Panthera R&D Program for Hemato-Oncology

An assay for simultaneous diagnosis of diagnosis of t(4;14), t(11;14), t(14;16)/t(14;20), DEL1P, ADD1Q, DEL13Q, DEL17P, MS/MF expression clusters, and the SKY92 high risk signature in multiple myeloma patients

M Van Vliet[1,] R Kuiper[2], A Broijl[2], Y de Knegt[2], W Hoos[1], L de Best[1], M van Duin[2], E Van Beers[1], P Sonneveld[2]*
[1]Skyline Diagnostics BV,
[2]Department of Hematology, ErasmusMC, Rotterdam, Netherlands

Introduction

Multiple Myeloma (MM) is a heterogeneous disease, with several recurring chromosomal aberrations including t(4;14), t(11;14), t(14;16)/t(14;20), del1p, add1q, del13q, and del17p. Gene expression profiling studies have revealed clusters of patients with distinct expression patterns including a signature that identifies high-risk patients (SKY-92) [1]. However, methodologies for assessing these markers have not been standardized yet. Lack of standardization hampers marker interpretation across cohorts, and limits the emerging strategies that combine these markers towards patient stratification and personalized medicine.

Aims

To develop a standardized assay for the detection of SKY-92, t(4;14), t(11;14), t(14;16)/t(14;20), del1p, add1q, del13q, and del17p.

SkylineDx

Methods

The MMprofiler assay uses standardized plasma cell purification, RNA and DNA extraction and proprietary sample labeling process for use with Affymetrix HG-U133 Plus2 and a Cytoscan HD chip and proprietary data preprocessing and analysis software. The assay includes ten markers, namely translocations t(4;14), and t(11;14), chromosomal copy number aberrations, del1p, add1q, del13q, and del17p, the MS, and MF gene expression clusters, and the SKY-92 high risk signature. A total of 329 patients from the HOVON65/GMMG-HD4 trial were used to train nearest mean classifiers for the translocation markers by means of a double loop cross validation protocol.

Results

The SKY-92 signature identifies high risk MM patients, and was previously shown to be a strong independent prognostic risk factor across multiple datasets, that outperforms signatures developed by others for the same goal [1]. The translocations t(4;14), and t(11;14) are associated with strong gene expression profiles, also reflected in their correlation with the clusters. Classifiers with high sensitivity and specificity were developed, as shown in Table 1. Classifiers for the prognostic MS and MF gene expression clusters were also developed. However, as there is no alternative method to compare against (e.g. no FISH etc.) their performance must be assessed by evaluation of their prognostic value in independent cohorts. Gene expression based classifiers performed reasonablefor the chromosomal aberrations add1q, and del13q, and performed poorly for the del17p. Since clinical implementation of these gene expression profiles is suboptimal we decided to detect clinically relevant variants of del1p, add1q, del13q, and del17p using the Cytoscan HD platform, at 99% sensitivity and specificity. An overview of all performances is provided in Table 1.

Conclusion

We report the development of a research use only assay for evaluation of ten different markers relevant for MM, which can ultimately be applied by qualified laboratories. The assay will be employed for further evaluation along the EMN-02/HOVON-95 clinical trial.

Reference

1. Kuiper R, et al. A gene expression signature for high-risk multiple myeloma. Leukemia, 2012, 26:2406–13.

Abstract at EHA 2013

Table 1. Assay performances for the ten markers in percentages.

Marker	Chip	Sensitivity (%)	Specificity (%)
t(4;14)	Expression	86.4	98.0
t(11;14)	Expression	89.7	95.3
t(14;16)/t(14;20)	Expression	82.9	89.2
del1p	Copy number	99.0	99.0
add1q	Copy number	99.0	99.0
del13q	Copy number	99.0	99.0
del17p	Copy number	99.0	99.0
Marker	Chip	HR	p-value
MS cluster	Expression	NA*	NA*
MF cluster	Expression	NA*	NA*
SKY-92	Expression	2.38 to 5.23†	p<0.0001†

* No alternative method available to compare against
† in four independent datasets [1]