FEASIBILITY OF NMR METABOLIC PROFILE OF NEWLY DIAGNOSED AML PATIENTS UNDERGOING CHEMOTHERAPY TREATMENT

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Introduction

Acute Myeloid Leukemia (AML) is the most common blood cancer in adults with a poor outcome. In AML, mutations in the blood-forming cells disrupt the process of differentiation and result in the accumulation of immature cells (blasts) with a reduction of functional blood cells in peripheral blood (PB) and bone marrow (BM). AML is a biologically variable disease with different molecular subtypes, risk factors, cytogenetics, clinical behaviours, and responses to treatment. Therefore, definition of biological characteristics of every single case of AML is essential to define the best therapeutic strategy.
Metabolomics investigates changes in metabolites and small molecules involved in biochemical processes offering a powerful tool for examining disease-related biochemical changes and identification of new biomarkers [1, 2]. In the present study we investigated the feasibility of a nuclear magnetic resonance (NMR) metabolomic approach to study metabolism in AML patients during intensive chemotherapy treatment. NMR is an indispensable analytical tool for metabolomics studies in the pharmaceutical and drug discovery areas. Combined with multivariate statistics, it allows a record of global changes in metabolites associated with phenotypic changes [3, 4].

Patients and Methods

Nine newly diagnosed AML patients undergoing intensive chemotherapy (FLAG-Ida CT) treatment were enrolled in a pilot clinical trial. Main inclusion criteria were: age 18+ years, untreated AML or AML relapsed >6 months after CT, potential eligibility to intensive treatments. All administered drugs and fluids were registered and the clinical monitoring of patients was strict (weight, fluid input and output, temperature) with blood tests taken daily. Clinical data were recorded in dedicated clinical report forms.
Plasma samples from peripheral blood (PB) and bone marrow (BM) were collected prior, during and after the first 2 CT cycles (**Figure 1**), and frozen within 2 hours by the OSR Leukemia Biobank facility. At the end of treatments, all patients were in complete remission, i.e. with less than 5% of blasts in bone marrow.

Figure 2 shows the workflow of our NMR metabolomic study. PB and BM samples were analyzed by 1D ^{1}H NMR spectroscopy at 310K on a Bruker Avance 600 MHz spectrometer equipped with a triple-resonance TCI cryoprobe. After processing, NMR data were subjected to multivariate analysis using Simca (Umetrics). Unsupervised principal components analysis (PCA) and supervised partial least squares-discriminate analysis (PLS-DA), or orthogonal PLS-DA (OPLS-DA) were used in order to correlate metabolomic markers with AML clinical and biological features.

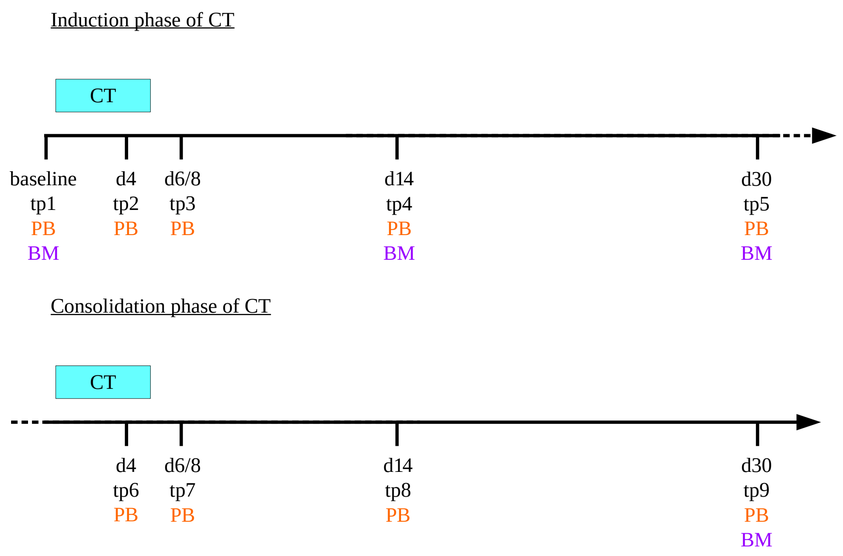

Figure 1: longitudinal study with 9 time points (tp)

NMR sample preparation
=> protocole reported by Dona et al. (2014) [5]
plasma + phosphate buffer pH 7.4 (50%/50% v/v)

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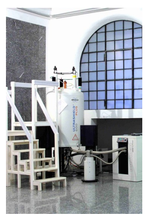

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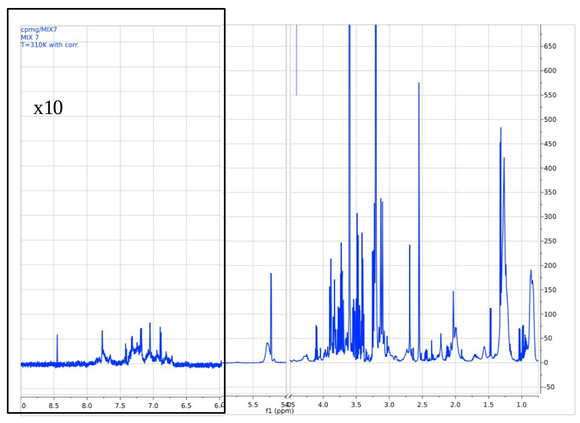
Data acquisition
600 MHz NMR spectrometer
T=310K (37°C)
Refrigerated SampleJet at 6°C
Acquisition in automation of the randomized samples
=> noesypr1d, cpmg, and Jres experiments for all the samples
+ 2D experiments (^{1}H-^{13}C HSQC and ^{1}H-^{1}H TOCSY) on selected samples to facilitate metabolite identification

Processing of the data
Baseline correction
Calibration: formate at 8.45 ppm
Exclusion of spectral regions: DSS, H_2O, EDTA
Alignment
Binning: 0.002 ppm
Normalization (total area)
Pareto scaling



^{1}H cpmg NMR spectrum of PB

Multivariate statistical analysis
1. Unsupervised PCA: global overview; outlier?
2. Supervised PLS-DA and OPLS-DA

In the next weeks

Metabolites identification
Metabolites identification (chenomx)
Quantification (deconvolution)
Univariate statistical evaluation

In the next weeks

Biological interpretation
Metabolic pathway analysis

Figure 2: workflow of our NMR metabolomic study

Results

107 blood samples were analyzed by NMR combined with multivariate statistics.
The metabolic profiles at tp2 and tp6 are very different from the other metabolic profiles due to NMR signals of the CT drugs. These data were not used for the following statistical analysis.
A global overview of all the data didn't allow us to discriminate the 9 patients as shown by the unsupervised PCA analysis (**Figure 3**). That's why, we decided to use supervised statistical analysis (PLS-DA and OPLS-DA) to detect the existence of underlying metabolic differences between groups of samples.
Several analysis were carried out:

- comparison of PB with BM samples;
- comparison of AML with remission samples (tp1 *vs* tp9);
- correlation of the metabolic profiles with biological features: number of blasts in BM and risk of relapse;
- correlation of the metabolic profiles with CT response: resistant patients.

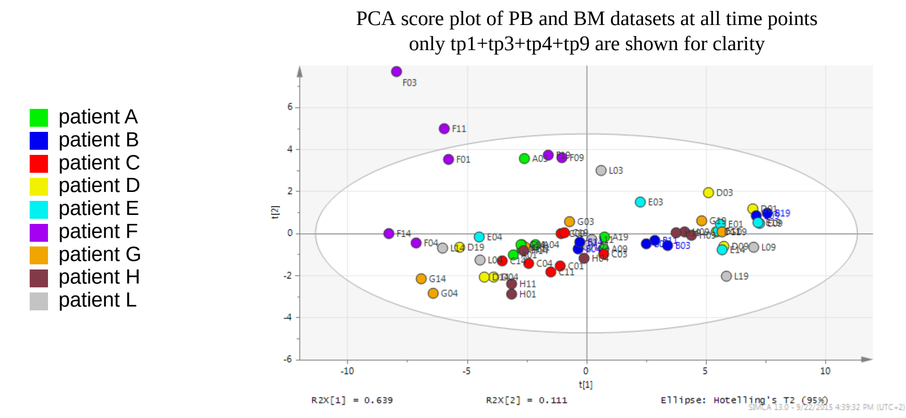
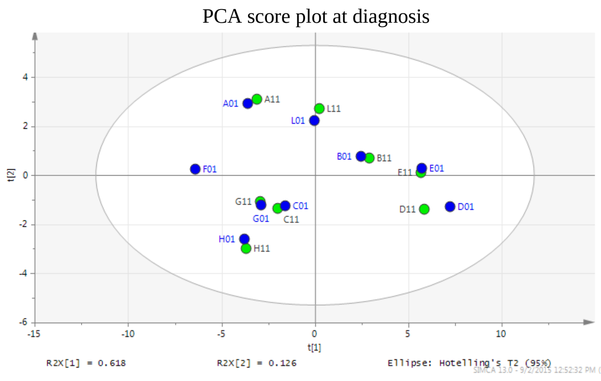


Figure 3: global overview of all the data

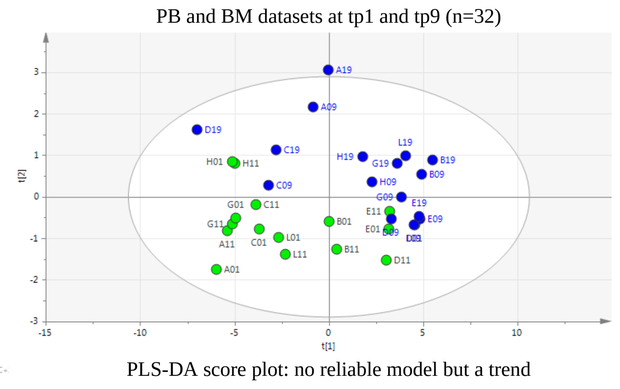
PB *vs* BM samples

The NMR metabolic profiles of PB and BM were very similar at diagnosis.



AML *vs* remission samples

The small sample size didn't allow us to get a reliable model discriminating AML from remission metabolic profiles.

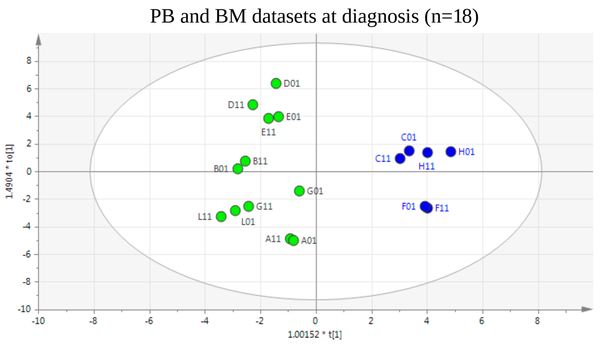


PLS-DA score plot: no reliable model but a trend

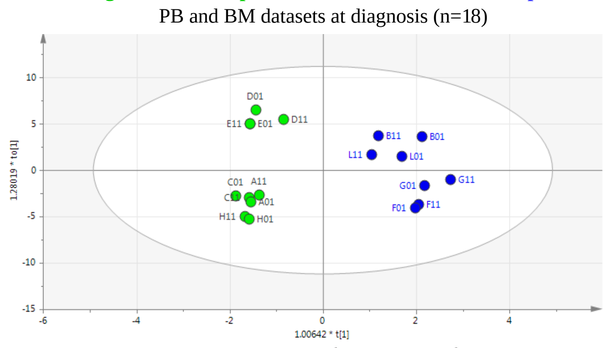
Correlation of the NMR metabolic profiles with biological features

Number of blasts at diagnosis:
The patients with more than 50% of blasts in BM clustered together versus the ones with 20-50% of blasts. The biggest contributions were associated with lipid metabolism according to what reported for other types of cancer [5].

Risk of relapse at diagnosis:
The patients with a high risk of relapse clustered together versus the ones with a low or intermediate risk of relapse.



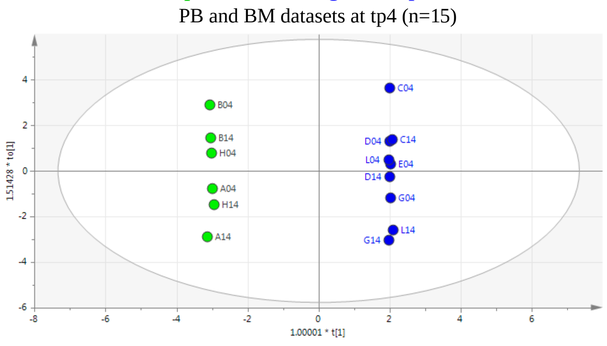
OPLS-DA score plot: $R^2X=0.29$ and $Q^2=0.76$



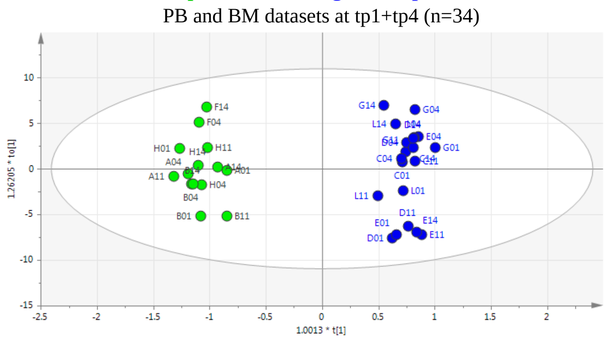
OPLS-DA score plot: $R^2X=0.11$ and $Q^2=0.84$

Correlation of the NMR metabolic profiles with CT response

4 out of 9 patients didn't respond well to the first CT cycle (the BM biopsie at tp4 revealed more than 5% of blasts), and hence started earlier the second CT cycle.
At tp4, the patients with a bad CT response clustered together versus the ones with a good CT response. Moreover, it was interesting to see that the discrimination remained with the addition of the data at tp1 even if the variance explained by this last model was very low ($R^2X=0.03$).



OPLS-DA score plot: $R^2X=0.37$ and $Q^2=0.85$



OPLS-DA score plot: $R^2X=0.03$ and $Q^2=0.72$

Conclusions and Perspectives

Our pilot study demonstrates the feasibility of an NMR metabolomic approach to study metabolism in AML patients during intensive CT. The present work suggests that metabolomics could give a readout of disease burden. Indeed, we were able to correlate the blood metabolic profiles with biological features of the patients like the number of blasts in BM and risk of relapse. Besides, our results suggest that we may associate metabolic changes with CT response. The identification of the metabolites that have changed under the studied condition will be done in the next weeks.
Considering the biological variability of AML (and hence the complexity of the NMR data), our future strategy is to use a mouse model as a clean system in order to understand the molecular changes associated with specific AML subtypes. Focusing on these changes, we will then go back to human data in order to validate and extend the results with patients. Preliminary results with a mouse model showed the feasibility of the project.

References
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Acknowledgments

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