# An assay for simultaneous diagnosis of t(4;14), t(11;14), t(14;16)/t(14;20), add1q, del13q, del17p, MS/MF expression clusters, and the SKY-92 high risk signature in Multiple Myeloma patients

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## Aim of the study

To develop a comprehensive, gene expression based, standardized assay for the detection of high risk SKY-92, t(4;14), t(11;14), t(14;16)/t(14;20), add1q, del13q, and del17p, MS cluster and MF cluster cases.

## MMprofiler™

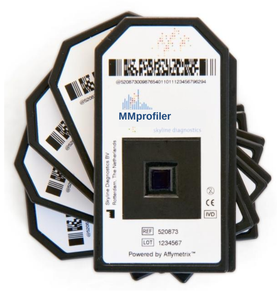
Risk classification in Multiple Myeloma (MM) is of vital importance since MM is a heterogeneous disease harboring distinct outcome categories [1]. MM subtypes are characterized by several different recurring chromosomal aberrations such as t(4;14), t(11;14), t(14;16), t(14;20), add1q, del13q, and del17p. Furthermore, gene expression profiling studies have identified other distinct biological subtypes some of which may or may not be correlated with clinical outcome [1,2]. One example is a high-risk subtype as detected by the SKY-92 gene expression classifier [3].

Lack of standardization of current methodologies hampers marker interpretation across cohorts and limits the applicability for patient stratification and development of personalized medicine in MM.

## Results (Development)

The MMprofiler assay uses standardized plasma cell ($CD138^+$) purification, RNA extraction and sample labeling for use with Affymetrix U133Plus2 GeneChips and proprietary data analysis software. The assay currently reports the markers; SKY-92 high risk, t(4;14), t(11;14), t(14;16)/t(14;20), add1q, del13q, del17p, MS and MF gene expression clusters.
A total of 329 patients from the HOVON-65/GMMG-HD4 trial were used to train classifiers for all these markers for single sample analysis. Gene expression based classifiers performed well for the chromosomal aberrations add1q, and del13q, and initially performed poorly for the del17p. For the del17p cases the percentage of positive cells scored between 10 and 100%, see Figure below. Classifier performance increased when retraining the classifier with cases with > 80% del17p positivity (see Table).

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 [3]. The translocations t(4;14), t(11;14), and t(14;16)/t(14;20), were found to be strongly associated with gene expression profiles (GEP), also reflected in their correlation with the GEP clusters. Classifiers with high sensitivity and specificity based on their FISH annotation were developed, as shown in Table. 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.



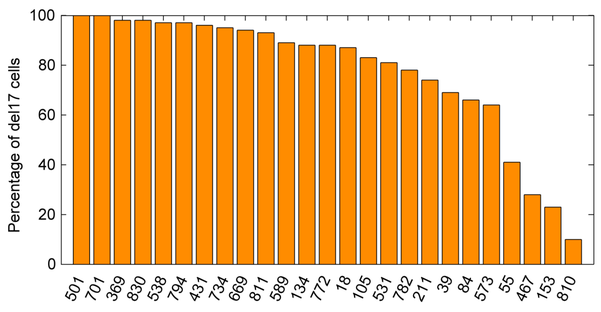




Figure 1. Del17 FISH percent age positive cells

## Results (Validation)

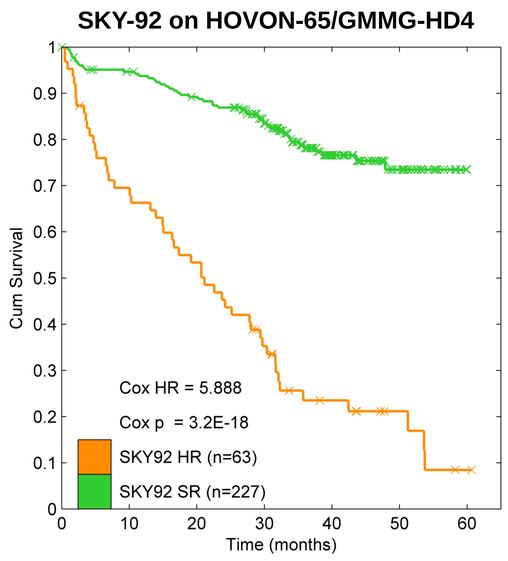



Figure 2. SKY-92 Risk Classification

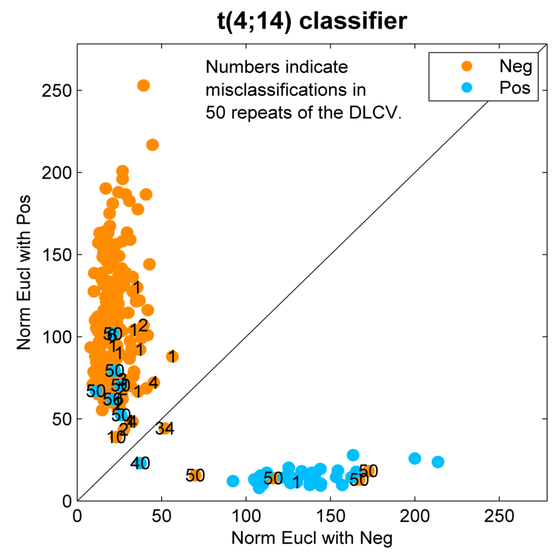



Figure 3. t(4;14) Classifier PCA

The performance of the classifiers that were developed on the HOVON-65 dataset have been assessed by means of a double loop cross validation (DLCV) protocol, providing realistic estimates of their performance on unseen data (Table). Next, we validated the classifiers on an independent cohort, the MRCIX dataset (n=247). This analysis resulted in highly similar performances, see Table, confirming the adequacy of the classifiers. An unresolved issue is that FISH remains an imperfect standard to compare against.

Table. Training and Validation Performances of all MMprofiler markers . HOVON-65 and MRC-IX cohorts.

| | Training HOVON-65/GMMG-HD4 | | Independent Validation MRCIX | |
|---|---|---|---|---|
| Marker | Sensitivity (%) | Specificity (%) | Sensitivity (%) | Specificity (%) |
| t(4;14) | 86,4 | 98,0 | 97,4 | 97,6 |
| t(11;14) | 89,7 | 95,3 | 86,0 | 97,5 |
| t(14;16)\t(14;20) | 69,6 | 96,1 | 77,8 | 95,7 |
| del13q | 79,9 | 88,2 | 89,4 | 90,8 |
| add1q | 73,5 | 91,1 | 77,1 | 87,7 |
| Hyperdiploid | 74,3 | 77,9 | 75,4 | 80,9 |
| del17p all | 83,4 | 65,9 | 89,5 | 61,4 |
| del17p ≥80% | 97,5 | 65,5 | 89,5 | 61,4 |

| | Training HOVON-65/GMMG-HD4 | | Indendent Validation MRCIX / TT2 / TT3 / APEX | |
|---|---|---|---|---|
| Marker | HR | p-value | HR | p-value |
| SKY-92 | 5,89 | p<0.0001 | 2.38-5.23 | p<0.0001 |
| MF cluster | 2.25 | p = 0.006 | NA* | NA* |
| MS cluster | 1.58 | p = 0.177 | NA* | NA* |

* Not Determined

## Conclusion

We report the development of a standardized assay for ten genetic markers in MM which can be applied for prognostic purposes and ultimately for therapeutic decision making.

## References

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2. Broyl A, et al. Blood. 2010 Oct 7;116(14):2543-53.
3. Kuiper R et al. Leukemia. 2012 Nov;26(11):2406-13.

## Participants

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The QR-code to download the MMprofiler brochure is provided here

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