Exploration of Early Signalling Events of CLL upon Exposure to Low Dose Combination of Ibrutinib and Venetoclax

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INTRODUCTION

Every year, 2500 elderly Canadians are diagnosed with Chronic lymphocytic leukemia (CLL). CLL places a significant strain on our healthcare system, as most patients require either life-long follow-up or expensive treatments. CLL patients are typically immunodeficient, leading to secondary malignancies or infections[1].

First-line targeted treatments cost up to $100,000 per patient per year and include Ibrutinib, an inhibitor of Bruton's Tyrosine Kinase (BTK), and Venetoclax, a BH3 mimetic, to induce apoptosis.

Moreover, toxicities associated with standard doses of each of these drugs often results in severe toxicites[2]. We have been exploring the impact Ibrutinib-Venetoclax treatment at reduced doses in an attempt to reduce toxicity and cost while improving patient tolerance and adherence.

Recent research finding from our lab demonstrates synergistic subduction of mitochondrial (MT) respiration by combination while CLL survival remains unaffected[1].

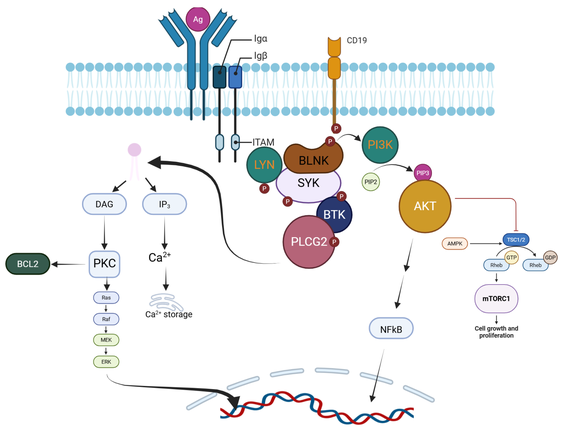

Figure 1: B cell receptor signalling cascade highlighting potential therapeutic targets in the management of CLL.

AIM

Leverage multi-omics to:

- Discover early signalling affected by combination of Ibrutinib (BTKi) and Venetoclax (BCL2i).
- Identify molecular basis of synergy of Ibrutinib (1μM) and Venetoclax (1.25 nM) at low doses.
- Identify **molecular signatures** predictive of drug action, alone or in combination.
- Discover or describe **regulatory networks** of each drug alone and in combination.

METHODS

Experimental design:

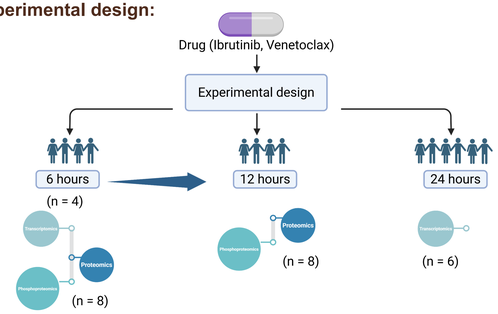

Figure 2: Experimental design to understand early effect of Ibrutinib & Venetoclax combination on CLL cells *ex-vivo*.

- Oroboros oxygraphy was used to mitochondrial respiration, while flow cytometry was used to assess cell viability, MMP and ROS. Cell titre glow was used to quantify ATP. Electron microscopy/immunofluorescence was used to characterize cellular impacts.
- Standard Illumina sequencing was performed to measure transcripts abundances.
- Bottom-up shot-gun proteomics protocol was employed to obtain quantitative proteome and phospho-proteome data. In brief: Cell lysis (3-4%SDS)→Reduction:Alkylation:Quenching (RAQ)→Purification (SP3)→Trypsin-digestion→ Desalting: i) tims-TOF proteome analysis ii) Phosphopeptide enrichment (Fe-NTA) → TIMS-TOF phospho-proteome analysis.
- Bioinformatics analyses & visualization involved R-based packages for differential analysis (Fold change = 1.5 & adjusted P-value < 0.05).

RESULTS

(Phospho)proteomics: tims-TOF

- Sensitively detected and quantified 6048 proteins and 7124 phosphorylation sites (613 sites representing 192 proteins being differentially expressed at adjusted p-value of 0.05).
- Identification of MAP3K1 as differentially expressed in Combination-treated samples (Figure 4).
- Phosphoproteomics (PhosX) predicts increased activity of kinases like DNA damage associated ATM, ATR, and AMPK with combination treatment.

Transcriptomics: Illumina RNAseq

- Combination downregulates adaptive (BCR signalling) and innate immune signalling (TLR signalling).
- Combination induced down-regulation of mitochondrial biogenesis (Figure 5).

Proposed insights:

- We hypothesize induction of MAP3K1-JNK-Caspase 8 apoptotic pathway as an early event.
- We postulate combination-induced bioenergetic-associated metabolite depletion as indicated by AMPK kinase activity.

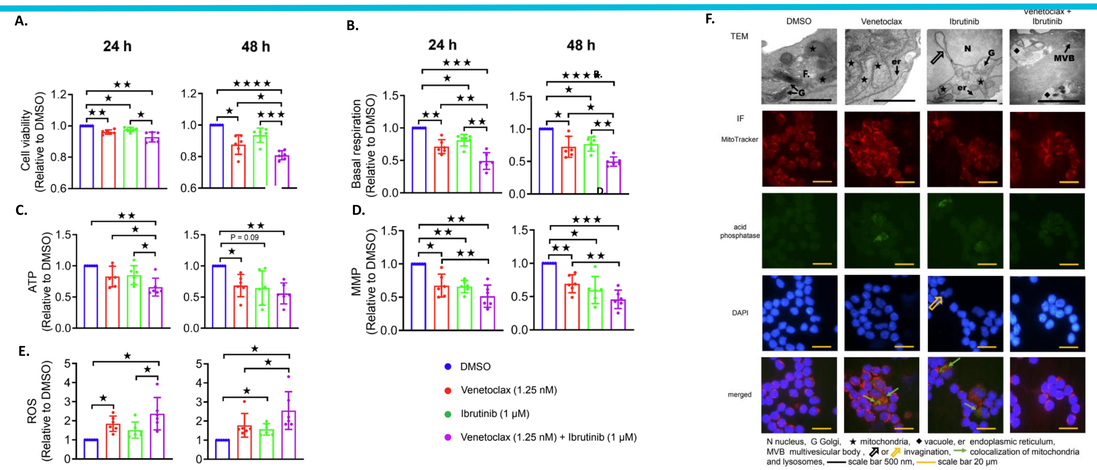

Figure 3: Combination effect: **A**. Reduced cell viability, **B**. Reduced basal respiration, **C**. ATP depletion **D**. reduced mitochondrial membrane potential (MMP) **E**. Elevation of reactive oxygen species (ROS) **F**. Transmission electron micrograph and immunofluorescence of CLL cells after treatment indicates mitochondrial changes.

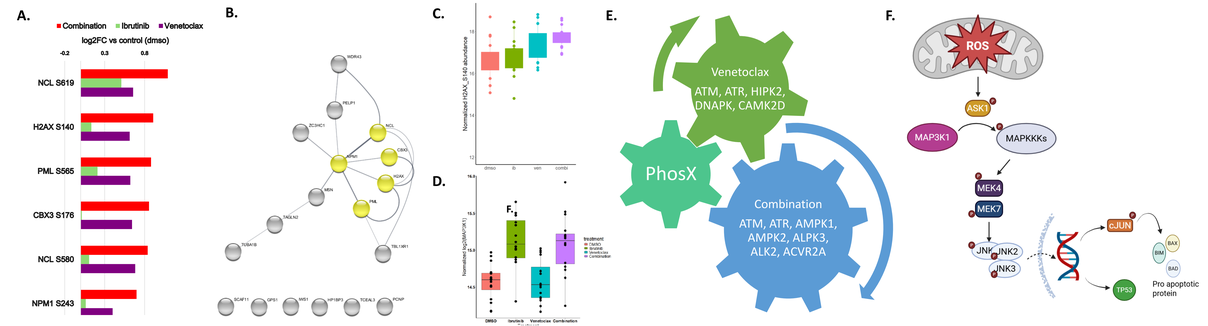

Figure 4: Combination effect on phosphoproteome: **A**. Bar plot of log2FC of DNA-damage related differential phosphosites by Ibrutinib, Venetoclax & combination treatment of CLL. **B.** STRING protein-protein interaction network of shared differential phosphoproteins between Venetoclax and Combination with hub gene cluster (yellow balls). C-D: Box plot of log2 Abundance of H2AX and MAP3K1. **E:** PhosX predicted upstream kinases in Venetoclax and combination. **F.** ROS induced MAP3K1-JNK- Caspase 8 based extrinsic apoptotic pathway (proposed).

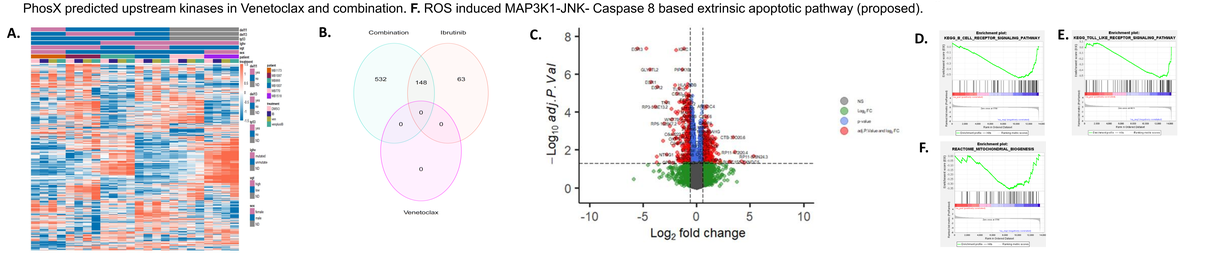

Figure 5: CLL transcriptomics profile 24-hours post treatments (n= 6). A: Heatmap of differentially expressed genes indicate heterogeneous response to therapy. B: Venn diagram of regulated genes indicates combination-driven response in terms of number of differential genes. C. Representative volcano plot showing differentially expressed genes in combination treated CLL cells versus DMSO. D-F: GSEA enrichment plot of downregulated pathways like innate and adaptive immune systems, & mitochondrial biogenesis.

CONCLUSION

- Heterogeneous responses to drug therapy
- Low dose combination possibly triggers JNK-mediated apoptotic pathway upon stress (ROS).
- Combination possibly shut down escape pathway of BTKi or BCL2i singular inhibition.

REFERENCES

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2. Canadian Cancer Society (https://cancer.ca/en/cancer-information/cancer-types/chronic-lymphocytic-leukemia-cll/treatment).

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