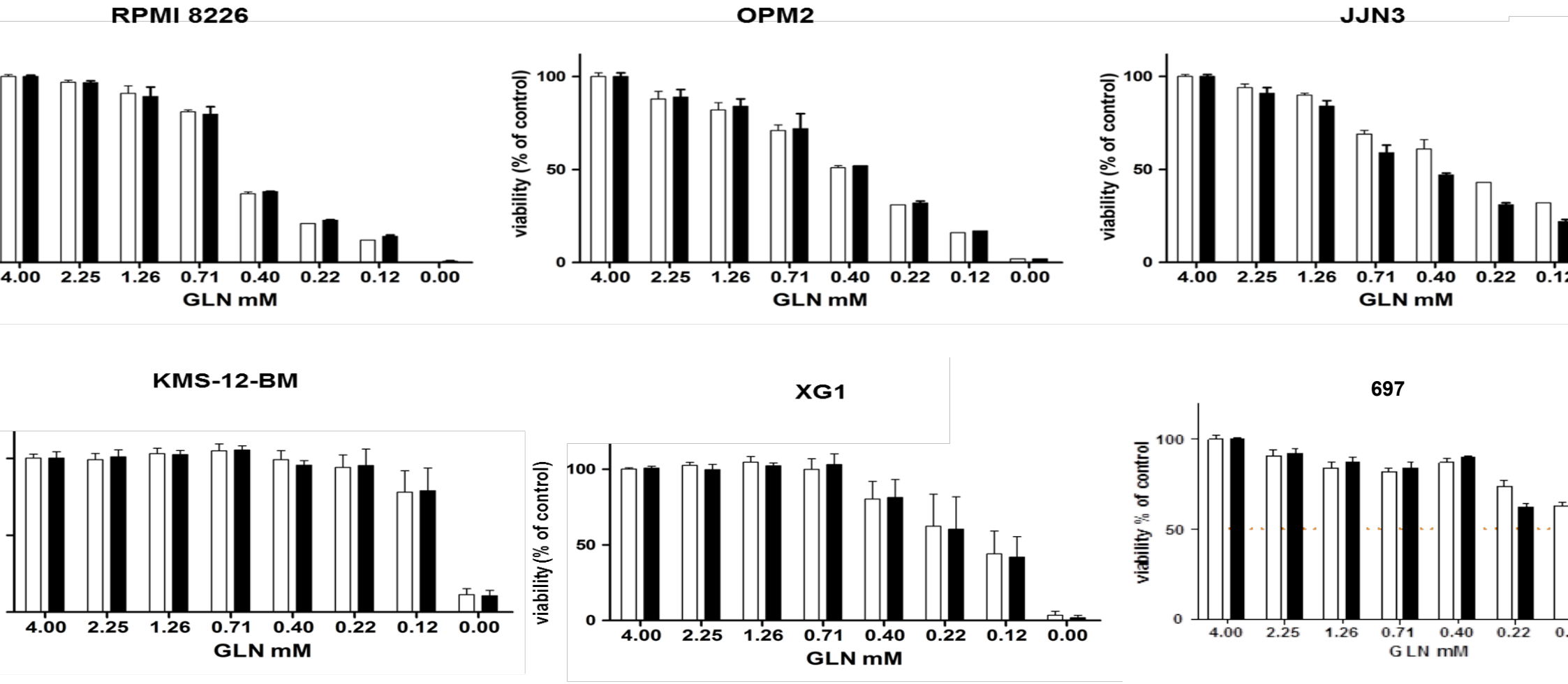
aksudul Alam†, Sneha Lal†, Keely E. FitzGerald and Li Zhang*





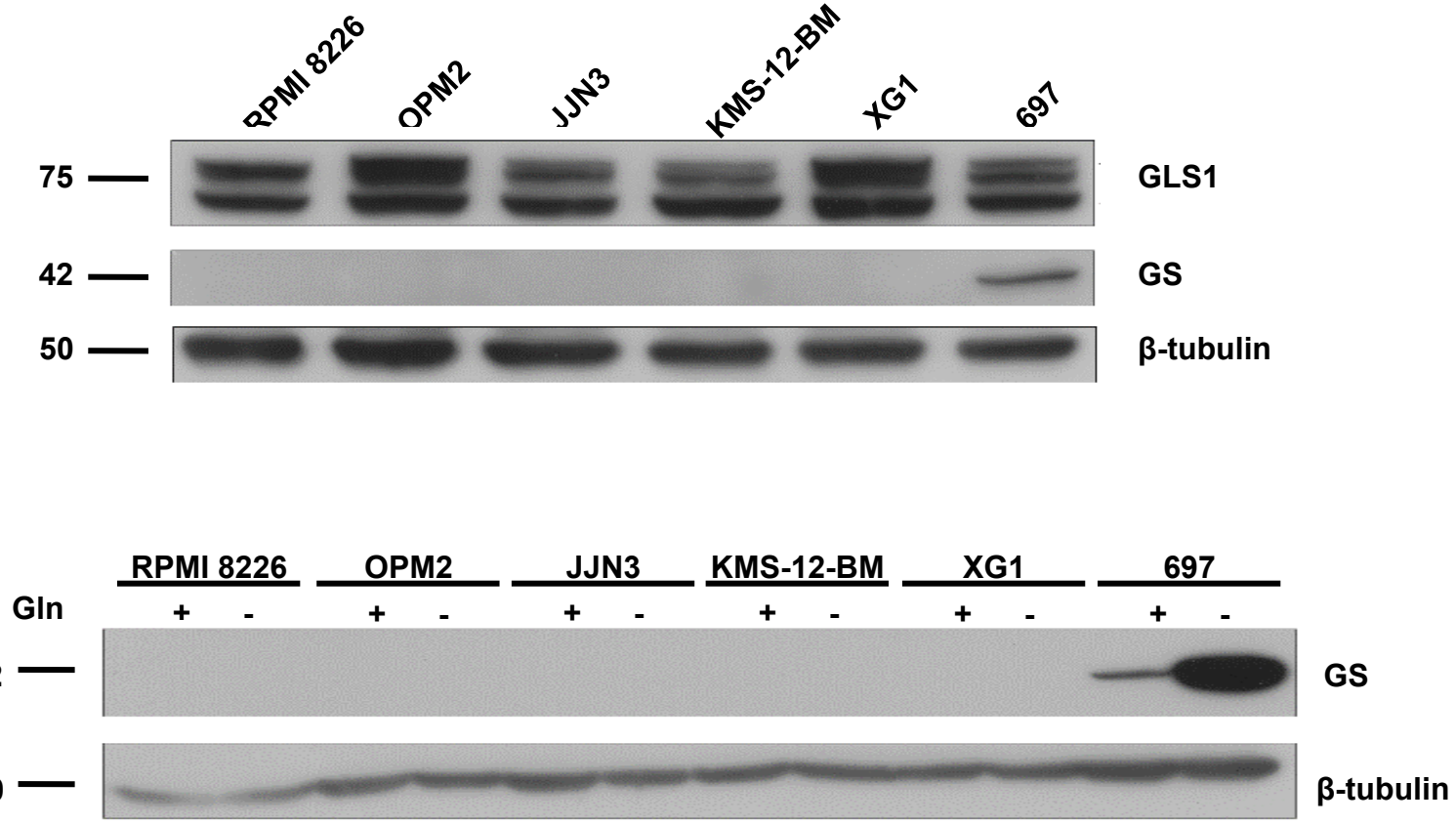
rans Med (2016) 5:3

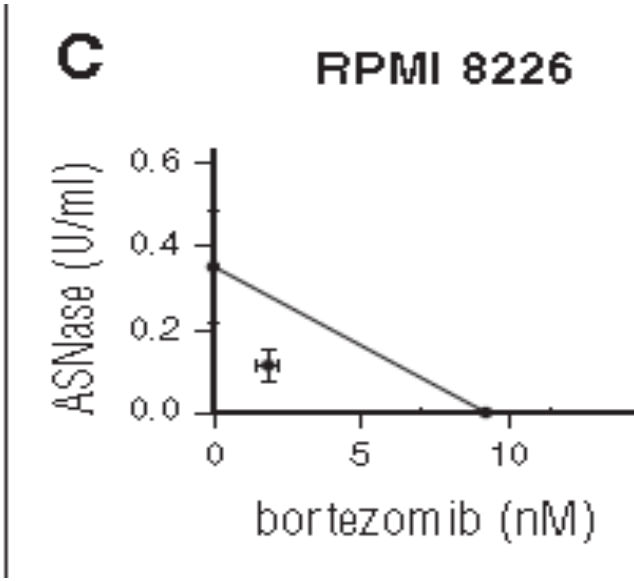
Gln-addiction of MM cells



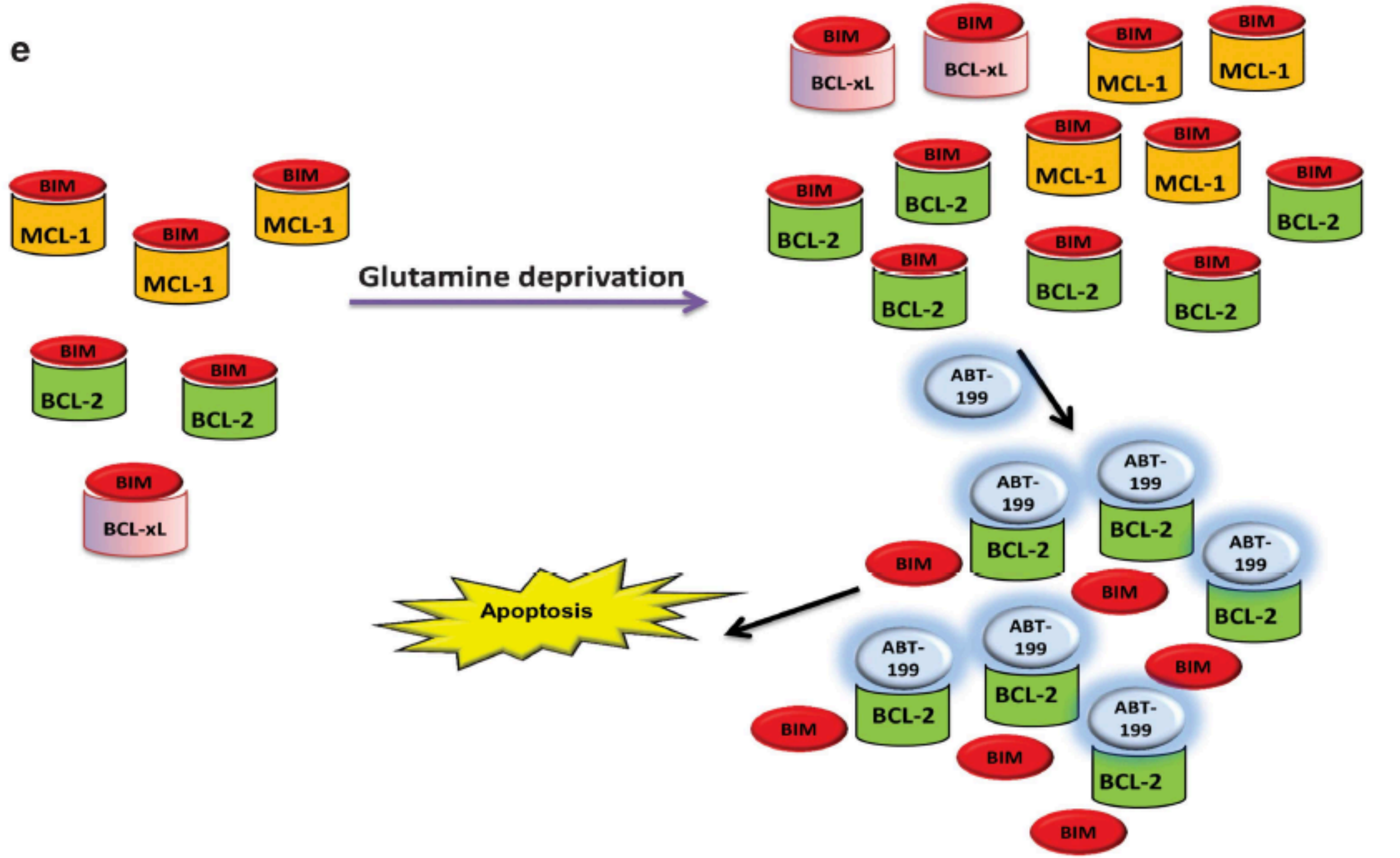
et al, Blood 2016

MM cells express high levels of GLS1 but not of GS





et al, Blood 2016

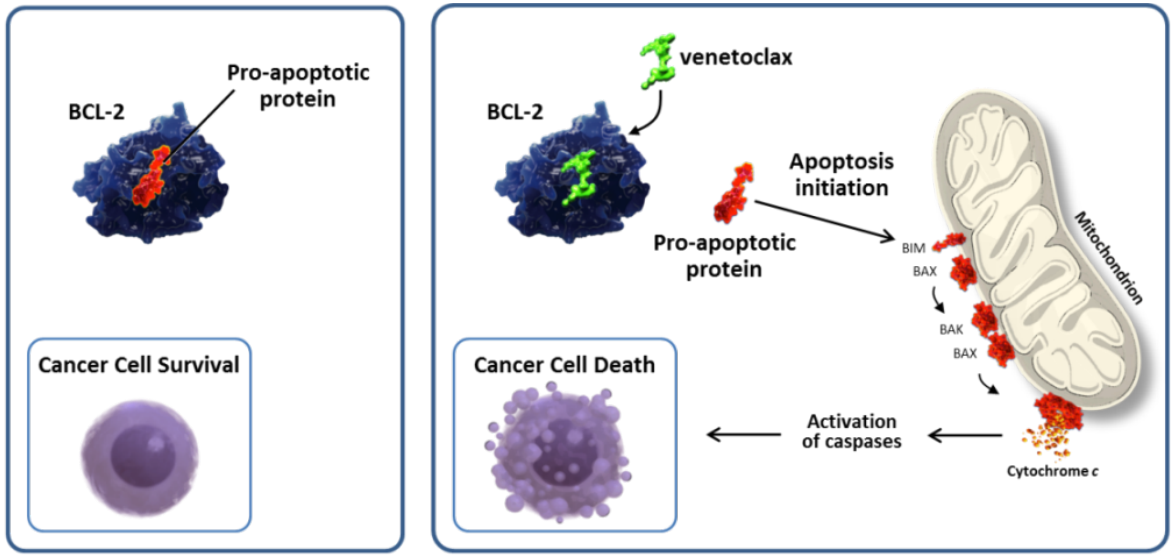

e
BIM
MCL-1
BIM
MCL-1
BIM
MCL-1
BIM
BCL-2
BIM
BCL-2
BIM
BCL-xL
Glutamine deprivation
BIM
BCL-xL
BIM
BCL-xL
BIM
MCL-1
BIM
MCL-1
BIM
MCL-1
BIM
BCL-2
BIM
BCL-2
BIM
BCL-2
BIM
BCL-2
BIM
BCL-2
BIM
BCL-2
ABT-199
ABT-199
BCL-2
ABT-199
BCL-2
ABT-199
BCL-2
BIM
BIM
BIM
ABT-199
BCL-2
ABT-199
BCL-2
ABT-199
BCL-2
BIM
BIM
BIM
BIM
Apoptosis

VENETOCLAX

nti-apoptotic proteins BCL-2 and MCL-1 promote multiple myeloma (MM) cell survival

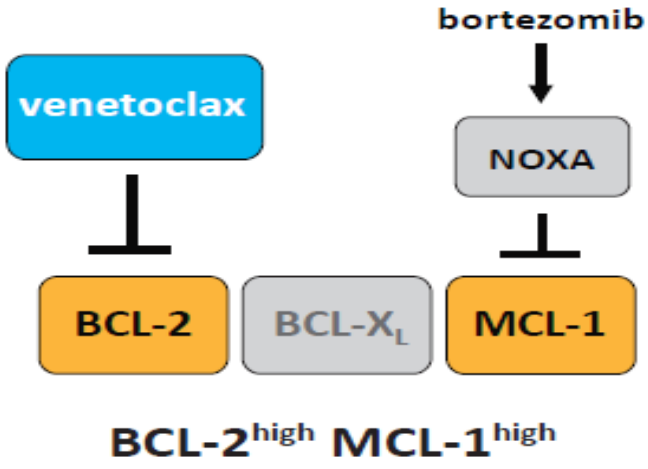
enetoclax is a selective, orally available small molecule BCL-2 inhibitor[1] and bortezomib can indirectly inh
CL-1

enetoclax enhanced bortezomib activity in vitro and in vivo[2]



BCL-2 overexpression allows cancer cells to evade apoptosis by sequestering pro-apoptotic proteins.[1-3]

Venetoclax binds selectively to BCL-2, freeing pro-apoptotic proteins that initiate programmed cell death (apoptosis).[4-6]



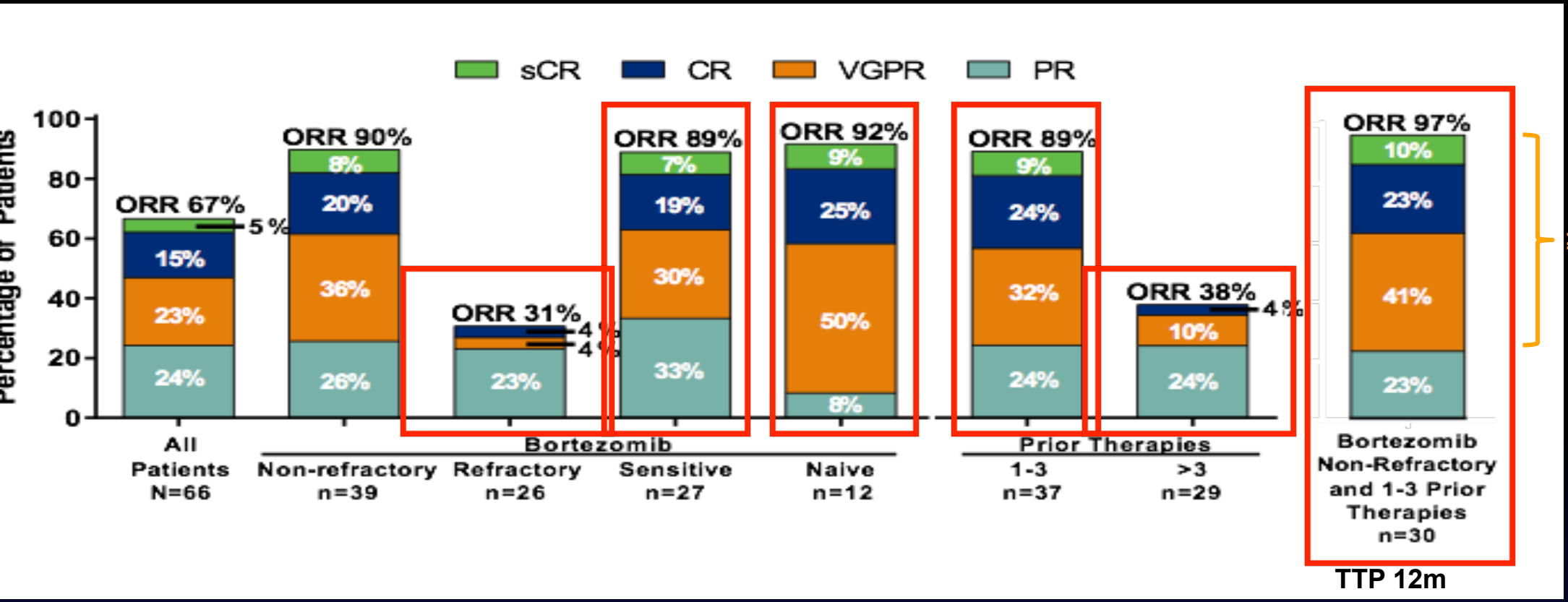
. Leverson JD, et al. *Sci Transl Med* 2015; 7:279ra40. 2. Czabotar, et al. *Nature Reviews* 2014;15:49-63. 3. Plati J, Bucur O, Khosravi-Far R. *Integr Biol (Camb)* 2011;3:279–296.
. Certo M, et al. *Cancer Cell*. 2006;9(5):351-65. 5. Souers AJ, et al. *Nat Med*. 2013;19(2):202-8. 6. Del Gaizo Moore V et al. *J Clin Invest*. 2007;117(1):112-21.

1. Roberts AW et al. *NEJM* 2015
2. Punnoose E et al. *Mol Cancer Ther* 2016

Venetoclax plus bortezomib an dexamethasone

-1200 mg oral daily + 1.3 mg/m^2 SC TW x cycles1-8, QW 9-11 + 20-20 mg x cycles 1-8

tients after >=1 prior lines of therapy (median 3). 61% refractory to the last line



Vd→ORR: 66% ≥VGPR: 37%

se events were manageable. G3-4 AEs: Thrombocytopenia (29%), anemia (15%), neutropenia (14%), diarrhea (6%), dyspnea (
a (5%), insomnia (5%), PN(3%), asthenia (2%), URTI (2%); **MTD not reached**

nale for a phase 3 trial: Vd +/- Venetoclax

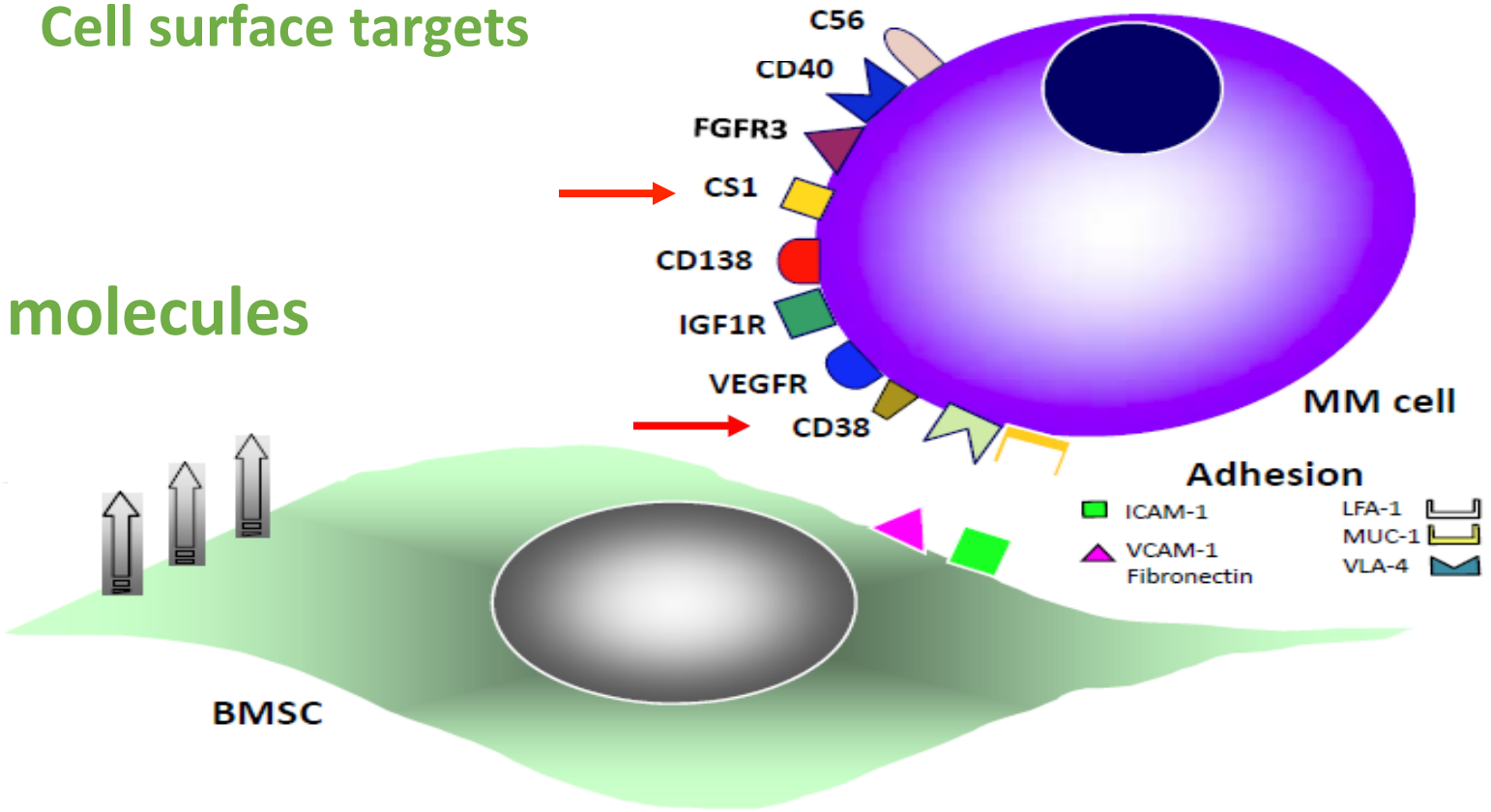
Moreau P, et al. Presented at ASH 2016 (Abstract 975), oral pr

Targets for monoclonal antibody therapy in MM

Cell surface targets

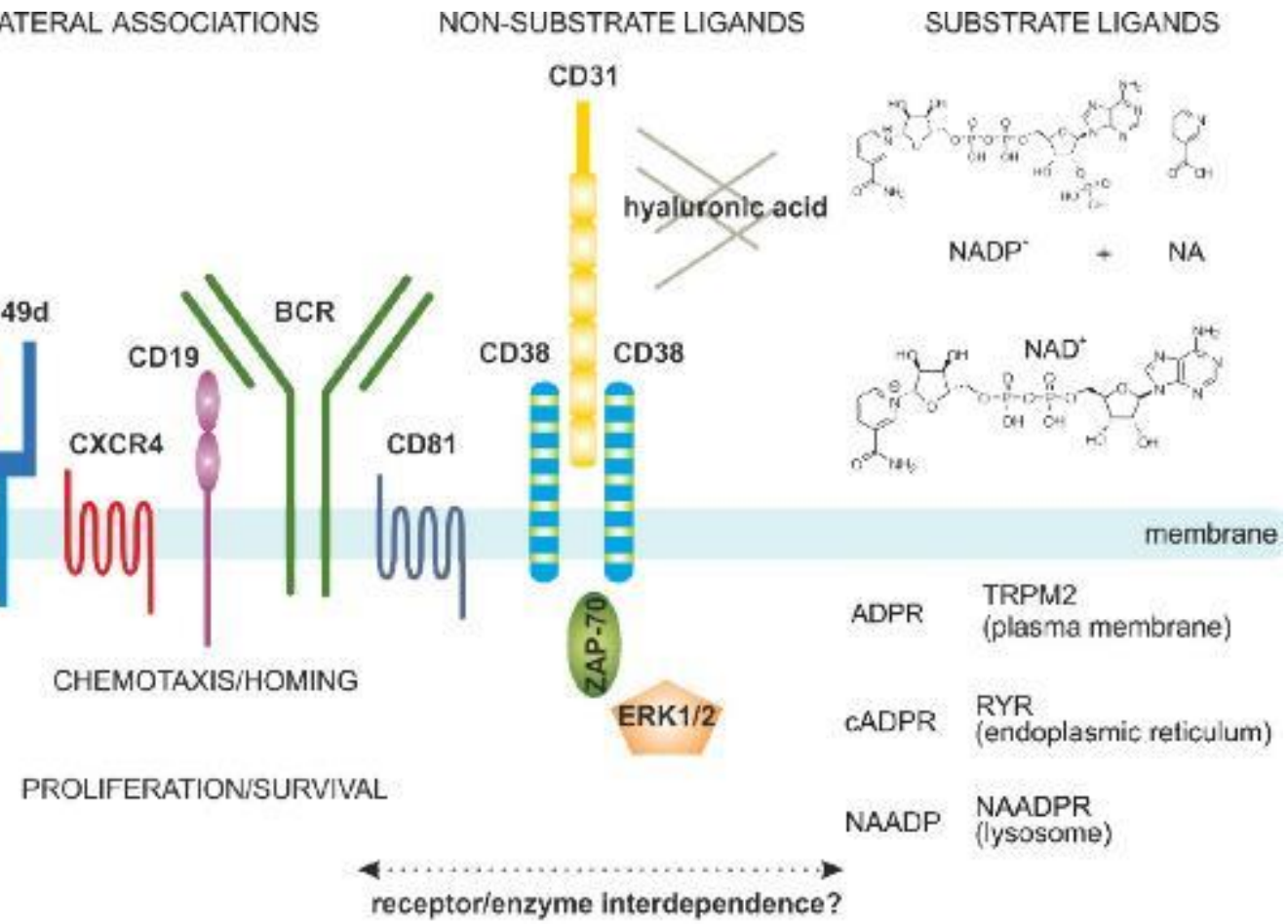
Signaling molecules

IL-6
RANKL
DKK1
VEGF
IGF-1
SDF-1α
BAFF, APRIL



rom: Anderson K. et al., J Clin Oncol 2012

CD38 is a Cell-surface Receptor and Ectoenzyme



- **As a receptor**
  - Regulates signaling, homing, adhe and migration in close contact BCR complex and CXCR4.
  - Engagement with CD31 or hyalur acid activate ZAP-70, ERK1/2 NFKB pathways and regul activation and proliferation of cell.
- **As an ectoenzyme**
  - CD38 interacts with NAD+ and N +, which are converted to cA ADPR, and NAADP, all intracel $Ca2^{+}$ mobilizing agents.

F. et al, Blood 2011

CD38 Expression

Lymphoid tissue	Cell population
Blood	T cells (precursors, activated) B cells (precursors, activated) Myeloid cells (monocytes, macrophages, dendritic cells) NK cells Erythrocytes Platelets
Cord blood	T and B lymphocytes, monocytes
Bone marrow	Precursors **Plasma cells**
Thymus	Cortical thymocytes
Lymph nodes	Germinal center B cells

- **Highly and uniformly expressed on myeloma cells**[1,2,3]
- **Relatively low expression on normal lymphoid and myeloid cells** and in some tissues of non-hematopoietic origin[4]

i F. et al, Physiol Rev 2008:
al, Am J Clin Pathol 2004;
cito A.M. et al, Leuk Res 2004;
S. et al, Leuk Res 2001.

Anti-CD38 monoclonal antibodies

Chimeric:

Isatuximab (SAR650984)

Fully human:

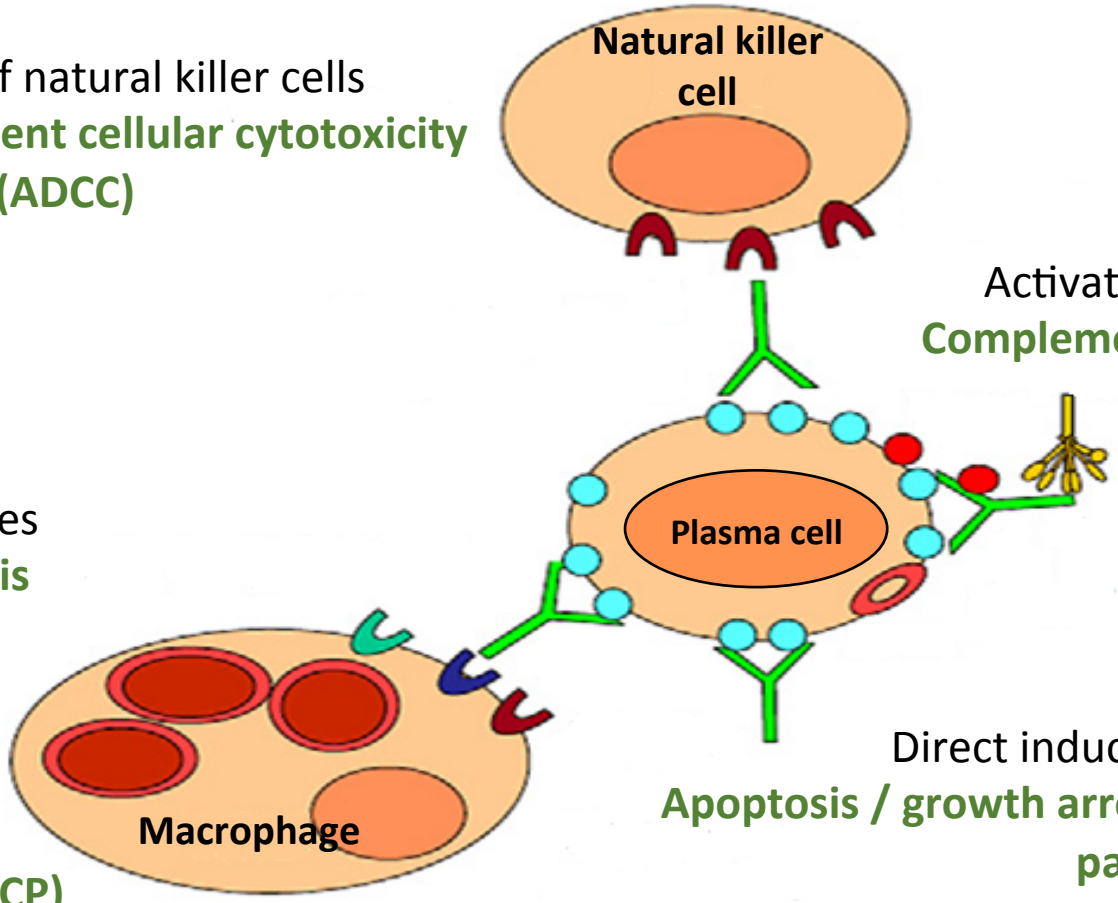
Daratumumab (DARA)

MOR202 (MOR)

M. et al, J Immunol 2011.
wnload.ama-assn.org/resources/doc/usan/x-pub/isatuximab.pdf
w.morphosys.com/pipeline/proprietary-product-portfolio/mor202

DARA: mechanisms of action

Activation of natural killer cells
Antibody-dependent cellular cytotoxicity (ADCC)



Activation of the complement system
Complement-dependent cytotoxicity (CDC)

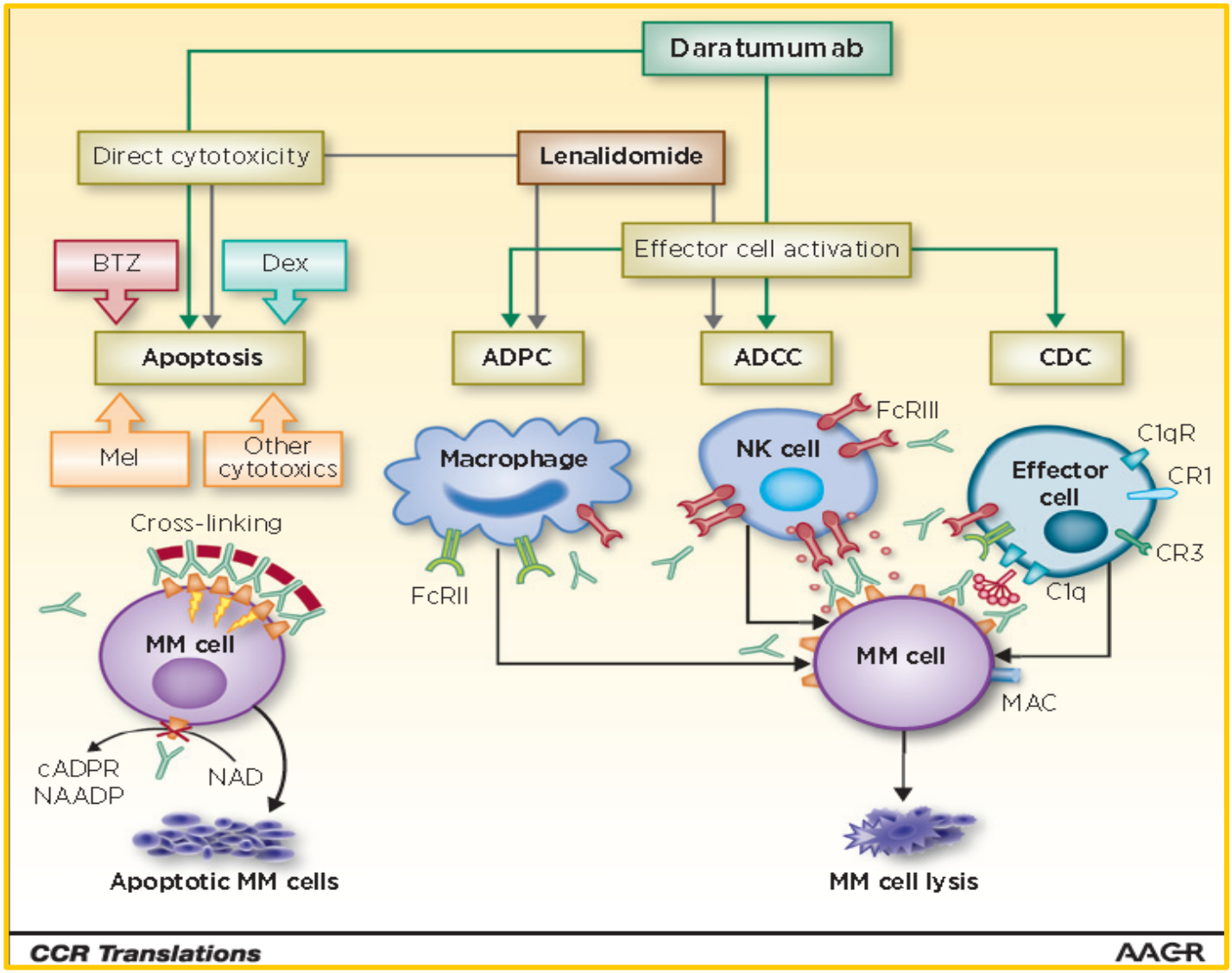
Activation of macrophages
Induction of phagocytosis

ibody-dependent cell-
iated phagocytosis = ADCP)

Direct induction of apoptosis
Apoptosis / growth arrest via targeting of signaling pathways

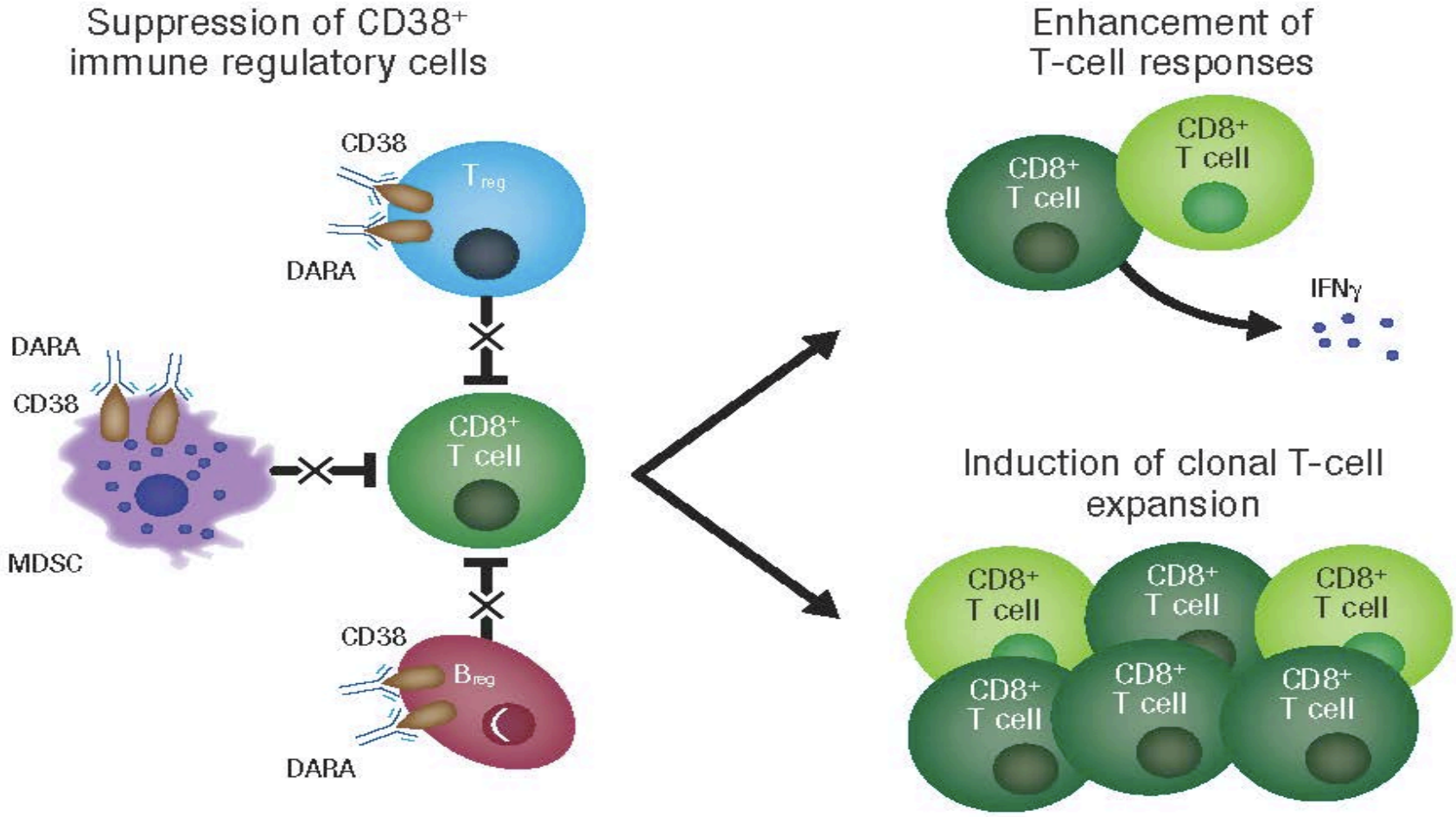
rom: Golay J. & Introna M., Arch Biochem Biophys 2012; Tai Y.T. & Anderson K.C., Bone Marrow Res 2011.

Mechanisms of DARA combination with other drugs



P. et al, Clin Cancer Res 2015

Potential immunomodulatory mechanism of action anti-CD38 mAbs



et al, Blood 2016

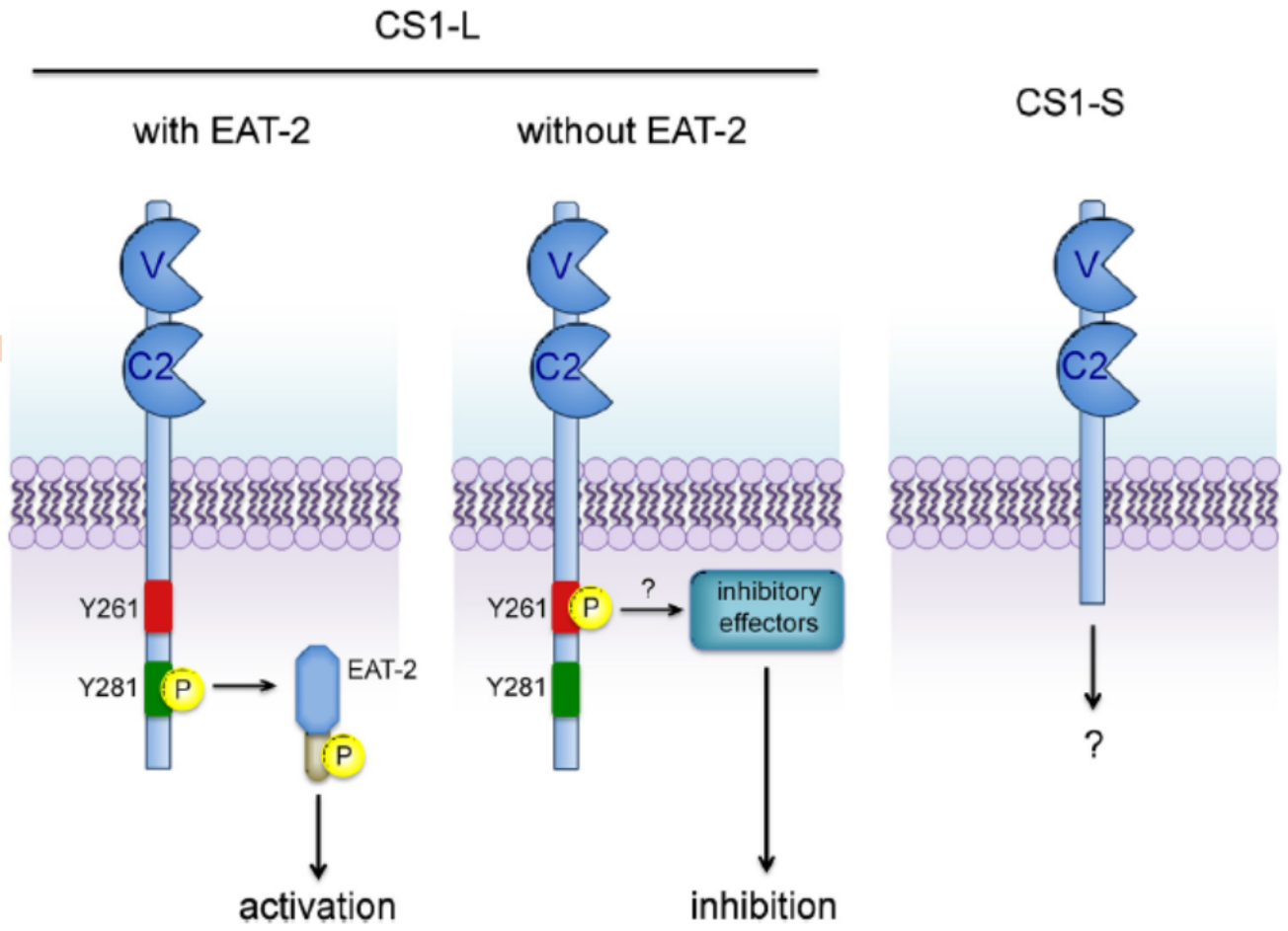
ummarized mechanisms of action of anti-CD38 mAb

	DARA	SAR	MOR
Origin	Human	Humanized	Human
Development phase	Phase III	Phase I/II	Phase I/IIa
Binding	+++	+++	++
CDC (max lysis)	+++	+	+
Phagocytosis	+++	nd*	++
ADCC (max lysis)	++	++	++
PCD direct	–	++	–
PCD crosslinking	+++	+++	+++
Modulation ectoenzyme function	+	+++	–

termined; PCD, programmed cell death.
n L. et al. ASH 2014.

SLAMF7/CS1: structure and function interplay

ediates self-adhesion



om: Veillette A. et al, Crit Rev Oncol Hematol 2013

LAMF7/CS1: expression profile on hemopoietic cell

Cell surface glycoprotein receptor

SLAM (Signaling Lymphocyte Activating Molecule) family:

- SLAM/CD150
- 2B4
- CD84
- NTB-A
- Ly-9

Cell type	CS1 expression
Non-hematopoietic cell	-
Activated monocytes	+
Immature dendritic cells	-
Mature dendritic cells	+
NK cells, NK-T cells	+
$CD8^+$ T lymphocytes	+
Activated B lymphocytes	+
Normal plasma cells	+
MM plasma cells	**++**

.. et al, Crit Rev Oncol Hematol 2013

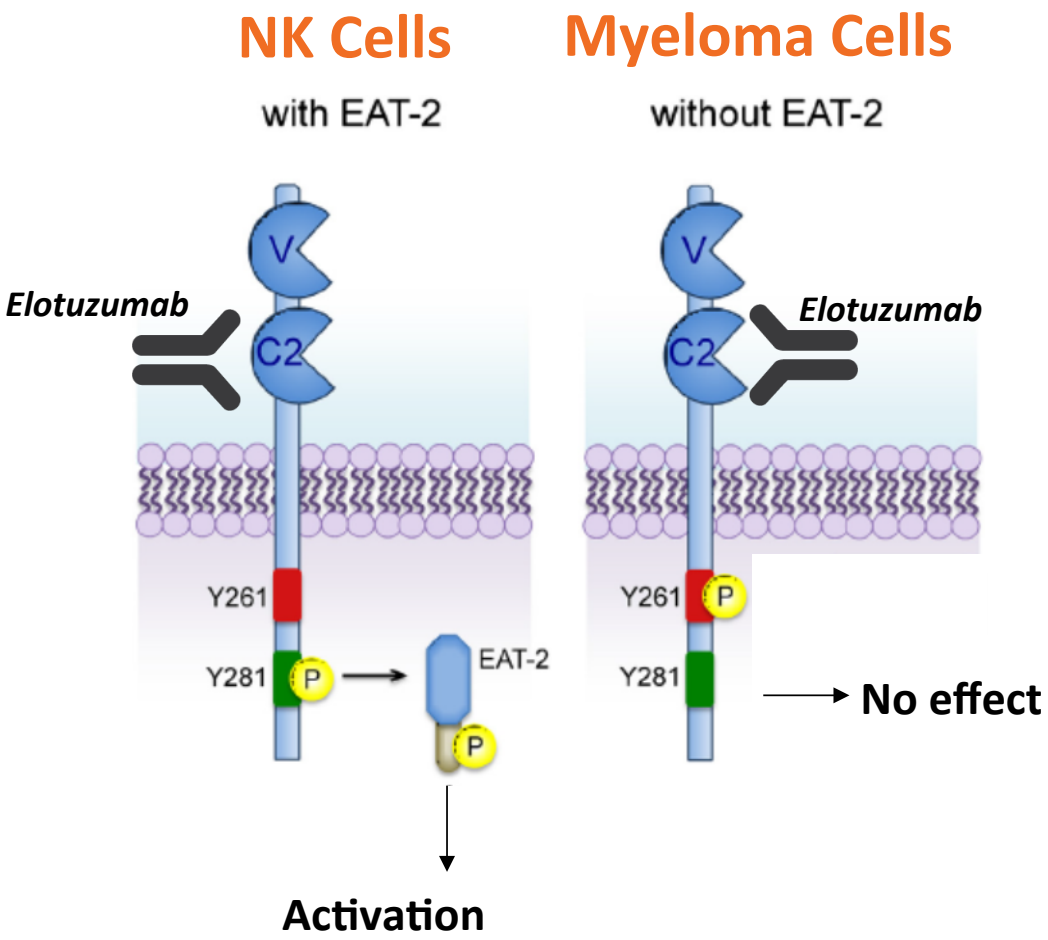
SLAMF7/CS1: an atypical SLAM family member

AM family receptors.

eptor	Alternative name	Physiological ligand	Number of ITSMs	Expression pattern	Interaction with		Phenotypes knock-out mice
					SAP	EAT-2	
AM	CD150 SLAMF1	SLAM	2	T, B, DC, Mϕ, plat	+	+	T, Mϕ, plat, NK-T
9	CD229 SLAMF3	Ly-9	1	T, B, NK, DC, Mϕ	+	+	$CD4^+$ T, innate-like $CD8^+$ T, NK-T
	CD244 SLAMF4	CD48	3	NK, $CD8^+$ T, DC, Mϕ, eos	+	+	NK
84	SLAMF5	CD84	2	T, B, NK, DC, Mϕ, gran, plat, mast, eos	+	+	T, B (GC)
B-A	Ly108 CD352 SLAMF6	NTB-A	2	T, B, NK, DC, neutro	+	+	T, B, neutro, NK-T
	CRACC CD319 SLAMF7	CS1	1	Human: NK, NK-T, DC, B, PC, T Mouse: NK, NK-T, DC, Mϕ, B, T	−	+	NK

. et al, Crit Rev Oncol Hematol 2013

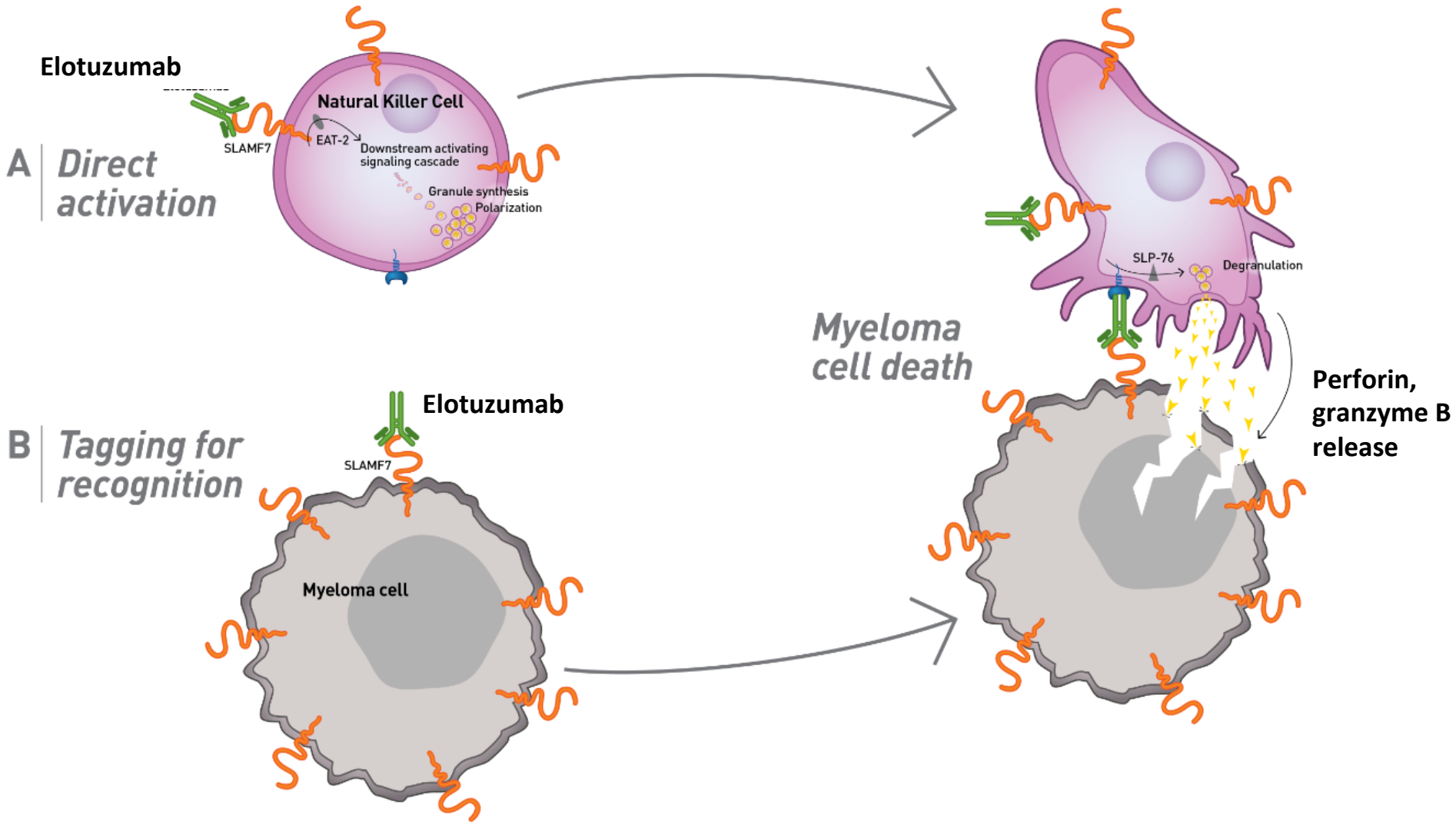
Elotuzumab: a monoclonal antibody targeting SLAMF7



- Humanized, IgG1 mab specific for human SLAMF7
  - No cross-reactivity with non-human homologues or other SLAM family members
- Binds to a membrane-proximal motif of SLAMF7
  - Critical for mediating killing of target c (*in vitro*)

. et al, Crit Rev Oncol Hematol 2013;
oz M,E. et al, Nat Immunol 2009;
al, Mol Cell Bio 2015

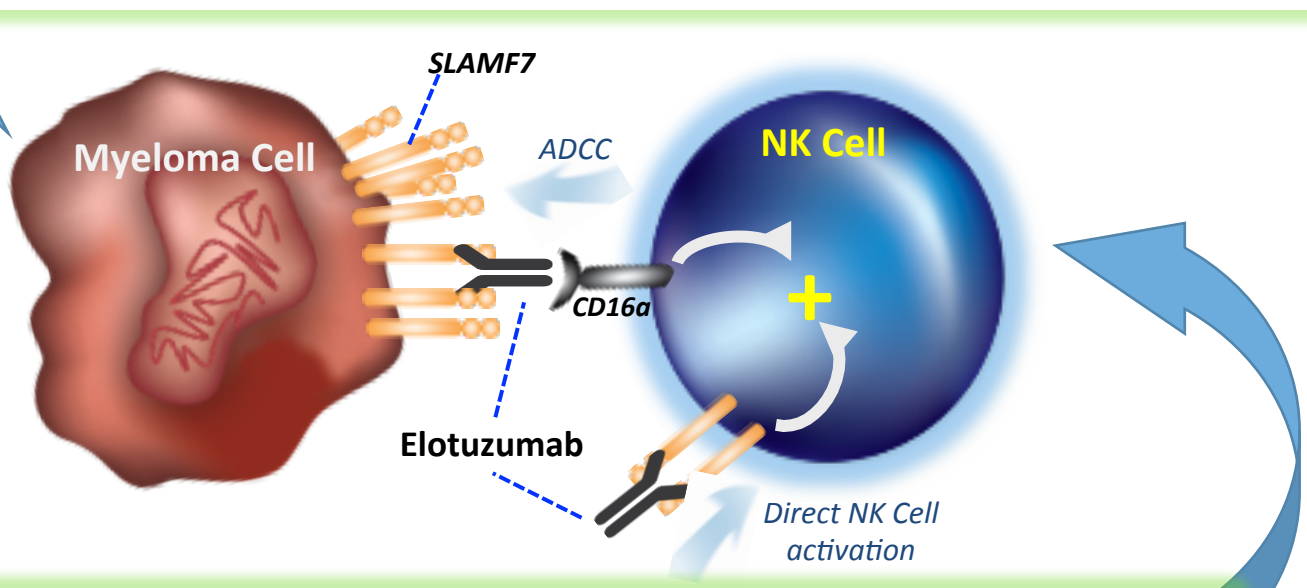
Elotuzumab: mechanisms of action in MM



. et al, Crit Rev Oncol Hematol 2013;
oz M,E. et al, Nat Immunol 2009

lotuzumab synergizes with Lenalidomide to enhan MM cell death

Lenalidomide
Induces myeloma cell injury and lowers threshold for NK cell-mediated killing of myeloma cells by Elotuzumab



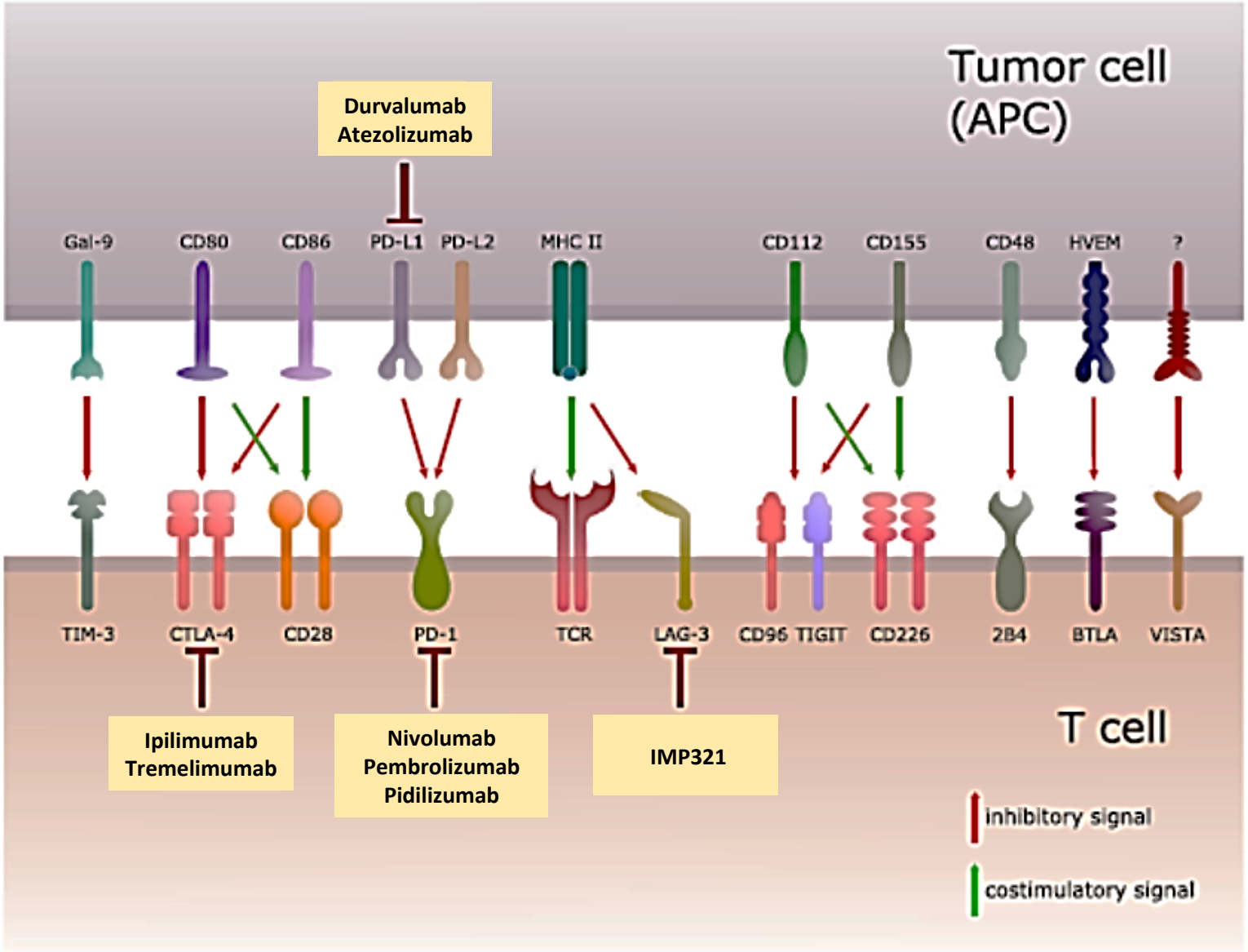
Lenalidomide
Enhances adaptive and innate immune system including production of IL2 to increase NK cell activity

t al, Cancer Immunol Immunother 2015

Monoclonal antibodies in MM

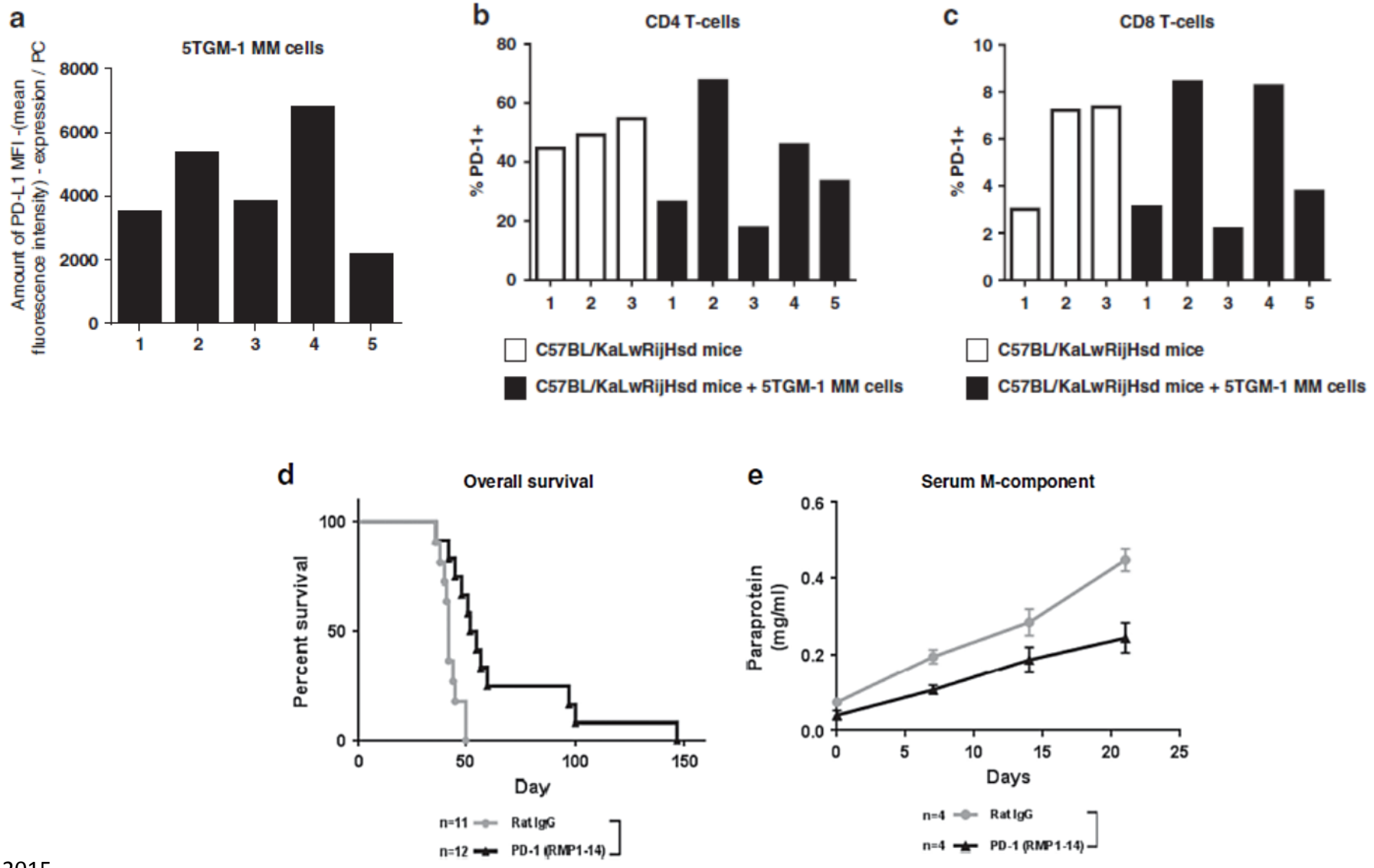
arget	Antibody	Mechanisms of action	Activity as mono-therapy	Activity/und evaluation i combo
./ AMF7	Elotuzumab (Humanized IgG1k)	• ADCC • Enhance NK activity • Interference with cell interaction	-	+ VD + Rd
38	Daratumumab (Fully human IgG1k)	• ADCC • CDC • ADCP • Direct induction of apopotosis • Modulation CD38 function	+	+ V-based + Rd + PomDex + VCD + Rd

Immune checkpoints in cancer



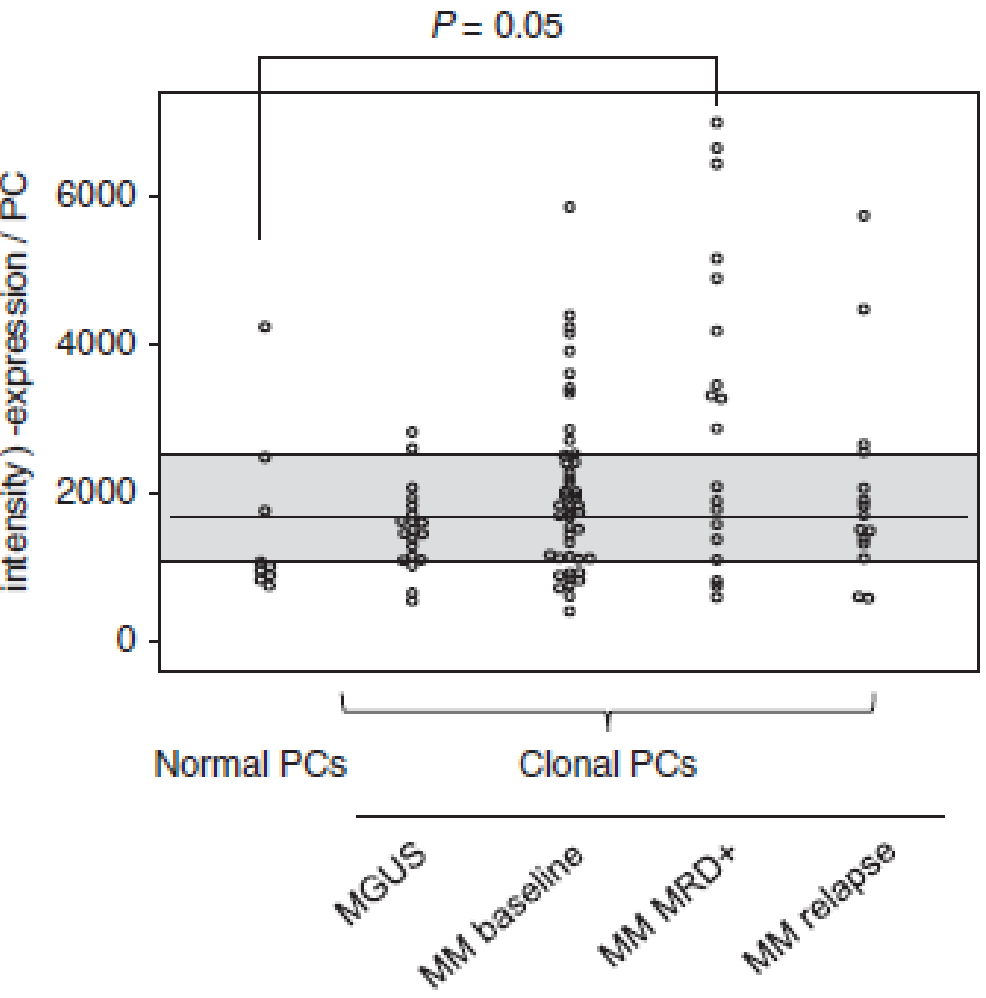
rom Catakovic K. et al, Cell Commun Signal 2017

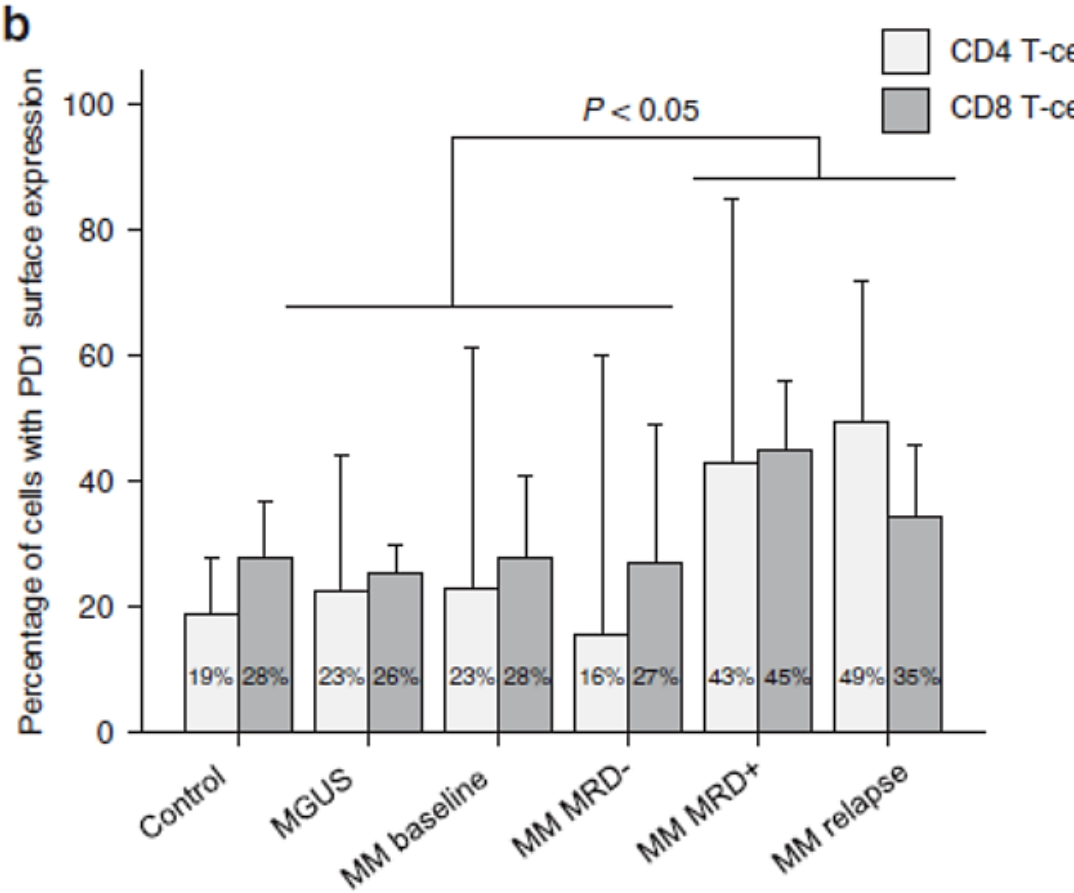
Blocking PD-1 prolonged survival in disseminated myeloma-bearing mice



al, Leukemia 2015

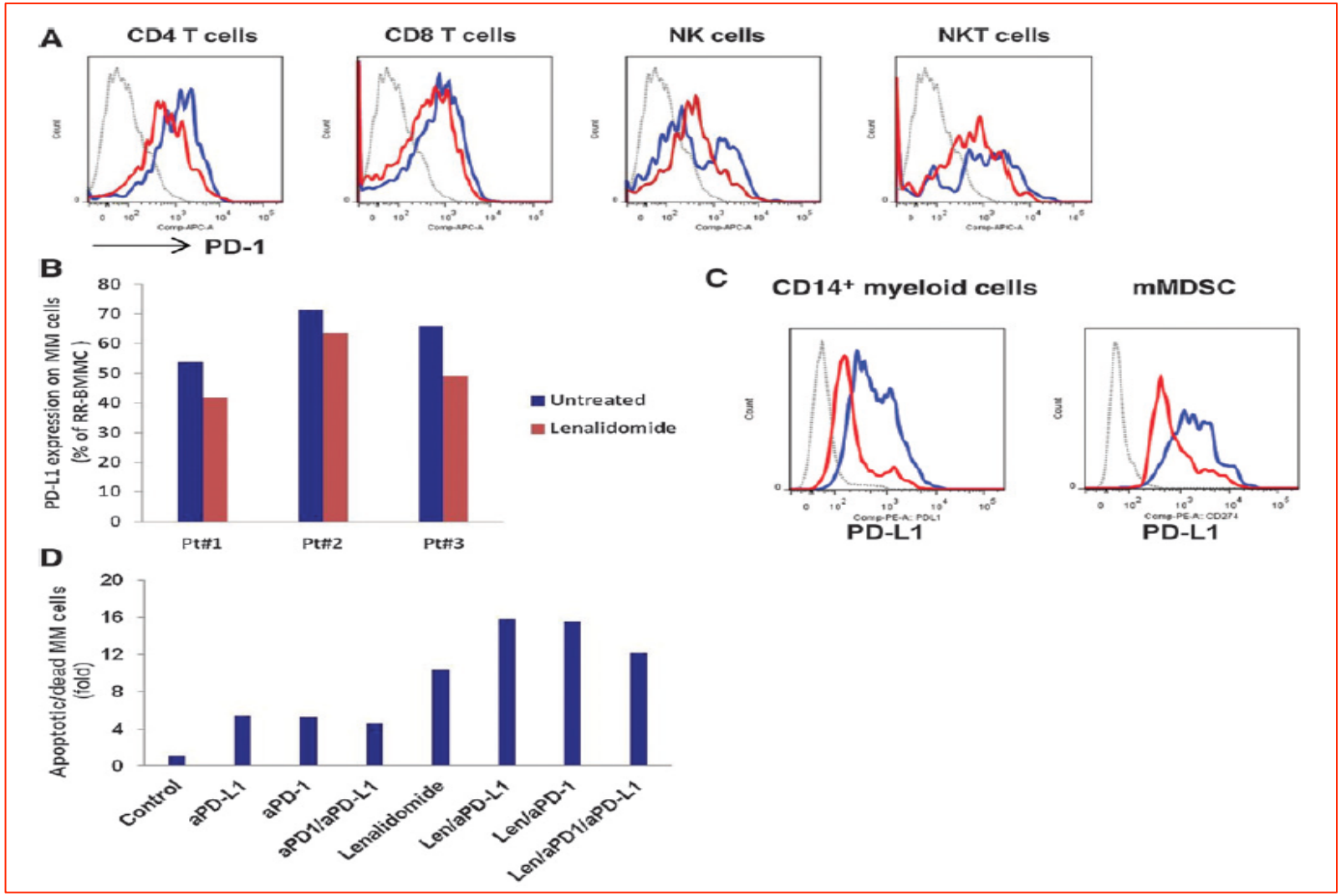
MRD positive MM patients: the best cohort for PD-1/PD-L1 blockade





al, Leukemia 2015

LEN enhances immune checkpoint blockade-induced immune response in MM



et al, Clin Cancer Res 2015

Lenalidomide and anti PD-1/PD-L1 antibodies combination: open clinical trials

Table 1: **MM, Multiple Myeloma; MDS,Myelodysplastic Syndrome; NHL, Non-Hodgkin's Lymphoma; FL, Follicular Lymphoma; PD-L1, Programmed Death Ligand-1**

Study	Therapy	Disease	Clinical trial	Status
A Study of Atezolizumab (Anti-Programmed Death Ligand 1 [PD-L1] Antibody) Administered With or Without Lenalidomide in Participants With Multiple Myeloma (MM)	Lenalidomide Atetolizumab	MM	NCT02431208	recruiting
A Study of Pembrolizumab (MK-3475) in Combination With Standard of Care Treatments in Participants With Multiple Myeloma (MK-3475-023/KEYNOTE-023)	Lenalidomide Pembrolizumab Dexamethasone	MM	NCT02036502	recruiting
Study of Lenalidomide and Dexamethasone With or Without Pembrolizumab (MK-3475) in Participants With Newly Diagnosed Treatment Naive Multiple Myeloma (MK-3475-185/KEYNOTE-185)	Lenalidomide Dexamethasone Pembrolizumab	MM	NCT02579863	recruiting
A Trial of Pembrolizumab (MK-3475) in Participants With Blood Cancers (MK-3475-013)(KEYNOTE-013)	Pembrolizumab Lenalidomide	MM NHL Lymphoma MDS	NCT01953692	recruiting
Phase 2 Multi-center Study of Anti-PD-1 During Lymphopenic State After HDT/ASCT for Multiple Myeloma	Lenalidomide Pembrolizumab	MM	NCT02331368	recruiting

. et al, Oncotarget 2017

ntitumor Activity
ntral Review (IMWG 2006)

Best Overall Response n (%)	Efficacy Population† (n = 40)	Len-Refractory (n = 29)
verall response rate	**20 (50)**	**11 (38)**
ringent complete response (sCR)	1 (3)	1 (3)
ry good partial response (VGPR)	5 (13)	3 (10)
rtial response (PR)	14 (35)	7 (24)
able disease (SD)	19 (48)	17 (59)
sease control rate (CR+PR+SD)	39 (98)	28 (97)
ogressive disease (PD)	1 (3)	1 (3)

ents NE by central review
ontinued within cycle 1 for reasons other than PD (2 no treatment assessments and 1 SD by investigator)
equate myeloma data for response assessment (5 PD and 3 SD by investigator)

Pembrolizumab-Pom-dex in RR Myeloma patients:

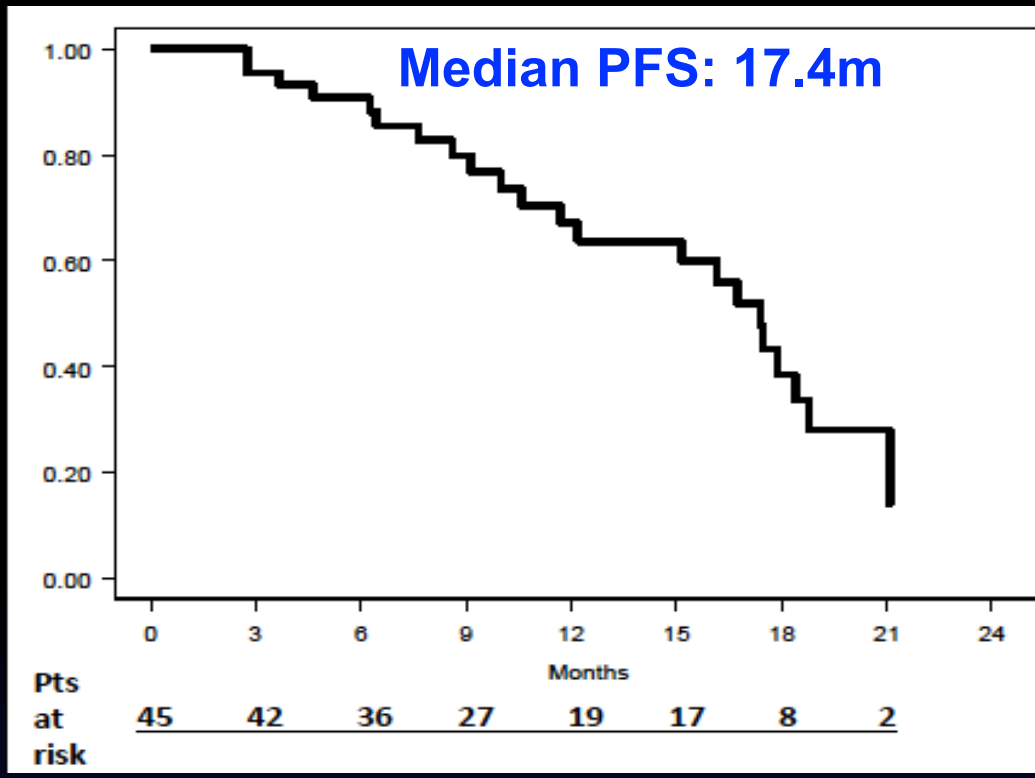
200 mg Q2W 4 mg (1-21) 40 mg QW

45pts refractoy a median of 3 prior lines; double refractory to PI&IMiD's 73%

Response category	Evaluable Patients (N=45)	Double refractory (N=32)
all response, n (%)	29 (65)	22 (68)
response, n (%)		
R	3 (7)	1 (3)
R	1 (2)	1 (3)
sPR	9 (20)	6 (18)
R	16 (36)	14 (44)
R	3 (7)	1 (3)
	11 (23)	7 (22)
	2 (5)	2 (4)

29%

24%



pts (12%) had G3-4 pneumonitis and 4 required discontinuation

Correlation between PD-L1 expression in PCs and ORR but no between PD-1&CD3 and ORR

Badros A, et al. Presented at ASH 2016 (Abstract 490), oral pres

Monoclonal antibodies in MM: a new era...

Target	mAb		Stage of development
Surface molecules			
SLAMF7 (CS1)	Elotuzumab	Humanized	Phase 1/2/3
CD38	Daratumumab	Fully human	Phase 1/2/3/4
	Isatuximab (SAR650984)	Chimeric	Phase 1/2
	MOR202	Fully human	Phase 1/2
CD138	Indatuximab ravtansine (BT062)		Phase 1/2
BCMA	J6M0-mcMMAF (GSK2857916)		Phase 1
Signaling molecules			
IL-6	Siltuximab		Phase 2
RANKL	Denosumab		Phase 3
VEGF	Bevacizumab		Phase 2
DKK1	BHQ880		Phase 2
Immune checkpoint inhibitors			
PD-1	Pembrolizumab		Phase 1/2/3
	Nivolumab		Phase 1/2
	Pidilizumab		Phase 1/2
PD-L1	Durvalumab, Atezolizumab		Phase 1
CTLA4	Ipilimumab		Phase 1/2
KIR	Lirilumab		Phase 1

et al, Blood 2015;
nk N.W. et al, Blood 2016

Grazie per l’ attenzione…..