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Mutations in CRBN and other cereblon pathway genes are infrequently associated with acquired resistance to immunomodulatory drugs

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Immunomodulatory drugs (IMiDs) are the current backbone of standard and experimental combination myeloma therapies at all stages of the disease. However, the majority of patients inevitably relapse and the mechanisms of resistance are still poorly understood. Previous studies looking for genetic drivers of resistance have looked for changes in core members of the CRL4CRBN E3-ubiquitin ligase complex (CUL4-RBX1-DDB1-CRBN) and identified mutations in the protein to which IMiDs bind, cereblon (CRBN), but at a rate that cannot account for resistance in the majority of patients [1, 2]. Several in vitro studies have now identified novel upstream and downstream regulators of CRBN activity that could have a role in IMiD resistance and have not been previously examined in patient samples [3–6]. In this study paired presentation and relapse samples from newly diagnosed patients recruited to a clinical trial of IMiD therapies were used to investigate the role of mutations in all genes currently implicated in IMiD activity. Infrequent mutations in CRBN itself were identified that could be a cause of IMiD resistance in some patients. CRBN and other genes in the IMiD response pathway were mutated at low frequency and, in many cases, at low clonal fraction suggesting that mechanisms other than mutations, for example post-translational modification or epigenetic alterations, may underlie resistance acquisition.

The mechanism of action of IMiDs in myeloma has been partly elucidated. Binding of the IMiDs within a tri-tryptophan pocket on the surface of CRBN alters substrate specificity of the CRL4CRBN E3-ubiquitin ligase complex, leading to the ubiquitination of neosubstrates such as the C2H2 zinc finger domain-containing B-cell transcription factors Ikaros (gene name: IKZF1) and Aiolos (IKZF3), which are then degraded via the proteasome [7–9]. Ikaros and Aiolos degradation results in the subsequent downregulation of their target genes, including interferon regulatory factor 4 (IRF4) and c-Myc, which are transcription factors regulating stages of B-cell development. IRF4 is thought to be the key driver of aberrant transcriptional regulation in myeloma, with its down-regulation causing cell death. Clinical response rates to IMiDs are high but the majority of patients will inevitably relapse. Understanding IMiD resistant and refractory states is therefore imperative to help us improve patient outcomes.

Recent in vitro studies have improved our understanding of the mechanisms of control of the cereblon IMiD response pathway implicating genes encoding components of the core CRL4CRBN E3-ubiquitin ligase complex and the COP9 signalosome, as well as IMiD-induced neosubstrates and downstream targets [3–6, 10]. Liu et al. [4], Sievers et al. [5], and Tateno et al. [6] performed genome-wide screens of myeloma cell lines to identify genes implicated in IMiD response. All three screens identified subunits of the CRL4CRBN complex as important for IMiD sensitivity, in line with the previously published IMiD mechanism of action [11]. The screens also all identified components of the COP9 signalosome as important in determining IMiD sensitivity, including COP51 [10], COP52, and COP54 [4, 6] and COP55 [3–6, 10]. Tateno et al. [6], in addition, identified NEDD8, which is critical for the activation of the CRL4 E3-ligase, as having a role in IMiD response and highlighted the influence of the subcellular localisation of CRBN. Sievers et al. [5] found that the loss of E2 ubiquitin-conjugating enzymes UBE2J3 and UBE2G1, as well as DEPDC5, a GAP Activity Towards Ras complex 1 (GATOR1) member, reduced degradation of the neosubstrate IKZF3. Zinc finger transcription factor crosses such as IKZF1, IKZF3, and SALL4 are well-described neosubstrates for CRBN in the presence of the IMiDs thalidomide, lenalidomide, and pomalidomide. Donovan et al. [3], using a proteomic screen, demonstrated the degradation of these neosubstrates upon IMiD treatment of cell lines and also identify a number of other neosubstrates, many of which are zinc finger proteins; ZNF827, ZNF98, and GZF1. They also identify non-zinc finger targets CSNK1A1 and DTWD1 [3].

Together these recent studies give a clearer understanding of the control of CRL4CRBN activity via neddylation and deneddylation
In this study, we applied this knowledge to investigate the role of mutations in these genes, as well as those of the core CRL4CRBN E3-ubiquitin ligase complex, in the acquisition of resistance to lenalidomide, the most widely clinically used IMiD. We have previously reported a study examining the impact of maintenance lenalidomide and depth of response on the genetics and sub-clonal structure of relapsed disease. Study samples were selected from newly diagnosed patients enrolled in the UK National Cancer Research Institute Myeloma XI trial (NCT01554852) [12, 13] for whom adequate DNA volumes were available at the time of study design. 56 patients who received immunomodulatory drug induction therapy followed by either lenalidomide maintenance (n=30) or observation (n=26), and subsequently relapsed, were selected. Whole exome sequencing analysis, median depth 122x for tumour samples and 58x for paired germline controls, had been performed as previously described [13] and summarised in Supplementary Methods.

From recent publications, a list of 42 genes involved in cereblon pathway regulation and IMiD response was curated, termed "CRBN/IMiD genes" (Fig. 1A and Supplementary Table 1). The frequency of non-synonymous mutations and deletions in CRBN/IMiD genes in the patient dataset was examined and the cancer clonal fraction (CCF) between the diagnosis and relapse samples was compared. 12/42 (29%) of these genes were found to be mutated in the dataset with a total of 17 mutations identified. With the exception of SALL4, which was mutated in three patients, no other CRBN/IMiD gene was mutated in more than two patients. 14/56 (25%) of patients had a mutation in a CRBN/IMiD gene either at presentation, relapse, or at both time points (Table 1). Three patients had mutations in two different genes. 6/14 of the patients with CRBN/IMiD mutations (43%) had received lenalidomide maintenance and 8/14 (57%) had been in the observation arm of the trial. Of the 17 mutations, 9 (53%) arose in patients who had received lenalidomide maintenance and 8 (47%) in patients who were observed. Importantly, in the patients receiving lenalidomide maintenance, 6 of the 9 (67%) mutations had a higher cancer clonal fraction (CCF) in the relapse sample, suggesting they may have been selected for by exposure to treatment. Two of these mutations were only detected at relapse and not at presentation, in CRBN (CCF 0.71 at relapse) and FAM83F (CCF 0.54 at relapse). Comparatively, in mutations identified in patients undergoing observation, only 3 of the 8 (38%) mutations had a higher CCF at relapse compared with the presentation. The only deletion in any of the CRBN/IMiD genes was in SETX in one patient at relapse.

A single patient in the study had a CRBN mutation identified only at relapse at g.3:3195148A > C, encoding a Cys326Gly sequence modification at the protein level. The absence of the CRBN mutation at presentation was confirmed by deep sequencing (769 reference mutations in the dataset with a total of 17 mutations identified. With the exception of SALL4, which was mutated in three patients, no other CRBN/IMiD gene was mutated in more than two patients. 14/56 (25%) of patients had a mutation in a CRBN/IMiD gene either at presentation, relapse, or at both time points (Table 1). Three patients had mutations in two different genes. 6/14 of the patients with CRBN/IMiD mutations (43%) had received lenalidomide maintenance and 8/14 (57%) had been in the observation arm of the trial. Of the 17 mutations, 9 (53%) arose in patients who had received lenalidomide maintenance and 8 (47%) in patients who were observed. Importantly, in the patients receiving lenalidomide maintenance, 6 of the 9 (67%) mutations had a higher cancer clonal fraction (CCF) in the relapse sample, suggesting they may have been selected for by exposure to treatment. Two of these mutations were only detected at relapse and not at presentation, in CRBN (CCF 0.71 at relapse) and FAM83F (CCF 0.54 at relapse). Comparatively, in mutations identified in patients undergoing observation, only 3 of the 8 (38%) mutations had a higher CCF at relapse compared with the presentation. The only deletion in any of the CRBN/IMiD genes was in SETX in one patient at relapse.

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Table 1. Mutations identified in CRBN/IMiD genes in patient samples.

| Ref. | Patient no. | Mutation | Mutation type | Amino acid change | Maintenance allocation | CCF at diagnosis | CCF at relapse |
|------|-------------|----------|---------------|-------------------|-----------------------|-----------------|---------------|
| 3,5,6 | 63          | 3:3195148A > C | Missense variant | Cys326Gly | Lenalidomide | 0 | 0.71 |
| 3,5,6 | 27          | 11:61070633A > C | Splice region variant | N/A | Observation | 0.99 | 0.69 |
| 3      | 106         | 21:46199134G > A | Splice region variant | N/A | Lenalidomide | 0.89 | 1.00 |
| 3      | 1          | 1:1203352C > G | Missense variant | Lys7Asn | Lenalidomide | 0.15 | 0 |
| 53     | 1:1190726C > T | Missense variant | Val229Ile | Observation | 0 | 0.18 |
| 6      | 9:135205643C > T | Missense variant | Asp448Asn | Lenalidomide | 1.0 | 0 |
| 6      | 9:135203409A > C | Missense variant | Asp1192Glu | Lenalidomide | 0.06 | 0.54 |
| 3      | 77          | 15:49935593G > C | Missense variant | Asp245His | Observation | 1.00 | 0.9 |
| 3      | 26          | 22:40417571G > A | Missense variant | Gly353Ser | Lenalidomide | 0 | 0.54 |
| 3      | 43          | 20:23345844A > C | Missense variant | Gln275Pro | Observation | 0.22 | 0.05 |
| 3      | 28          | 11:58378474G > C | Missense variant | Glu223Asp | Observation | 0.93 | 1.00 |
| 3      | 75          | 19:22575634G > T | Missense variant | His135Asn | Observation | 0.95 | 0.95 |
| 6      | 9:135203409G > A | Missense variant | Pro162Ser | Observation | 1.00 | 0.24 |
| 3      | 28          | 11:58378474G > C | Missense variant | Glu223Asp | Observation | 0.93 | 1.00 |

**DATA AVAILABLE**

Original sequencing data are available via https://ega-archive.org/datasets/EGAD00001004846/.

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AUTHOR CONTRIBUTIONS

J.R.J., A.B., G.J.M., and C.P designed this analysis; G.J.M., G.H.I., and F.E.D were Chief Investigators of the Myeloma XI trial; B.A.W., M.F.K., and G.J.M coordinated the central laboratory sample collection for the trial; J.R.J., N.W., C.A., B.A.W., C.W. performed the original sequencing sample preparation and analysis; J.R.J., A.B., Y.-V.L.e.B., H.W., F.E.D., R.C., G.J.M., and C.P analysed and interpreted the data for this analysis; J.R.J., A.B., and C.P drafted the paper. All authors contributed to critically revising the paper and approved the final submitted version.

COMPETING INTERESTS

J.R.J: Celgene Corporation—honoraria, research funding. M.F.K: Chugai-consultancy; Bristol-Myers Squibb, Takeda—consultancy, travel support; Janssen, Amgen—consultancy, honoraria; Celgene Corporation—consultancy, honoraria, research funding. G.H.I: Roche, Amgen, Janssen, Merck Sharp, and Dohme—consultancy, honoraria, speakers bureau; Celgene Corporation, Takeda—consultancy, honoraria, travel support, research funding. G.S.: Adaptive—consultancy; Celgene Corporation—consultancy, honoraria, research funding; Janssen, Oncopertide, Roche, Sanofi, Takeda—consultancy, honoraria. R.C: Celgene Corporation—previous employment, Monte Rosa Therapeutics - founder. G.M.: Janssen—research funding; Bristol-Myers Squibb, Takeda, Roche, Amgen, GSK, Karyopharm—consultancy, honoraria; Celgene Corporation—consultancy, honoraria, research funding. C.P.: Amgen, Takeda—consultancy, travel support; Janssen—honoraria, travel support; Celgene Corporation—consultancy, honoraria, travel support; Sanofi—consultancy, honoraria, the ICR has a financial interest in the development of compounds targeting CRL4-CRBN E3 ubiquitin ligase. Y.V.L.e.B., H.W., R.C, are in collaborative projects with Monte Rosa Therapeutics. All other authors declare no competing interests.

ADDITIONAL INFORMATION

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