Interrogating the Ikaros Pathway in Multiple Myeloma IMiD Drug Resistance

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Drug Resistance in Multiple Myeloma

- Multiple myeloma (MM or myeloma) is a malignant plasma cell neoplasm
- Second most prevalent adult hematologic malignancy
- MM remains an incurable disease, as nearly all patients will eventually relapse and develop drug or multidrug resistance
- Acquired drug resistance, leading to relapse/refractory disease, is the root cause of treatment failure and remains the greatest obstacle to successfully curing MM
- The IMiDs lenalidomide (Len) and pomalidomide (Pom) are frequently included in MM treatment regimens
- Patients exposed to IMiDs eventually become resistant, and the mechanisms of acquired resistance remain largely unknown
- IMiDs exert direct anti-myeloma effects via multiple mechanisms of critical MM transcription factors Ikaros (IKZF1) and Aiolos (IKZF3)3

Methods

Myeloma cell line and primary multiple myeloma sample drug treatment

Mononuclear cells from patient bone marrow aspirates were Ficoll-separated and cryopreserved. Patients are shown by identification number. All samples were treated (Tx) with 10 µM IMiD (Len or Pom) for 24 hr ex vivo unless otherwise indicated. IMiD sensitivity was classified by ex vivo Myeloma Drug Sensitivity test (My-DST, Sherbenou lab). Cell lines were plated at 96-100 cells/well and primaries at 25×10⁶ cells/well. All conditions were performed in triplicate.

Measuring Ikaros pathway via intracellular flow cytometry

An intracellular staining assay was performed on MM cell lines and patient samples for IKZF1, IKZF3, IRF4, and c-MYC on the BD FacsCanto. Samples were fixed and permeabilized with FoxP3 transcription factor kit. Patient samples included CD38 and CD138 as myeloma markers. IKZF pathway protein levels were analyzed in FlowJo by geometric mean (GM) and normalized to untreated. P values were determined using 2-way ANOVA multiple comparisons in Graphpad Prism 8 (p < 0.05; *p < 0.01; **p < 0.001; ***p < 0.0001; ns = not significant).

Mass cytometry

Patient 1089.2 bone marrow was thawed and treated with 10 µM Len for 24 hrs. Cells were stained for a panel of immune and immune cell markers, IKZF1, proliferative, and apoptotic intracellular markers. Prepared CyTOF sample was sent to University of Rochester Medical Center. Data was analyzed by visNE in Cytobank and CyTOF median in FlowJo.