An in-depth investigation of the causes of treatment failure in AML

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Objectives

Relapsed acute myeloid leukemia (AML) has a poor outcome. Allogeneic stem cell transplantation is in most of these patients the only curative option, but it carries a high risk of morbidity and mortality. Therefore, the goal should be to prevent relapse by identifying high-risk patients at diagnosis, and reliably detecting minimal residual disease (MRD) after chemotherapy. The generally accepted risk stratification is based on the 2017 ELN risk stratification criteria and classifies patients into favorable, intermediate and adverse risk categories based on molecular and genetic tests. Flow cytometry has also shown its importance in classification and MRD detection of AML. Currently, flow cytometry data are analyzed manually and thus carry a higher probability of bias. Therefore, we wanted to develop a new, objective method for analyzing flow data, using computational models and bio-informatics. By linking these data to clinical outcome data of patients, we aim to identify new prognostic markers which could help us determine the optimal treatment strategy for each individual AML patient. Furthermore, these methods will allow more reliable detection of MRD.

Methods

Flow cytometry data (EuroFlow panel) and clinical data were collected from 138 AML patients diagnosed at Ghent University Hospital between 2015 and 2018. Flow data were first subjected to quality control using PeacoQC, which automatically evaluates flow signal over time and eliminates regions that show irregular behavior. Further computational pre-processing of the files was used to automatically remove debris and doublets. The FlowSOM computer model (Fig 1A), which automatically clusters cells with similar phenotypes, was used to analyze the pre-processed flow data for each tube of the EuroFlow panel (acute leukemia orientation tube + 6 additional tubes to further characterize the AML). Statistical analyses were used to compare clinical outcomes of patients.

Results

The percentage of cells present in each cluster of the FlowSOM tree was compared between groups of patients with different ELN risk profiles or clinical outcomes. Leukemic stem cells (LSC) show a CD34+ CD38- phenotype in AML patients (except for monocytic AML) (Fig 1B, pink). A statistically significant higher percentage of LSC at diagnosis could be observed in patients who had morphological MRD after the second induction cycle, as compared to patients with no MRD at this point. Also, adverse risk patients (according to ELN) had a significantly higher percentage of LSC at diagnosis as compared to favorable risk patients. These preliminary results are promising and we are currently also analyzing other cell populations with significantly higher expression levels at diagnosis in patients with worse outcomes.

Conclusion

Computational analysis is a promising method for objective analysis of flow cytometry data. Moreover, combining computational data with clinical data could result in the detection of specific cell populations (like the leukemic stem cell) present at diagnosis and responsible for MRD or relapse in AML, and therefore linked to a worse prognosis. This would help us optimize risk stratification and treatment strategy for patients with AML. Furthermore, markers expressed on these cell populations could be identified as novel therapeutic targets for immunotherapy of AML.

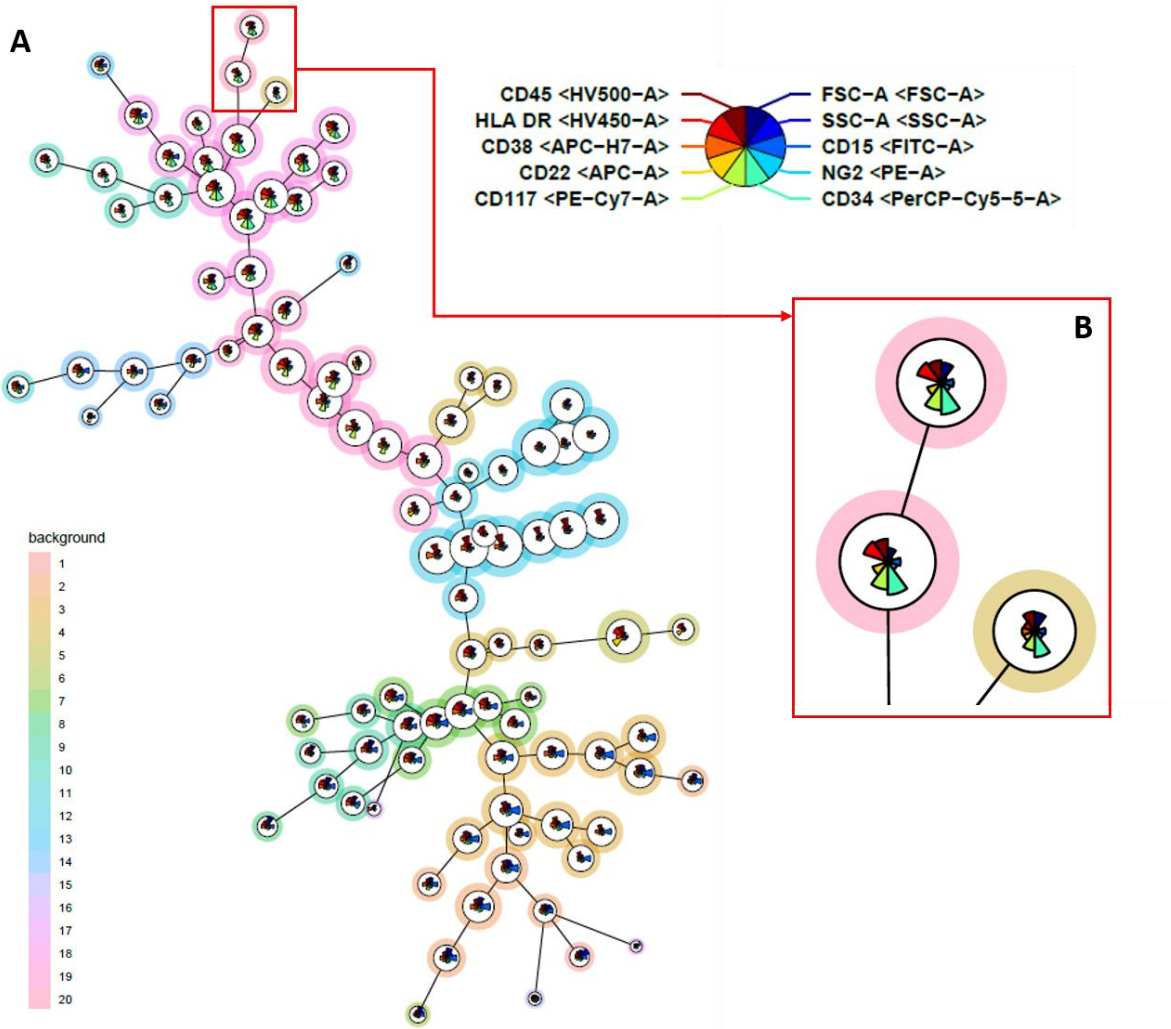

Figure 1. FlowSOM. **(A)** As an example, we show here the FlowSOM tree of tube 5 of the EuroFlow panel. The markers included in this tube are shown in the top right corner. FlowSOM aggregates flow cytometry data of all patients to cluster cells with similar marker expression. Background colors indicate metaclusters and are similar to cell types or populations in a manual gating strategy. **(B)** For each cluster (i.e. circle) expression levels for all markers are indicated.