

The Ikaros Axis is Heterogeneously Expressed in Multiple Myeloma Subpopulations and Does Not Drive IMiD Resistance

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Multiple myeloma (MM) is a malignant plasma cell neoplasm that remains incurable due to the widespread prevalence of acquired drug resistance. Immunomodulatory drugs (IMiDs) are a cornerstone of MM therapy, yet the mechanisms of acquired IMiD resistance remain largely unknown. IMiDs possess direct anti-MM effects by promoting the degradation of Ikaros (IKZF1) and Aiolos (IKZF3), which in turn downregulates the expression of the critical MM transcription factor IRF4 and consequently MYC (collectively known as the Ikaros axis). Based on the known IMiD mechanism of action, we hypothesized that the Ikaros axis differentially responds to IMiD treatment in patients with IMiD resistance. We investigated how expression of these proteins responds to *ex vivo* IMiD treatment in IMiD-sensitive and -resistant patient MM cells (CD38⁺CD138^{dim/+}) by employing flow cytometry. Our results reveal that the levels of IKZF1, IKZF3, and IRF4 are equivalently decreased in IMiD-sensitive and -resistant cell lines and patient samples (n = 6) following *ex vivo* IMiD exposure. However, in the subset of patients examined thus far, MYC was not downregulated in IMiD-resistant MM. We further assessed the expression of the Ikaros axis in diverse MM subpopulations via mass cytometry. Interestingly, we discovered that IKZF1, IKZF3 and IRF4 are not expressed in MM cells with a less plasma cell phenotype, whereas MYC is expressed in all MM cells. These results suggest that IMiD-resistant MM cells may be independent of IKZF1, IKZF3, and IRF4 through possessing a less plasma cell phenotype. Further, our data suggest that IMiD-resistant MM is likely still dependent on MYC through Ikaros axis-independent mechanisms and thus targeting MYC may be a promising therapeutic strategy for IMiD-refractory patients.