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Lenalidomide: deciphering mechanisms of action in myeloma, myelodysplastic syndrome and beyond

Andrew A Guirguis[1,2] and Benjamin L Ebert[1,2]

Lenalidomide and its related 'analogues' modulate the substrate specificity of the $CRL4^{CRBN}$ E3 ubiquitin ligase complex. Polyubiquitination and subsequent proteasomal degradation of IKZF1 and IKZF3 in multiple myeloma and CK1α in del(5q) MDS has recently been linked to therapeutic efficacy of this class of compounds. Harnessing ubiquitin ligase substrate specificity, may in time facilitate the degradation of other 'undruggable' proteins and allow for separation of detrimental side effects of IMiD compounds from those associated with therapeutic efficacy.

Addresses
[1] Brigham and Women's Hospital, Division of Hematology, Boston, MA 02115, USA
[2] Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

Corresponding author: Ebert, Benjamin L (benjamin_Ebert@dfci.harvard.edu)

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Introduction

Lenalidomide is a member of a class of molecules that have been termed immunomodulatory drugs (or IMiDs). It exhibits significant therapeutic activity in diseases such as multiple myeloma [1,2], myelodysplastic syndrome with del(5q) [3–5] and mantle cell lymphoma [6,7•]. Despite its demonstrated efficacy however, the basis for lenalidomide's mechanism of action has only recently been elucidated.

History of lenalidomide and clinical use

Following the association of 'phocomelia' (congenital deformity with arm/leg shortening) and 'amelia' (absence of arms and/or legs) with thalidomide use as an anti-emetic during pregnancy in the 1950s, it seemed unlikely that this agent, or its derivatives, might subsequently be repurposed for therapeutic use in human disease. Almost six decades later, lenalidomide, a thalidomide analogue, is FDA-approved for use in multiple myeloma, del(5q) myelodysplastic syndrome and more recently, mantle cell lymphoma (Figure 1 and Table 1). Further emerging evidence suggests, that lenalidomide may have therapeutic potential particularly as combination therapy in other B-cell malignancies (such as DLBCL) [8•].

Over the past decade, studies in multiple myeloma have demonstrated a therapeutic benefit of lenalidomide treatment (typically in combination with steroids or additional agents) in the disease relapse or refractory setting [1,9•,10•,11], in the context of a new diagnosis [2,12,13] and potentially following stem cell transplantation [14,15]. These studies build on previous findings associated with thalidomide use in myeloma and are pertinent for more recently derived analogues such as pomalidomide [16•,17].

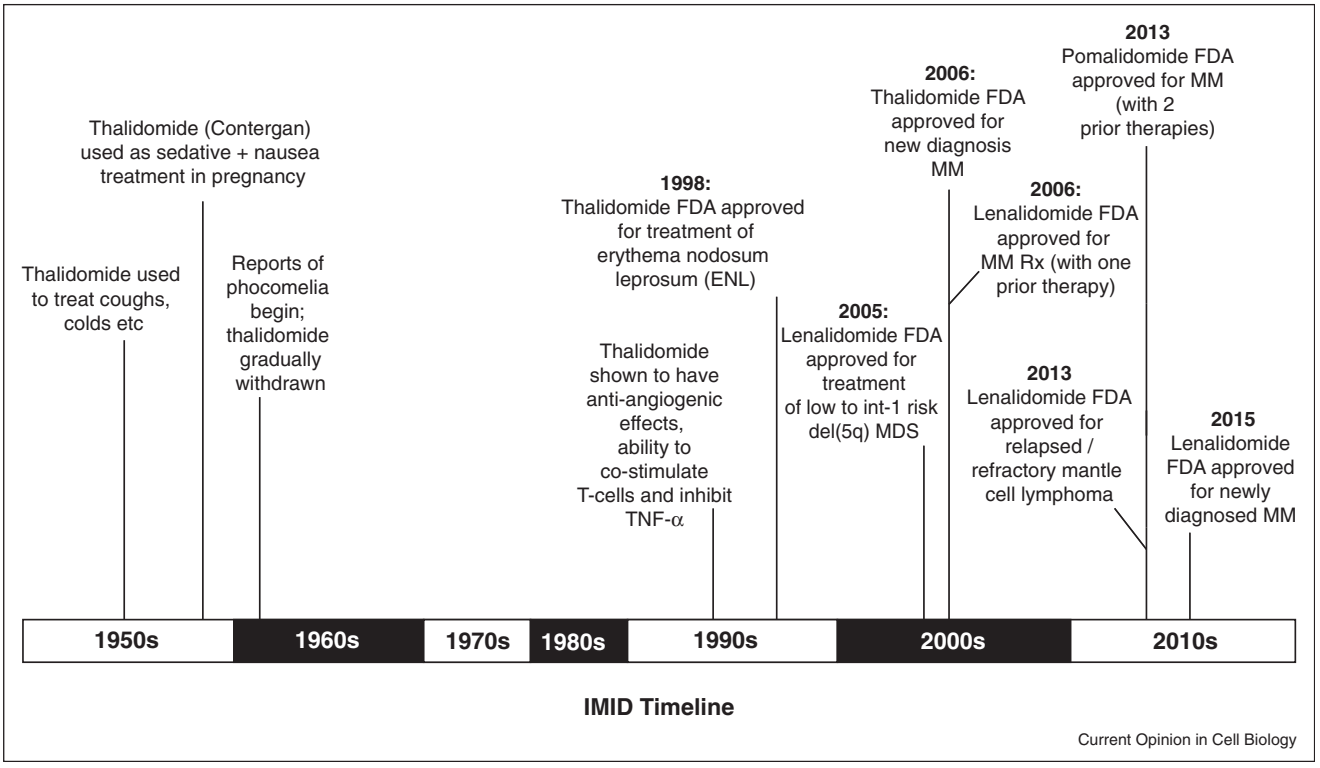
In MDS, transfusion-dependent patients harboring a chromosome 5q31 deletion (comprising 15–20% of MDS subtypes [18]) derive significant therapeutic benefit from lenalidomide with over 65% achieving transfusion independence [3–5]. Whilst more than 50% of patients achieve cytogenetic remissions through the selective apoptosis of abnormal MDS clones, the presence of additional mutations such as those within p53 have been associated with lenalidomide resistance [19]. By contrast, although responses to lenalidomide are still evident in those patients who lack the chromosome 5q deletion, these usually occur at significantly lower frequencies [20].

Lenalidomide dictates the substrate specificity of the E3 ubiquitin ligase

In spite of the demonstrated efficacy of the IMiD compounds in various disease entities, the mechanistic basis of their pleiotropic effects has only recently become apparent.

For a long time, the IMiD compounds have been most notable for their effects on cytokine modulation (through TNF-α inhibition in activated monocytes [21]) and T-cell co-stimulation through IL-2 production [22•].) Additional suggested mechanisms of action particularly in myeloma have included induction of cell cycle arrest through increased expression of the cyclin-dependent kinase inhibitor p21 *(Cdkn1a)* [23], decreased expression of interferon regulatory factor 4 (IRF4) [24], induction of apoptosis and inhibition of angiogenesis [25,26].

Figure 1



Timeline outlining the history of IMiD compound development and use over the past six decades.

More recent work has demonstrated that lenalidomide, and the other IMiDs, bind cereblon (CRBN) through their common glutarimide ring and modulate the substrate specificity of the $CRL4^{CRBN}$ E3 ubiquitin ligase complex [23,27••,28••,29••] (Table 1). Formed by the binding of RING finger protein regulator of cullins 1 (ROC1) and DNA damage binding protein-1 (DDB1) to the cullin ring 4 ligase (CUL4A), the $CRL4^{CRBN}$ E3 ubiquitin ligase complex uses CRBN as a substrate adaptor to polyubiquitinate specific substrates tagging these proteins for degradation (Figure 2). In this context, the IMiDs are the first clinically-approved drugs that target an E3 ubiquitin ligase and thus represent a novel mechanism of action.

The interaction with the E3 ligase complex is notable for a number of findings:-

- binding of CRBN to a particular substrate is increased in the presence of these drugs [28••,30••];
- increased binding of substrates and subsequent polyubiquitination and degradation is dependent on the presence of CRBN. In this context, inactivation of CRBN abrogates substrate degradation causing lenalidomide and pomalidomide resistance [23,31], as demonstrated in myeloma cell lines [28••,29••];
- substrate specificity is governed by individual compounds within this class of drugs and is highly specific [32••,33••];
- The binding of CRBN to substrates in the presence of IMiDs varies between human and mouse CRBN [30••].

From a structural perspective, the binding site of the IMiD compounds' glutarimide ring lies within CRBN's putative substrate-binding pocket [33••]. By contrast, the variable phthaloyl ring that makes each IMiD compound unique, likely confers substrate specificity for proteins that are recruited to the CRBN ligase complex. This model may shed light on why lenalidomide is more efficacious in del(5q) MDS in comparison with thalidomide. Of significance, as the chemical structure of this variable ring is amenable to alteration, this suggests it may be possible to direct the specificity of substrates recruited to the E3 ligase complex for subsequent degradation.

In myeloma, the B-cell lymphoid transcription factors, IKZF1 and IKZF3 are targeted for degradation in the presence of lenalidomide

Using two distinct approaches (SILAC-based quantitative mass spectrometry and a luciferase-based ORF screen), we and others have recently shown that lenalidomide induces the specific ubiquitination and proteasomal degradation of two B-cell lymphoid transcription factors, IKZF1 (Ikaros) and IKZF3 (Aiolos) in multiple myeloma cells [23,28••,29••]. Homologous members of