Detection of CCND1 Overexpression By RNA-Seq from TNA Samples As a Surrogate for t(11:14)Translocation Traditionally Measured By FISH in Multiple Myeloma Patients for Improved Patient Care

Abhisek Ghosal [1], Francys Alarcon [1], Samuel Koo [1], Grace Kang [1], Archana Ramesh [2], Tibor Gyuris [2], Segun C Jung [1], Brad Thomas [3],Rudy Fabunan [1], Christophe Magnan [2], Hyunjun Nam [2], Paris Petersen [1], Fernando Lopez-Diaz [2], Susan Yamahata[1], Ryan Bender [2], Sally Agersborg [1], Fei Ye [2], Vincent A. Funari [1]

[1]NeoGenomics Laboratories, Inc., Aliso Viejo, CA; [2]NeoGenomics Laboratories, Inc., Carlsbad, CA; [3]NeoGenomics Laboratories, Inc., Houston, Texas

2961

Introduction

- Multiple Myeloma (MM) is a blood cancer type affecting plasma cell in bone marrow. MM is heterogenous in nature but t(11;14)(q13;q32) translocation is a common prognostic marker among MM patients. CCND1 (Cyclin D1) translocation, resulting from the t(11:14) with immunoglobulin heavy chain (IGH) causes over expression (OE) of CCND1, which leads to cell cycle abnormalities, thus oncogenesis.
- Currently, FISH is the gold standard for detection t(11:14) translocations at the DNA level but it cannot detect the downstream molecular events that result in RNA stability/RNA turn-around rate. It is reported that CCND1 can be upregulated independent of t(11:14) translocations, therefore obtaining CCND1 expression levels is important for diagnostic purposes.
- Heme NGS test for TNA panel from NeoGenomics (Neo Heme) can simultaneously detect RNA expression. Taking advantage of the existing panel, in this study we evaluated the need for in-use NeoLab Heme NGS assay for detection of the CCND1- OE in relation to the FISH data.

Overview of Method

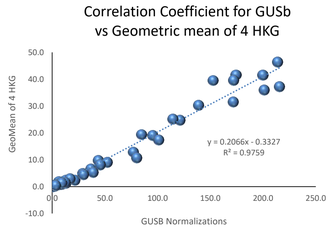
- NeoHeme RNA panel was used for NGS and TPM (transcript per million) was determined by TPM Calculator processed by RNA pipeline
- Refined the cutoff for FISH positive and negative samples for sensitivity and specificity by ROC curve fitting; also used FISH positive samples to evaluate the sensitivity and specificity
- Established and validated qRT-PCR using synthetic controls for evaluating the correctness for call
- For the confirmation of t(11:14) based cutoff for NGS based CCND1 expression qRT-PCR was established and used

Key Finding

- Using NGS we observed CCND1 over expression (verified by qRT-PCR) which may be because of downstream molecular event/mutation on accessory gene which FISH can not detect justifying the need of NGS to supplement FISH.

Result

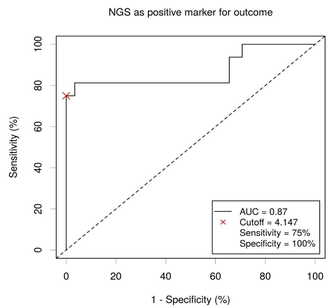
A. House-Keeping Gene



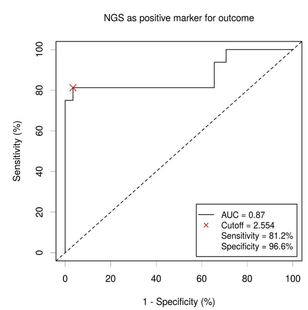
B. ROC curve fitting for cutoff for over expression

ROC curve fitting model	AUC	Cutoff	Sensitivity	Specificity	P value
Fisher Exact test	0.87	4.14	75%	100%	8.3e-11
Euclidean Distance	0.87	2.55	81.2%	96.6%	5.1e-10
Manhattan Distance	0.87	2.55	81.2%	96.6%	5.1e-10
Mixture of model	0.87	3.07	75%	100%	8.3e-11

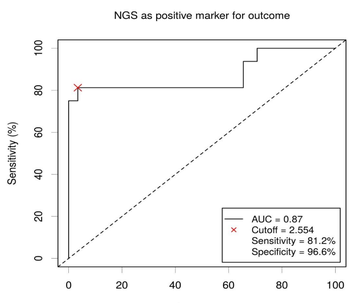
Fisher Exact test



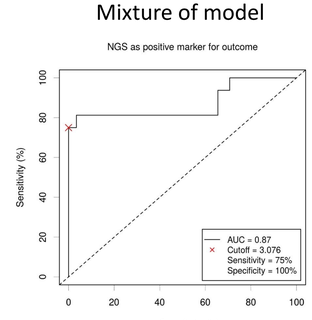
Euclidean Distance



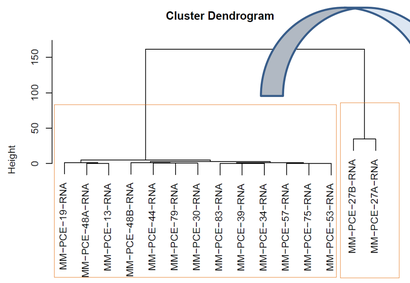
Manhattan Distance



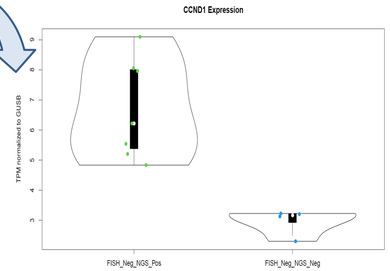
Mixture of model



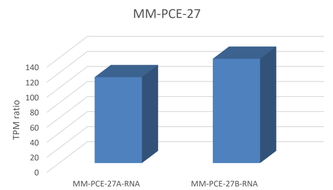
C. Clustering for FISH negative sample for NGS Exp

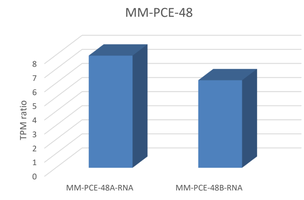


D. Clustering for FISH negative sample in low expression cluster in NGS



E. Expression cutoff does not change on extraction method





E. Case Study for FISH Neg and NGS high positive

FISH Result: MM-PCE-27	FISH Data
Del(1P)	Not Detected
Del(1q)Gains(5,9,15)	Not Detected
Del(13q)/-13	Not Detected
Del(17P)(TP53)	Not Detected
IGH rearrangement	Not Detected
T(4:14) translocation	Not Detected
T(11:14) translocation	Not Detected
T(14:16) translocation	Not Detected
T(14:20) translocation	Not Detected

Cytogenetics: MM-PCE-27	Comment
First clone with unbalanced translocations of the long arms of chromosomes 7 and 13 and a loss of a copy of chromosome 16.	Abnormal
The second clone (3/20 cells) is evolved from the first clone and exhibits a near tetraploid version of the first clone.	Abnormal
The third clone (5/20) shows the loss of the Y chromosome	Abnormal