Network-based Bayesian inference revealed critical ncRNAs signal drivers from transcriptomics in CLL

*Vasileios Vasileiou[1, 2], George I. Gavriilidis[1], Antonis Giannakakis[2], Psomopoulos Fotis[1]

[1]Institute of Applied Biosciences (INAB), Centre for Research and Technology Hellas (CERTH), Thessaloniki 57001, Greece
[2]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, 68100, Greece

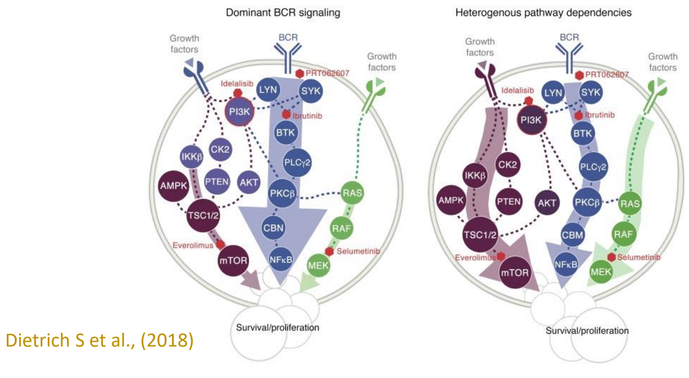
*Contact: vasileioubill95@certh.gr

Abstract

- **NetBID** is a data driven, network-based Bayesian inference workflow that reveals molecular drivers of biological interest.
- The study reveals critical drivers expression in comparison with samples that differentiate in drug response with Ibrutinib.
- Analysis specified 5 significant ncRNAs, which regulate critical molecules in the BCR pathway.
- Finally, analysis unveils one novel ncRNA that is not recorded in databases, and it needs further analysis.

Background

- **Chronic lymphocytic leukemia** (CLL) is a Non-Hodgkin's lymphoma characterized by the accumulation of mature **B-lymphocytes** in lymphoid organs, bone marrow and peripheral blood [1]. CLL arises due to genetic/epigenetic perturbations, within a permissive malignant microenvironment.
- **B-cell receptor** (**BCR**) facilitates interactions of CLL-bystander cells triggering proliferation, drug resistance and metabolic rewiring of leukemic clones [2].
- **Ibrutinib** (**BTKi**) is an inhibitor of Bruton Tyrosine Kinase (BTK), that operates downstream of BCR, conferring significant clinical results in otherwise aggressive cases [3].



Dietrich S et al., (2018)

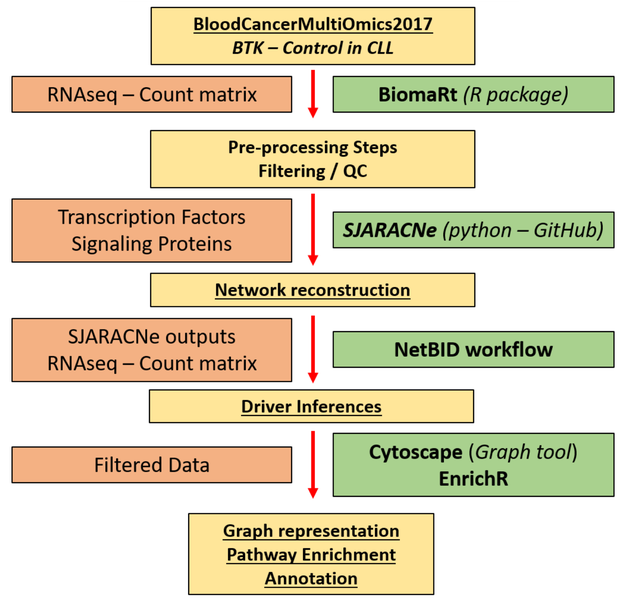
- **Notwithstanding, certain CLL cases exhibit chemorefractoriness to Ibrutinib foreshadowing fatal outcomes.**
- **In Systems Immunology,** research in **gene regulatory networks (GRN)** (RNA-seq datasets) aspires to unveil novel biomarkers and drug targets for leukemic patients with unfavorable prognosis.
- **However, these studies are often overlooking non-coding RNAs (ncRNAs) which could reveal unknown nascent regulatory motifs.**

Aim

To perform gene co-expression analysis on BloodCancerMultiOmics2017 RNA-seq data from CLL cases [4], that positively respond to Ibrutinib (Re) or not (NonRe), to detect regulons among genes and ncRNAs, with potential translational significance.

Methods

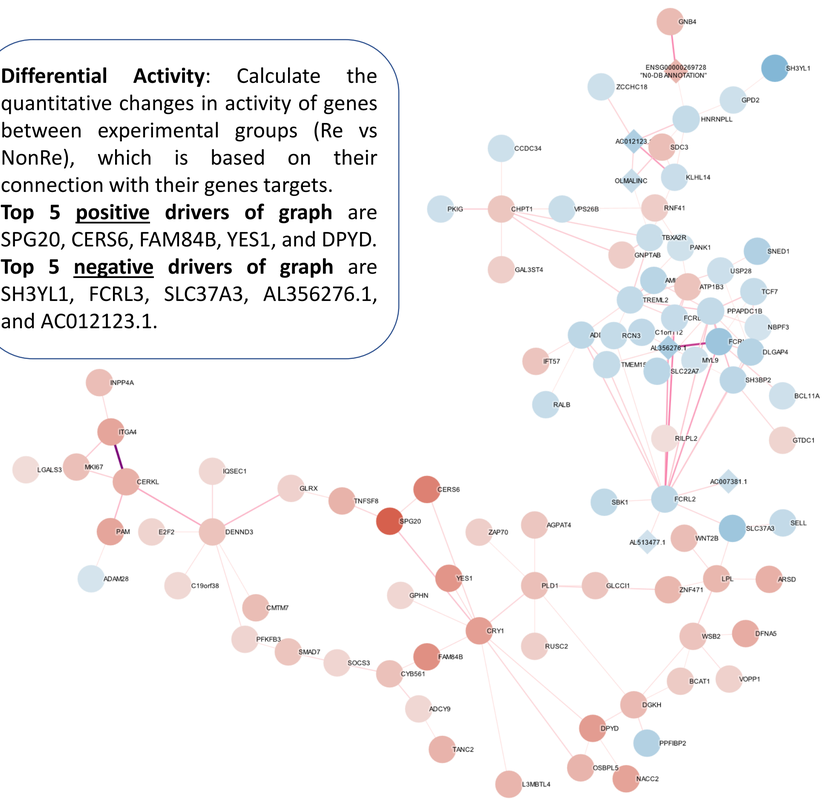
Workflow of Analysis



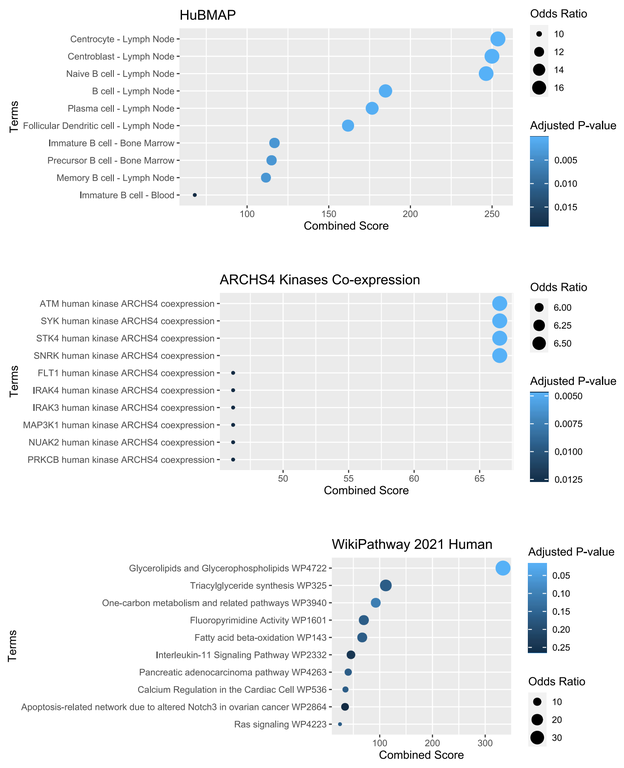
Results

Co-expression Network of drivers

Differential Activity: Calculate the quantitative changes in activity of genes between experimental groups (Re vs NonRe), which is based on their connection with their genes targets.
Top 5 positive drivers of graph are SPG20, CERS6, FAM84B, YES1, and DPYD.
Top 5 negative drivers of graph are SH3YL1, FCRL3, SLC37A3, AL356276.1, and AC012123.1.



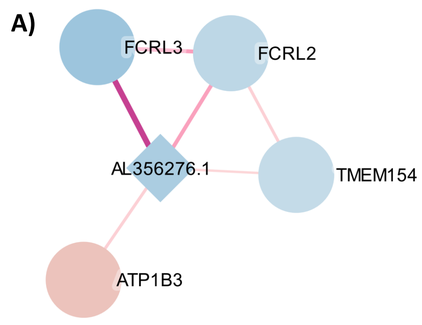
Over Representation Analysis



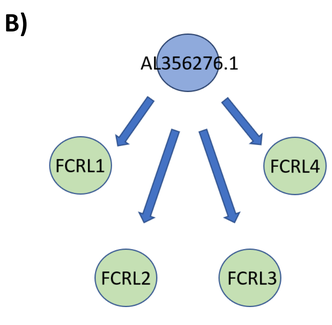
ncRNAs Annotation

ncRNA – ENSEMBL ID	HGCN SYMBOL	LogFC - DA	Adjusted P-value – DA
ENSG00000269728	***NA***	0.142493015	0.00241287
ENSG00000227217	AL356276.1	-0.139384633	0.003802401
ENSG00000228835	AC012123.1	-0.131406529	0.012807195
ENSG00000235823	OLMALINC	-0.08852756	0.018284138
ENSG00000228590	AC007381.1	-0.082240445	0.004665612
ENSG00000269896	AL513477.1	-0.071683163	0.020374079

Table of significant ncRNAs that exist in graph: ncRNAs were annotated through Biotools and SynGoPortal. 5 out of 6 ncRNAs were identified in those databases, whereas the most significant one, by both LogFC and p-value, is not exist. The most critical recorded negative ncRNA driver is **AL356276.1**.



SJARACNe co-expression module



LncRNADisease in silico validation

Presentation of SJARACNe graph: Differential Activated transcripts are depicted into a co-expression graph, by considering |LogFC| >= 0.1 and mutual connected information (MI) > 0.45. Drivers that are colored with red have higher activity in case where BTK is suppressed (Re group), whereas with blue are colored those drivers that have higher activity in case non-responsive to Ibrutinib (NonRe).

Enrichment Analysis by using EnrichR: The graph reveals molecules that participate in B-cell Lymph, validating our analysis in cells. Moreover, ARCHS4 unveils kinases that are correlated with genes of the graph. Finally, Wikipathway reveals that Glycerolipids and Glycerophospholipids metabolic pathways is critical in case where BTK kinase is suppressed by its inhibitor (BTKi).

NetBID identify that AL356276.1 ncRNAs regulate significant proteins that participate in BCR signaling pathway in NonRe patient group. (A) In this case, AL356276.1 was loaded into **LncRNADisease** database, and extract that it is high correlated with FCRL (B).

Conclusion

- NetBID pipeline reconstructs an accurate SJARACNe co-expression network for CLL pathobiology which can facilitate inference of biological insights for cases that respond to Ibrutinib (Re) or not (NonRe).
- The top positive driver in (Re) group was 5 protein-coding genes [SPG20, CERS6, FAM84B, YES1, and DPYD], whereas the top negative driver was 3 protein-coding genes [SH3YL1, FCRL3, and SLC37A3], and 2 ncRNAs [AL356276.1, and AC012123.1].
- Among ncRNAs, AL356276.1 emerged as a critical driver decreased in (Re) group [increased in (NonRe) group] that is not referred in the current bibliography with CLL.
- The SJARACNe network is ongoing to characterize ncRNAs like the unknown ENSG00000269728 that needs to be studied further.

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