Combination strategies to overcome doxorubicin-induced senescence in triple-negative breast cancer

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WOMEN'S CANCER DEVELOPMENTAL THERAPEUTICS

BACKGROUND:

Triple-negative breast cancer (TNBC) is a subtype of breast cancer that lacks the expression of the estrogen receptor, progesterone receptor, and human epidermal growth factor-2 over-expression. Compared to other subtypes of breast cancer, TNBC is associated with a higher risk for metastatic recurrence. While immunotherapy, PARP inhibitors, and sacituzumab govitecan have shown benefit in a subset of TNBC patients, chemotherapy with doxorubicin remains a mainstay of treatment. Senescence is a cellular phenomenon where cells are committed to an arrested state, however, senescent cells are able to secrete pro-tumorigenic factors which can help to promote tumor progression and invasion. It has been suggested that senescence is a potential mechanism of resistance in doxorubicin treated cells. Transitioning senescence mediated resistance to apoptosis is crucial to the treatment of TNBC. The objective of this study was to evaluate the combination of the BCL-2 inhibitor venetoclax and the novel class I HDAC inhibitor OKI-005 with doxorubicin in order to analyze if resistant cells could transition to apoptosis.[1]

METHODS:

- TNBC cell lines resistant and sensitive to doxorubicin were identified using a CellTiter-Glo Viability Assay.
- To assess proliferation, cells were plated in a 96-well plate and then exposed to control (no drug), doxorubicin or doxorubicin in combination with venetoclax or OKI-005. Cellular proliferation was then analyzed using the BioSpa live cell analysis system.
- Apoptosis at 48 hours on cells treated with vehicle control, doxorubicin or doxorubicin in combination with venetoclax or OKI-005 was analyzed by flow cytometry using Annexin V staining.
- Immunoblotting was performed to evaluate the downstream effects of apoptosis and senescence in cells treated in combination.

RESULTS:

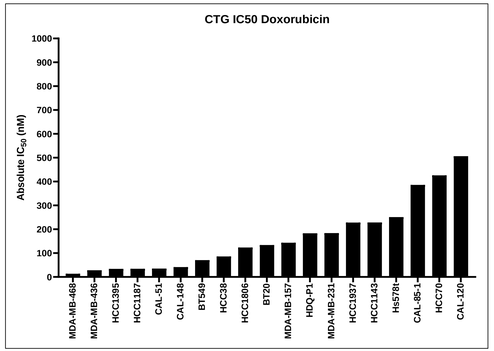

Figure 1: CellTiter-Glo Viability Assay indicating more resistant and sensitive cells lines to doxorubicin. Absolute IC_{50} >100 nM considered more resistant.

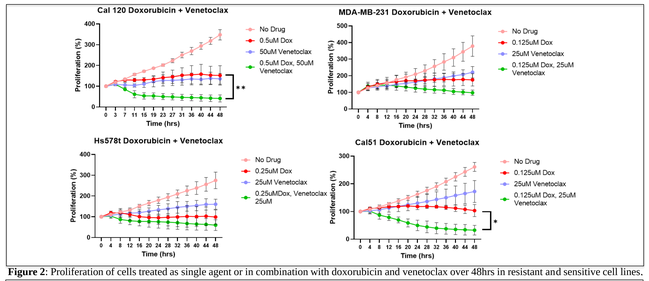

Figure 2: Proliferation of cells treated as single agent or in combination with doxorubicin and venetoclax over 48hrs in resistant and sensitive cell lines.

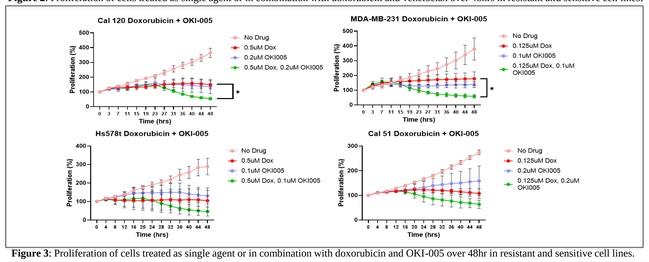

Figure 3: Proliferation of cells treated as single agent or in combination with doxorubicin and OKI-005 over 48hr in resistant and sensitive cell lines.

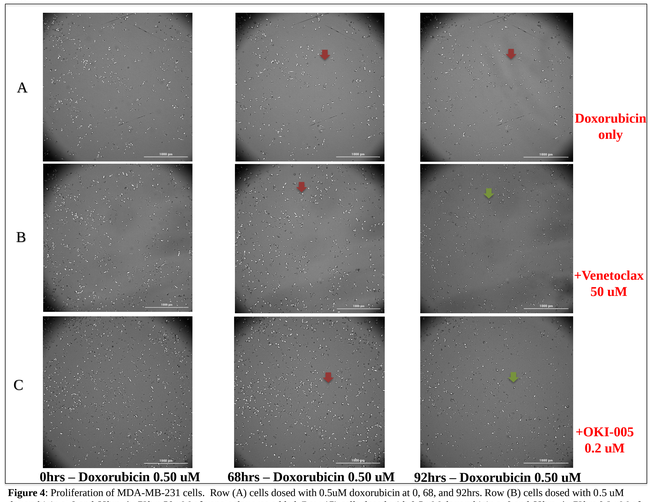

Figure 4: Proliferation of MDA-MB-231 cells. Row (A) cells dosed with 0.5uM doxorubicin at 0, 68, and 92hrs. Row (B) cells dosed with 0.5 uM doxorubicin at 0 and 68hrs. At 72hrs 50 uM of venetoclax was added. Row (C) cells dosed with 0.5 uM doxorubicin at 0 and 68hrs. At 72hrs 0.2 uM of OKI-005 was added. Red arrows indicate senescent cells. Green arrows indicate the absence of senescent cells.

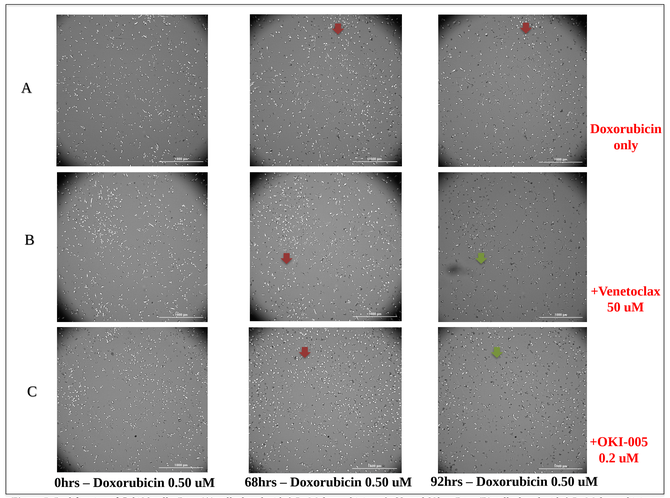

Figure 5: Proliferation of Cal120 cells. Row (A) cells dosed with 0.5 uM doxorubicin at 0, 68, and 92hrs. Row (B) cells dosed with 0.5 uM doxorubicin at 0 and 68hrs. At 72hrs 50 uM of venetoclax was added. Row (C) cells dosed with 0.5 uM doxorubicin at 0 and 68hrs. At 72hrs 0.2 uM of OKI-005 was added. Red arrows indicate senescent cells. Green arrows indicate the absence of senescent cells.

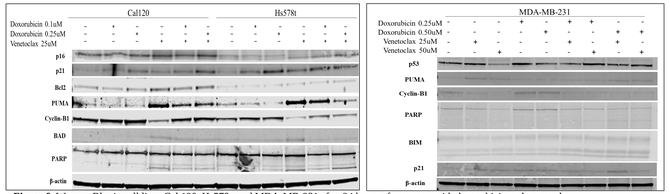

Figure 6: Western Blot in cell lines Cal 120, Hs578t and MDA-MB-231 after 24 hours of treatment with doxorubicin and venetoclax.

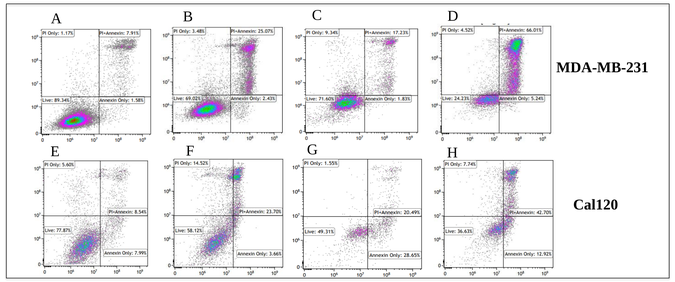

Figure 7: Annexin V Apoptosis assay in MDA-MB-231 and Cal120 at 48hrs. (A) No Drug (B) Venetoclax 25 uM (C) Doxorubicin 0.50 uM (D) Venetoclax 25 uM, Doxorubicin 0.50 uM (E) No Drug (F) Venetoclax 12.5 uM (G) Doxorubicin 0.25 uM (H) Venetoclax 12.5 uM, Doxorubicin 0.25 uM

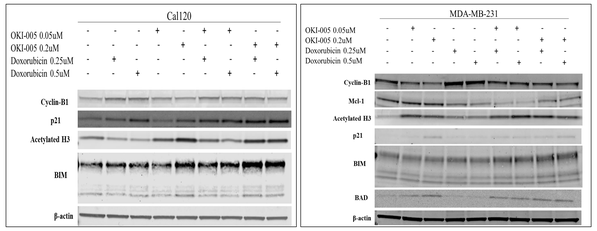

Figure 8: Western Blot in cell lines Cal 120 and MDA-MB-231 after 24 hours of treatment with doxorubicin and OKI-005.

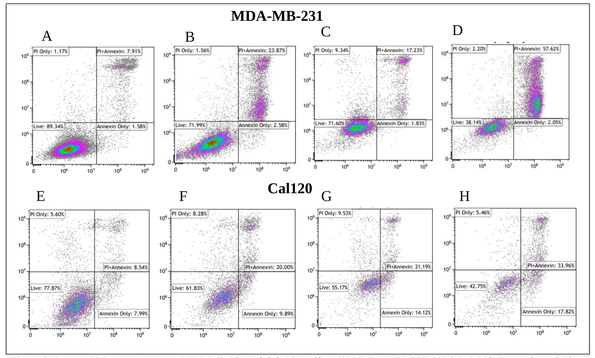

Figure 9: Annexin V Apoptosis assay in MDA-MB-231 and Cal120 at 48hrs. (A) No Drug (B) OKI-005 0.2 uM (C) Doxorubicin 0.50 uM (D) OKI-005 0.2 uM, Doxorubicin 0.50 uM (E) No drug (F) OKI-005 0.2 uM (G) Doxorubicin 0.5 uM (H) OKI-005 0.2 uM, Doxorubicin 0.5 uM

CONCLUSIONS/NEXT STEPS:

The combination of doxorubicin with venetoclax and doxorubicin with OKI-005 resulted in decreased cellular proliferation and increased apoptosis when compared to single agents. Further evaluation of these combinations across resistant and sensitive cell lines is planned. These combinations represent an exciting potential to overcome doxorubicin resistance in TNBC and warrant further investigation.

ACKNOWLEDGEMENTS/FUNDING:

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REFRENCES:

1. Diamond JR, et al. Preclinical Development of the Class-I-Selective Histone Deacetylase Inhibitor OKI-179 for the Treatment of Solid Tumors. Mol Cancer Ther. 2022 Mar 1;21(3):397-406. doi: 10.1158/1535-7163.

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