Evaluating the impact of Aurora kinase inhibition on immunogenic cell death (ICD) in HPV+ murine models

Onyinye F Ejiaka[1,2], Soma Ghosh[2], Lacin Yapindi[2], Faye M Johnson[2]

1. Undergraduate Student, Fisk University, Nashville, TN.

2. Department of Thoracic Head and Neck Medical Oncology, the University of Texas, MD Anderson Cancer Center, Houston, TX.

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

BACKGROUND

- Human Papillomavirus (HPV) causes over 694,000 new cancer cases annually and is a leading cause of cancer in women in developing countries.
- HPV positive models express viral oncoproteins *E*6 and *E*7 that bind and degrade tumor suppressor proteins p53 and Rb respectively, leading to abnormal cell growth and division, reduced cell differentiation and an increased risk of cancer development.
- Although HPV-positive tumors are molecularly distinct from HPV negative tumors, their treatments are identical.
- Standard treatments for HPV-driven cancers includes radiotherapy and chemotherapy that have chronic side effects and are not effective in 20% of HPV+ cancers.
- Therefore, there is an urgent need for therapies that are less toxic and more effective.
- Previously the Johnson lab conducted a high-throughput drug screen (HTDS) evaluating 864 unique compounds. In that HTDS, Aurora kinase inhibitors were more effective in HPV+ than in HPV- human cancer cells.

HYPOTHESIS

Aurora Kinase Inhibition in HPV+ cells will disrupt cell cycle process by delaying mitosis, lead to apoptosis and induce immune cells to target such cells.

METHOD

Since syngeneic mouse models are needed for understanding tumor micro-environment and ICD, murine cell lines,C3.43 and TC-1 cells lines were treated for 24 and 48hrs with a concentration of 300nM of Alisertib with vehicle(DMSO) control.

RESUTS

1. Aurora kinase A inhibition leads to apoptosis in murine HPV+ cancer cell lines.

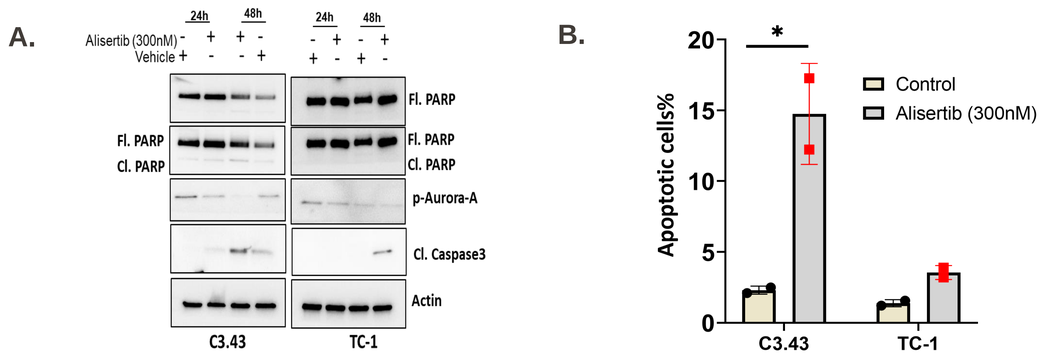

Figure 1: A, Western blot showing murine HPV+ cancer cell lines (C3.43 and TC-1) which were treated with 300nM alisertib for 24 and 48 hours and subjected to immunoblotting with the indicated antibodies. **B,** Orthogonal method(Annexin flow cytometry) was used to test for apoptosis in which C3.43 and TC-1 were treated with Alisertib(300nM) or vehicle(DMSO) for 48 hours and stained with propidium Iodide and Annexin V. Values are the means ± the SDs for three independent experiments (*, $P < 0.05$).

2. Aurora kinase A inhibition results in Immunogenic cell death in murine HPV+ cancer cell lines.

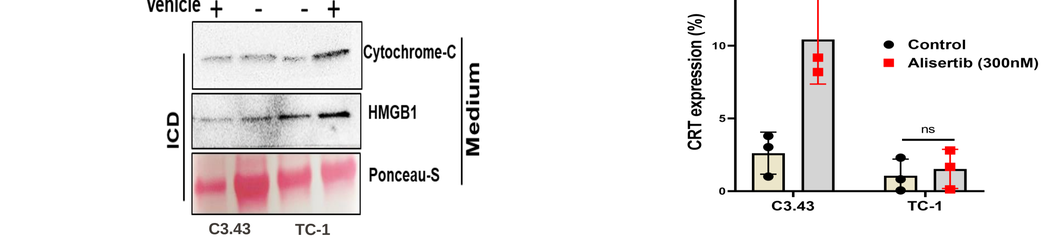

Figure 2: A, The two murine models(C3.43 and TC-1) were treated with Alisertib for 48hours and the cell surface calreticulin expression was analyzed by flow cytometry. Aurora Kinase Inhibition induced increase in surface CRT expression in the C3.43 cells but not the TC-1 cells. The experiment was conducted 3 times and yielded consistent results. Values are the means ± the SDs for three independent experiments (*, $P < 0.05$). **B,** To determine the release of HMGB1 and Cytochrome C, Equal volumes of media free from these proteins were used. HMGB1 and Cytochrome C were normalized to Ponceau S which was used as the loading control.

3. Inhibition of the ATP-binding cassettes (ABC) transporters increases alisertib-induced aurora inhibition and apoptosis in TC-1.

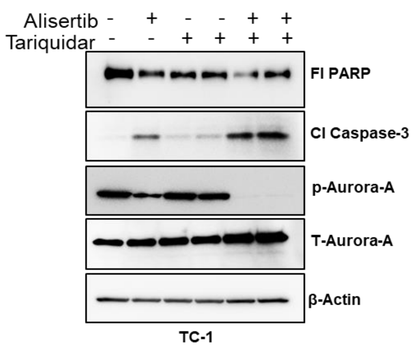

Figure 3: Western blot showing treated with100 and 300nM of Tariquidar (ABC inhibitor), in addition to 300nM of Alisertib in TC-1. The loading control used for the immunoblotting was B-actin. Alisertib leads to decreased levels of p-Aurora A and apoptosis (Cl.Caspase3), Tariquidar showed no difference, and the combination of both showed more decrease levels of p-Aurora A and apoptosis.

CONCLUSIONS

1. Alisertib in is more effective at inhibiting Aurora Kinase A in C3.43 than in TC-1 cells.
2. The effective inhibition of Aurora kinase A in C3.43 results in apoptosis and markers of immunogenic cell death.
3. Drug efflux via ATP-binding cassettes (ABC) transporters may account for resistance to alisertib in TC-1.

.

REFERENCES

- Ghosh S, Mazumdar T, Xu W, Powell RT, Stephan C, Shen L, Shah PA, Pickering CR, Myers JN, Wang J, Frederick MJ, Johnson FM. Combined TRIP13 and Aurora Kinase Inhibition Induces Apoptosis in Human Papillomavirus-Driven Cancers. Clin Cancer Res. 2022 Oct 14;28(20):4479-4493. doi: 10.1158/1078-0432.CCR-22-1627. PMID: 35972731; PMCID: PMC9588713.
- Tang A, Gao K, Chu L, Zhang R, Yang J, Zheng J. Aurora kinases: novel therapy targets in cancers. Oncotarget. 2017 Apr 4;8(14):23937-23954. doi: 10.18632/oncotarget.14893. PMID: 28147341; PMCID: PMC5410356.
- Sinha, Pragya, "Preclinical evaluation of immunomodulatory effects of Aurora kinase inhibition in HPV+ cancers." (2023). The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences Dissertations and Theses (Open Access). 1281.

ACKNOWLEDGEMENT

- OE is supported by the 1R25CA240137-01A1 UPWARS Training Program (Underrepresented Minorities Working Towards Research Diversity in Science).
- OE is supported by the CPRIT Research Training Award CPRIT Training Program (RP210028).