Targeting NF-kB Signaling using a Novel Inhibitor in DLBCL

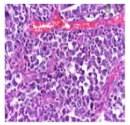
Julia Ceniceros, Alexandria Eiken and Dalia El-Gamal
Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE

Abstract

- Diffuse large B-cell lymphoma (DLBCL) is an aggressive B-cell malignancy that can be categorized by cell of origin, molecular features and reoccurring mutations.
- The transcription factor "NF-κB" plays a key role in cell survival, inflammation and immune responses; in cancer it promotes malignant cell proliferation.
- Targeting pathways such as the NF-κB is a viable option to treat B-cell malignancies.
- A novel NF-κB inhibitor was evaluated in DLBCL cell lines (OCI-LY3, RI-1, and Pfeiffer).
- The NF-κB inhibitor showed cytotoxic effects in DLBCL cells, especially those dependent on NF-κB.

Introduction

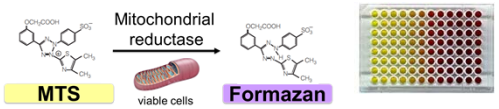
DLBCL



- DLBCL is the most common type of non-Hodgkin lymphoma in adults, with an annual incidence > 25,000 cases.
- Patients have enlarged lymph nodes in the neck, groin, or abdomen with abundant infiltrating B-cells.
- The most common treatment is chemoimmunotherapy such as R-CHOP, which has a 5-year survival rate of 58%.
- DLBCL is clinically heterogeneous in part due to the diversity in gene expression and/or molecular features.
- Based on cell of origin, there are two prominent subtypes of DLBCL (85% of cases): geminal center B-cell "GBC-DLBCL" and activated B-cell "ABC-DLBCL".
- These subtypes have distinct gene signatures/molecular patterns indicating different stages of B-cell differentiation and activation.
- ABC-DLBCL are characterized with active B-cell receptor-dependent NF-κB signaling.
- These molecular differences between GBC and ABC-DLBCL subtypes translate to clinical differences, with ABC-DLBCL having a poorer therapeutic outcome.

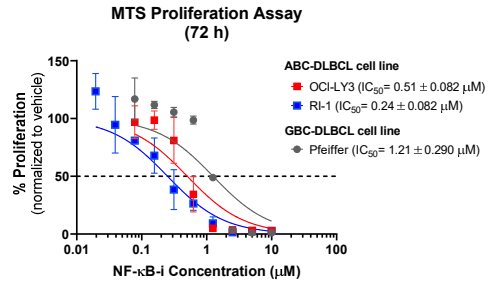
Methodology

Cell Proliferation Assay



- MTS assay is a colorimetric method used to determine the number of viable cells in proliferation using a tetrazolium salt (MTS).
- Cells that are metabolically active will convert the MTS salt into a purple formazan product.
- The amount of formazan measured at 490 nm absorbance is directly proportional to the number of living/proliferating cells.
- To determine half maximal inhibitory concentration (IC_{50}) of drug-of-interest, cells are treated with various amounts of drug/inhibitor starting from high concentration to low (i.e., serial dilution).
- After drug treatment, the MTS salt solution is added, and absorbance is measured on a plate reader 3-4 hours later.
- **To evaluate the effects of our novel NF-κB inhibitor (NF-κB-i),** GBC- and ABC-DLBCL cell lines were treated with NF-κB-i for 72 hours and cell proliferation was measured by MTS assay.

Results



- Experiments repeated 3 times per cell line.
- Dotted line represents IC_{50} value.

Conclusion and Future Directions

NF-κB-i decreased DLBCL cell proliferation especially for the ABC cell lines with the RI-1 cell line having the lowest IC_{50}.

ABC-DLBCL cell lines are more dependent on NF-kB signaling compared to GBC which may be the reason why our novel NF-κB-i had a greater anti-proliferative effects (~2-6 folds).

This approach may be promising to treat DLBCL, especially more aggressive subtype.

Future studies are needed to validate the drugs mechanism of action and preclinical efficacy.

Acknowledgments

- El-Gamal lab members
- Eppley Institute for Cancer Research, UNMC
- SURP Program

References

- Alizadeh AA et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000; 403:503–511.
- Feugier P et al. Long-Term Results of the R-CHOP Study in the Treatment of Elderly Patients with Diffuse Large B-Cell Lymphoma: A Study by the Groupe d'Etude des Lymphomes de l'Adulte. *J Clin Oncol*. 2005; 23 (18): 4117–4126.
- Liu Y, Barta SK. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. *Am J Hematol*. 2019;94:604–616.
- Vaisitti T et al. Targeting Metabolism and Survival in Chronic Lymphocytic Leukemia and Richter Syndrome Cells by a Novel NF-KB Inhibitor. *Haematologica*. 2017; 102 (11): 1878–1889.