Multiple Myeloma Drivers of High Risk and Response to Stem Cell Transplantation

Identified by Causal Machine Learning: Out-of-Sort-And and Experimental Validation


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Introduction

We previously developed a high-dimensional network model of MM based on data from 645 patients in the Interim Analysis 9 (IA9) MMRF CoMPass trial dataset (NCT0145429). This model, developed using the REFSM causal inference engine, consists of an ensemble of 256 Bayesian networks, each representing the inferred causal relationships between 30,084 clinical and genomic variables. Key results from this model include:

1. Identification of a pathway driving high-risk disease (progression or death within 18 months)
2. Characterization of a subpopulation of patients with increased progression-free survival (PFS) after stem cell transplantation (SCT).

We have now tested the overall IA9 model and its key results using the IFM/DFCI 2009 dataset (NCT01191060), as well as performed experimental pre-clinical validation of core molecular drivers in the high-risk status pathway.

Methodology

RNA-Seq, demographic and clinical data modalities in the IFM/DFCI dataset were processed and normalized using the same pipeline as the IA9 dataset. Among the 30,084 variables in the IA9 model, 24,559 were present in the IFM/DFCI dataset, and a total of 333 patients had complete clinical and molecular data. High Risk was defined as progression or death before 18 months.

Global Validation of IA9 Model

- The IA9 model contains 121,708 edges (causally enriched statistical associations) that appear in at least 25% of the inferred networks in the ensemble. 93,636 of these edges (77%) were tested in the IFM/DFCI dataset and 81,155 edges (87%) had significant q-value (< 0.05) and effect sizes of the same sign between the two datasets.

Subnetwork Validation

- Simulation of the IA9 model also revealed a patient subpopulation with increased PFS in response to stem cell transplantation and decreased PFS in its absence. The top driver of this subpopulation in the IA9 model was expression of CHEK1; other drivers included RUNX2 and MYB2.

Figure 1. IA9 Network Constraints

Figure 2. IA9 Consensus Network Graph

Figure 3. Effect sizes in IA9 vs DFCI edges in the causal model

- The effect sizes between datasets were highly correlated: among all edges, Pearson’s r = 0.89; among validated edges, r = 0.93.

Conclusions

- Together, these results confirm key predictive results of the IA9 computational model in an out-of-sample dataset. 87% of edges that could be tested were validated, and the effect sizes are highly correlated.
- MEK, PIK4, and TTK can be confirmed as associated high-risk status in both CoMPass and IFM/DFCI datasets, and in multiple myeloma cell lines.
- CHEK1 may be confirmed as a potential driver of population stratification with regard to response to stem cell transplantation.
- This model should now help researchers to focus on the most promising targets and pathways, as well as to address unanswered questions and unmet needs in myeloma, especially high-risk disease.

References


Conflict of Interest


Experimental Validation of High-Risk Drivers

The functional relevance of these potential drivers of high risk was confirmed pre-clinically in myeloma cell lines using targeted small molecule inhibitors of MEK, CDK1, PIK4, and TTK.

Figure 5. Subnetwork of molecular drivers of High Risk in the IA9 causal model.

- Edges represent causal relationships; the width of each edge is proportional to the frequency of that edge in the networks in the ensemble (edges with frequencies between 0.05 and 1 are shown). All edges were confirmed in the IFM/DFCI dataset (q < 0.05).
- Drivers of high risk confirmed in the IFM/DFCI dataset (q < 0.05) are shown with a green outline; others are shown with red outline. Drivers of high risk that were confirmed pre-clinically, in myeloma cell lines using targeted small molecule inhibitors, are shaded in green.

Figure 6. Experimental validation of TTK as novel drug target in myeloma.

(A) TTK protein expression was observed in all MM cell lines analyzed. (B) Single dose treatment with IC70 concentrations of TTK inhibitors CI-400257 (250M) and BAY1217389 (75M) impaired the growth of OPM2 cells. (C) DNA content analysis demonstrated a time-dependent accumulation of polyplid MM cells post treatment with TTK inhibitors. (D) Max-Garma (Gloradzetti) staining of OPM2 cells 48h post treatment with BAY1217389 confirmed the formation of polyplid cells (green arrowheads). (E) Formation of polyplid cells led to the induction of apoptosis 96h post treatment with BAY1217389. Importantly, this persisted in the presence of bone marrow stromal cells. Graphs represent the mean±SD of three independent experiments.

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