IMiDs; multiple pathways but how do they really work?

Gareth Morgan

Royal Marsden Hospital and ICR

London

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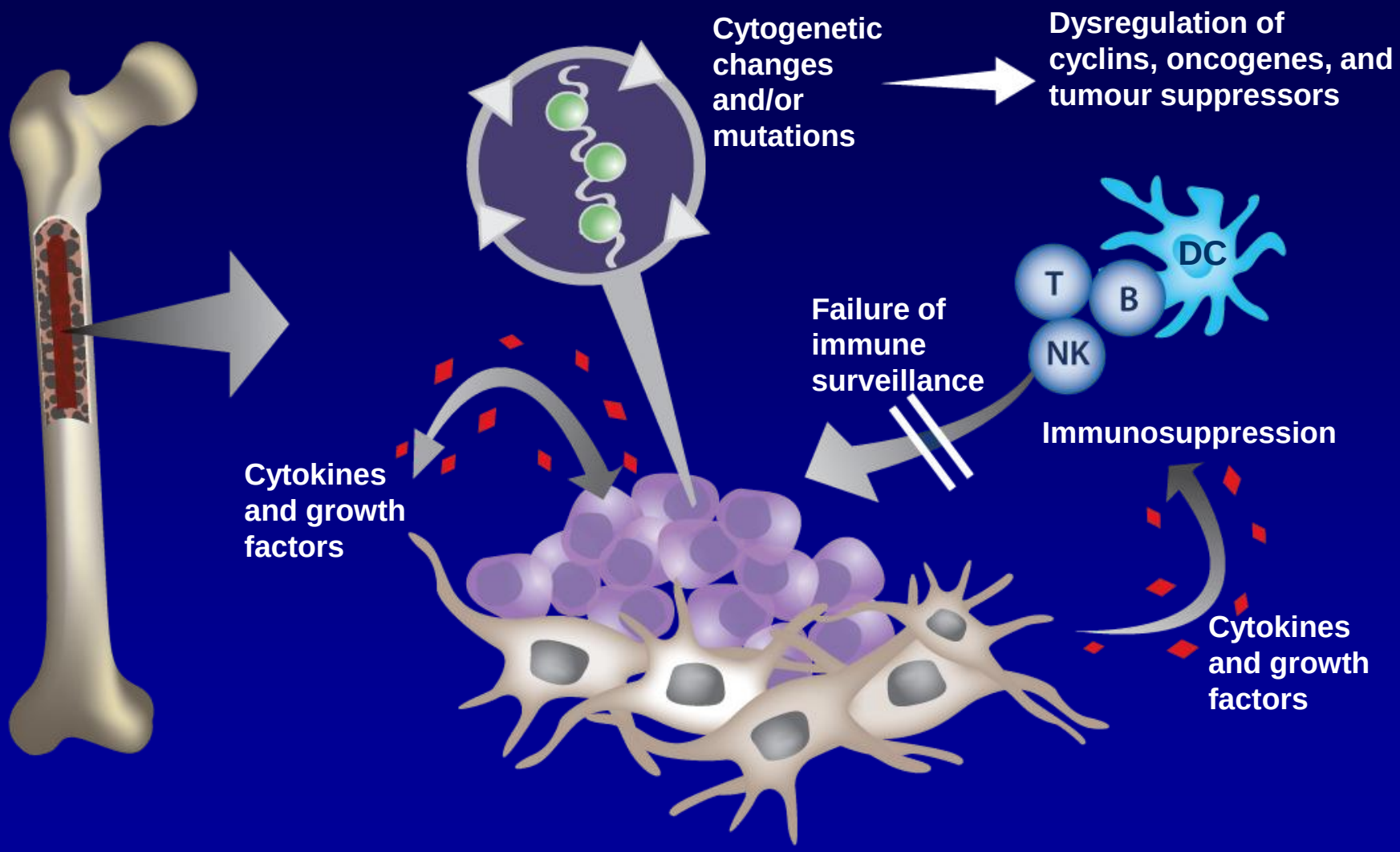
Outline

- Pathogenesis of myeloma
- IMiD drugs
- Mechanism of action of lenalidomide
  - Tumouricidal effects
    - Preclinical data
  - Immunomodulatory effects
    - Preclinical data
- New insights based on CRBN
- Conclusions

Multiple Myeloma Pathogenesis



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Cytogenetic Changes in MM Cells

FISH abnormality	Feature*	Frequency
Translocation		
IgH translocations (14q32)		40%
Cyclin D translocation		
t(11;14)	Upregulation of cyclin D1	16%
t(6;14)	Upregulation of cyclin D3	2%
MMSET translocation		
t(4;14)[a]		15%
MAF translocation	Upregulation of FGFR3 and MMSET	
t(14;16)[a]		3%
t(14;20)[a]	Dysregulation of c-maf	1%
Other translocations		
t(8;14)	Overexpression of c-myc	1%
Deletion		
del(13q)[a]	Loss of Rb1, NBEA	50%
del(17p)[a]	Loss of p53	10%
Hyperdiploid	Favorable prognosis	45%
1q and 1p abnormality	Unfavorable prognosis	

[a]high risk mutation/abnormality.

1. Fonesca R, et al. *Leukemia*. 2009;[Epub].
2. Pratt G, et al. *Mol Path.* 2002;55(5):273-83.
3. Stewart AK, et al. *Leukemia*. 2007;21:529-534.

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Long-Term Treatment Strategies are Needed

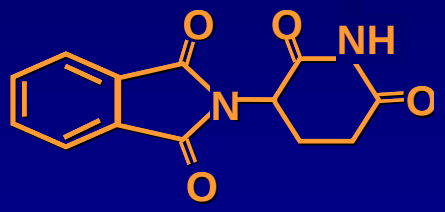
- Multiple myeloma is a chronic disease characterized by regrowth of residual tumour and immune suppression, thus it requires a long-term treatment strategy
  - The majority of patients with MM will eventually relapse within 3 years regardless of therapy
  - Continued therapy until disease progression may help prolong PFS
- An ideal therapy should target both tumour growth and concomitant immunosuppression while being an orally-administered drug that is well tolerated during long-term use

PFS, progression-free survival.

1. Attal M, et al. *Blood.* 2006; 108: 3289-3294.

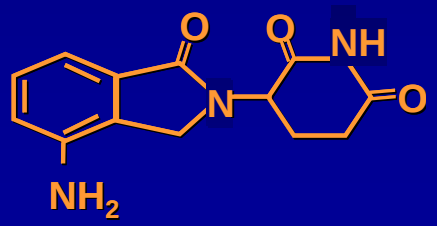
IMiD Drugs

- Lenalidomide and pomalidomide represent a novel class of immunodulatory agents known as IMiDs®
- They are structurally related to thalidomide. All three agents are currently being used or being evaluated in the treatment of multiple myeloma
- Unlike chemotherapy, IMiDs bolster the immune response while also demonstrating tumouricidal activity



Thalidomide

IMiDs



Lenalidomide

Pomalidomide

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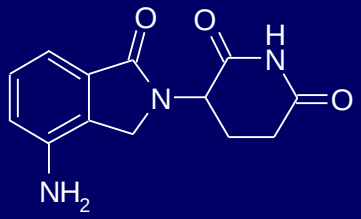
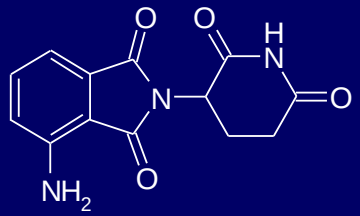
1. Chanan-Khan A, et al. *J Clin Oncol*. 2008;26:1544-1552.
2. Lenalidomide product insert. Celgene Corp. Summit, NJ. Jan 2009.

Thalidomide, Lenalidomide and Pomalidomide

IMiDs





EC_{50} (μM)	Thalidomide	Lenalidomide	Pomalidomide
Tumoricidal (MM.1S)	>100	**0.1-1**	**0.01-0.1**
Immunomodulation (T cell IL-2)	>100	**0.15**	**0.010**
Anti-angiogenesis (human explant)	**0.17**	1.8	0.33
Plasma Cmax	6.8	2.2	0.19

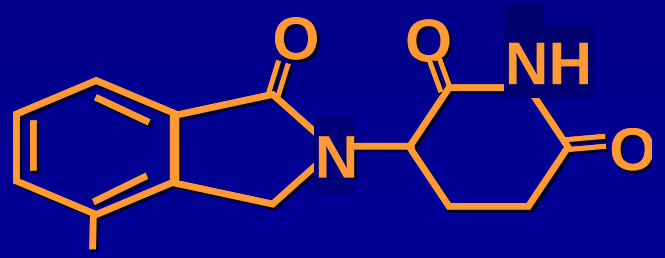
List AF, et al. Semin Oncol. 2005;32 Suppl 5:S31-5.
Teo ST, et al. Drug Discovery Today. 2005;10:107-14.
Chen N, et al. J Clin Pharmacol. 2007. 47(12):1466-75

Hideshima et al. Blood 2000
Corral et al. J. Immunol 1999
Lu et al. Microvascular Research 2009

Lenalidomide

- Lenalidomide is an oral IMiD® with a mechanism of action that consists of dual effects:
  - Tumouricidal: treatment leads to direct tumour cell death
  - Immunomodulatory: treatment improves the immune system

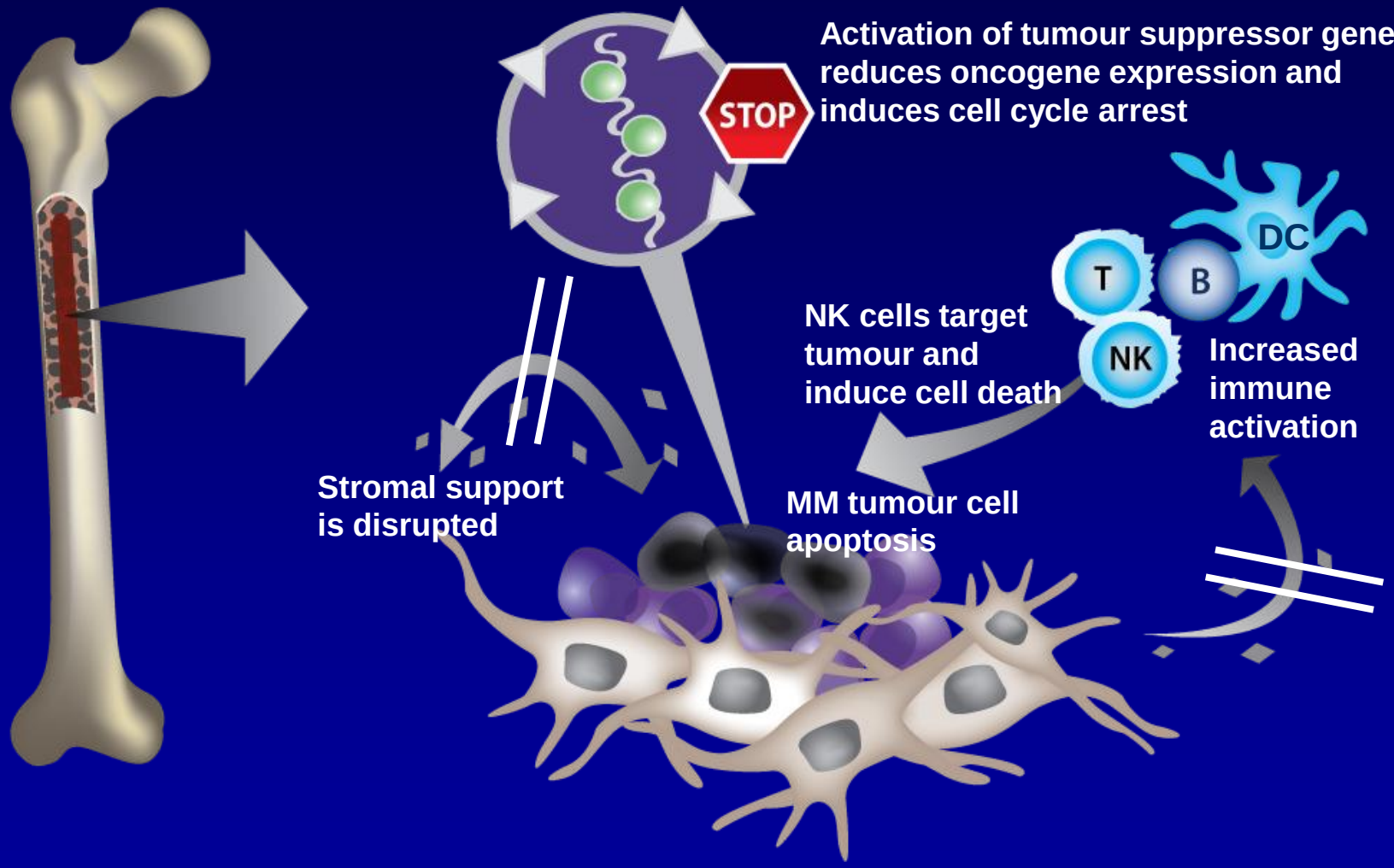
Lenalidomide is currently indicated in combination with dexamethasone for the treatment of multiple myeloma (MM) patients who have received at least one prior therapy



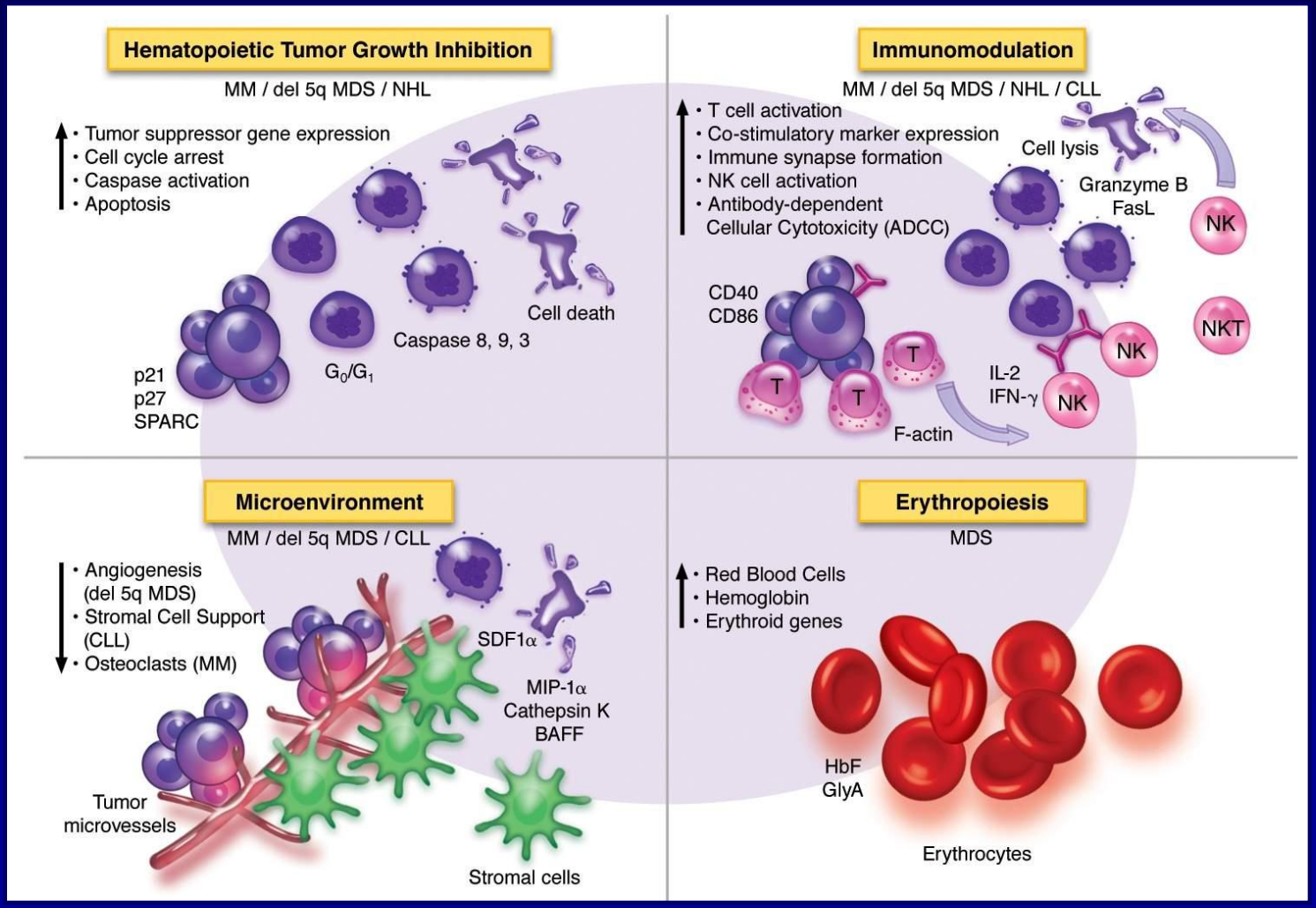
Lenalidomide

1. Chanan-Khan A, et al. *J Clin Oncol*. 2008;26:1544-1552.
2. Lenalidomide product insert. Celgene Corp. Summit, NJ. Jan 2009.

Lenalidomide Mechanism of Action



Lenalidomide activity in myeloma and other hematological malignancies



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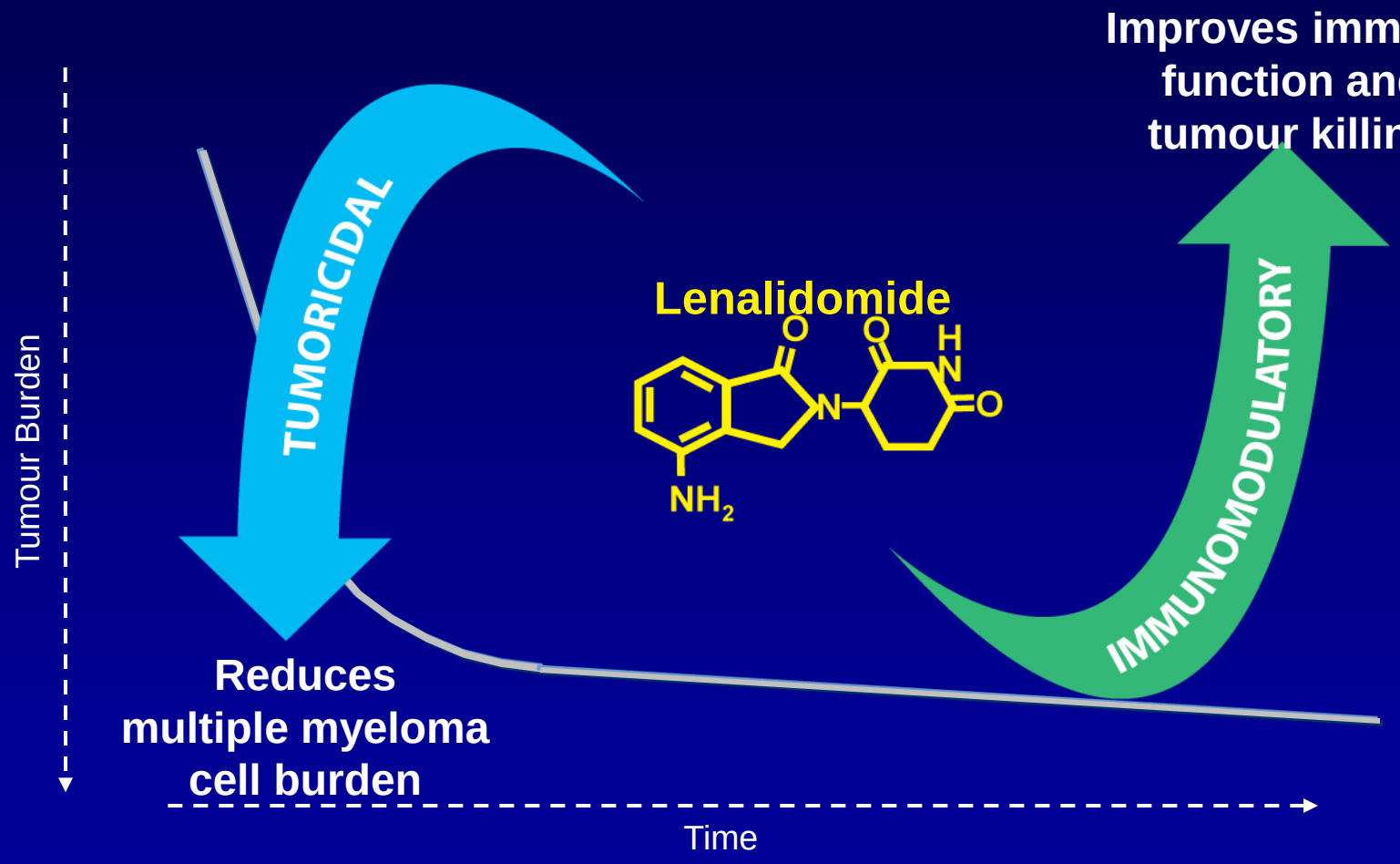
Heise et al., Expert Rev Anticancer Ther, 2010; 10: 1663

Tumouricidal and Immunomodulatory Effects of Lenalidomide



These dual effects make lenalidomide the optimal foundation therapy for the necessary long-term treatment of multiple myeloma

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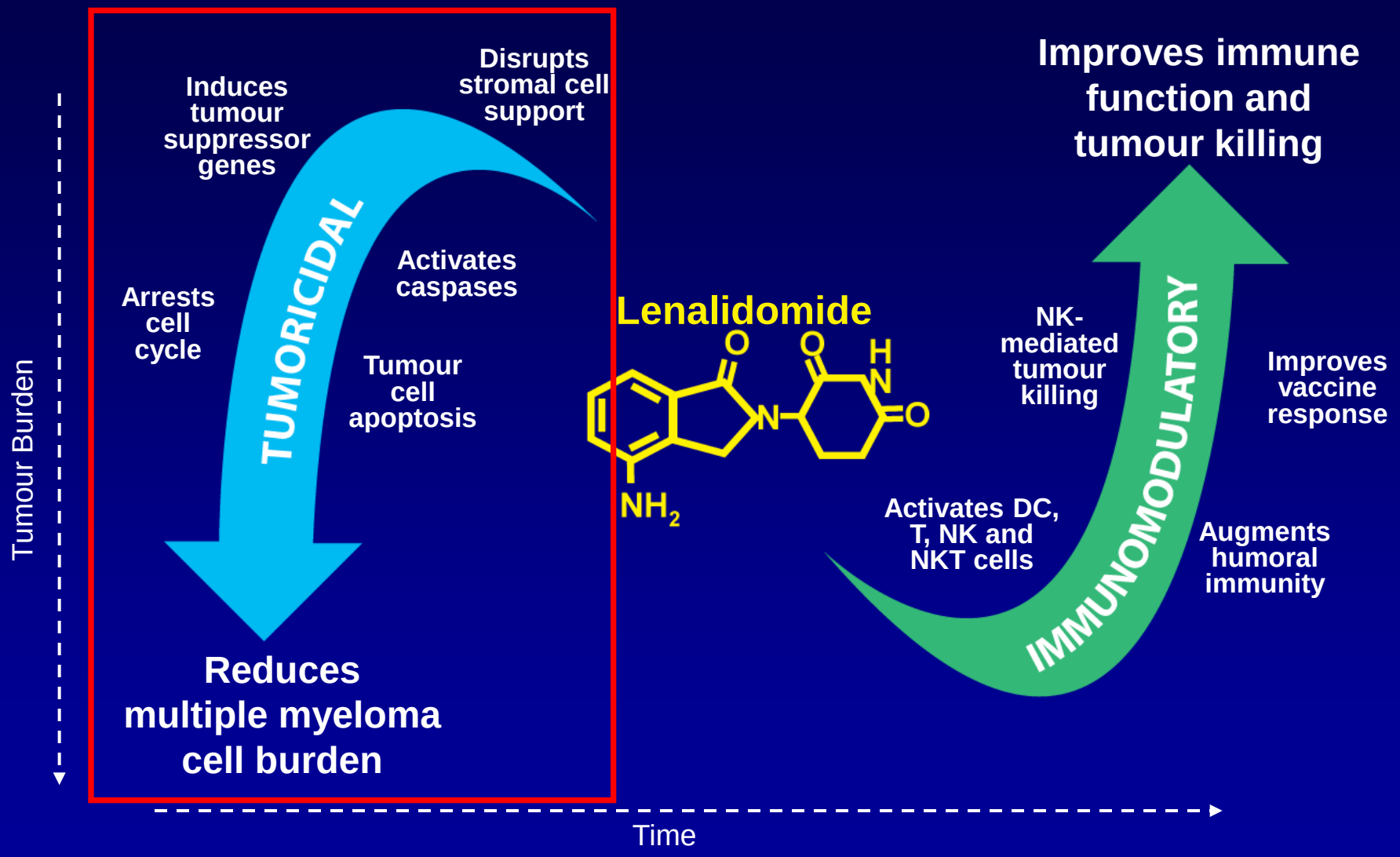
Lenalidomide Has Potent Tumouricidal Effects



These effects contribute to the rapid and high quality responses that are seen when treating multiple myeloma

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Tumouricidal effects are enhanced by targeting stromal support in MM

- MM cells interact directly with bone marrow stromal cells (BMSC) through receptors and cell adhesion molecules (e.g. ICAM) and indirectly through cytokines (eg. IL-6, VEGF).[1]
- These interactions result in increased production of VEGF by MM cells, thus stimulating IL-6 production by BMSC. These paracrine effects lead to increased MM proliferation and cell survival[2]
- IMiDs disrupt stromal support by decreasing cytokine production from MM and stromal cell interactions[3]

1. Raab MS, et al. Lancet. 2009;374(9686):324-339.
2. Gupta D, et al. Leukemia. 2001;15:1950-1961.
3. Hideshima T, et al. Semin Oncol. 2001;28(6):607-12.

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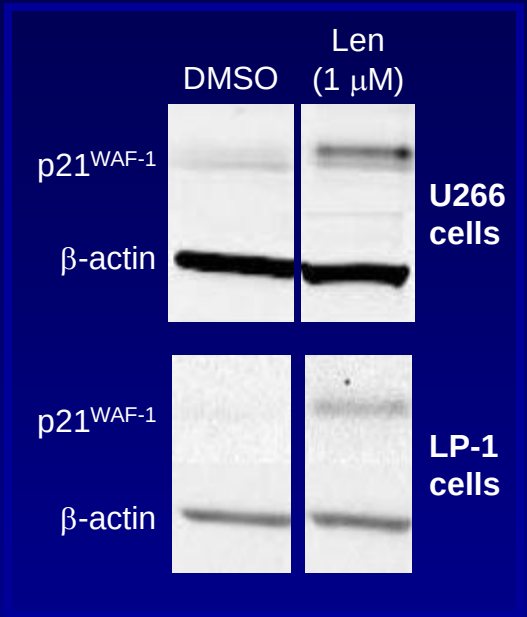
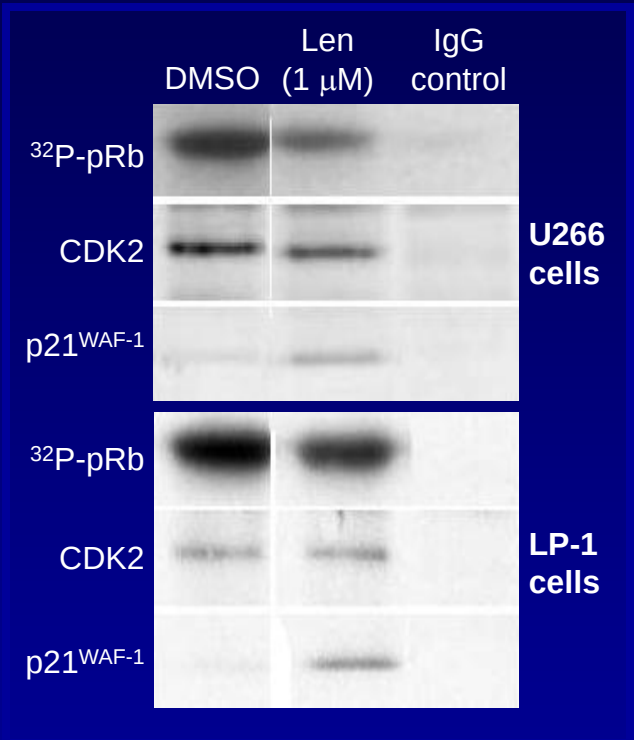
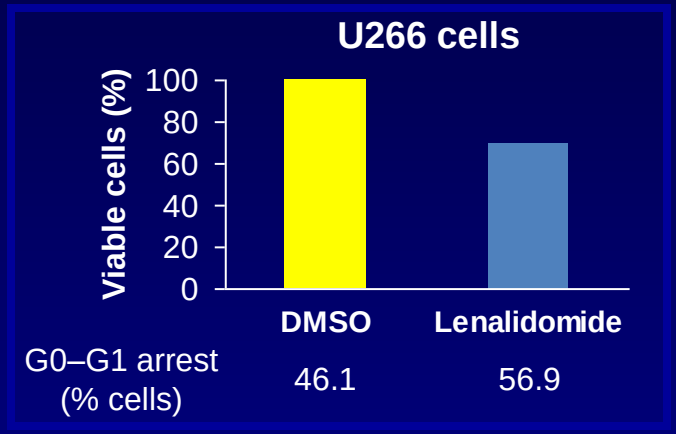
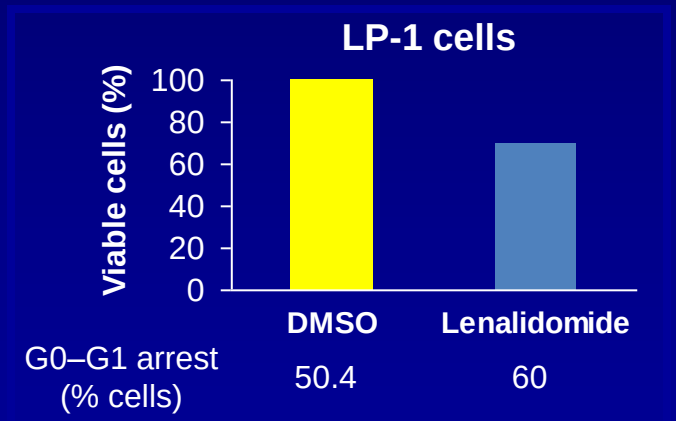
Lenalidomide Induces Cell-Cycle Arrest in MM Cells via p21 Increase and CDK2 and pRb Inhibition









Lenalidomide enhances p21 expression, CDK inhibition which leads to cell-cycle arrest and cell death

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Verhelle D, et al. *Cancer Res*. 2007;67:746-55.

CDK2 = cyclin-dependent kinase 2; DMSO = dimethyl sulfoxide; Len = lenalidomide: pRb = retinoblastoma protein.

Lenalidomide Induces Transcription of Tumour-Suppressor Genes in Myeloma Cells

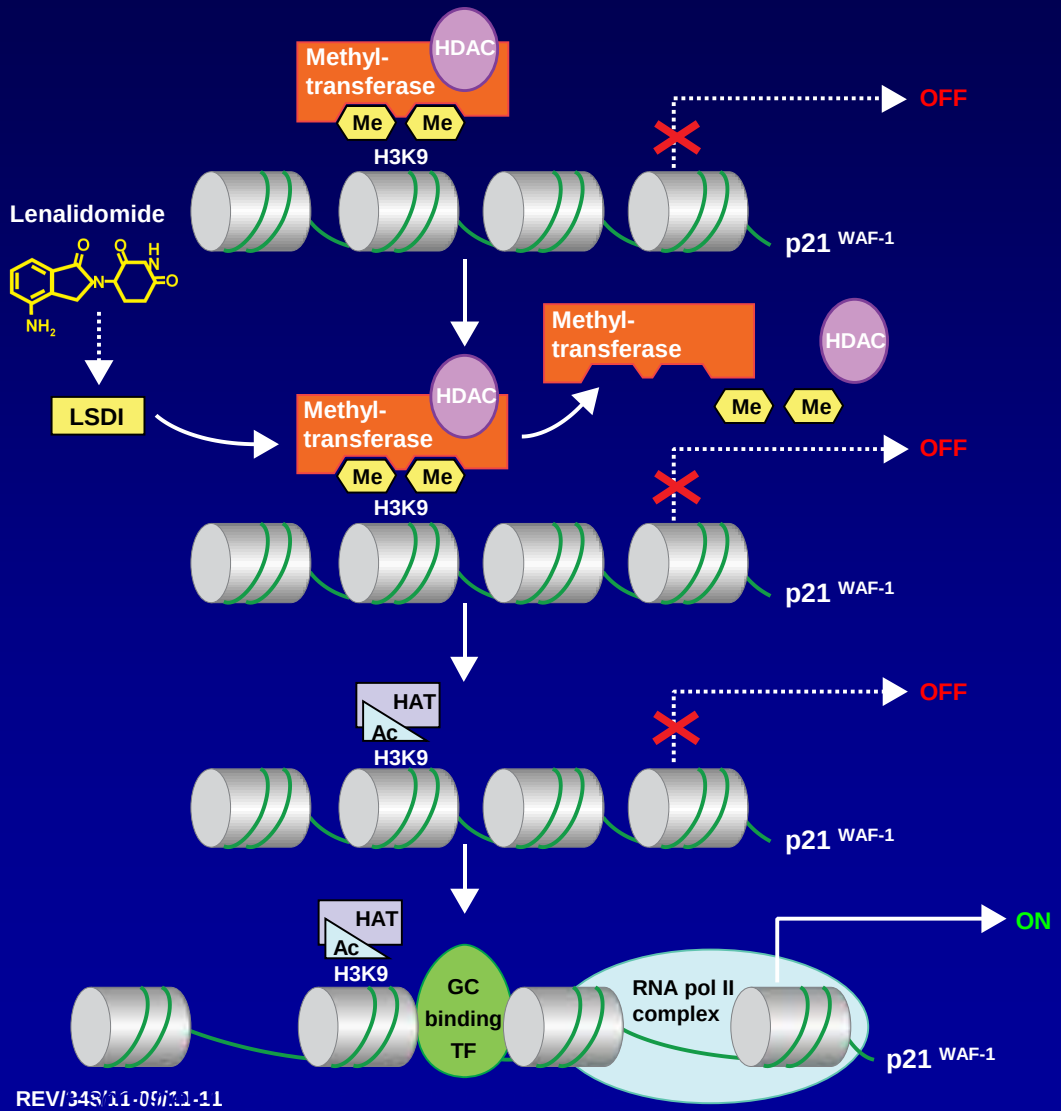
MM Cell Line	Time (hr)	Lenalidomide	Dex	Combination
KARPAS-620	6	Egr3	-	Egr1[a], p15[a]
	24	Egr1, p15, p21	-	Egr1[a], Egr3[a], p15, p21
NCI-H929	6	Egr2, Egr3	Egr1, p15	Egr1, p15, p21[a]
	24	-	-	Egr3[a], p27[a]
LP-1	6	Egr2, Egr3	p21	p21[a]
	24	p21	p21	p21[a]
U266B1	6	p21	-	p21[a]
	24	p21	-	p21
JJN-3	6	-	Egr1	Egr1[a]
	24	Egr3, p15	Egr1	Egr1[a], Egr2[a], Egr3[a], p21[a]
RPMI-8226	6	Egr3	p21	Egr2[a], p21[a], p27[a]
	24	-	-	-

[a]Synergistic combination

- Induction of tumour suppressor genes contributes to cell cycle arrest of tumour cells

1. Schafer et al. 2008. *ASH Abstract #2761.* American Hematology Society.

Lenalidomide Activation of LSD1 Mediates the Epigenetic Regulation of Tumour Suppressor Genes



- Lenalidomide upregulates p21 through a novel epigenetic mechanism of histone demethylation via LSD-1 activation
- Lenalidomide-induced upregulation of p21 can occur in p53-independent fashion which leads to inhibition of cell growth in p53 mutated or deleted tumours
- This epigenetic mechanism may play a role in the activation of other tumour suppressor genes by lenalidomide

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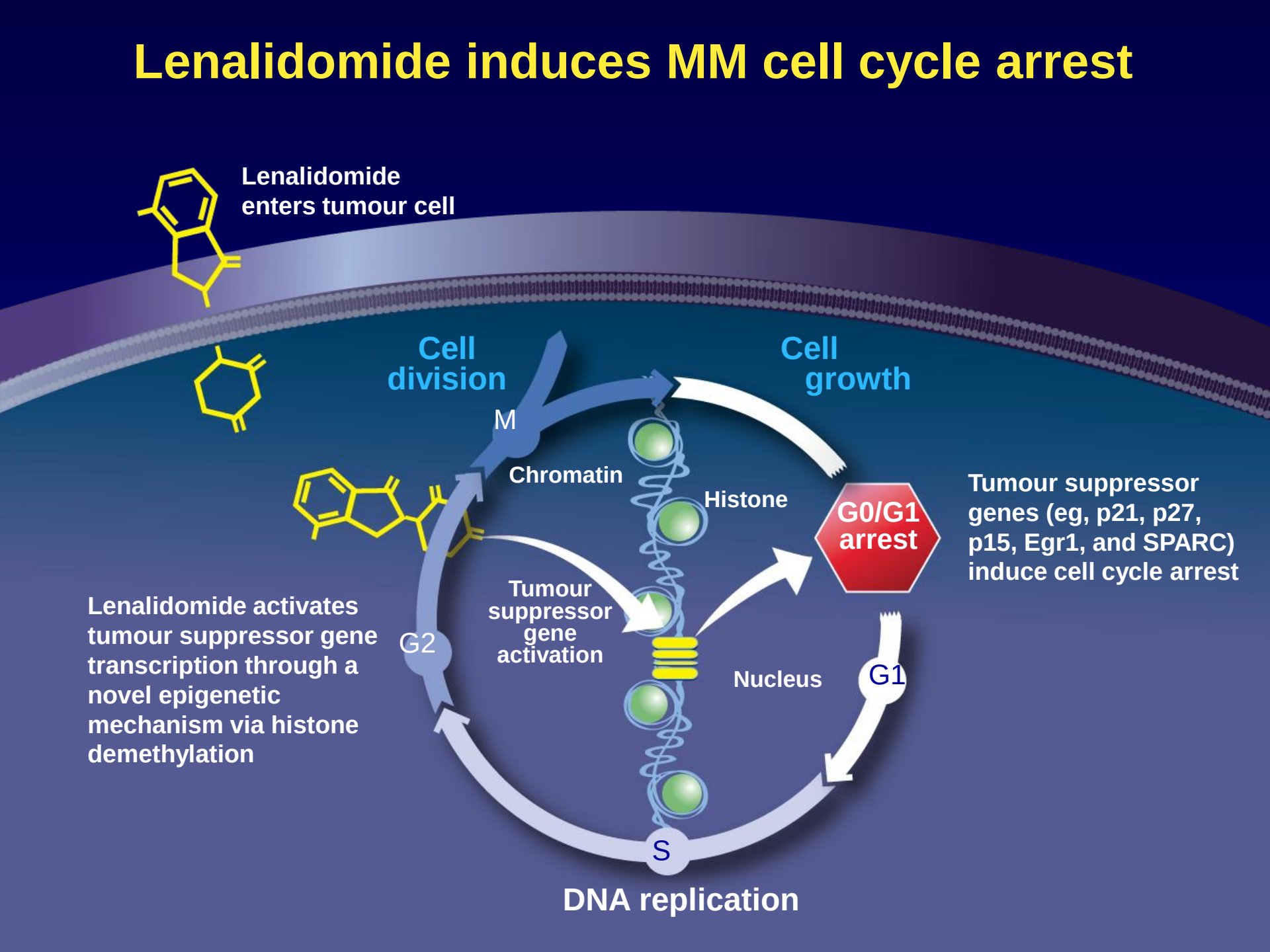
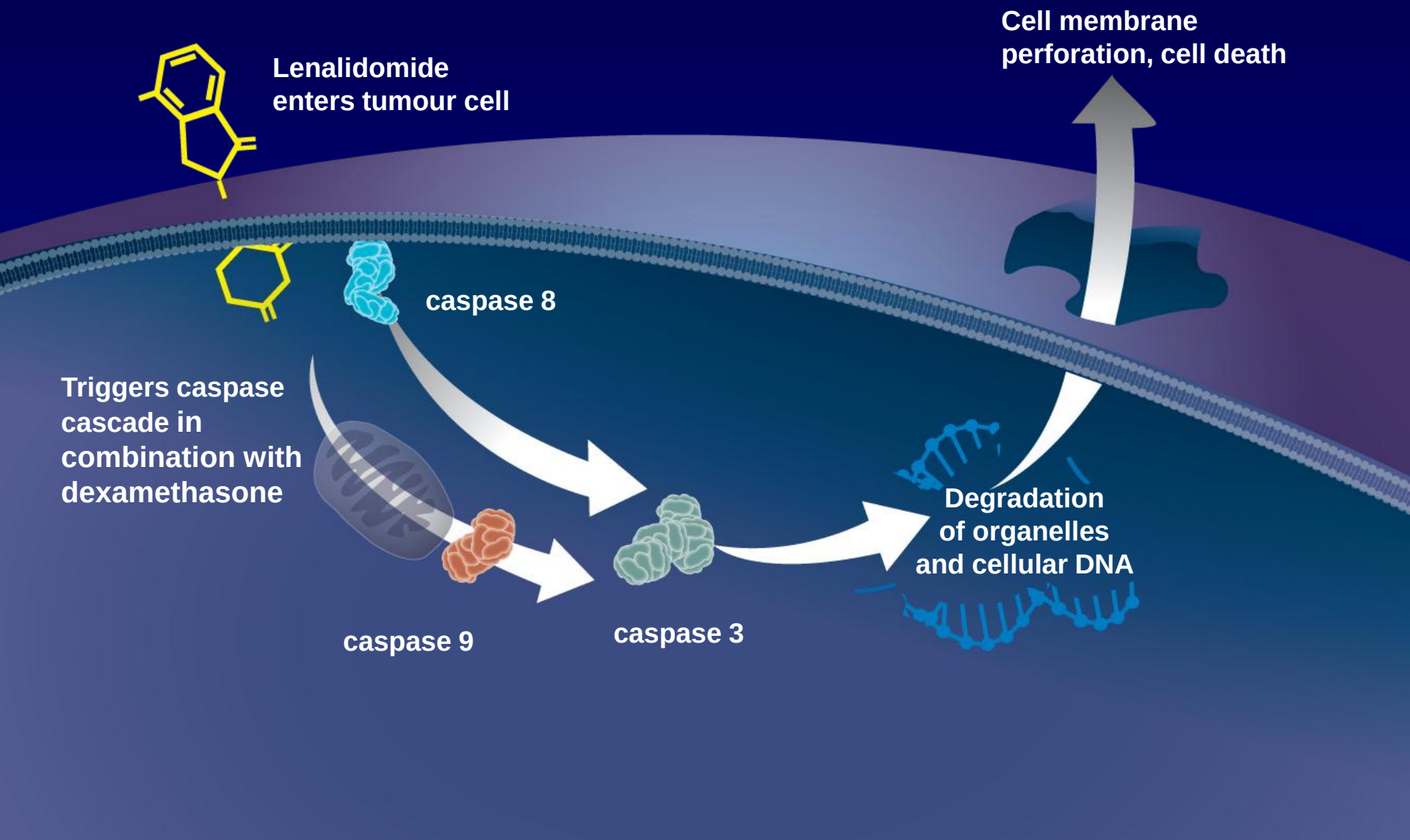
1. Escoubet-Lozach et al. *Cancer Res.* 2009;69(18):7347-56.

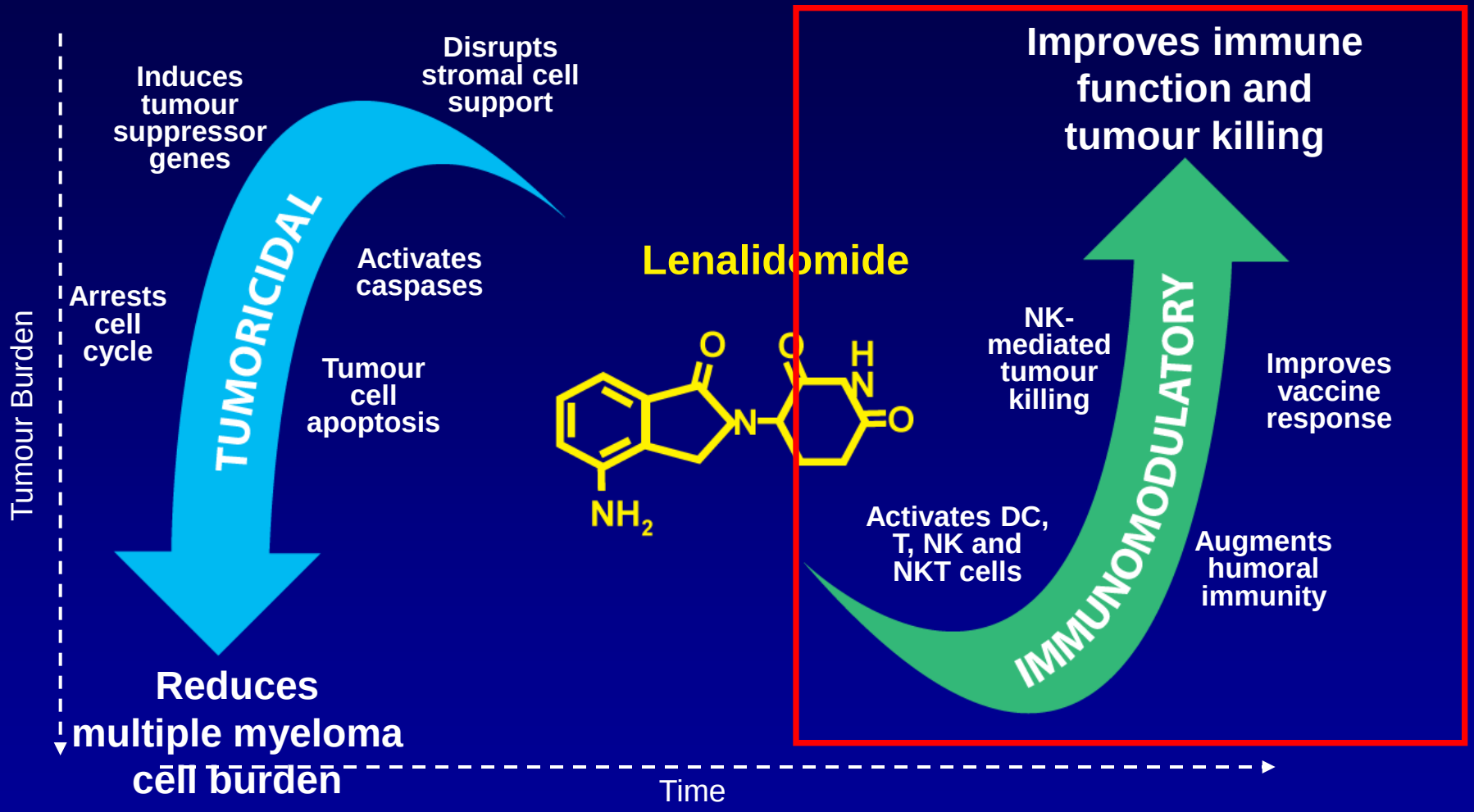
Lenalidomide induces MM cell cycle arrest



Lenalidomide Activates Caspases, Triggering Tumour Cell Apoptosis



Lenalidomide Has an Immunomodulatory Effect



Lenalidomide Immunomodulatory Activity

- Lenalidomide enhances antigen-specific $CD8^+$ T-cell cytolysis[1]
- Lenalidomide increases death effector molecules in NK cells[2]
  - Lenalidomide enhances antibody-induced NK-cell expression of the potential effector molecules granzyme B and FasL
- Lenalidomide enhances cytokine production and T-cell activation in patients with advanced cancer[3]

1. Haslett PAJ, et al. *J Infect Dis*. 2003;187:946-55.
2. Wu L, et al. *Clin Cancer Res*. 2008;14(14):4650-4657.
3. Bartlett JB, et al. *Br J Cancer*. 2004;90:955-61.

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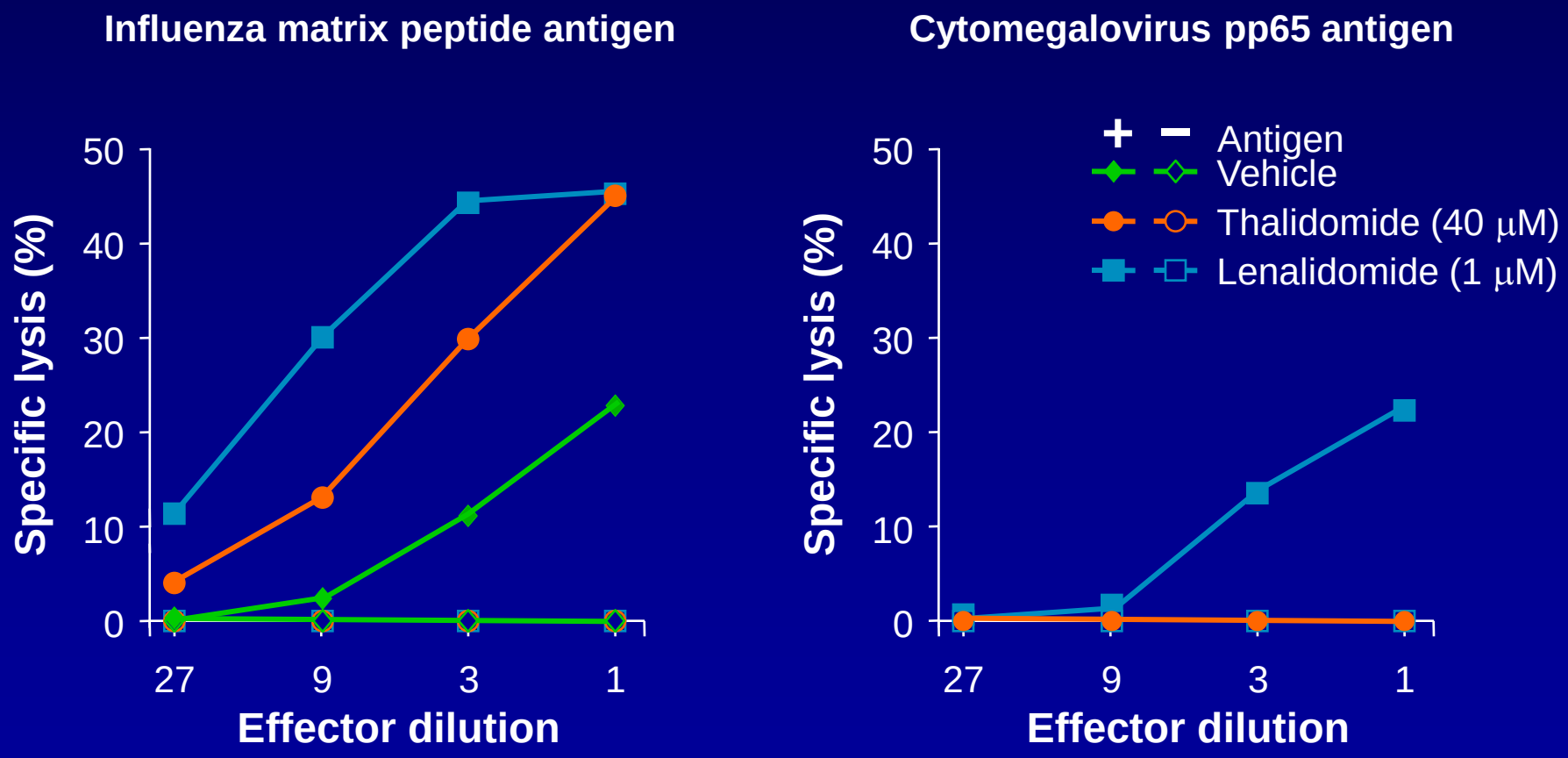
FasL, Fas ligand; NK, natural killer.

Lenalidomide Enhances Antigen-Specific CD8+ T-Cell Cytolysis

Cytolytic activity of CD8+ T-cells against autologous HLA-A*0201 dendritic cells pulsed with:



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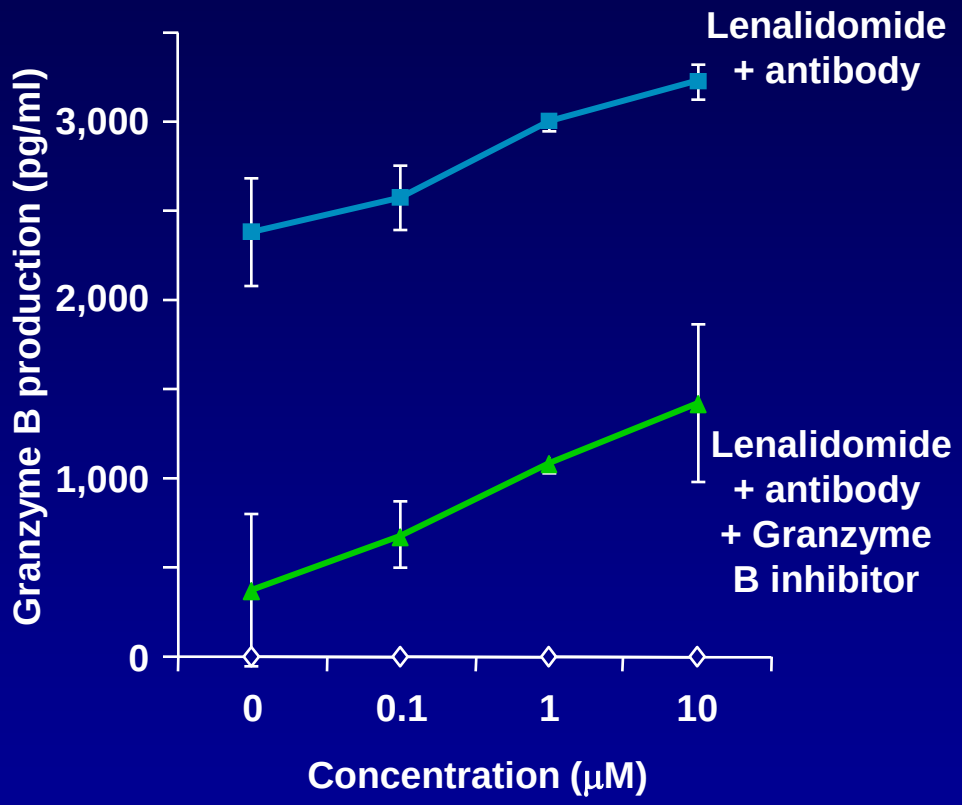
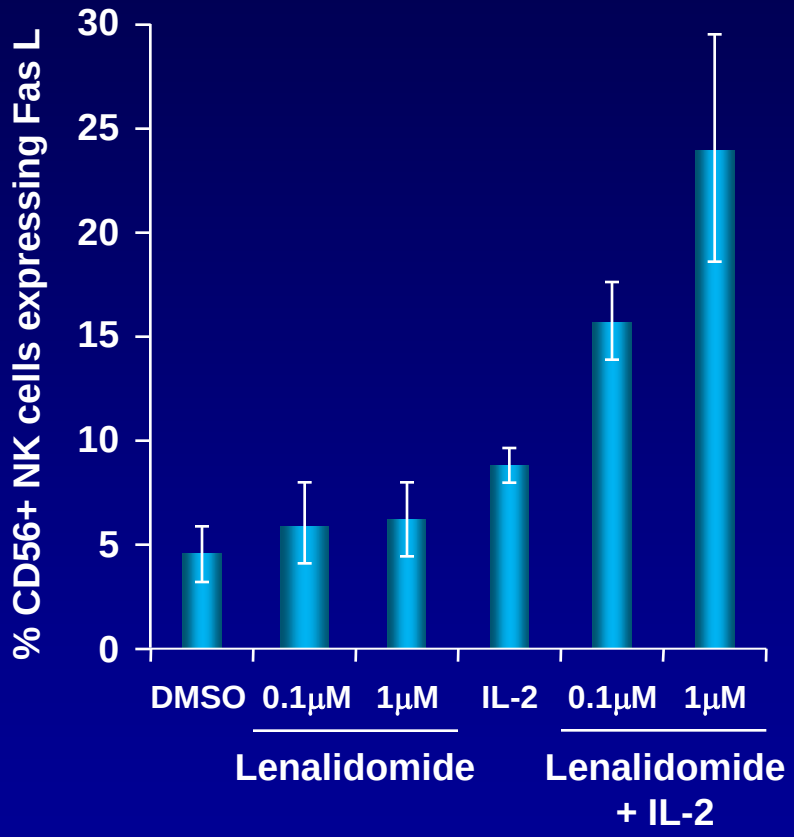
Haslett PAJ, et al. *J Infect Dis*. 2003;187:946-55.

Effect of Lenalidomide + Antibody on NK Cell Granzyme B Production & Fas L Expression





Lenalidomide enhances antibody-induced NK-cell expression of the potential effector molecules Granzyme B and Fas L

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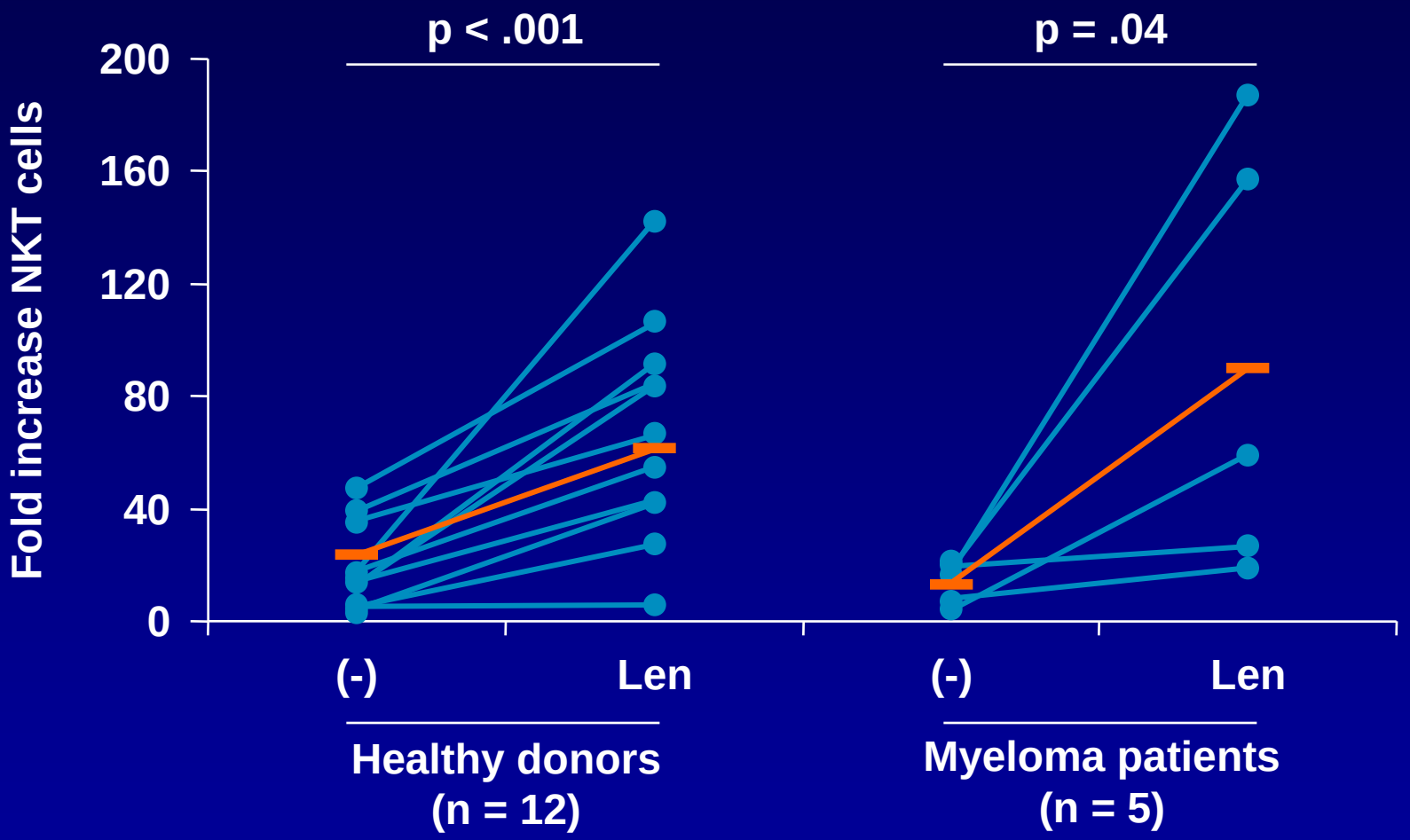
Wu L, et al. *Clin Cancer Res*. 2008;14(14):4650-4657.

Lenalidomide Boosts Expansion of NKT Cells from MM patients



Lenalidomide boosts expansion of NKT cells by DCs pulsed with -GalCer in both healthy donors and patients with myeloma

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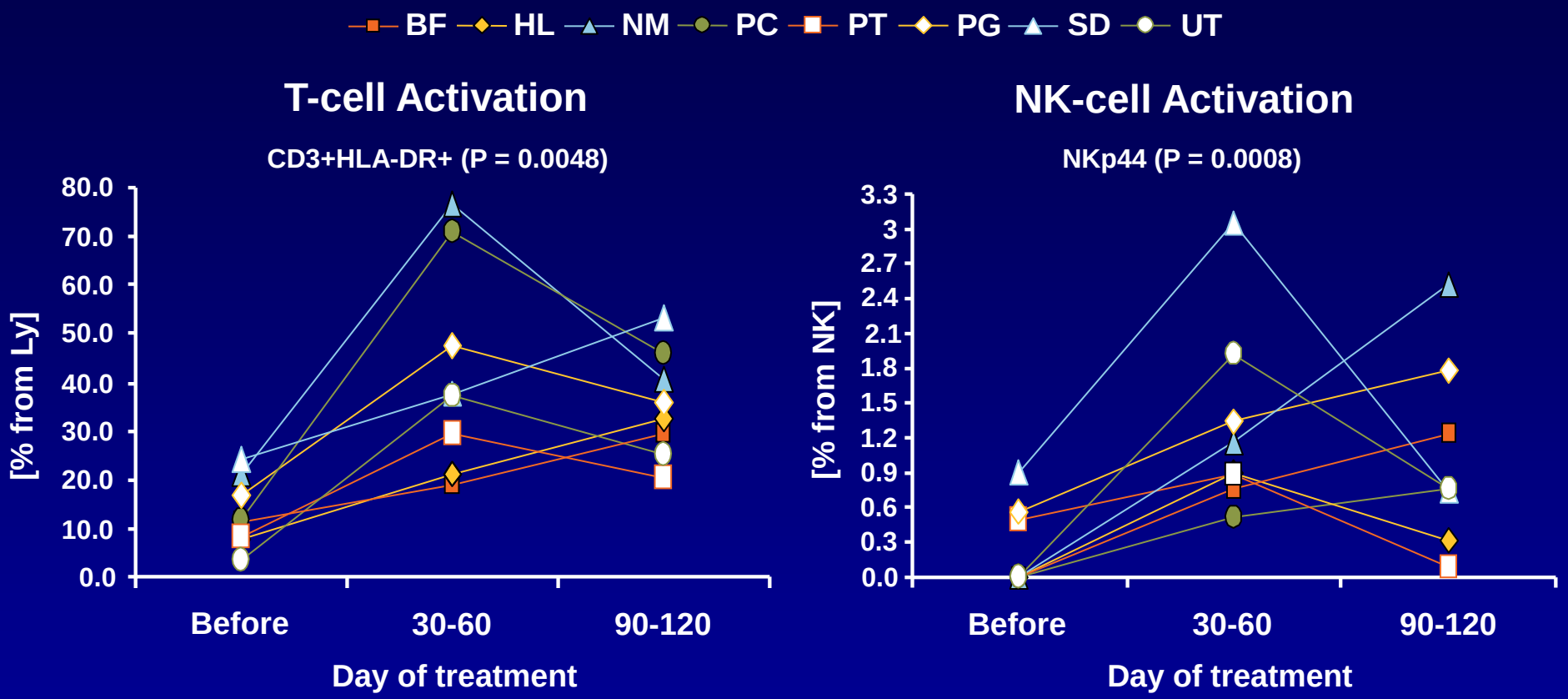
Chang, et al. *Blood*. 2006;18(2):618-621.

Lenalidomide Increases the Frequency of Activated T and NK Cells in Patients with Multiple Myeloma



- Patients received lenalidomide post-relapse following SCT
- Median number of 6 earlier chemotherapy regimens (range: 2-13)
- Immunomonitoring by flow cytometry before and after initiation of lenalidomide based therapy

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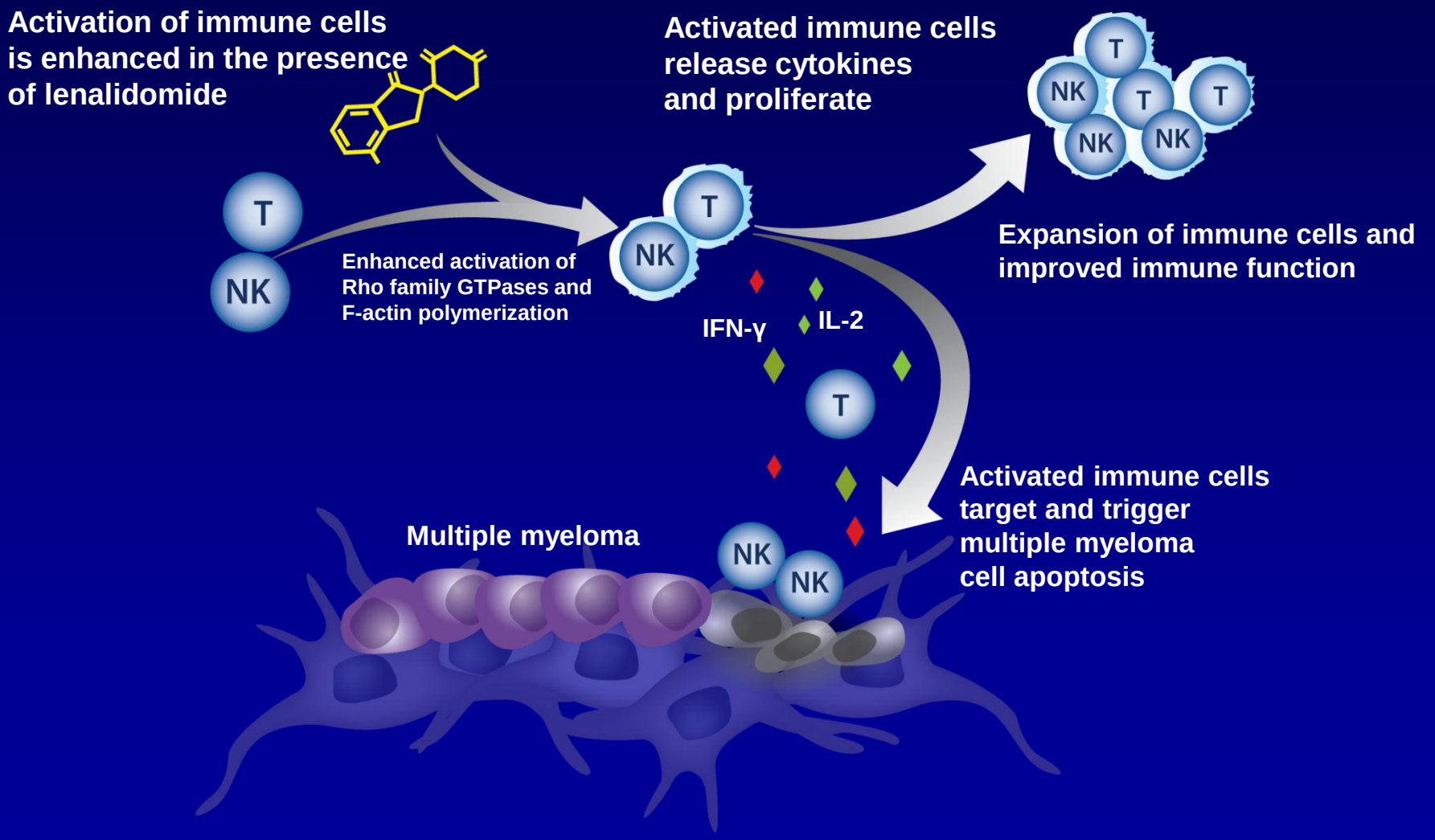
NK, natural killer; SCT, stem cell transplant.

1. Lioznov M, et al. *Bone Marrow Transpl.* doi: 10.1038/bmt.2009.155.

Lenalidomide Improves Immune Function and Facilitates Tumour Cell Killing



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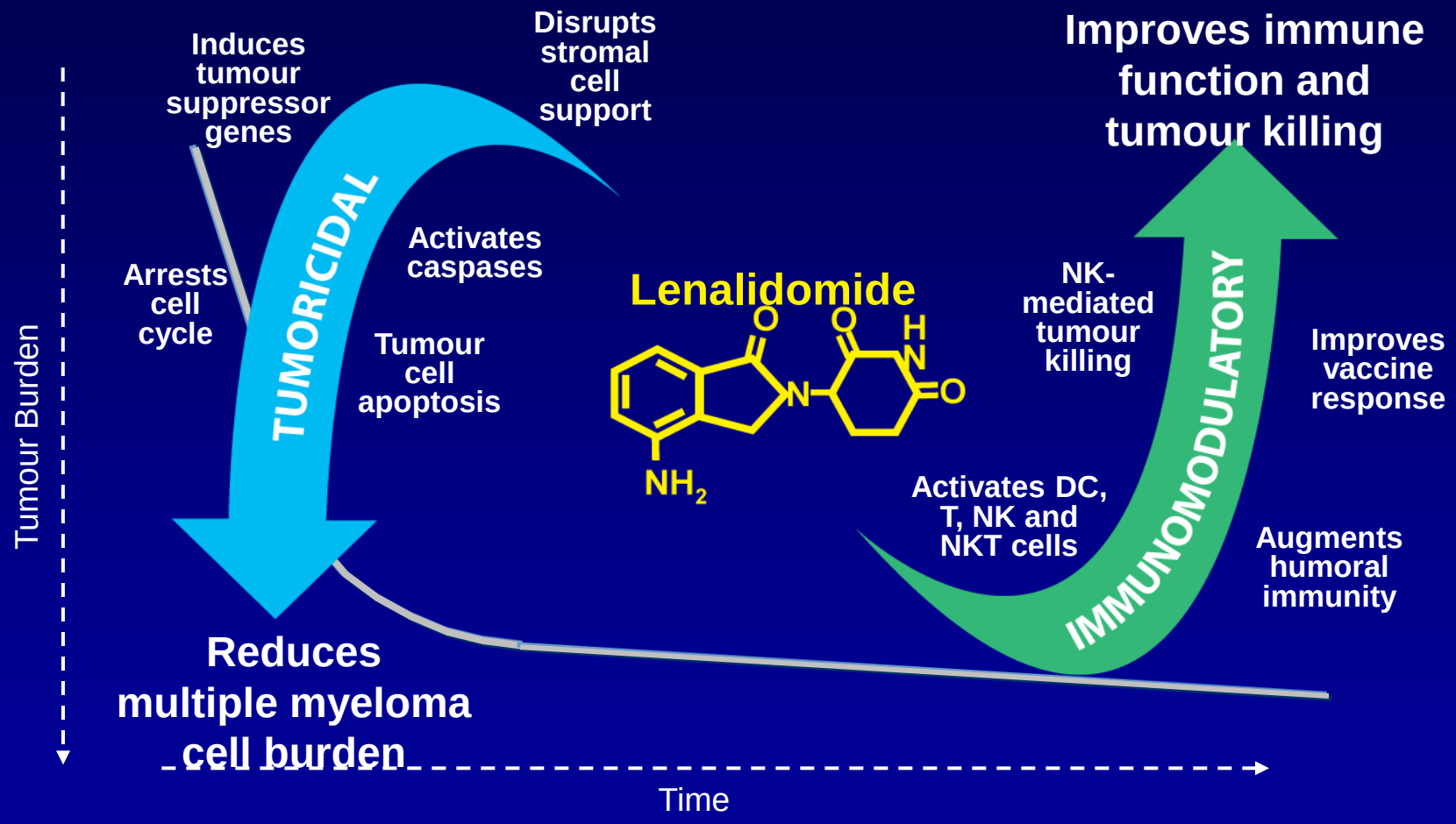
The Tumouricidal and Immunomodulatory Effects of Lenalidomide Induce Rapid and Sustained Responses



These dual effects make lenalidomide the optimal foundation therapy for the necessary long-term treatment of multiple myeloma

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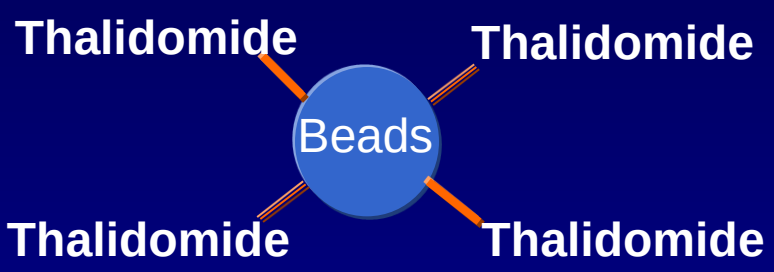
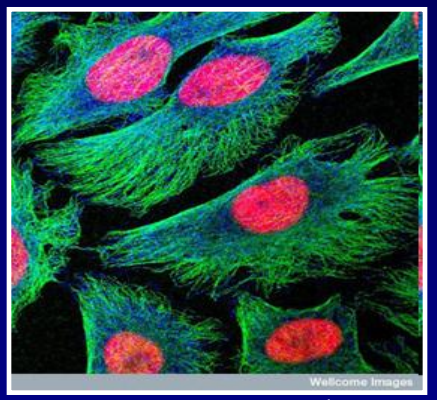
Cereblon Identified as a Thalidomide Binding Protein





binding to

- **Cereblon (CRBN)**
- **Damged DNA binding protein (DDB1)**

Ito T, et al. Science. 2010;327:1345-50.

Identification of Cereblon: Implications for IMiD MOA

**Takumi Ito, Hideki Ando, Takayuki Suzuki,
Toshihiko Ogura, Kentaro Hotta, Yoshimasa Imamura, Yuki Yamaguchi,
Hiroshi Handa**

- Half a century ago, thalidomide was found to be teratogenic, causing multiple birth defects…….Here, we identified cereblon (CRBN) as a thalidomide-binding protein.
- CRBN forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 (DDB1) and Cul4A that is important for limb outgrowth and expression of the fibroblast growth factor Fgf8 in zebrafish and chicks.
- Thalidomide initiates its teratogenic effects by binding to CRBN and inhibiting the associated ubiquitin ligase activity.

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Ito T, et al. Science. 2010;327:1345-50.

Cereblon (CRBN) and the Mode of Action of Thalidomide and IMiD Drugs

- Thalidomide binds to CRBN, a protein required for the teratogenic effects of Thal in zebrafish and chicken embryos[1]
  - CRBN forms an ubiquitin E3 ligase complex with DDB1, Cul4A, and Roc1
    - CRBN is ubiquitously expressed across cell types and is highly conserved across species
  - Thal treatment has been shown to inhibit the ubiquitin ligase activity of the complex
- Is CRBN required for immunomodulatory and antiproliferative responses of Len and Pom[2]

CRBN, cereblon; Cul4A, cullin 4A; DDB1, DNA damage-binding protein 1; Len, lenalidomide; Pom, pomalidomide; Roc1, regulator of cullins 1; Thal, thalidomide.

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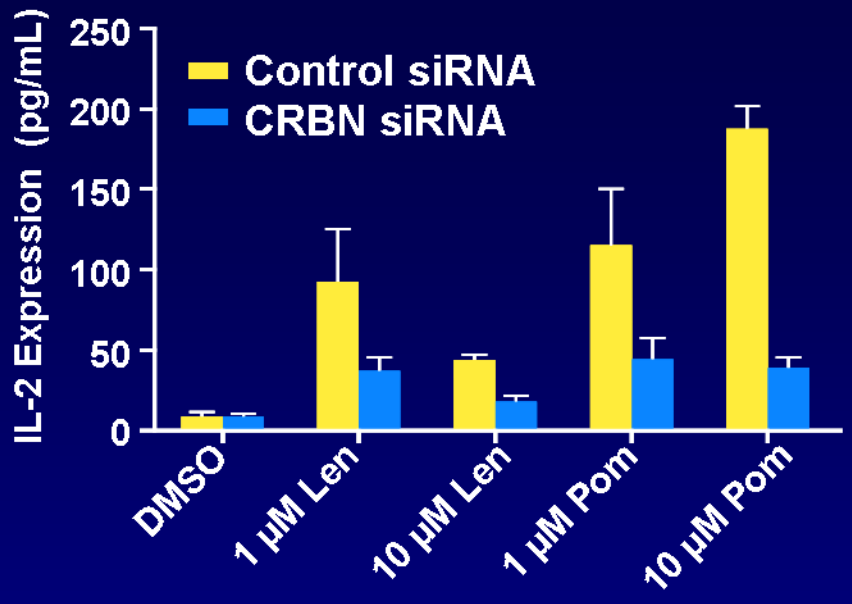
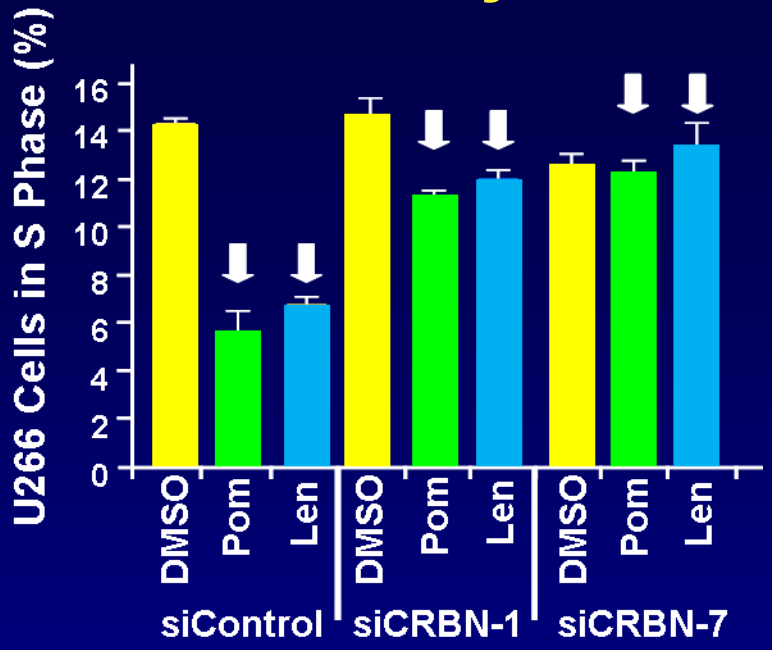
1. Ito T, et al. *Science.* 2010;327:1345-1350.
2. Lopez-Girona A, Mendy D, Miller K, et al. Direct binding with cereblon mediates the antiproliferative and immunomodulatory action of lenalidomide and pomalidomide. *Annual Meeting and Exposition of the American Society of Hematology.* 2011; December 10-13; San Diego, CA. Abstract 738.

CRBN Effects on Len and Pom Activity





- Len and Pom bind to CRBN via the agents' glutarimide moiety[a]

Knockdown of CRBN via siRNA had the following effects in:

- T cells: Abrogated T-cell induction of IL-2 by Len and Pom
- U266 MM cell line:
  - Decreased Len- and Pom-induced inhibition of cell-cycle progression
  - Attenuated Len- and Pom-mediated downregulation of the oncogene IRF4[a] and upregulation of the tumor suppressor gene p21[a]

[a] Data not shown.

CRBN, cereblon; DMSO, dimethyl sulfoxide; IL-2, interleukin-2; IRF4, interferon regulatory factor-4; Len, lenalidomide; Pom, pomalidomide; siRNA, small interfering RNA.

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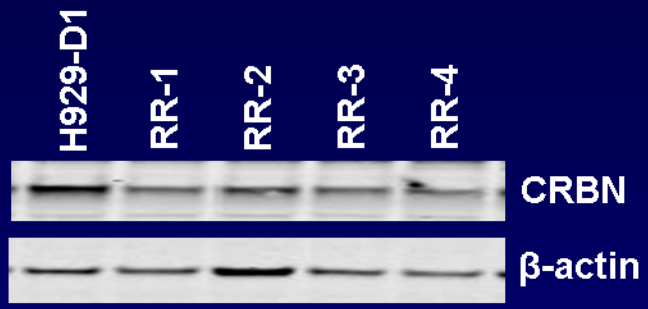
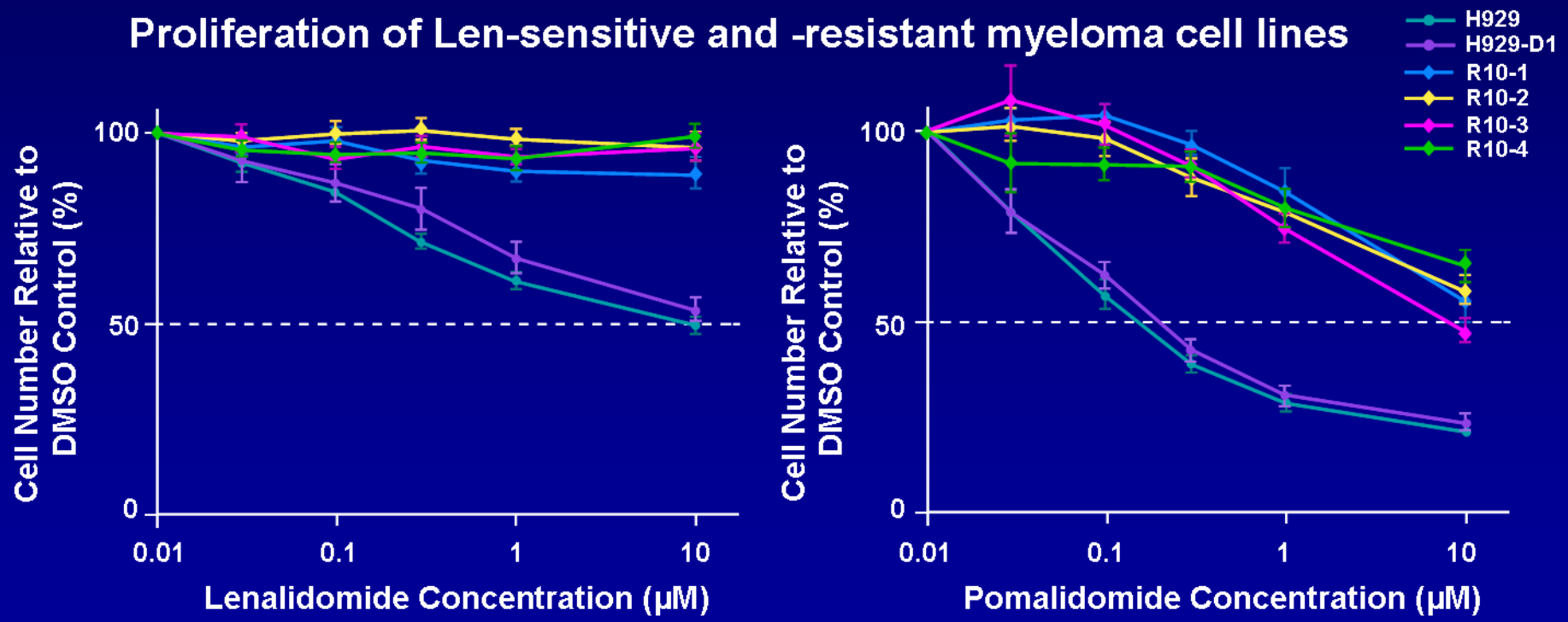
Lopez-Girona A, Mendy D, Miller K, et al. Direct binding with cereblon mediates the antiproliferative and immunomodulatory action of lenalidomide and pomalidomide. *Oral presented at: Annual Meeting and Exposition of the American Society of Hematology.* 2011; December 10-13; San Diego, CA.

Selection for Lenalidomide Resistance in MM Cell Lines Correlates With Reduced CRBN Expression



Lenalidomide-resistant myeloma cell lines demonstrate reduced levels of CRBN protein

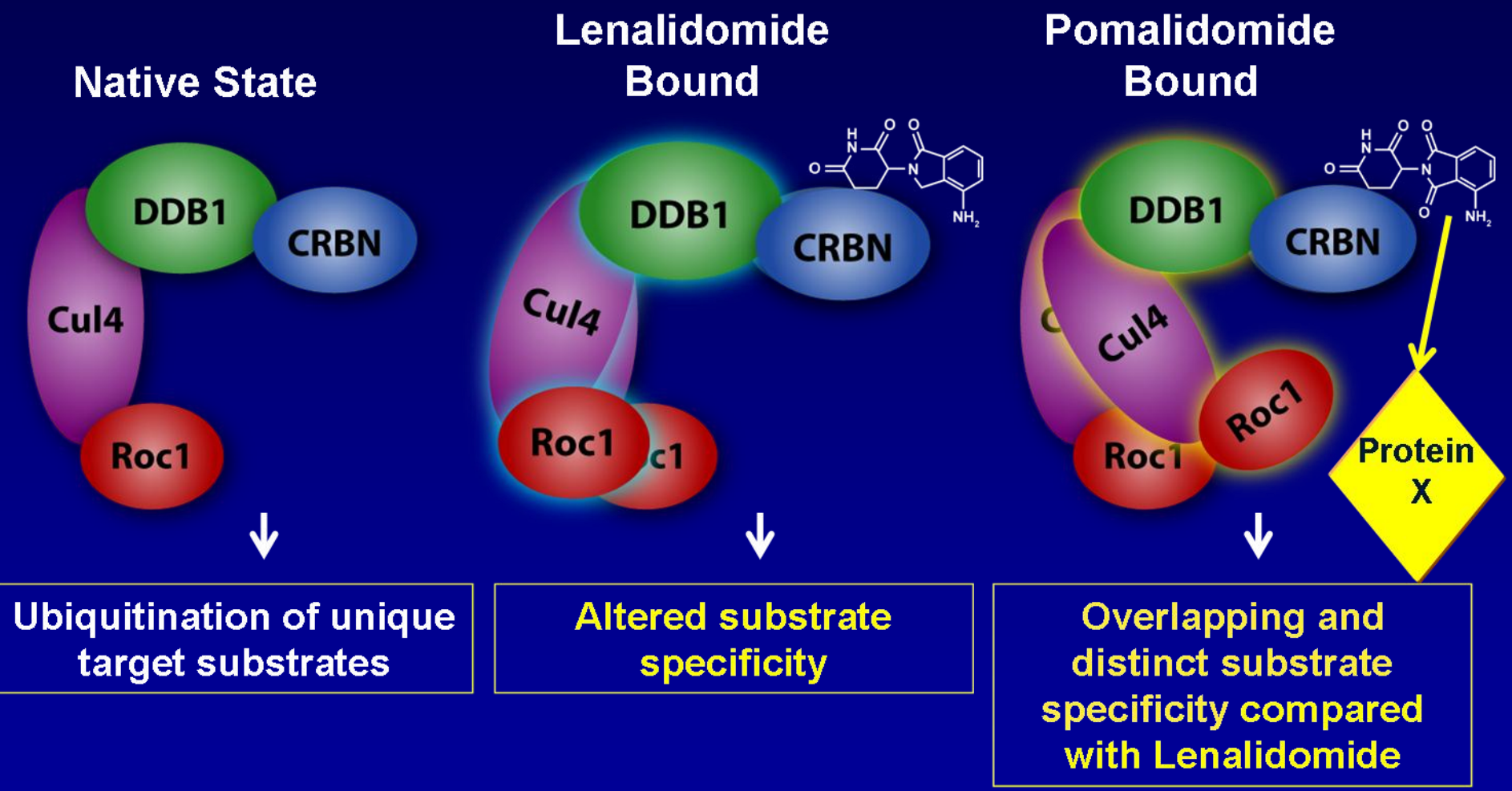


CRBN, cereblon; DMSO, dimethyl sulfoxide; Len, lenalidomide; MM, multiple myeloma.

REV/348/11-09/11-11

Lopez-Girona A, Mendy D, Miller K, et al. Direct binding with cereblon mediates the antiproliferative and immunomodulatory action of lenalidomide and pomalidomide. *Oral presented at: Annual Meeting and Exposition of the American Society of Hematology.* 2011; December 10-13; San Diego, CA.

Hypothesis: Differences in mode of action of lenalidomide and pomalidomide are due to differences in complex conformation which affects interactions with target substrates



Cul4, cullin-4; CRBN, cereblon; DDB1, damaged DNA binding protein 1; Roc1, regulator of cullins 1.

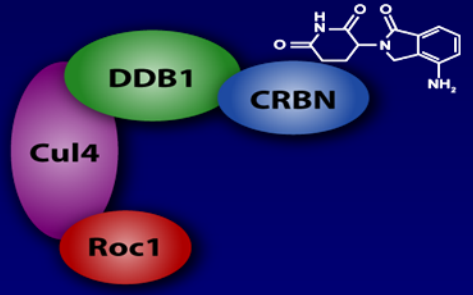
REV/348/11.09/16.11

Lopez-Girona A, Mendy D, Miller K, et al. Direct binding with cereblon mediates the antiproliferative and immunomodulatory action of lenalidomide and pomalidomide. *Oral presented at: Annual Meeting and Exposition of the American Society of Hematology.* 2011; December 10-13; San Diego, CA.

Downstream Consequencees of Binding Either Lenalidomide or Pomalidomide are Different

Proposed model

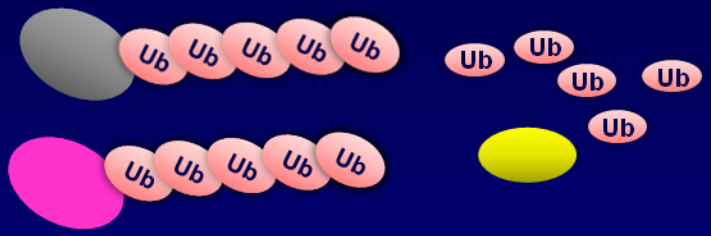
Direct binding of drug



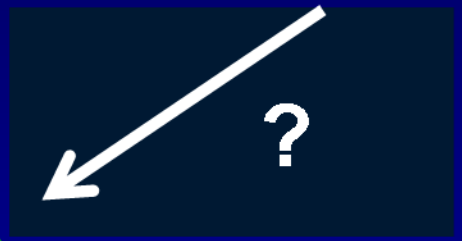
Modulation of ubiquitin ligase

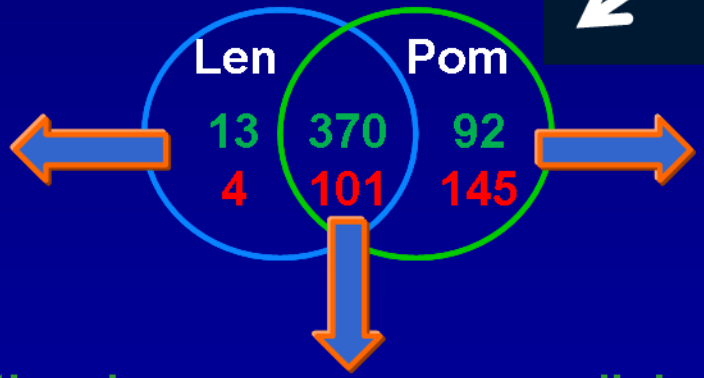
→

activity and/or substrates



GEP probes altered by Len and Pom[a]





Calcium ion sequestration

Mitosis / cell-cycle and DNA damage response

Antigen presentation, immune response, cell death, proliferation

Green — high expression; Red — low expression

[a] Values represent numbers of microarray probes, not numbers of genes.

Cul4, cullin 4; CRBN, cereblon; DDB1, DNA damage-binding protein 1; DNA, deoxyribonucleic acid; GEP, gene expression profile; Len, lenalidomide; Pom, pomalidomide; Roc1, regulator of cullins 1; Ub, ubiquitin.

REV/348/11.09/11-11

Lopez-Girona A, Mendy D, Miller K, et al. Direct binding with cereblon mediates the antiproliferative and immunomodulatory action of lenalidomide and pomalidomide. *Oral presented at: Annual Meeting and Exposition of the American Society of Hematology.* 2011; December 10-13; San Diego, CA.

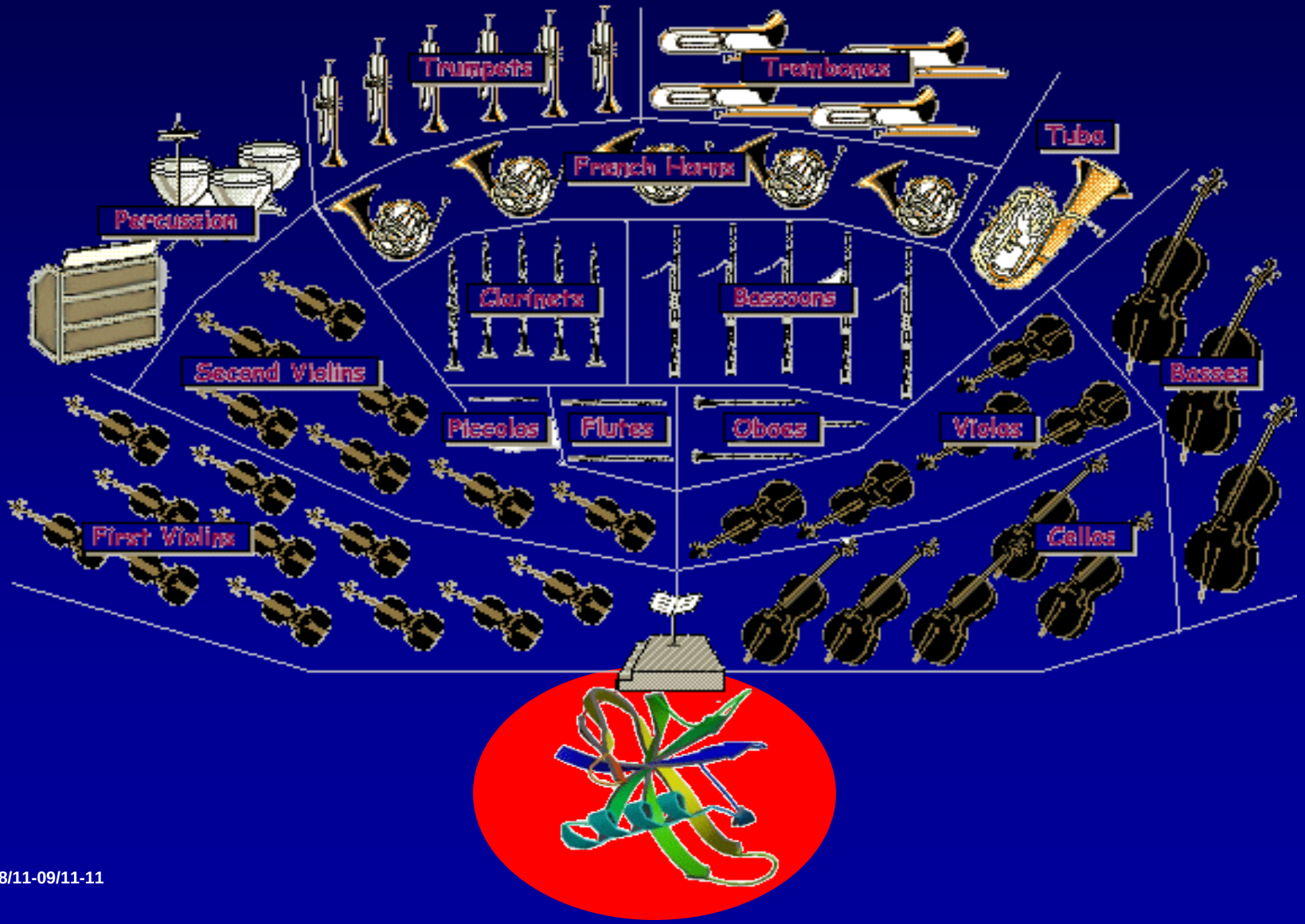
Conclusions

- Thalidomide, lenalidomide, and pomalidomide bind CRBN through the glutarimide moiety in each compound
- CRBN expression correlates with clinically relevant responses to lenalidomide in human T cells and multiple myeloma cells
  - Lenalidomide resistance correlates with reduced CRBN
- Lenalidomide and pomalidomide induce an overlapping but distinct mRNA profile in U266 myeloma cells
- Pomalidomide retains antiproliferative activity in CRBN-depleted lenalidomide-resistant myeloma cells

CRBN, cereblon; mRNA, messenger ribonucleic acid.

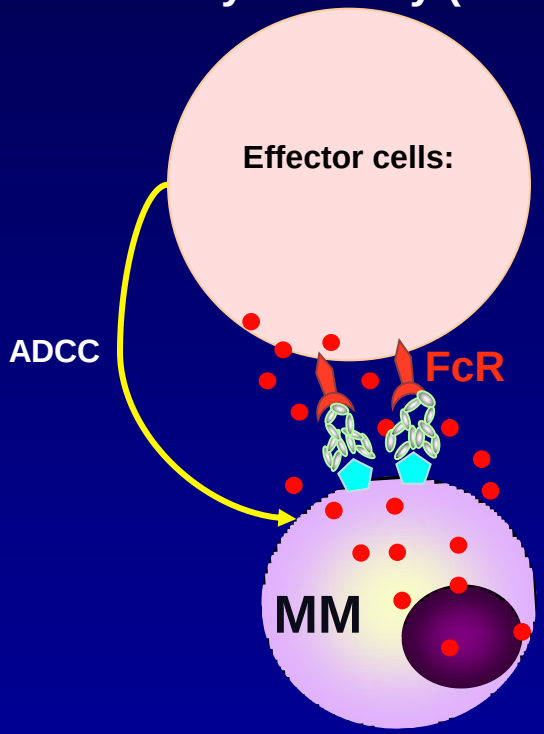
Lopez-Girona A, Mendy D, Miller K, et al. Direct binding with cereblon mediates the antiproliferative and immunomodulatory action of lenalidomide and pomalidomide. *Oral presented at: Annual Meeting and Exposition of the American Society of Hematology.* 2011; December 10-13; San Diego, CA.

CRBN is a Central Orchestrator of Lenalidomide and Pomalidomide Activity



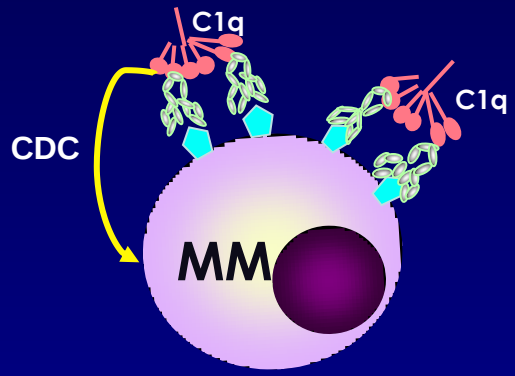
Monoclonal antibody-based therapeutic targeting of myeloma

Antibody-dependent Cellular cytotoxicity (ADCC)



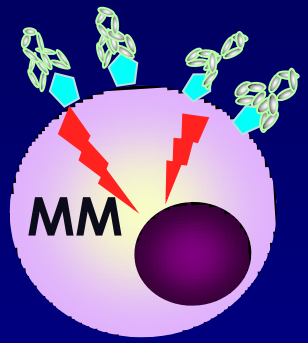
- Lucatumumab or Dacetuzumab (CD40)
- Elotuzumab (CS1)
- Daratumumab (CD38)
- XmAb®5592 (HM1.24)

Complement-dependent Cytotoxicity (CDC)



- Daratumumab (CD38)

Apoptosis/growth arrest via targeting signaling pathways



- huN901-DM1 (CD56)
- nBT062-maytansinoid (CD138)
- 1339 (IL-6)
- BHQ880 (DKK1)
- RAP-011 (activin A)
- Daratumumab (CD38)

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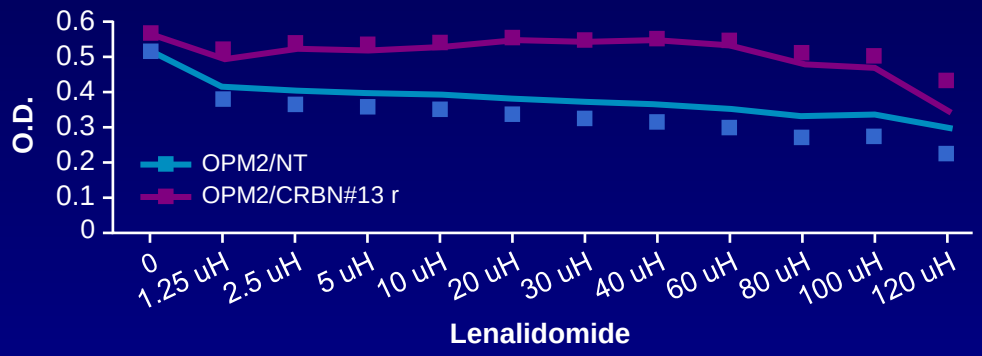
Myeloma Mechanism of Disease and MoA of Lenalidomide
Summary

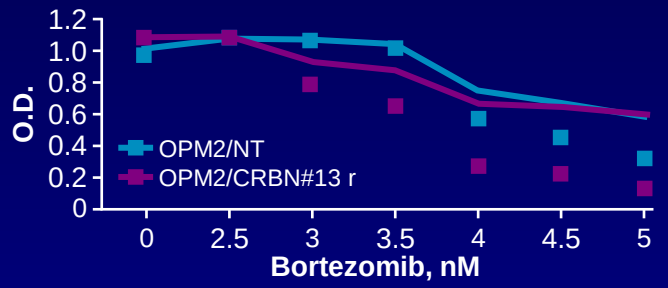
- Multiple myeloma is a chronic disease that requires effective, long-term treatment strategies
  - Disease recurrence and immunosuppression represent two fundamental challenges for management of the disease
- Lenalidomide has a MOA with a duality of effects: it directly leads to tumour cell death and improves the immune system to keep the tumour in remission
  - These effects provide rapid and sustained control of multiple myeloma when used long term
- Unlike chemotherapy, lenalidomide bolsters the immune response while also demonstrating tumouricidal activity

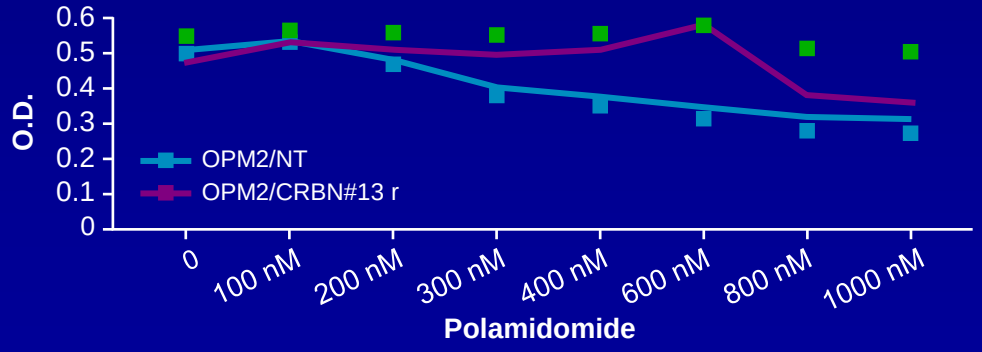
MOA, mechanism of action

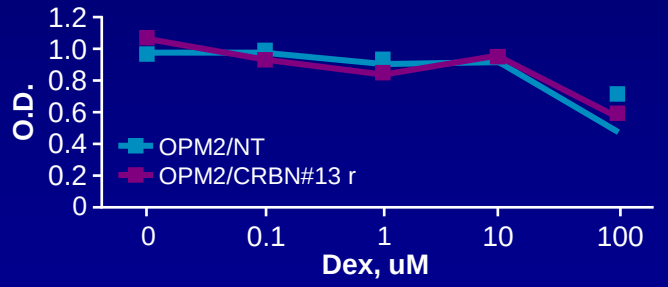
CRBN silencing confers resistance to lenalidomide and pomalidomide, but not other agents

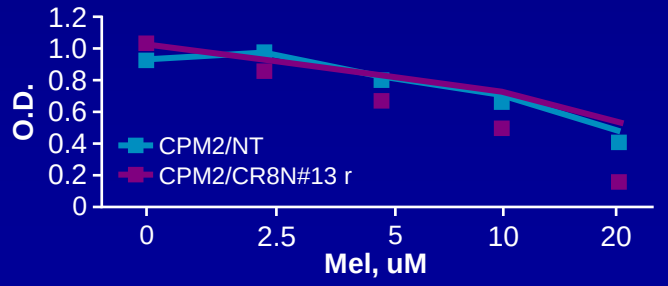
MTT analysis at day 3 of OPM2 cells with or without CRBN knockdown, and exposure to various anti-myeloma agents











MTT = 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium;
NT = non-targeting.

REV/34811-49/1111

Zhu YX, et al. Blood. 2011;118:4771-9.

Features of Symptomatic Multiple Myeloma

Common features of symptomatic myeloma include:

C – Calcium elevation (> 10 mg/L)[1,2]

- Due to myeloma-associated bone destruction

R – Renal dysfunction (creatinine > 2 mg/dL)[1,3]

- Caused by hypercalcemia and accumulation of light chains

A – Anemia (hemoglobin < 10 gm/dL)[1,4]

- Caused by inhibition of erythropoiesis due to renal failure and cytokines such as TNF-α

B – Bone disease (lytic lesions or osteoporosis)[1,2]

- Characterized by increased osteoclast generation mediated by cytokines and chemokines such as IL-6, TNF-α, and RANKL

1. The IMWG. *Br J Haematol.* 2003;121:749-757.; 2. Roodman GD. *Leukemia.* 2009; 23:434-441; 3. Dimopoulos MA. *Leukemia*. 2008; 22:1485-1493; 4. Ludwig H. *Clin Adv Hematol Oncol.* 2004; 2:233-241.

MM is associated with the development of Immunosuppression

- MM tumour cells induce immunosuppression
  - Suppression of normal B cell and HSC proliferation[1]
  - Induction of T and NK cell apoptosis[1]
  - Impaired cytokine production following cellular activation[2]
  - Overall immunosuppression[3]

HSC, haematopoetic stem cell; MGUS, monoclonal gammopathy of unknown significance; NK, natural killer.

1. Cook G and JDM Campbell. *Blood Reviews*. 1999;13:151-162.
2. Mozaffari F, et al. *Brit J Haematol.* 2004;124:315-32.
3. Schütt P, et al. *Leukemia and Lymphoma.* 2006;47(8):1570-1582.
4. Bernal M et al. *Human Immunol.* 2009; Epub:Jul 4.

REV/348/11-09/11-11

Lenalidomide Activates a Caspase Cascade in Myeloma Cells

MM cell line	Time (hr)	Lenalidomide	Dex	Combination
KARPAS-620	24	3, 8, 9	-	3[a], 8[a], 9[a]
	48	3, 8, 9	3, 8	3, 8, 9
KMS-12-BM	24	3, 8, 9	8	3
	48	3, 8	3, 8	3
NCI-H929	24	-	-	-
	48	-	3, 8	3[a], 8
LP-1	24	-	-	-
	48	-	-	3[a]
U266B1	24	8	-	-
	48	-	-	3[a], 9[a]
JJN-3	24	-	3, 8, 9	3, 8, 9
	48	-	3, 8, 9	3[a], 8[a], 9[a]
RPMI-8226	24	8	9	8
	48	-	-	3

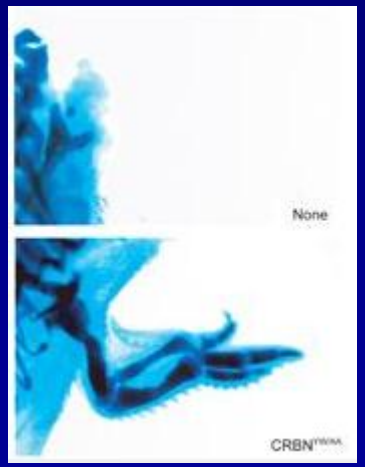
[a]Synergistic combination

- Synergistic tumouricidal activities of Len + Dex contribute to rapid responses

1. Schafer et al. 2008. *ASH Abstract #2761.* American Hematology Society.

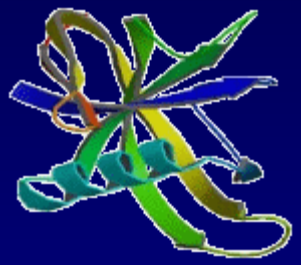
Thalidomide induced inhibition of limb growth still poorly understood

Ubiquitination and degradation leads to accumulation of Fibroblast growth factor 8 and 10 (which are reduced with thalidomide treatment) and to impaired limb development.



Ito T, et al. Science. 2010;327:1345-50.

Cereblon



442 amino acids
location in nucleus, cytoplasm, cell membrane
gene located on 3p

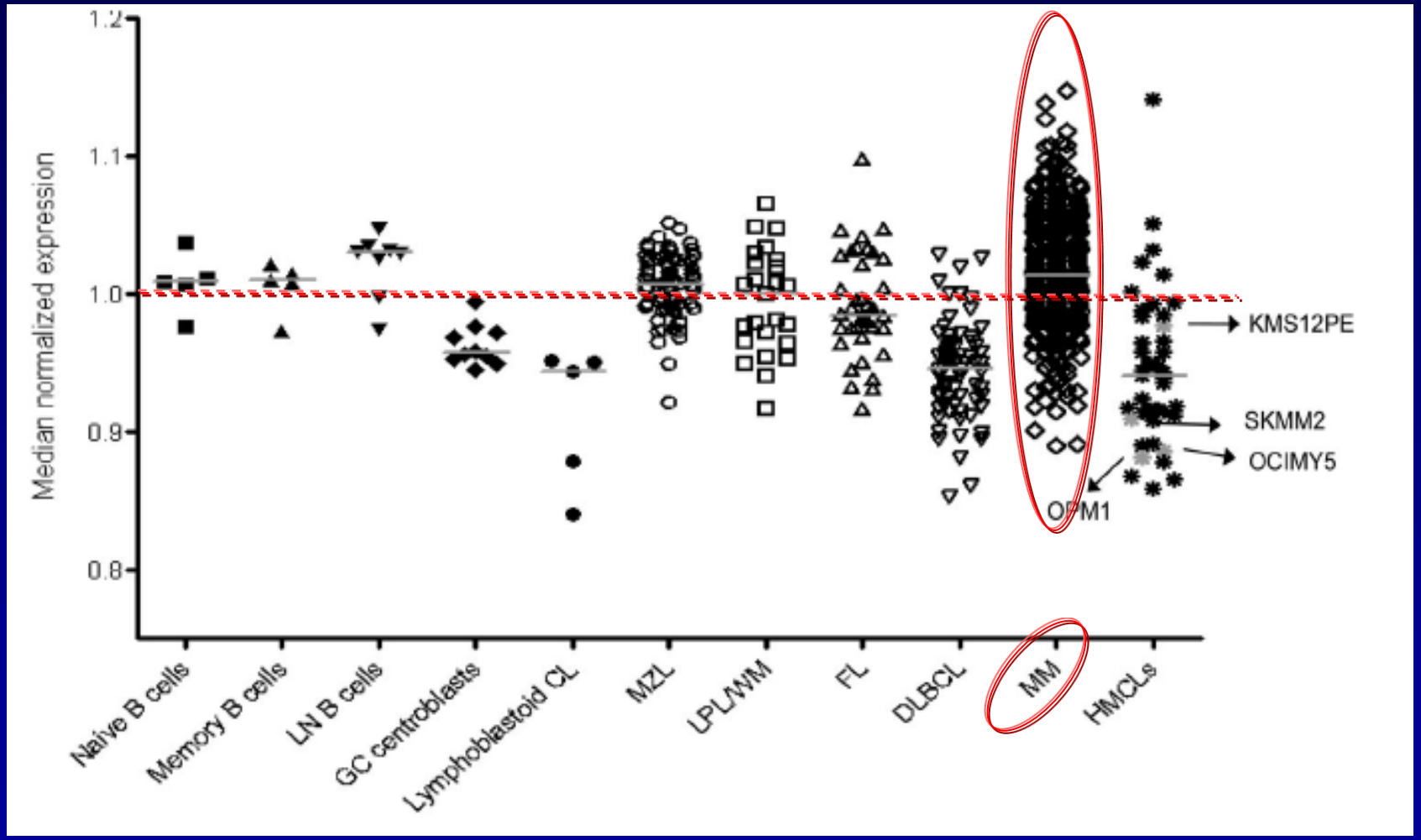
- Highly expressed in Cerebellum, hypocampus, Dorsal ganglion
- CD 4- and CD 8 positive T – cells
- B-cells
- Oocytes

- localized in cytoplasm with a calcium channel membrane protein

- A specific mutation of CRBN results in mild mental retardation with a standard IQ between 50-70

[1]UniProt Consortium

Cereblon expression in different stages of B cell development and B cell tumors



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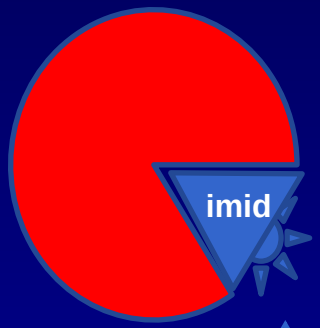
Zhu YX, et al. Blood. 2011;118:4771-9.

Target the immune system

Cereblon



Chemically modifiable group

Downstream effects

Cytotoxicity
NK activation
Immune system
Anti TNF

Diverse anti tumour effects