MYC Inhibition Overcomes Immunomodulatory Drug Resistance in Multiple Myeloma

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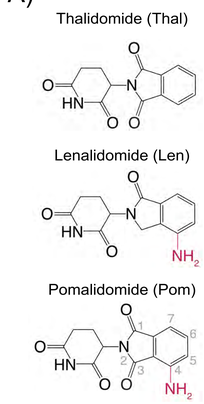
IMiD Resistance in Multiple Myeloma

- Multiple Myeloma (MM) is a cancer of plasma cells, predominately in the bone marrow
- Considered incurable, as nearly all patients endure a relapse-remitting disease course
- Drug resistance increases with relapses, making it more difficult to treat patients using currently available agents.
  - Median overall survival (OS) for triple drug class refractory MM is 5.6 months
- Immunomodulatory drugs (IMiD) (**A**) based regimens are frequently utilized and given in all disease settings
- IMiD binding to the E3 ubiquitin ligase receptor Cereblon leads to the proteasomal degradation of Ikaros (IKZF1) and Aiolos (IKZF3). This leads to the downregulation of the Ikaros axis overall (**B**)
- Resistance mechanisms driven by Cereblon or Ikaros axis gene abnormalities have been discovered in MM cell lines, but functional consequences have not been investigated in primary samples

We hypothesize that IMiD treatment fails to downregulate the Ikaros axis in IMiD resistant MM in patients.

A)



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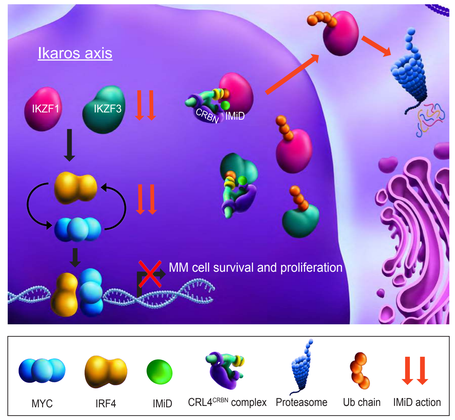
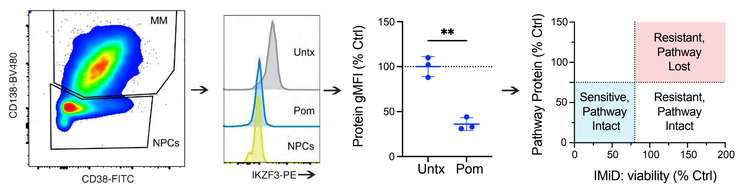


Characterizing the Ikaros axis in cell lines and patient samples

Intracellular flow cytometry to measure IMiD-induced Ikaros axis degradation

An intracellular flow cytometry assay was performed on MM cell lines (MM1S and H929) and patient samples for IKZF1, IKZF3, IRF4, and MYC. MM cell lines or patient samples were treated with 10 μM Pom for 24h (IKZF1/IKZF3) or 48h (IRF4/MYC) before being fixed and permeabilized. The relative IKZF1, IKZF3, IRF4, and MYC protein levels in CD38+CD138+ MM cells were assessed by normalizing the geometric mean (gMFI) to untreated (Untx) controls.



Mass cytometry to interrogate Ikaros axis expression in MM subpopulations

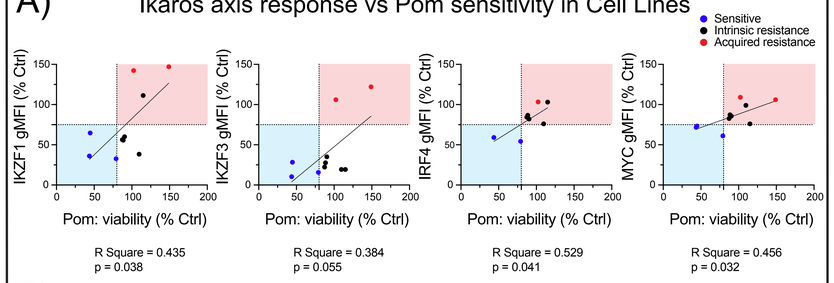
MNCs from patient BM biopsies were thawed and cells were stained for a panel of MM and immune cell surface markers, as well as light chains (kappa/lambda), IKZF1, IKZF3, IRF4, MYC, proliferative and anti-apoptotic markers, and dead cell stain. The samples were run on the Helios mass cytometer and analyzed in R.

Assessing ex vivo drug response in MM cell lines and patient samples

Acquired resistant MM1S and H929 cell lines were generated by increasing doses of Pom until cells were resistant to 40μM. Mononuclear cells (MNCs) from patient bone marrow biopsies were Ficoll-separated and cryopreserved previously. Cell line viability was assessed by flow cytometry at 120h and growth rate inhibition by the MYC inhibitor MYCi975 was calculated (http://www.grcalculator.org/grcalculator/). Patient samples were treated with the indicated drug concentration for 48h and assessed for MM viability via flow cytometry using a panel of MM markers (CD38, CD138, CD46, BCMA, FCRL5, CD45). Samples with <80% viability compared to the untreated controls were considered drug sensitive.

IMiD resistance positively correlates with Ikaros axis dysregulation in cell lines, but not in patient samples

A)



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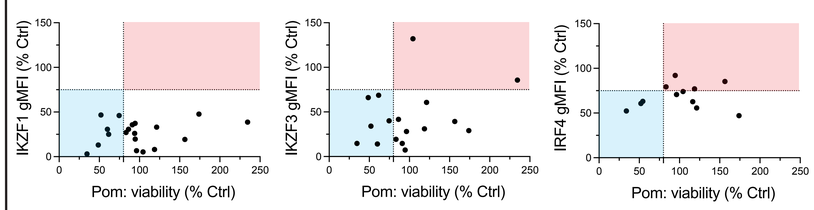
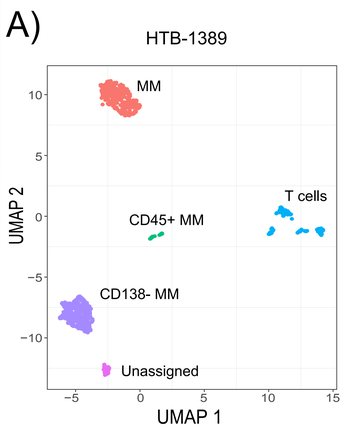


Figure 1. IMiD-induced Ikaros axis downregulation. (A) The relationship between MM cell line Pom sensitivity and Ikaros axis protein levels after 10μM IMiD treatment. IKZF1 and IKZF3 were measured at 24h, IRF4 and MYC at 48h, Pom sensitivity at 120h. Each point is the average of 3 replicates (N = 10). Blue boxes indicate sensitive samples with protein decrease and red boxes indicate resistant samples without protein decrease. (B) Pom sensitivity at 48h vs Ikaros axis protein levels in CD38+CD138+ MM cells from patients. Each point is the average of a patient sample tested in triplicate (N = 19 for IKZF1/3, N = 12 for IRF4). MYC decrease could not be detected in patient samples. Pearson r is shown when a signficant correlation was found.

Ikaros axis proteins are differentially expressed between MM subpopulations

A)



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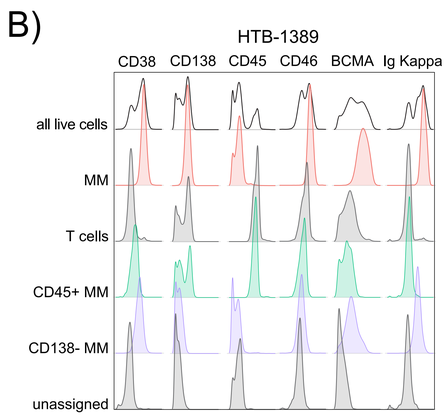

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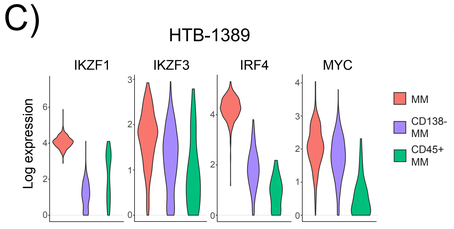

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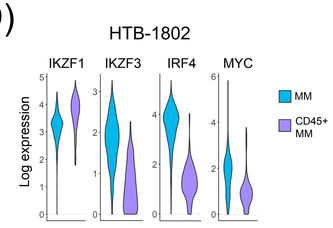

Figure 2. Mass cytometry of Ikaros axis proteins. (A) UMAP of patient sample HTB-1389 showing MM phenotypic heterogeneity with three subpopulations. (B) Histograms of markers used to determine immunophenotype of MM subpopulations in HTB-1389. (C-D) Violin plots of Ikaros axis protein expression in MM subpopulations (UMAP clusters) from patient samples HTB-1389 (relapsed) and HTB-1802 (new diagnosis).

MM Subpopulations Have Differential IMiD Sensitivity

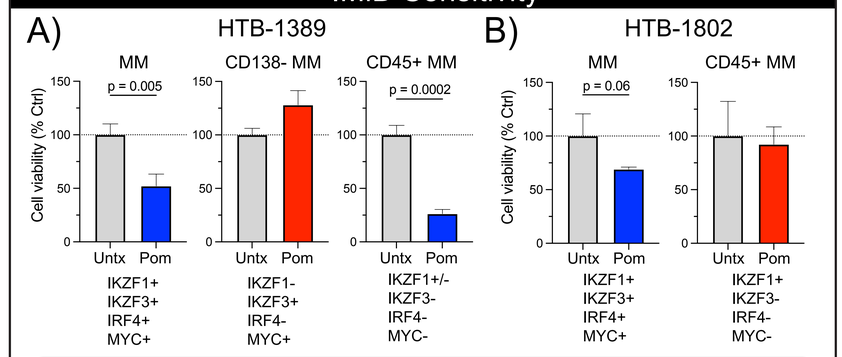

Figure 3. Ex vivo Pom sensitivity of MM subpopulations. Samples were treated with 10μM Pom for 48h and relative viability for each MM subpopulation was assessed. Relative Ikaros axis protein expression summarized below each subpopulation. (A) Patient HTB-1389. (B) Patient HTB-1802.

IMiD Resistant MM is Sensitive to MYC Inhibition

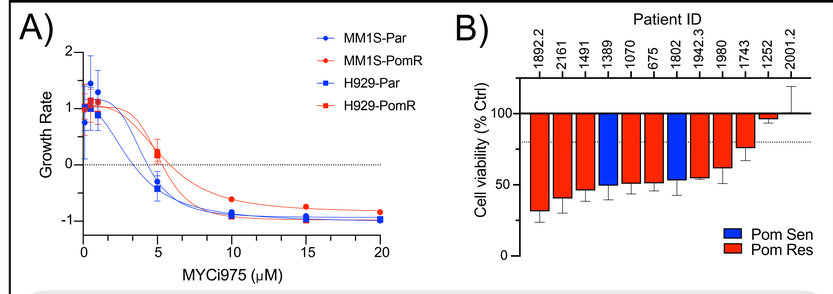

Figure 4. Ex vivo MYC inhibitor sensitivity of bulk IMiD resistant MM. (A) Growth rate curve of Pom sensitive (Par) and resistant (PomR) cell lines treated with MYCi975 for 120h. Growth rate of <0 means drug is cytotoxic. (B) Waterfall plot showing the relative viability of bulk MM cells in patient samples treated with 1 μM MYCi975 for 48h. Cutoff between a sample being sensitive or resistant is 80% (dashed line).

Conclusions

- While Pom resistant cell lines lose the ability to downregulate the Ikaros axis, this resistance mechanism has not been observed in most of our patients
  - IKZF1 and IKZF3 degradation intact in most resistant MM, but many resistant samples do not downregulate IRF4 - suggesting resistance mechanisms primarily downstream of IKZF1/3
- Ikaros axis proteins are heterogeneously expressed in MM intratumoral subpopulations, and subpopulations exist that have abnormal Ikaros axis protein levels and can display IMiD resistance
  - suggests Ikaros axis may not be operational in some MM subpopulations
- IMiD resistant MM is sensitive to MYC inhibition

Continuing to interrogate IMiD resistance

- Mutation/CNV analysis in patient MM where IMiD-induced Ikaros axis downregulation is perturbed
- Correlate Ikaros axis protein expression with IMiD sensitivity of MM subpopulations and relapses
- Characterize phenotypes of IMiD resistant populations using mass cytometry and single-cell RNA sequencing
- Determine the necessity of individual Ikaros axis proteins in resistant patient MM transcription and survival

Acknowledgments

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