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Single Sample Application of the EMC92/SKY92 Signature Using the MMprofiler

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Introduction

Multiple Myeloma (MM) is a heterogeneous disease with diverse gene expression patterns (GEP) across patients. This has led to the development of various signatures allowing virtual karyotyping, defining different clusters of patients, and prognostication by high risk signatures (e.g. EMC92/SKY92). Several clinically well annotated GEP datasets exist, but may have scaling/offset differences (batch effects) in the data, e.g. due to differences in reagents used, location, etc. Batch wise normalization approaches can enable cross-cohort comparisons, and have allowed successful validation of those signatures across independent datasets (see Figure 1). Batch wise normalization requires groups of patients that have a similar distribution of clinical characteristics, and hence cannot be applied on single patients. Here we demonstrate the validity of applying GEP algorithms on single patients using the MMprofiler, enabling the application of GEP in a routine clinical setting.

Materials and Methods

The MMprofiler GEP assay is a standardized assay from bone marrow to data analysis and result reporting. It was used for 77 MM patients that were prospectively enrolled in the HOVON87/NMSG18 trial (73 patients) or HOVON95/EMN02 trial (4 patients) and processed using the MMprofiler sample work-up procedure. A representative reference set of 30 HOVON87 patients was selected from which new normalization parameters were derived, to be used for (future) normalization of a single sample against. The remaining 47 samples served as an independent set of samples. In addition, we have also used the publicly available GEP data from 247 patients (MRC-IX trial) as independent samples. This MRC-IX dataset has been produced using different reagents and sample work-up procedures. Therefore, it is likely that a batch effect will exist relative to the HOVON reference dataset, which may influence correctness of single sample analyses.

The GEP data from the 47 and 247 independent samples were normalized using two approaches. Firstly, by batch wise mean variance normalization (i.e. across the 47 and 247 patient batches separately). And secondly, by single sample normalization using the normalization parameters from the initial 30 HOVON samples. Subsequently, several classifiers (EMC92/SKY92 etc.) were applied to the data, and their results were compared between the two normalization approaches (see Figure 2).

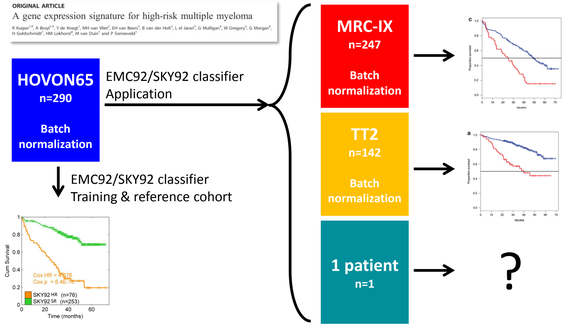


Figure 1. The EMC92/SKY92 GEP signature was previously developed on the HOVON65 dataset (Kuiper et al. Leukemia 2012), and validated on five independent datasets (e.g. MRC-IX, TT3). Since those independent datasets used different sample work up procedures (as indicated by the different colors), batch normalization across 25+ MM patients is required. This setup does not allow a single patient to be used.

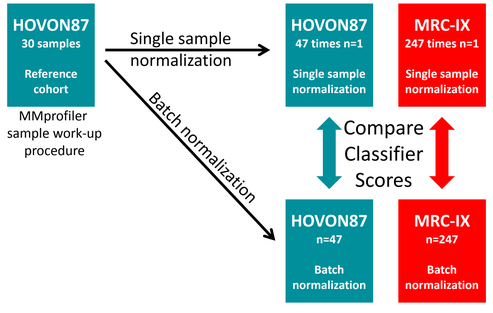


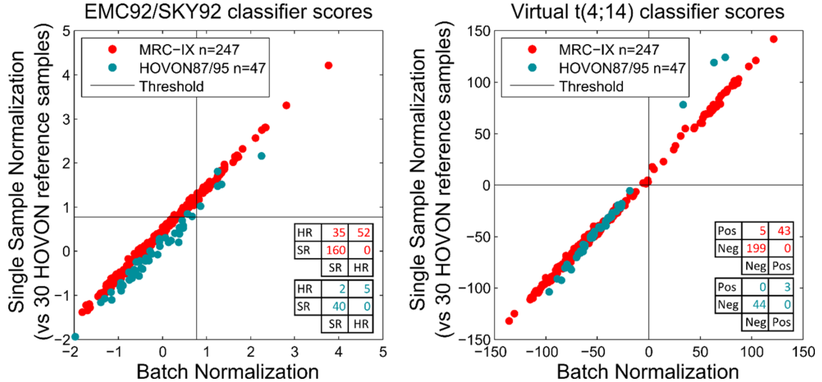
Figure 2. GEP data from the HOVON87 and MRC-IX trials were processed using batch normalization and single sample normalization (relative to the reference cohort of 30 HOVON87 samples processed using the MMprofiler sample work-up procedure). Next, classifier scores (EMC92/SKY92) were calculated and compared (Figure 3).

Results

Figure 3 shows the EMC92/SKY92 scores that were obtained after batch normalization (x-axis) and single sample normalization (y-axis). For the 47 HOVON samples there is a high degree of concordance with data points close to the identity line (y=x). Only 2 out of the 47 samples would switch assignment, which is not unexpected since those 2 samples are really close to the threshold (e.g. might also switch due to technical variation). For the MRC-IX dataset, based on single sample normalization more patients would be predicted as high risk (87 (35.2%) instead of 52 (21.0%), see Figure 1), which is caused by a positive offset (i.e. intersect with the y-axis) due to the batch effect. For the Virtual t(4;14) classifier, both datasets have a very high concordance with 0 out 47 HOVON samples, and 5 out of 247 MRC-IX samples (but really close to the threshold) switching assignment (see Figure 3). Hence, even in the presence of a potential batch effect in the MRC-IX dataset, the single sample predictions are accurate. These data suggest that single sample normalization of microarray GEP is essential, its extent is different per marker, and requires the strict standardization of the MMprofiler assay sample work-up process and algorithms.

Conclusions

Scores for the EMC92/SKY92 signature were nearly equivalent when derived from the data following single sample normalization and batch normalization in the data generated using the MMprofiler process. In the external dataset, a much higher discrepancy was found, highlighting the need to use highly standardized methods to generate consistent results. Further validation of this method is planned, and will include replicate runs systematically controlled for various conditions.



HOVON87: Concordant results
MRC-IX: Unrightfully too many HR for single sample

HOVON87: Concordant results
MRC-IX: Concordant results

Figure 3. Scatterplots and confusion matrices of the classifier scores obtained after batch normalization (x-axis, columns) and single sample normalization (y-axis, rows) of the EMC92/SKY92 signature (left), and Virtual t(4;14) classifier (right). Scores above/below the threshold correspond to high risk(HR)/standard risk(SR) (EMC92/SKY92) and positive/negative (Virtual t(4;14)).

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Disclosures

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