Interrogating the Ikaros Axis and MM Heterogeneity in IMiD resistance

Lorraine N. Davis1,2, Zachary J. Walker1,2, Brett M. Stevens1,2, Beau Idler1,2, Tomer M. Mark1,2, Peter A. Forsberg1,2, Daniel W. Sherbenou1,2

1Division of Hematology, University of Colorado Cancer Center, 2Department of Medicine, University of Colorado Anschutz Medical Campus

Drug Resistance in Multiple Myeloma

Multiple myeloma (MM or myeloma) is a malignant plasma cell neoplasm that affects more than 30,000 Americans each year and is the second most prevalent adult hematologic malignancy. Over the last decade, incidence has continued to increase, with an estimated 12,960 deaths occurring this year. MM is characterized by the infiltration and rampant clonal proliferation of plasma cells and the overproduction of immunoglobulin (Ig) myeloma proteins in the bone marrow. Treatment options for MM have significantly improved over the past two decades, with the emergence of modern and potent anti-myeloma drugs increasing the average life expectancy to 7.6 years. However, this remains an largely incurable disease, as nearly all patients will eventually relapse and develop drug or multiform resistance. This acquired drug resistance, leading to refractory/relapsing disease, is the most cause of treatment failure and remains the greatest obstacle to successfully curing MM.

Immunomodulatory drugs (IMiDs) are a cornerstone of MM therapy. IMiDs are a pleiotropic drug class with several mechanisms of action. Most importantly, IMiDs possess direct antitumor effects by promoting the proteosomal degradation of critical MM transcription factors (IKZF1 and IKZF3) (Fig 2). Although most patients initially respond, the majority of patients exposed will become resistant or cross-resistant to IMiDs, and the mechanisms of acquired IMiD resistance remain largely unknown. Based on the known mechanism of action in MM cells, we have investigated whether the Ikaros axis shows a differential response to IMiD treatment in patients with MM resistance.

Methods

MM cell line and primary MM sample drug treatment

For cell viability, MM cell lines were treated with the indicated IMiD concentrations for 96 hours and relative live cell count with assessed by flow cytometry. Mononuclear cells from MM cell line and primary MM sample drug treatment were generated in FlowJo v9.

Figure 1. Disease course of Patient 614. Bottom axis is time and vertical axis is a low light chair (FLC), indicative of disease burden. Cancer patients are treated with both IMiDs and 45 cycles of high dose chemotherapy (HDCT) as per protocol of the study. MM 1 = MM diagnosis, MM 2 = primary plasmaoma, MM 3 = second relapse, MM 4 = fourth relapse, MM 5 = sixth relapse, MM 6 = seventh relapse, MM 7 = eighth relapse, MM 8 = ninth relapse, MM 9 = tenth relapse, MM 10 = eleventh relapse.

Figure 2. Myeloma Ikaros axis and IMiD mechanism of action. (A) Ikaros (IKZF1) and Aiolos (IKZF3) promote multiple myeloma survival and proliferation through inducing the upregulation of IRF4, which in turn upregulates MYC (MYC) to form a positive autoregulatory feedback loop. In IMiD-sensitive cells, this axis is necessary for MM proliferation and survival. (B) IMiDs modulate and promote the degradation of IKZF1 and IKZF2 by modulating the conformation of the substrate specificity receptor Cereblon (CRBN) in the CUL4b-APC E3-ubiquitin ligase. This in cooperation with an E2-ubiquitin ligase results in efficient posttranslational degradation of IKZF1 and IKZF2 and subsequent downregulation of IRF4 and MYC, leading to MM cell death.

Conclusions and Future Directions

• Ikaros (IKZF1) and Aiolos (IKZF3) are directly stabilized by IMiDs, leading to decreased IRF4 expression and MYC expression.
• The Ikaros Axis is expressed in more PC-like MM subpopulations and the Kessenich Donation

Acknowledgments

This work was supported by:· NIH (K08-Sherbenou), CCTSI Junior Faculty CO-PILOT
• University of Colorado Cancer Biology Graduate Program, University of Colorado Anschutz Medical Campus

Figure 3. IMiD sensitivity in MM cell lines. Five MM cell lines were treated with increasing doses of three different IMiDs for 96 hours. MM 15 and MM 20 were loosely sensitive to all IMiDs, while MM 1 and MM 6 were largely insensitive to all IMiDs tested.

Figure 4. Ikaros axis IMiD response and expression in primary MM patients. (A) Representative gating scheme of two cell primary samples. (B) Relative protein expression of IKZF1 or IKZF3 following 48h IMiD treatment. Each dot represents an individual patient. Each dot represents the relative expression of an IMiD resistant or sensitive patient. (C) Relative IRF4 expression in primary samples after 48h IMiD treatment. Each dot represents a technical replicate, and relative MFI is calculated compared to respective untreated controls. (D) Relative MYC expression is maintained in IMiD-resistant primary samples after 48h IMiD treatment. Each dot represents a technical replicate, and relative MFI is calculated compared to respective untreated controls. Re-expression data from bruk RNA-sequencing in patients enrolled in the MMRF CoMMpass research study. No response was classified as a patient who received either Len or Pom treatment. Each dot represents a technical replicate, and relative MFI is calculated compared to respective untreated controls. For cell viability, MM cell lines were treated with the indicated IMiD concentrations for 96 hours and relative live cell count with assessed by flow cytometry. Mononuclear cells from MM cell line and primary MM sample drug treatment were generated in FlowJo v9.

Figure 5. MYC protein is maintained in IMiD-resistant MM cell lines and primary samples. (A) Sensitive vs. resistant primary MYC expression. (B) Sensitive vs. resistant primary MYC expression. A) B) C) D) E) F) G) H) I) J) K) L) M) N) O) P) Q) R) S) T) U) V) W) X) Y) Z)