Targeting CLL and MCL cells with DNA repair and B cell receptor inhibitors

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ABSTRACT

Background: Patient-specific responses and the development of ibrutinib resistance remain significant challenges to the treatment of mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL). The poly ADP ribose polymerase (PARP) inhibitor, olaparib, targets cells with high levels of DNA damage, which have the mutational potential to become drug resistant.

Methods: To improve treatment efficacy and delay ibrutinib resistance, we tested ibrutinib treatment with olaparib on CLL and MCL cells in vitro. Primary CLL cells from patients were obtained via collaboration with the Harold Alfond Center for Cancer Care. These and MCL cell lines were analyzed by indirect immunofluorescence, cell counting, and flow cytometry.

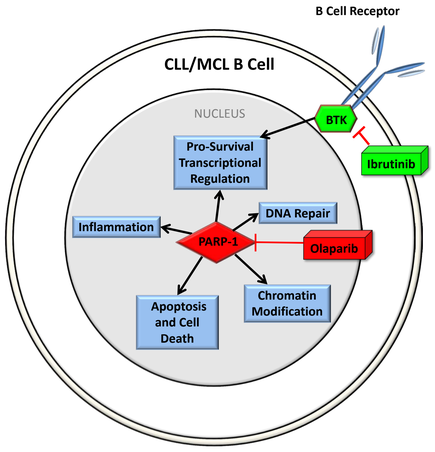
Results: CLL cells were found to have significant patient-to-patient differences in their levels of DNA damage. These differences correlate with increased copies of the gene, AICDA. Limitations in primary CLL cell culture have prevented testing of ibrutinib plus olaparib thus far. However, tests of these drugs on MCL cell lines revealed either additive or synergistic inhibition of culture growth. These effects correlate with the absence and presence of the DNA repair protein, ATM, respectively.

Conclusions: Our data provide a strong case for investigation of olaparib-ibrutinib combination therapy in MCL and CLL animal models. The additive and synergistic inhibitions of tumor cell growth support a therapeutic strategy using lower doses of each drug in combination to reduce side effects. Our data also suggest that the expression of AID and ATM might be useful biomarkers for this combination therapy in CLL and MCL, respectively.

BACKGROUND

Chronic Lymphocytic Leukemia (CLL) and Mantle Cell Lymphoma (MCL):

- Incurable diseases
- Arise in B cells in lymph nodes
- Relatively high mutational load for heme malignancies
- Treated with ibrutinib, but **develop genetic resistance**



Ibrutinib:
- B-cell specific
- Inhibits Bruton's Tyrosine Kinase (BTK), pro-survival signaling
- First line CLL treatment
- Second line MCL treatment

Olaparib:
- Poly ADP ribose polymerase (PARP) inhibitor
- Synthetic lethality
- FDA approved for second-line BRCA mutated ovarian cancer

❖ **STRATEGY:** Improve ibrutinib therapy for CLL and MCL with the **addition of olaparib**, which targets DNA repair deficient cells and may limit tumor evolution.

❖ **GOAL of STUDY:** Initial, **pre-clinical testing** of the effects of combined ibrutinib plus olaparib on CLL and MCL cell growth in culture.

METHODS

❖ **STUDY DESIGN:**

1. Measure **DNA damage load** in disease cells using fluorescence microscopy and cytogenetic data from clinical diagnostics.
2. Measure **growth of CLL and MCL cells** cultured with olaparib plus ibrutinib, ibrutinib alone, olaparib alone, or DMSO vehicle control.
3. Assess **mechanisms of culture growth inhibition** using flow cytometry.

❖ **SELECTED CELLS:**

- **Primary CLL bone marrow cells** with high and low DNA damage levels
- **MCL cell lines**: Granta-519 (DNA damage high), Z-138 (DNA damage low)

Amounts of DNA damage in CLL cells vary among patients and correlate with trisomy 12

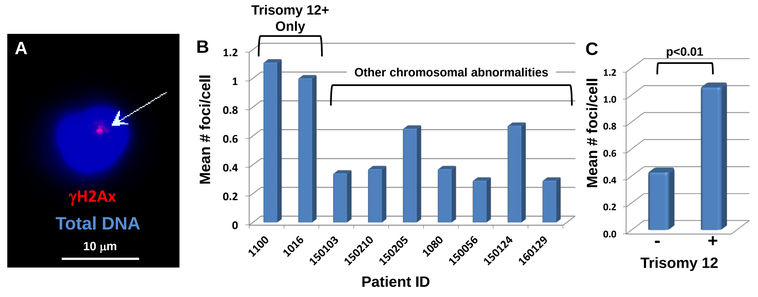


Figure 2: DNA damage levels were evaluated in primary bone marrow cells from CLL patients were evaluated using antibody against γH2Ax, which accumulates on broken DNA ends. (A) Fluorescence microscopy image of a representative CLL specimen (150210). Arrow indicates focus of γH2Ax (red). Total DNA, stained with DAPI (blue), delimits the cell nucleus. **(B)** The number of γH2Ax foci per cell was counted in 2 replicate experiments per patient for 9 patient specimens. N=100 cells per patient per replicate. **(C)** Mean γH2Ax foci numbers of patients from (B) without (-) and with (+) trisomy 12 were compared. P-value was derived from 2-tailed Student's t-tests. N=7 and 2 patients, respectively.

CLL bone marrow cells fail to stimulate in vitro

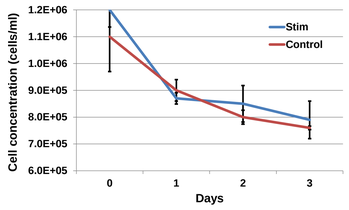


Figure 3: Primary CLL bone marrow cells were cultured in the presence of stimulating agents, IL4 and CD40 ligand (stim, blue) or vehicle control (DMSO, red). Cell concentration was determine by cell counting at the beginning of culture (Day 0) and for 3 subsequent days. Data from triplicate experiments are shown. Error bars represent standard deviations.

The addition of olaparib to ibrutinib at least additively inhibits MCL culture growth

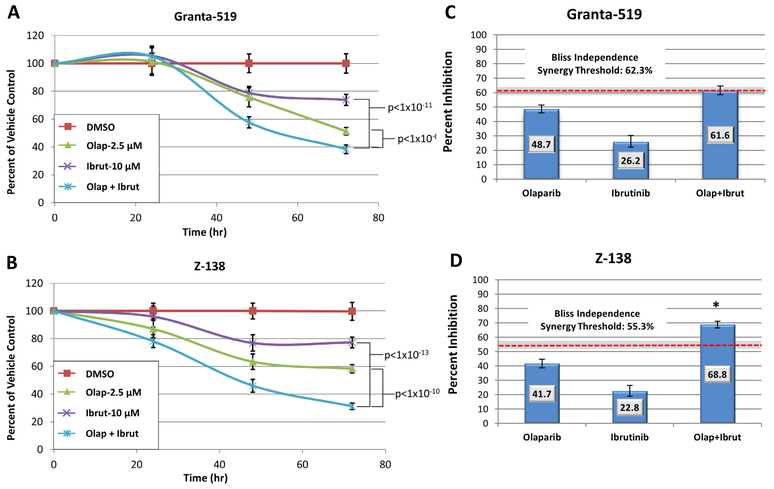


Figure 4: MCL cell lines Granta-519 **(A, C)** and Z-138 **(B, D)** were cultured in the presence of vehicle (DMSO), 2.5 μM olaparib, 10 μM ibrutinib, or both drugs in combination. Viable cell concentrations were determined every 24 hours. **(A,B)** Cell concentrations were normalized to vehicle control on each indicated day. **(C, D)** The Bliss Independence model of additivity indicates an additive effect of the drug combination for Granta-519 and a synergistic effect for Z-138 . Error bars and shaded areas indicate 95% confidence intervals of 3 independent experiments and the Bliss Independence threshold, respectively. P-values were derived from Student's t-tests.

Olaparib increases apoptosis and MCL cell death

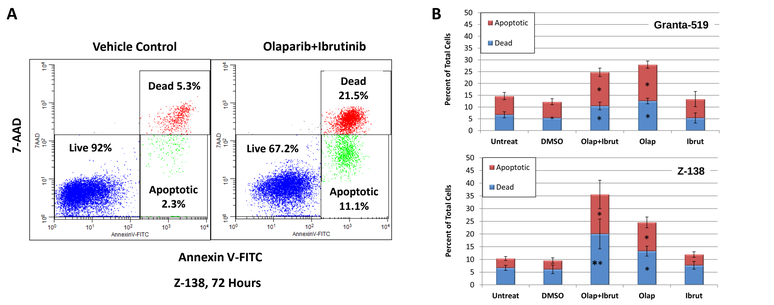


Figure 5: (A) Increased levels of apoptotic and dead cells are revealed in a representative flow cytometry profile of Z-138 cells treated with DMSO or olaparib plus ibrutinib for 72 hr, stained with Annexin V and 7-AAD, and analyzed by WinList 3D. **(B)** Data from 3 independent experiments show significant increases in dead and apoptotic cells treated with olaparib (*, p<0.05), and a further increase in cell death for Z-138 cells treated with both drugs (**, p<0.005). Error bars represent 95% confidence intervals from 3 independent experiments.

Dual drugs cause cell line-dependent cell cycle arrest

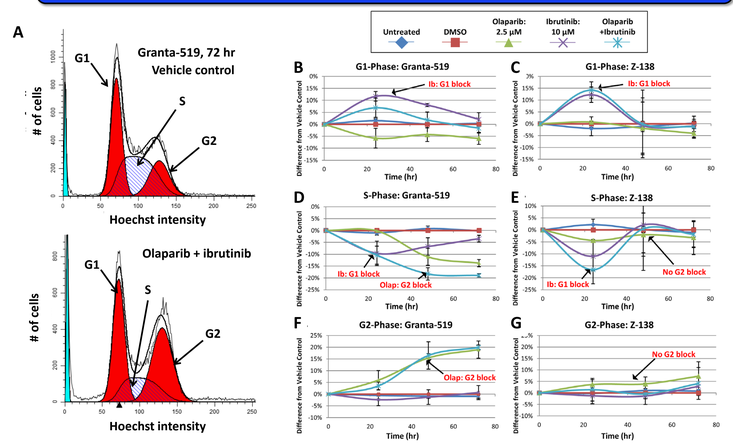


Figure 6: Flow cytometry measurements of Hoechst stained viable cells indicate G2 arrest for Granta-519 but not Z-138 MCL cells. (A) Representative cell cycle analysis of Granta-519 cells using ModFit LT. **(B-G)** Cumulative data from 3 independent experiments confirm G2 arrest for Granta-519 cells treated with olaparib plus ibrutinib for 72 hr, mimicking olaparib-only treatment. In contrast, Z138 cells show temporary G1 arrest that mimics effects of ibrutinib alone.

CONCLUSIONS

1. Addition of olaparib to ibrutinib additively/synergistically inhibits MCL cell growth in culture.
2. Olaparib-ibrutinib combination affects either cell cycling or cell death, depending on genetics of MCL cells.
3. Primary CLL cells exhibit significant differences in DNA damage but are difficult to grow in culture.
4. Findings support the testing of olaparib-ibrutinib combination on animal models of MCL and CLL.

ACKNOWLEDGEMENTS

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