Toxicology or Exaggerated Pharmacology
The MOA of the IMiD Drugs
The IMiD Drugs: Structural Analogs of Thalidomide

Thalidomide

Pomalidomide

Lenalidomide
Serious Side Effects Associated with IMiDs

- Fetal risk (Teratogenicity - most infamous)
- Peripheral neuropathy
- Risk of new cancers (2nd primary malignancies)
- Hematologic toxicity (cytopenias)

Challenging to understand toxicities if you don’t understand the mechanism...
Drug Development Targeting the UPS

- E1 Activating Enzyme (2)
- E2 Conjugating Enzyme (~40)
- E3 Ligase (>600)
- Deubiquitinase (DUB) (~100)
- Chaperone

Ubiquitin (Ub) and Protein Substrate (S) interact with various enzymes and complexes to facilitate proteasomal degradation.
Drug Development Targeting the UPS

**Proteasome Inhibitors**
- Bortezomib (Velcade)
- Carfilzomib (Kyprolis)
- Ixazomib (Ninlaro)
- Marizomib
- Oprozomib
- Delanzomib

**Ubiquitin**
- Ub

**Protein Substrate**
- S

**Substrate**

**E1**
- ATP
- ADP + Pi

**E2**
- Ub

**E3**
- Ub

**DUB**

**p97**

**MLN7243**
- MLN4924

**CB-5083**

**VLX1570**

**Thalidomide**

**IMiD® Compounds**
- Lenalidomide
- Pomalidomide

**CELMoD® Compounds**
- CC-122, CC-220
- CC-90009, CC-92480
Thalidomide, the First Drug Targeting the UPS

**Patented 1954 – Chemie Grunenthal**

**Sedative – alternative to barbiturates**

**Contergan/Distaval - 1957**

**Leprosy**

**Anti-myeloma**

**Unknown mechanism**
Unraveling a 50 Year Old Mystery
Identification of Cereblon as the Target for Thalidomide

Cereblon is proposed to function as a novel substrate receptor for a Cullin E3 ubiquitin ligase

Ito et al. Science 327,1345-50, March 12, 2010
Cereblon at the Center of a 50 Year Old Mystery

Takumi Ito, et al.  

JJ Higgins et al.  

Antonia Lopez-Girona et al.  
Cereblon Crystal Structures Show How Lenalidomide Binds in the E3 Ligase Machinery
Subtle Sequence Differences Provide Rationale for Species-Specific Effects of IMiDs

**Primate - Sensitive**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Human</td>
<td>LIGRPSTEHSWFPGYAWTV AQCKICASHIGWKFTA</td>
</tr>
<tr>
<td>Monkey</td>
<td>LIGRPSTEHSWFPGYAWTV AQCKICASHIGWKFTA</td>
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</table>

**Rodent - Resistant**

<table>
<thead>
<tr>
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<th>Sequence</th>
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<tbody>
<tr>
<td>Mouse</td>
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</tr>
<tr>
<td>Rat</td>
<td>LIGRPSTVHSWFPGYAWTIAQCKICASHIGWKFTA</td>
</tr>
</tbody>
</table>
A New Paradigm: Unlocking the Cereblon Mechanism

Lenalidomide acts like “molecular glue” to induce the binding of Ikaros/Aiolos to CRBN and drive the ubiquitination and ultimate degradation by the proteasome.

Jan Kronke et al.  

Gang Lu et al.  

Anita Gandhi et al.  
The Ikaros (IKZF) Family of Transcription Factors

- C2H2 Zinc-finger containing transcription factors
- Expression predominantly restricted to hematopoietic cells
- Function in blood cell development and survival

What toxicities/activities are associated with the loss of Ikaros and Aiolos?
Mutations at the SALL4 locus on chromosome 20 result in a range of clinically overlapping phenotypes, including Okihiro syndrome, Holt-Oram syndrome, acro-renal-ocular syndrome, and patients previously reported to represent thalidomide embryopathy.

J Kohlhase, L Schubert, M Liebers, A Rauch, K Becker, S N Mohammed, R Newbury-Ecob, W Reardon

We have recently shown that Okihiro syndrome results from mutation in the putative zinc finger transcription factor gene SALL4 on chromosome 20q13.13-13.2. There is considerable overlap of clinical features of Okihiro syndrome with other conditions, most notably Holt-Oram syndrome, a condition in part resulting from mutation of the TBX5 locus, as well as acro-renal-ocular syndrome. We analysed further families/patients with the clinical diagnosis of Holt-Oram syndrome and acro-renal-ocular syndrome for SALL4 mutations. We identified a novel SALL4 mutation in one family where the father was originally thought to have thalidomide embryopathy and had a daughter with a similar phenotype. We also found two novel mutations in two German families originally diagnosed as Holt-Oram syndrome and a further mutation in one out of two families carrying the diagnosis acro-renal-ocular syndrome. Our results show that some cases of “thalidomide embryopathy” might be the result of SALL4 mutations, resulting in an increased risk for similarly affected offspring. Furthermore we confirm the overlap of acro-renal-ocular syndrome with Okihiro syndrome at the molecular level and expand the phenotype of SALL4 mutations.
SALL4 mediates teratogenicity as a thalidomide-dependent cereblon substrate


Unlikely related to IKZF1/3 loss. SALL4 degradation is a likely contributor...
Mechanism of Neuropathy...

Unlikely related to IKZF1/3 degradation. Mechanism is currently unknown...
Mechanism of 2nd Primary Malignancies...
in multiple myeloma patients

Ikaros: master of hematopoiesis, agent of leukemia

Kara L. Davis

Abstract: Ikaros is the founding member of a family of zinc finger transcription factors whose function during early hematopoietic development is required for differentiation into the three major hematopoietic lineages. Ikaros deletions have been described in human malignancies, particularly precursor B-cell leukemia. Deletions of this transcription factor appear to mediate leukemogenesis, although the exact mechanism is unclear. This article reviews the structure and function of Ikaros proteins in chromatin remodeling and gene expression as well as the current knowledge of Ikaros deletions in human malignancies. A new proteomic platform, mass cytometry, is introduced which allows measurements of greater than 30 parameters at the single-cell level and should thus provide a greater level of detail to unravel the mechanistic consequences of Ikaros dysfunction in leukemia.

Keywords: cell signaling, chromatin remodeling, hematopoiesis, Ikaros, leukemia
Genetic Loss of Ikaros Function Associated with Acute Lymphoblastic Leukemia

The many faces of IKZF1 in B-cell precursor acute lymphoblastic leukemia

René Marke,1 Frank N. van Leeuwen1 and Blanca Scheijen1,2

1Laboratory of Pediatric Oncology, Radboud University Medical Center, and 2Department of Pathology, Radboud University Medical Center; Radboud Institute for Molecular Life Sciences (RIMLS), Nijmegen, the Netherlands

ABSTRACT

Transcription factor IKZF1 (IKAROS) acts as a critical regulator of lymphoid differentiation and is frequently deleted or mutated in B-cell precursor acute lymphoblastic leukemia. IKZF1 gene defects are associated with inferior treatment outcome in both childhood and adult B-cell precursor acute lymphoblastic leukemia and occur in more than 70% of BCR-ABL1-positive and BCR-ABL1-like cases of acute lymphoblastic leukemia. Over the past few years, much has been learned about the tumor suppressive function of IKZF1 during leukemia development and the molecular pathways that relate to its impact on treatment outcome. In this review, we provide a concise overview on the role of IKZF1 during normal lymphopoiesis and the pathways that contribute to leukemia pathogenesis as a consequence of altered IKZF1 function. Furthermore, we discuss different mechanisms by which IKZF1 alterations impose therapy resistance on leukemic cells, including enhanced cell adhesion and modulation of glucocorticoid response.
Apparent Mechanistic Disconnect...
IMiD induced vs genetic defect loss of Ikaros
Mechanism of 2nd Primary Malignancies...

in multiple myeloma patients

AML w/ Myelodysplastic features

Age (IMiD-treated patients live longer)
Prior ASCT
Melphalan
GM-CSF

Ikaros degradation may contribute, but other factors are likely the main drivers.
Ikaros/Aiolos degradation is likely related to the clinically observed cytopenias.
Mechanism of Cytopenias... and clinical activity

**“Toxicology”**
- Neutrophils
- B-cells
- Long-lived plasma cells
- PC-dendritic cells
- T-cells

**“Pharmacology”**
- Myeloma & Lymphomas
  - Plasma cell and B-cell malignancies appear to maintain dependence on Aiolos.
  - T-cell (and NK cell) activation plays a role in clearing malignant cells.
- Lupus
  - IKZF1/3 are susceptibility loci for SLE.
  - Reduced PC-dendritic cells may lower IFN-α.
  - B-cell and Plasma cell reduction may lower auto-Ab titers.
Thank you