Notable

CASE STUDY

Profiling of Rigosertib Identifies Distinct Classes of Responders in Myelodysplastic Syndrome

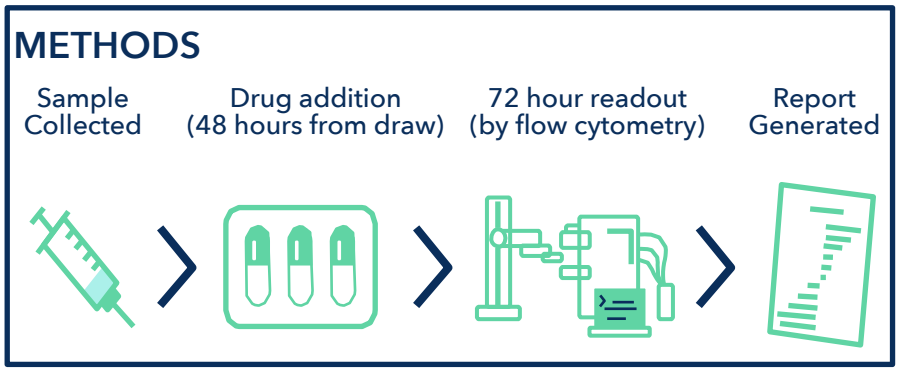
PROBLEM

Myelodysplastic syndromes (MDS) are clonal neoplasms with dysfunctional hematopoietic stem cells characterized by ineffective hematopoiesis, cytopenias, and have a substantial risk of transformation to AML. The hypomethylating agents (HMAs) azacytidine and decitabine are the standard frontline therapy for most patients with higher risk MDS. Limited treatment options are available for patients with HMA-refractory MDS, and there are no approved therapies in this setting. The prognosis is very poor in these patients with the median overall survival less than 6 months. This is an area of high unmet medical need, and new treatments are desperately needed. A precision oncology platform to enrich clinical trial populations to those most likely to benefit from a specific therapy would be of great value and speed the drug development process.

SOLUTION

Notable Labs has developed an automated, high-throughput, drug sensitivity testing platform based on multi-parameter flow cytometry to analyze peripheral blood and bone marrow samples from patients with hematologic malignancies. Primary samples undergo red blood cell lysis and are resuspended in media supplemented with various cytokines to support cells in culture for the duration of the assay. In addition to dysplastic cells and malignant blasts, normal immune cell components are retained in culture to preserve the tumor microenvironment. Cells are aliquoted into 384-well microtiter plates for drug screening. The automated system then dispenses nanoliter volumes of investigational and FDA-approved compounds and combinations into each well, in replicates, and incubates for a specified period of time. Upon assay readout, the drugged aliquots are stained with a flow cytometry antibody panel to quantify surface proteins to define specific immune cell populations. Drugged conditions are normalized to vehicle-only controls in an individual patient sample.

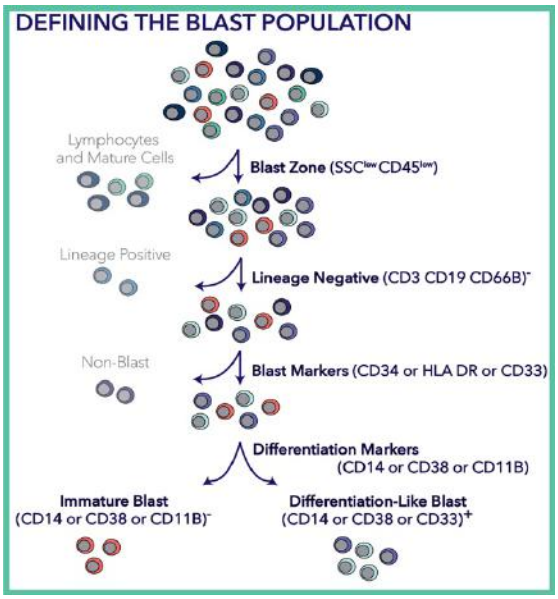
METHODS



SCOPE OF PROJECT

Notable Labs' ex vivo drug sensitivity screening platform was used to stratify responses (based on reduction of the dysplastic cell [blast] population) in primary MDS samples to rigosertib. Rigosertib is an investigational small molecule that disrupts the binding between RAS and RAS effector proteins that is in late stage clinical development in patients with high risk MDS refractory to HMAs.

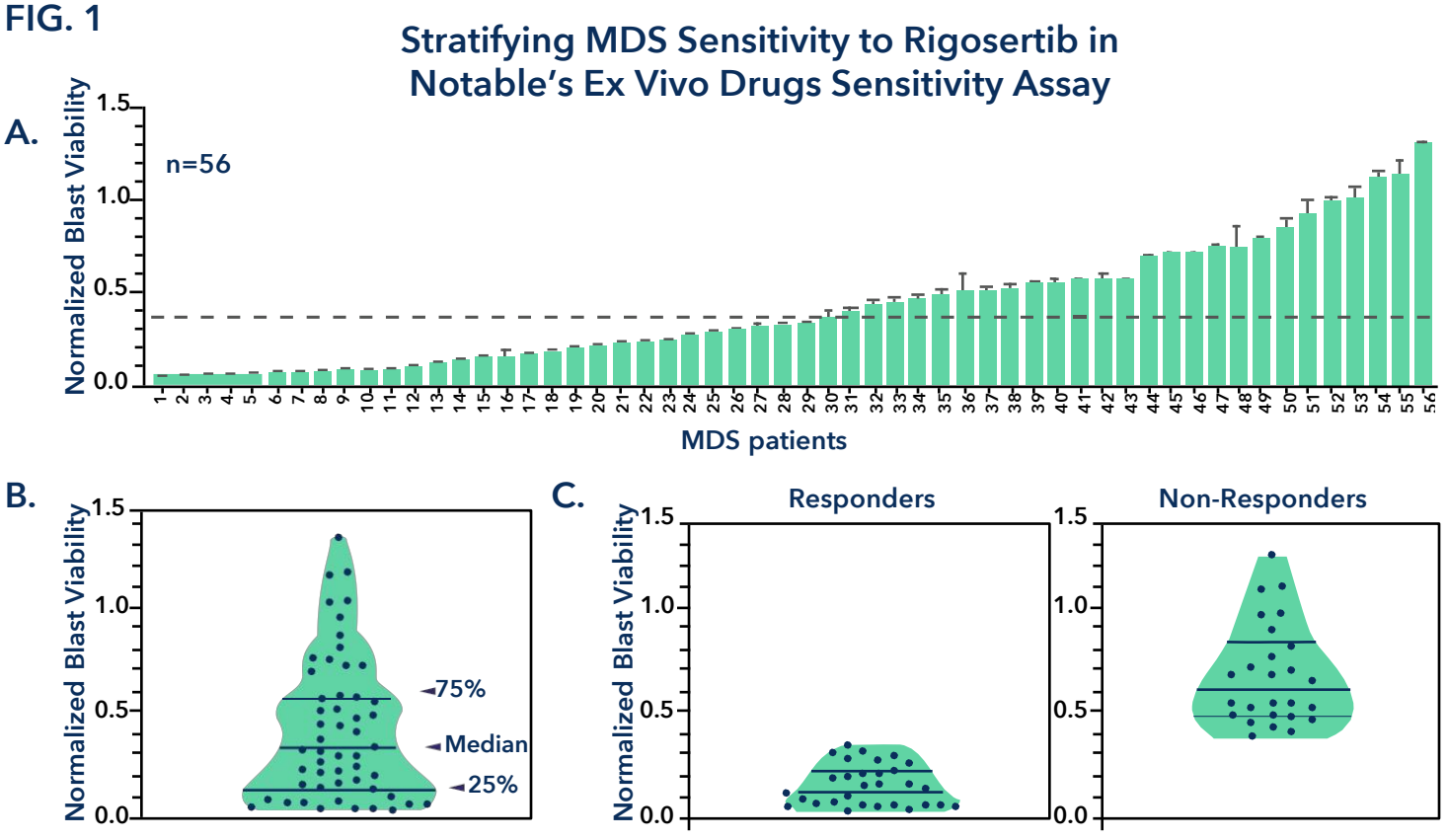
Notable Labs screened 56 primary MDS samples for sensitivity to a clinically achievable dose of rigosertib (70nM). Samples were identified as responders and non-responders based on sensitivity to rigosertib relative to the median response of the population. Sensitivity to rigosertib was further segregated into sensitive, reduced-sensitive and insensitive groups by measuring dose responses of 12 samples to increasing concentrations of rigosertib in the assay. The gating strategy used to identify the sensitive dysplastic cell populations is shown to the right.



OUTCOME

The mean drug sensitivity-response of rigosertib in the assay was 38.3% normalized blast viability with a 75th and 25th percentile response of 70% and 20% normalized blast viability, respectively (Figure 1).

FIG. 1

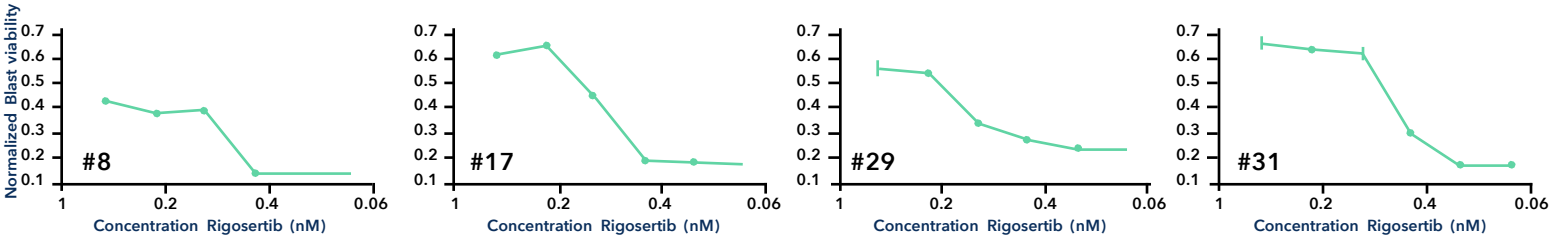


Additionally, the 12 samples screened in a 6-point dose response (2.6 nM to 620 nM) identified into three subgroups: a sensitive population, a population with reduced sensitivity and an insensitive population. Increasing concentrations of rigosertib identified samples with reduced viability below 30% as the sensitive population (n=4; Figure 2A). A reduced sensitivity group plateaued without reducing viability below 50% at any concentration (n=5; Figure 2B). An insensitive group was identified that did not demonstrate reduction in viability at any rigosertib concentration tested (n=3; Figure 2C).

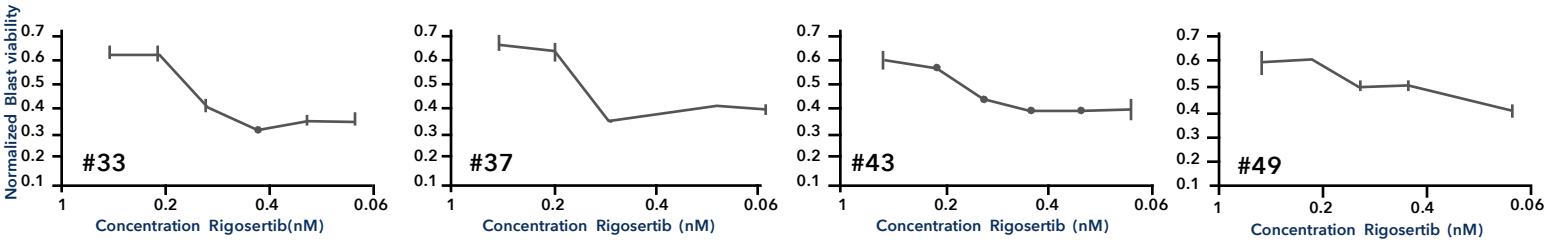
FIG. 2

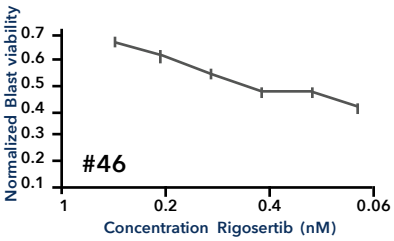
MDS Patient Sensitive Grouping to Dose Response of Rigosertib

A. Sensitive Group

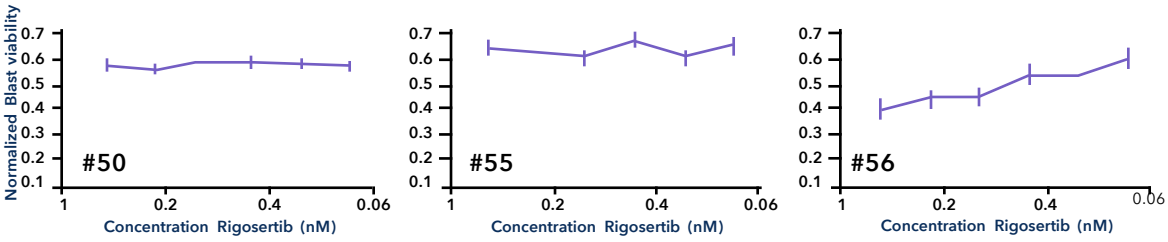


B. Reduced-sensitive Group





C. Insensitive Group



IMPACT

These data represent the most comprehensive ex vivo analysis of rigosertib sensitivity to date. Notable Labs has established an ex vivo drug sensitivity assay for rigosertib using primary patient-derived samples that segregates patient responses into three groups: sensitive, reduced sensitivity and insensitive. This ex vivo assay has potential utility as a patient selection tool to enrich clinical trial populations to those most likely to benefit from therapy, as well as identify potential synergistic drug combinations; further validation with clinical outcomes is necessary.

REFERENCES

1. M. Santaguida, E. Anderson, M. Maniar, S. Fruchtman, M. De Silva and D. Heiser. Ex vivo Response Profiling of Rigosertib Identifies Distinct Classes of Responders in Myelodysplastic Syndrome. Myelodysplastic Syndrome Foundation International Symposium (poster) 2019.