Mass spectrometry imaging discerns the metabolome of CD19+ lymphocytes in untreated and treated subjects with chronic lymphocytic leukemia

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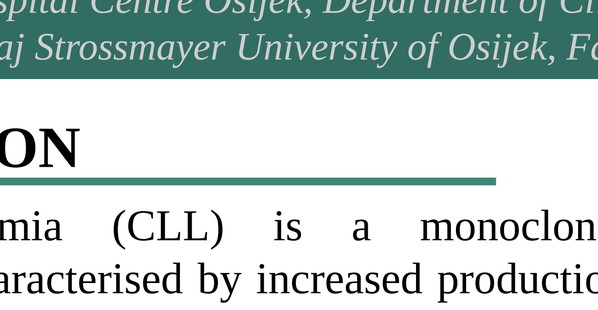
1 INTRODUCTION

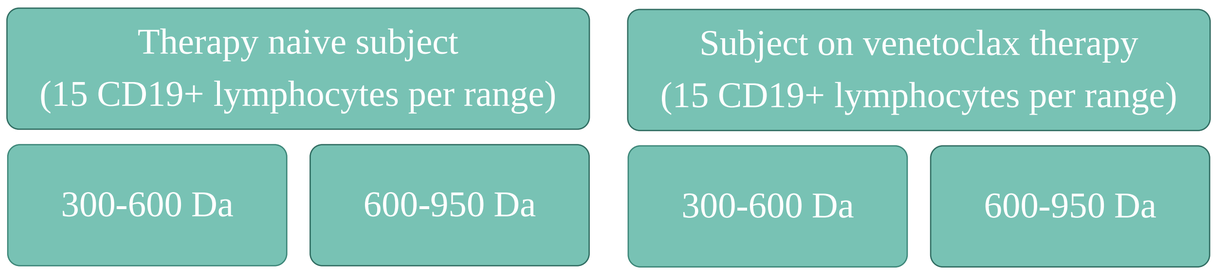
Chronic lymphocytic leukemia (CLL) is a monoclonal lymphoproliferative disease characterised by increased production of dysfunctional CD19+ B lymphocytes and heterogenus clinical sympthoms between patients. Bcl-2 inhibitors, the most common of wich is venetoclax, are used in CLL treatment. Even though there are potent and selective drugs available for targeted therapy, course of the disease is often unpredictible and therapy is not always effective. Therefore, more research is needed to elucidate the causes behind such therapy outcomes. Mass spectrometry imaging (MSI) is a powerfull toll in bioanalytics due to its ability to detect biomolecules as well as their spartial resolution. The aim of this study was to compare the metabolome of CD19+ lymphocytes from therapy naive subject to a subject on venetoclax therapy using MALDI-TOF-MSI technology.

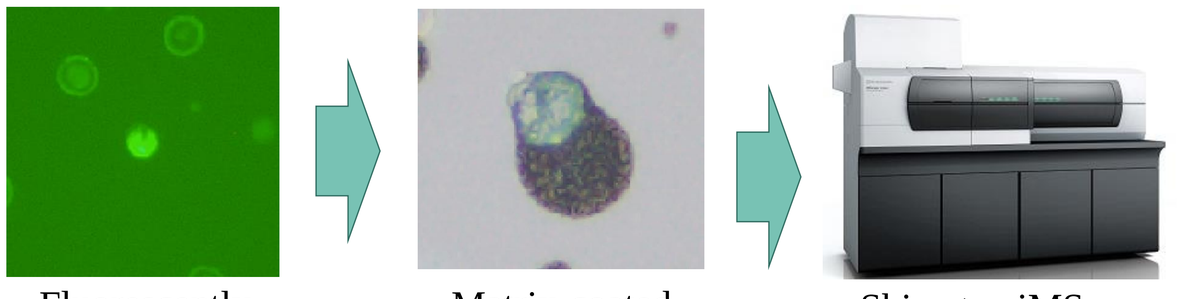


Venetoclax structure

2 METHODS

A single-cell MSI method for CD19+ B lymphocyte metabolic profiling has previously been developed [1]. Human CD19+ B lymphocytes were collected from a subject on venetoclax therapy and from a therapy naive subject. The isolates were transfered on two ITO slides and covered with a CHCA matrix using an iMLayer sublimator device (Shimatzu, Kyoto, Japan). After matrix recrystallisation, CD19+ lymphocytes were selected using fluorescent microscope integrated in the IMScope TRIO MALDI-TOF IMS instrument (Shimatzu, Kyoto, Japan). The metabolome of CD19+ lymphocytes (n=15 per mass range) from a CLL subject on venetoclax therapy was compared to a CLL therapy naive subject. One pixel per cell ROI was taken into account and the differences in signal intensities between the groups of CD19+ lymphocytes were evaluated using the t- test implemented in IMAEREVEAL v1.1 software (Shimadzu, Kyoto, Japan). Principal component analysis (PCA) was used for dimensionality reduction and for visualisation of the cell groups separation.





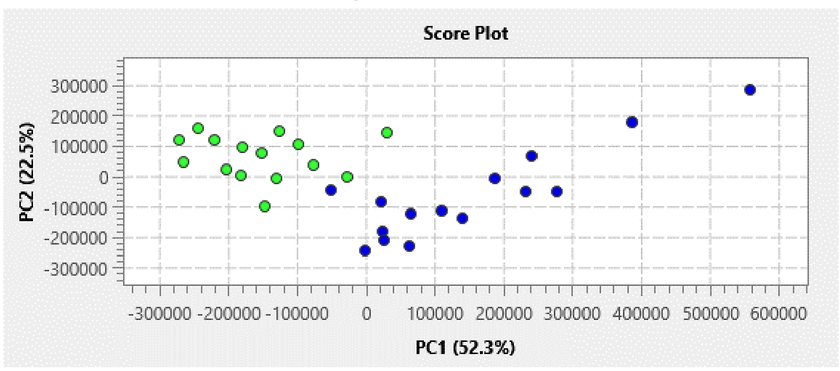
Fluorescently labeled CD19+ lymphocyte

Matrix-coated CD19+ lymphocyte

Shimatzu iMScope TRIO mass microscope

3 RESULTS

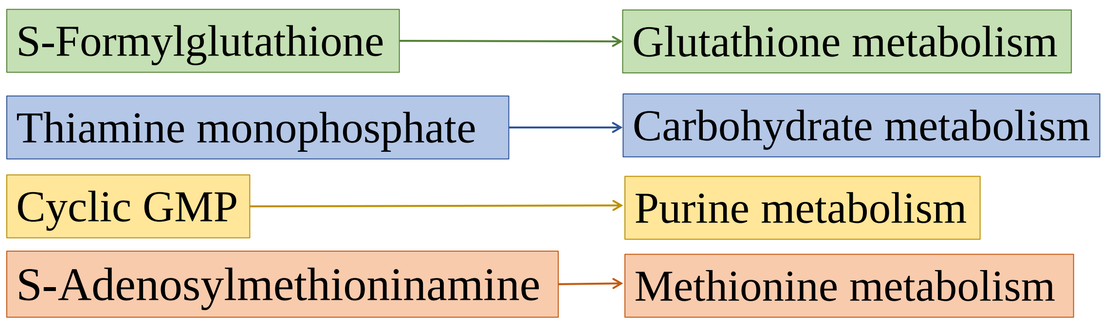
P<0,05 and 2-fold change in signal intensities between cell groups were considered significant. Significant differences between the subject on venetoclax therapy and the untreated subject were observed on 117 m/z signals. 70 m/z signals were more expressed in a subject on venetoclax therapy, while 47 m/z signals were more expressed in the untreated subject.



PCA analysis of CD19+ lymphocytes metabolome in treated and untreated subject

Human Metabolome Database (HMDB) was used for putative annotation of the following m/z signals:

Measured m/z	Database hit m/z	Mass aduct	Compound name
335.10	335.10	$M+NH_4-H_2O$	S-Formylglutathione
345.08	345.08	M+H	Thiamine monophosphate
345.08	345.07	$M+NH_4-H_2O$	Cyclic GMP
393.11	393.11	M+K	S-Adenosylmethioninamine



Metabolic pathways that are changed in CD19+ lymphocytes on venetoclax therapy

4 CONCLUSION

- MALDI TOF MSI method was able to discern different CD19+ lymphocyte populations based on their metabolome.
- There were statistically significant differences in expression of m/z signals among groups of B lymphocytes.
- Changes in glutathione, carbohydrate, purine and methionine metabolism were detected.

REFERENCES

[1] I. Marković, Ž. Debeljak, B. Dobrošević, M. Lukić et al., Metabolic profiling of CD19+ cells in chronic lymphocytic leukemia by single-cell mass spectrometry imaging, *Clin Chim Acta*. 561 (2024) 1119758

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