Understanding the cellular targets of IMiDs® and the relevance to clinical efficacy

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Molecular Structure of Thalidomide, Lenalidomide and Pomalidomide

**Thalidomide**
- 100–200 mg/d
- Neuropathy
- Constipation
- Sedation
- DVT

**Lenalidomide**
- 15–25 mg/d
- Myelosuppression
- Skin rash
- DVT

**Pomalidomide**
- 2–4 mg/d
- Myelosuppression
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.

Thalidomide initiates its teratogenic effects by binding to CRBN and inhibiting the associated ubiquitin ligase activity.
Cereblon Levels Are Highest in Myeloma, Leukemias and Neuroblastoma

Lenalidomide Resistant Myeloma Cells Lack Cereblon

Cereblon Knockdown Confers Complete Lenalidomide and Pomalidomide Resistance

Low Cereblon Expression is Common in Lenalidomide Refractory Patients

Truncating Mutation of Cereblon in Drug Resistant patient

Pomalidomide: Measurable Parameter From Baseline (Serum, Urine, FLC)

N = 331

Gene Expression Levels of Cereblon Predict Response to Pomalidomide

Gene Expression Levels of Cereblon Predict PFS of Pomalidomide Treated Patients

P=0.0001

3 months versus 17 months

Gene Expression Levels of Cereblon Predict OS of Pomalidomide Treated Patients

9.1 months versus 27 months

P=0.01

P=0.005

Cereblon Expression in HOVON-65/GMMG-HD4

A-B: thal-treated, C-D: bort-treated

IMiDs® Bind to a Cereblon Mediated E3 Ligase Resulting in Pleiotropic Clinically Relevant Effects

IMiD

 Ubiquitin E3 ligase

DDB1

Cul4

Roc1

IMiDs®® Bind to a Cereblon Mediated E3 Ligase Resulting in Pleiotropic Clinically Relevant Effects

Ubiquitin E3 ligase

DDB1

Cul4

Roc1

Substrates?

Proteasome

•↑Signaling (Rho, IL2)
•↓TNFα
•↑F-Actin/capping
•↑Antigen Presentation

•↓Cell Cycle/Prolif
•↑Apoptosis
•↑Tumor Suppr
•↓Oncogenes
•↓Migration/invasion
•↓Angiogenesis

•↑Progenitor cell expansion/fate
•↑Hb

Immunomodulation

Tumoricidal

Differentiation and side effects

UBiquitin E3 ligase

Impairment of Response to Lenalidomide After Cereblon Knockdown (Gene Expression Profile)

Control Lenalidomide 48h

Cereblon k/d Lenalidomide 48h

636 ↓
637 ↑

27 ↓
136 ↑

30 ↓
150 ↑

Lenalidomide Treatment and Cereblon Knockdown Reduce IRF-4 Expression in MM Cells

Overexpression of IRF4 Protects MM cells From Lenalidomide Induced Cytoxicity

Pathway Analysis of Genes Regulated by IMiD-Cereblon
IMiDs Synergize With Dexamethasone to Overcome Resistance

[Image of gel electrophoresis results showing expression levels of C-Myc, IRF4, and Actin in different cell lines treated with various combinations of lenalidomide (Len), pomalidomide (Pom), dexamethasone (Dex), and Len-Dex, Pom-Dex treatments.]

Len sensitive

Len resistant

Celgene data on file. Courtesy of Dr. R. Chopra
Cereblon is Not the Only Story in Drug Resistance

Do Lenalidomide and Pomalidomide Have Different Substrate Specificities After Binding the Cereblon E3 Ligase Complex?

Pomalidomide Proteome

Lenalidomide Proteome

Celgene on file. Courtesy of Dr. R. Chopra
Assay Development and Clinical Role of Cereblon Measurement

Current Status

• Lack of standardization of reagents and assays
• Uncertainty over what to measure
  o RNA (RNA seq, GEP)
  o Protein (immunohistochemistry, flow cytometry, western blot)
  o Splice variants

What we need

• Gold standard reagents and methods
• Reproducibility in multiple laboratories for confirmation of results
• Large clinical trial sample sets
Cereblon: IMiDs Next Steps

1. What are the substrates that link Cereblon to MM cell death

2. How does Cereblon regulate immune activity of IMiDs

3. What are the relevant differences between IMiDs

4. Extend clinical data into prospective studies

5. Determine value (or not) as a clinical biomarker

6. New drugs targeting the Cereblon - IRF4 pathway