Utilizing a novel HDAC inhibitor bocodepsin (OKI-179) to overcome doxorubicin resistance in triple-negative breast cancer

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BACKGROUND:

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with limited therapeutic treatment options and a high rate of metastatic recurrence. TNBC is defined by lack of estrogen and progesterone receptor expression with non-amplified HER2, and accounts for 10-15% of all breast cancer cases. While pembrolizumab, sacituzumab govitecan and PARP inhibitors are beneficial for a subset of TNBC patients, chemotherapy including doxorubicin continues to be the primary treatment. Bocodepsin is a novel, orally bioavailable clinical stage Class 1-targeting HDAC inhibitor with activity in preclinical models of TNBC. The purpose of this study was to investigate OKI-005 (in vitro) and bocodepsin (in vivo) in combination with doxorubicin to overcome resistance.

METHODS:

- TNBC cell lines were exposed to doxorubicin (0-1 µM) and OKI-005 (0.1 µM, 0.2 µM, 0.4 µM) for 72 hours and cell proliferation was determined using the Cell Titer Glo assay. Bliss scores were calculated using Synergy Finder+.
- Annexin-V staining was performed to assess apoptosis after 48 hours of either ND, 0.5 µM DOX, 0.2 µM OKI-005, or combination.
- Cells were treated no drug (ND), 0.5 µM doxorubicin, 0.2 µM OKI-005, or combination for 24 hours and western blotting was performed for mediators of apoptosis.
- Cells were treated with no drug (ND), 0.1 µM, 0.5 µM doxorubicin, 0.2 µM OKI-005, or combination for 6 days and senescence was characterized by ß-galactosidase staining.
- Athymic nude xenograft mouse model with the cell line MDA-MB-231 were treated with vehicle, 1.5mg/kg IP QW doxorubicin, 80 mg/kg PO QD bocodepsin, or the combination for 36 days.

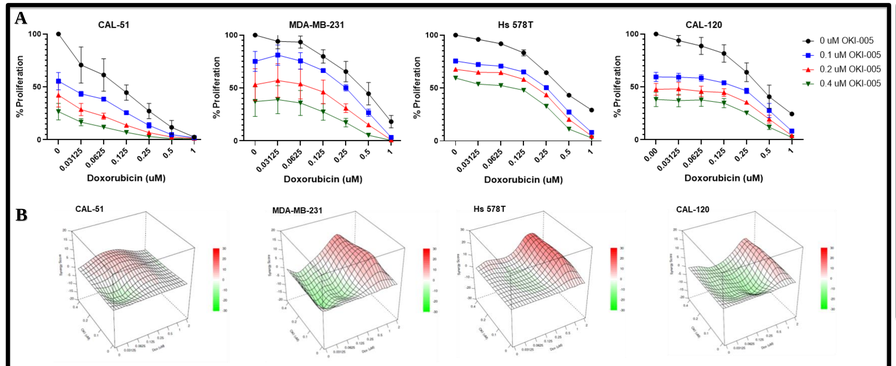

Figure 1: (A) Cell Titer-Glo Viability Assay of CAL-51, MDA-MB-231, Hs 578T, CAL-120 after 72 hours of drug treatment. (B) Bliss scores calculated using Synergy Finder+.

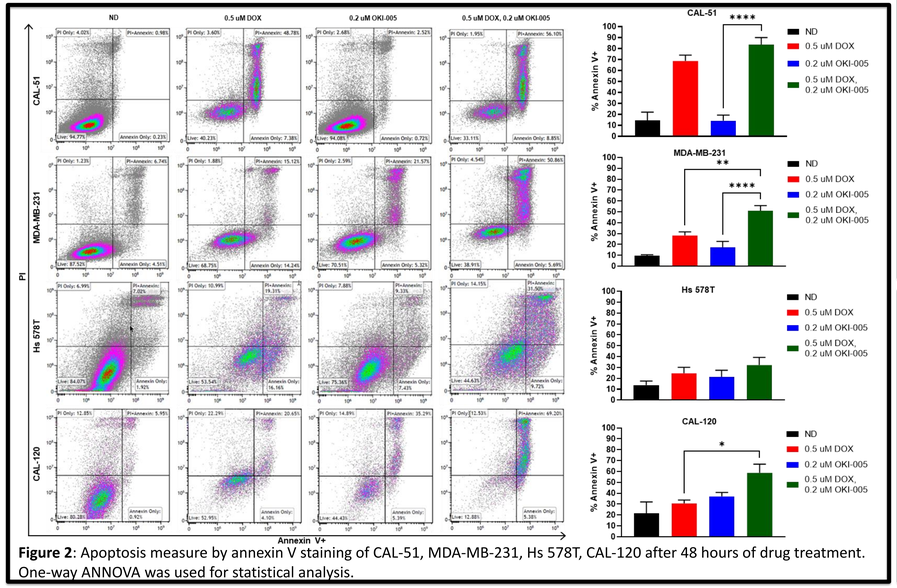

Figure 2: Apoptosis measure by annexin V staining of CAL-51, MDA-MB-231, Hs 578T, CAL-120 after 48 hours of drug treatment. One-way ANNOVA was used for statistical analysis.

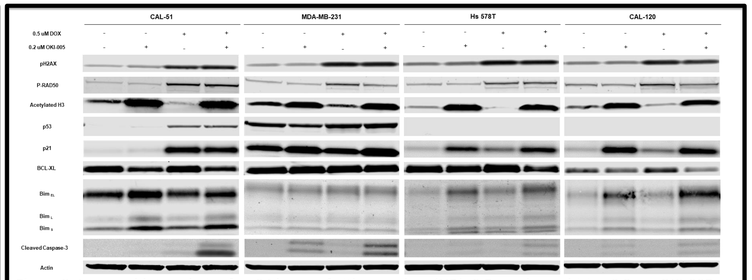

Figure 3: Immunoblot analysis after 24 hours of either no drug (ND), 0.5 µM doxorubicin, 0.2 µM OKI-005, or the combination.

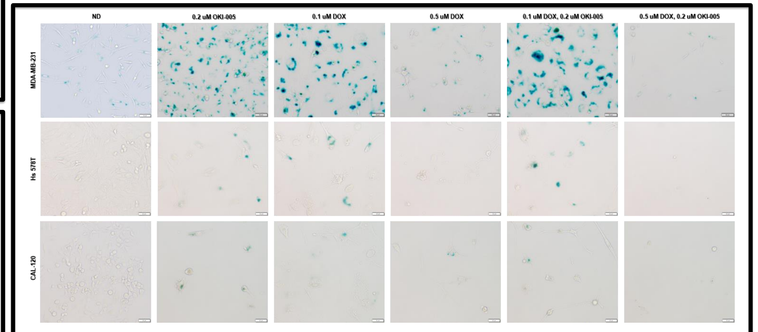

Figure 4: Senescence associated beta-galactosidase (SA-β-Gal) staining of MDA-MB-231, Hs 578T, and CAL-120 after 6 days drug treatment. Blue staining represents senescence cells.

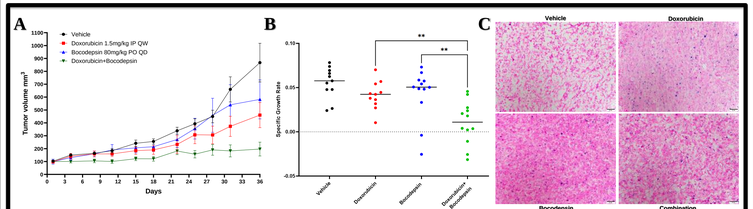

Figure 5: Athymic nude xenograft mouse model using the TNBC cell line MDA-MB-231. (A) Mice treated with either vehicle, 1.5 mg/kg doxorubicin IP QW, 80 mg/kg bocodepsin PO QD, or the combination for 36 days. (B) Specific growth rates showing overall tumor growth inhibition. (C) SA-β-Gal staining of tissue collected at day 36.

CONCLUSIONS:

- Cell Titer Glo revealed synergy with the combination of doxorubicin and OKI-005.
- The combination resulted in statistically significant increased apoptosis measured by annexin V compared to single agent drug treatment in CAL-51, MDA-MB-231 and CAL-120 cell lines.
- An increase in the pro-apoptotic protein BIM and decrease in anti-apoptotic protein BCL-XL was observed in the combination.
- The cyclin dependent kinase inhibitor p21 was increased by OKI-005 independently of p53 and doxorubicin for the cell lines MDA-MB-231, Hs 578T, and CAL-120.
- The combination resulted in increased cleaved caspase-3 for all cell lines evaluated.
- Senescence-associated beta-galactosidase showed an increase in senescent cells for both doxorubicin and OKI-005, which was eradicated in combination.
- In a xenograft mouse model, doxorubicin and bocodepsin generated significant tumor growth inhibition in combination compared to doxorubicin and bocodepsin.
- Senescence-associated beta-galactosidase showed an increase in senescent cells for both doxorubicin and bocodepsin in vivo, which was decreased in combination.

DISCUSSION:

The addition of OKI-005 (in vitro) or bocodepsin (in vivo) to doxorubicin resulted in improved anti-proliferative and anti-cancer activity with evidence of increased apoptosis and decreased senescence. As a result, bocodepsin is a promising combination partner for doxorubicin to overcome doxorubicin resistance and promote apoptosis in TNBC. Bocodepsin has a favorable toxicity profile compared to other HDAC inhibitors and this work supports the potential clinical investigation of doxorubicin and bocodepsin in patients with metastatic TNBC.

ACKNOLWEDGEMENTS:

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