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Blood 142 (2023) 3301–3304

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Selinexor Disrupts Epigenetic Programing and Modulates Immunogenicity in Multiple Myeloma

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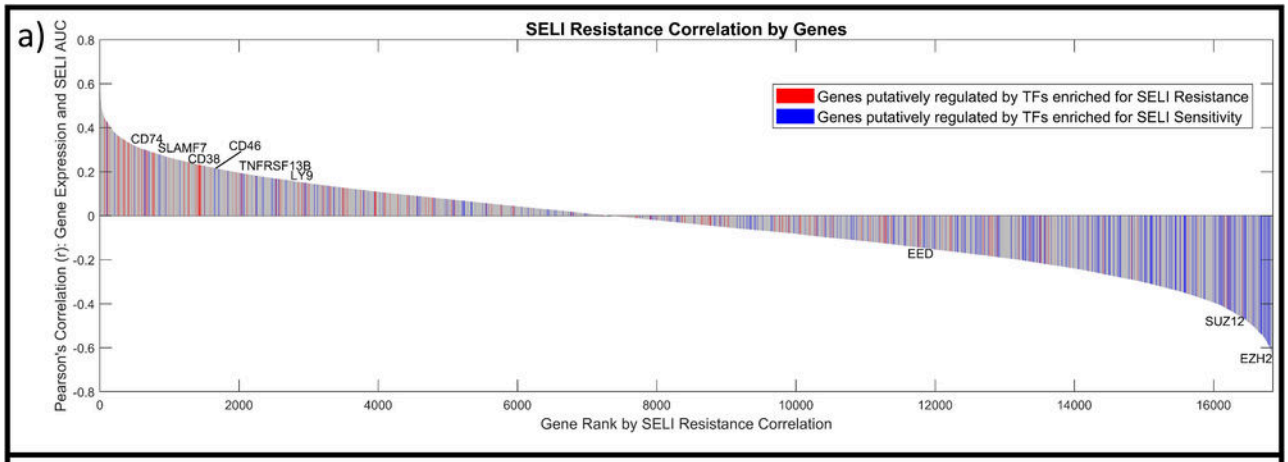
Introduction: Multiple myeloma (MM), remains an incurable, but treatable, malignancy that has benefitted from the integration of novel immunologic agents in clinical use, such as monoclonal antibody, bispecific T cell engager (TCE) and chimeric antigen receptor T (CAR-T) cell and antibody-drug conjugate based therapies. While these agents have shown great promise in treating relapsed/refractory MM patients, there is heterogeneity in response to therapy due to differences in patients' immune tumor microenvironments (iTME) and immunogenicity of the tumor cells. Previously (Sudalagunta et. al., ASH 2021), we used functional genomic screening of 53 MM samples to characterize the transcriptomic profile associated with sensitivity to the selective nuclear export inhibitor, selinexor (SELI), which was shown to be inversely correlated with the transcriptomic profile associated with sensitivity to an anti-CD38 monoclonal antibody in 106 MM samples. In this study, we investigate the cellular mechanisms of SELI resistance leading to an immunogenic cell state.
Methods: Primary cells from an additional set of MM patients (n=65) were used for paired bulk RNAseq and an organotypic ex vivo drug sensitivity assay. Cellular viability was assessed using digital analysis of brightfield longitudinal images, and drug sensitivity was assessed as area under the dose-time-response curve (AUC) for each sample. We conducted gene set enrichment analysis (GSEA) on paired RNAseq data and ex vivo AUC of SELI. Individual genes and their respective correlations with ex vivo AUC of SELI were ranked from highest to lowest. We used RNAseq to examine the role of acute exposure to SELI on CD138-selected cells from paired pre/post treatment bone marrow specimens of five MM patients enrolled in the NCT02186834 clinical trial, taken prior to treatment with SELI and dexamethasone (DEX) and one/two weeks post-induction. Paired t-tests were carried out to quantify the changes.
Results: In our previous analysis of 844 MM samples, a subset of the genes whose expression is positively correlated with SELI AUC are controlled by H3K27me3, which is catalyzed by nuclear accumulation of EZH2. Tripathi et. al. (AACR Annual Meeting 2022 & 2023) have demonstrated that XPO1 and EZH2 form a complex, and inhibition of XPO1 activity blocks export of EZH2. GSEA of a new cohort of 65 MM patient samples demonstrated increased ex vivo SELI sensitivity (lower AUC, hence negative correlation) in samples with higher expression of known epigenetic regulators of the polycomb repressive complex 2 (PRC2), such as EZH2, SUZ12, and EED; as shown in Figure a, which led to lowering of expression of genes bound by these proteins due to gene silencing via H3K27me3, shown in red in Figure a, according to GSEA of ChIPseq databases. Furthermore, gene expression profiles of paired pre/post SELI/DEX treatment samples showed reduced expression of genes associated with H3K27me3 histone modification (paired t-test p=2.06e-83, 1.1061e-81, 2.56e-80, 5.15e-12, 6.22e-4) in all five patients. These observations suggest that XPO1 inhibition leads to nuclear accumulation of epigenetic modulators like EZH2, SUZ12, and EED, which catalyze H3K27me3 activity leading to silencing of H3K27me3-regulated genes. GSEA of paired ex vivo AUC and RNAseq data for an EZH2 inhibitor (EPZ-6438) resulted in negative correlation between expression of genes in PRC2 and ex vivo sensitivity, similar to SELI. Interestingly, we observed that this epigenetic mechanism of resistance to SELI is inversely associated with key immunological targets in MM such as CD74, SLAMF7, CD38, CD46, TNFRSF13B, and LY9 as shown in

Figure a. In Figure b, we present a graphical visualization of intracellular changes due to exposure to SELI in a naïve and a SELI-resistant MM cell.
Conclusions: Here we propose that SELI disrupts EZH2 subcellular localization, dysregulating H3K27me3-gene suppression. Sequential BM biopsies from SELI treated patients demonstrate decreased expression of H3K27me3-regulated genes, suggesting increased EZH2 activity by nuclear accumulation. Finally, SELI-resistant primary cells have increased expression of EZH2 regulated immunotherapy target genes (e.g. CD38, SLAMF7), suggesting a possible therapeutic strategy of sequential treatment of SELI followed by immunotherapy. Using multiparameter flow cytometry of samples pre/post SELI/DEX induction, we will study SELI's effect on iTME.

Disclosures Hampton: *Aster Insights:* Current Employment. **Freeman:** *Janssen:* Consultancy, Honoraria, Research Funding; *ONK Therapeutics:* Consultancy, Honoraria; *Celgene:* Consultancy, Honoraria; *Bristol Myers Squibb:* Consultancy, Honoraria, Research Funding. **Wu:** *Karyopharm:* Current Employment. **Ellero:** *Karyopharm:* Current Employment. **Peres:** *Bristol-Myers Squibb:* Research Funding; *Karyopharm:* Research Funding; *National Institute of Health/National Cancer Institute:* Research Funding; *Pentecost Family Myeloma Research Center:* Research Funding. **Hansen:** *Bristol Myers Squibb:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Karyopharm:* Consultancy, Research Funding; *Janssen:* Consultancy; *Pfizer:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *International Myeloma Society Young Investigator Award:* Research Funding; *Pentecost Family Myeloma Research Center:* Research Funding; *OncLive:* Honoraria; *Survivorship:* Honoraria. **Mark:** *Karyopharm:* Current Employment. **Walker:** *Karyopharm Therapeutics Inc.:* Consultancy, Current Employment, Current equity holder in publicly-traded company. **Taverna:** *Karyopharm:* Current Employment. **Baz:** *BMS:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *AbbVie:* Research Funding; *Janssen:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Karyopharm:* Research Funding; *Pfizer:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *GSK:* Honoraria; *Regeneron:* Research Funding; *HIKMA Cancer Network:* Honoraria; *Curio Science:* Honoraria; *AHOMPR:* Honoraria; *ASH:* Honoraria. **Shain:** *Takeda:* Honoraria; *Sanofi:* Honoraria; *GlaxoSmithKline:* Honoraria; *Adaptive:* Honoraria; *Amgen:* Honoraria; *Amgen:* Honoraria; *Janssen:* Honoraria; *Bristol Myers Squibb:* Honoraria; *Karyopharm:* Research Funding; *AbbVie:* Research Funding.



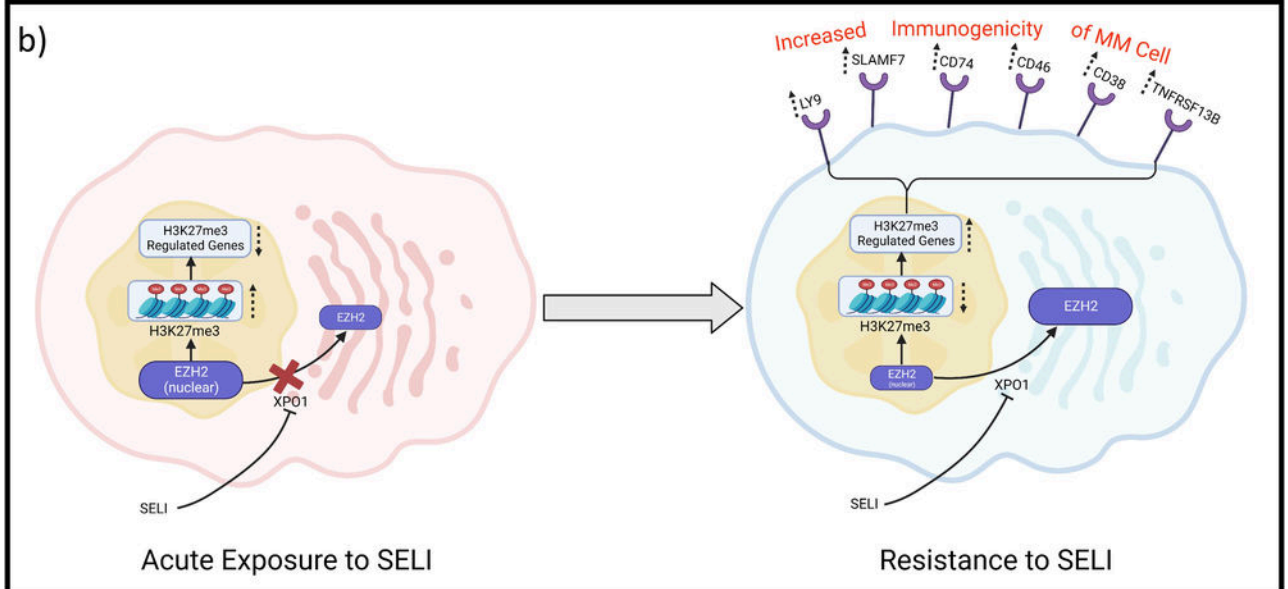


Figure a is a bar plot of Pearson's correlation coefficient (r) of ex vivo AUC (higher AUC implies resistance, lower AUC indicates sensitivity) to SELI and z-normalized expression of genes from matched samples with RNA-seq data shown on y-axis and x-axis shows ranked list of genes from most positively correlated to most negatively correlated. Genes highlighted in red are putatively regulated by EZH2, SUZ12, IRF8, ZEB1, VDR, ESR1, and FOXA2; which are transcription factors enriched for SELI resistance using GSEA from ENCODE and ChEA. Similarly, genes highlighted in blue are putatively regulated by FOXM1, NR2C2, YY1, ATF3, TAF7, CBX3, and BHLHE40. However, expression of genes part of PRC2 (EZH2, SUZ12, and EED) are negatively correlated with SELI AUC. On the other hand, novel immunological markers in MM are positively correlated with SELI AUC, which suggests resistance to SELI leads to increased immunogenicity of MM cells via loss of expression of EZH2 (catalyzes H3K27me3).

Figure b is a visualization, which depicts acute exposure to SELI inhibits XPO1 thereby inhibiting the export of several proteins from the nucleus (including EZH2), which leads to nuclear accumulation of EZH2 (indicated by a larger symbol) that catalyzes H3K27me3 via PRC2, which leads to silencing of genes regulated by H3K27me3. On the other hand, a SELI-resistant MM cell continues to export EZH2 from the nucleus to the cytoplasm leading to lower nuclear accumulation (compared to SELI-sensitive MM cells). This results in lower catalysis of H3k27me3, which results in increased expression of downstream genes. This leads to higher expression of some novel immunological markers in MM (CD74, SLAMF7, LY9, CD46, CD38, TNFRSF13B), which could make a SELI-resistant MM cell primed for greater response to an immunological agent.

Figure 1

https://doi.org/10.1182/blood-2023-190658