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Investigating Drug Repositioning as a New Treatment for Triple Negative Breast Cancer

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ABSTRACT

Triple-negative Breast Cancer (TNBC) is defined by the lack of expression of estrogen and progesterone receptors as well as the absence of human epidermal growth factor receptor 2 over-expression/amplification. The lack of proven therapeutic drug targets for TNBC poses great challenges in the discovery of anticancer agents with efficacy in TNBC. This study conducted to identify compounds that have been approved by the FDA for non-oncology diseases and which may selectively inhibit TNBC cells either individually or in combination and to test combinations of these compounds for synergistic activity for killing or inhibiting the proliferation of selected TNBC cell lines. A 96- well homogenous cell-based viability assay utilizing the TNBC cell lines MDA-MB-231, MDA-MB-468 and HCC1806 were employed. Using this assay, emetine, pyrvinium pamoate and disulfiram were confirmed to possess inhibitory activities with average IC50 values of 4.5 µM, 138 nM, 181 nM, 238 nM and 138 nM in HCC1806, respectively. No synergies were identified for combinations of these compounds, except that of digoxin and emetine which may have synergistic effects in HCC1806 cells at some concentrations. Some selected FDA approved non-oncology drugs have cytotoxic/anti-proliferative properties against two TNBC cell lines.

Key words: Breast cancer, non-oncology, synergistic activity, cytotoxic, TNBC

INTRODUCTION

Breast Cancer (BC) is a tumor that initiates in breast tissue of women and can initiate also in breast tissue of men with low percentage. It ranks amongst the most common cancer disease that affects women[1]. According to the American cancer society, there will be an estimated 1,665,540 new cancer cases diagnosed and 585,720 cancer deaths in 2014 in the US[2]. Worldwide, it is estimated that the numbers of women who are diagnosed with BC reach about 1 million every year; with approximately 400,000 women expected to die from this disease. Triple Negative Breast Cancer (TNBC) is a subtype of breast cancer that is characterized by absence of estrogen receptor and progesterone receptor expression as well as absence of HER-2 amplification[3]. About 20% of breast cancer cases can be considered to be triple negative subtype[4]. It is characterized by its biological aggressiveness, short survival time, poor prognosis and lack of a proven therapeutic target, in contrast to hormone receptor positive and

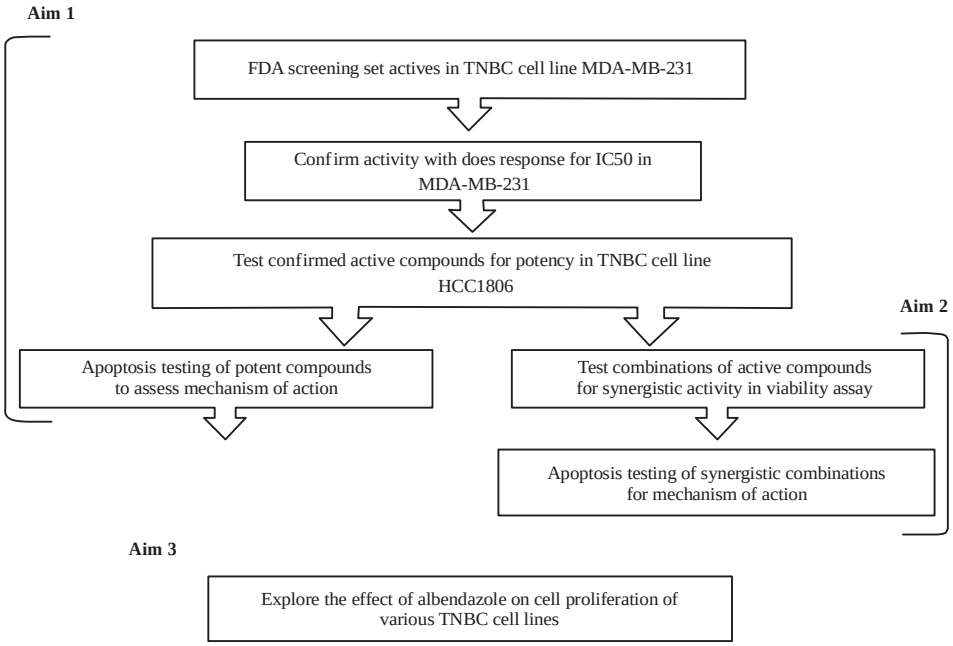

Fig. 1: Project flow chart

ERBB2+ breast cancers[5]. The clinical features of TNBC patients include an increased body weight, metabolic syndrome, a worse prognosis, larger tumor size, rare histologies, elevated mitotic count, aggressive relapses and no specific proven therapeutic target[5]. TNBC poses a great drug discovery challenge because TNBC lacks a specific therapeutic target[4]. Consequently, more and immediate research is required to develop an effective therapy for treating TNBC patients[5]. Six TNBC subtypes have been described by Chen *et al.*[4] based on gene expression studies of 587 TNBC samples, where each subtype displays a unique gene expression pattern and ontology. They also reported existing cell lines as models representing each of the TNBC subtypes. These subtypes of TNBC also displayed different sensitivities to targeted therapeutic agents[4]. One study conducted by Dent *et al.*[6] compared women with different types of breast cancer; those with triple-negative breast cancer had an increased possibility of recurrence and consequently dying within 5 years of first diagnosis, but not thereafter. Furthermore, they found that the pattern of recurrence was also qualitatively different; among the triple-negative group, the risk of recurrence is higher at approximately 3 years and declined sharply thereafter[6]. So far, there are limited options for treating triple negative breast cancer[5]. It has been noted that triple negative breast cancer subtype is unresponsive to chemotherapy and other anticancer drugs such as ixabepilone, anthracyclines, platinum and taxanes[5]. Due to the heterogeneity of TNBC and absence of a proven molecular target, treatment of patients with triple negative breast cancer was challenging. There were many clinical trials currently on-going to test the effectiveness of therapeutic compounds such as small molecules and recombinant proteins as possible treatments for triple negative breast cancer. Presently, standard chemotherapy is the only treatment option available for treating TNBC[7]. Recent studies by Perou *et al.*[8] have distinguished five distinct BC subgroups; luminal-A, luminal-B, Basal-Like Breast Cancer (BLBC), HER-2 and normal-like. Drug combinations are widely used in treating disease. Using combination of drugs with different mechanisms or action may be beneficial in treating diseases more effectively. For these therapeutic benefits, drug combinations have been widely used and became an alternative choice for treating the most harmful diseases, such as cancer and AIDS[9]. Therefore, the objective of this research project was to identify selected FDA-approved compounds with activity against TNBC cell lines and identify combinations of these compounds that show synergistic activity as also shown in Fig. 1.

MATERIALS AND METHODS

Cell culture maintenance: Triple negative human breast cancer cell lines, MDA-MB-231, MDA-MB-468 and HCC 1806 were obtained from American Type Culture Collection (ATCC, Manassas, VA) and cultured according to specifications. This research was conducted from October, 2013 to October, 2014.

The cells were grown at 37°C in a humidified 5% CO_2 atmosphere. MDA-MB-231 and MDA-MB-468 adherent cell line cells were cultured in 75 cm^2 vented tissue culture flasks (Corning Inc., Corning, NY) and maintained in high glucose Dubecco's Modified Eagle's Media (DMEM) obtained from Life Technologies (Grand Island, NY, USA) and supplemented with 10% Fetal Bovine Serum (FBS), 100 units/mL penicillin G-streptomycin and 2.0 mM glutamine. HCC1806 adherent cell line cells were cultured in 75 cm^2 vented tissue culture flasks (Corning Inc., Corning, NY) and maintained in RPMI medium obtained from Life Technologies (Grand Island, NY, USA) and supplemented with 10% fetal bovine serum (FBS), 100 units mL^{-1} penicillin G-streptomycin and 2.0 mM glutamine. Cells were routinely passages at a frequency of two times per week and cell lines harvested with trypsin following washing with Ca/Mg free Dulbecco's Phosphate-Buffered Saline (DPBS).

Dose response curves and IC_{50} determination: Cells were seeded into 96 well assay plates in 90 µL medium and cultured overnight at 37°C in humidified 5% CO_2 atmosphere. Due to its rapid growth; HCC1806 cells were seeded in triplicate at 3000-5000 cells per well (90 µL) in a 96-well plate. MDA-MB 231 and MDA-MB-468 cells were plated in triplicate at 10,000 cells per well (90 µL) in a 96-well plate. Compound preparation was done using the following procedure: 3 fold serial dilutions of initial 100% DMSO stock compound solutions were performed in 100% DMSO. 1:100 intermediate dilutions were performed by the addition of 5 µL of diluted compound to 495 µL of suitable growth media i.e., 1:100 dilution factor. The cells were treated with 10 µL of a corresponding intermediate dilution providing a dilution factor of 1:10 and a total dilution factor of 1:1000 (0.1% final DMSO). Cells then incubated at 37°C in humidified 5% CO_2 atmosphere for 48-72 h. After 48-72 h incubation period, plates were treated with 10 µL of resazurin (PrestoBlue® reagent). Plates were shaken briefly for 30 sec using IKA MTS4 plate shaker before the plates were returned to 37°C in 5% CO_2 for 90 min to allow viable cells to convert resazurin into fluorescent resorufin. Fluorescence was determined using 560 nm excitation and 590 nm emissions filters using BMG PheraStar plate reader. The data obtained from plate reader were normalized using the following formula:

$$\text{Activity}(\%) = \frac{\text{Experimental} - \text{Min}}{\text{Max} - \text{Min}} \times 100\%$$

where, experimental represents the effect of the compound dose maximum represents the average fluorescence of DMSO only treated wells (solvent control). Minimum represent the average fluorescence of a "0 cell" control. The half-maximum inhibition concentrations (IC_{50}) were calculated using non-linear regression analysis with 3- or 4-parameter curve fits (GraphPad Prism v.4.0, GraphPad Software, San Diego, CA, USA).

Synergy assays: HCC1806 cells were seeded in triplicate into 96-well plates and cultured overnight using the standard protocol outlined above. Cells were treated with compounds; equal-molar mixtures of compounds were generated by mixing equal volumes of the 10 mM 100% DMSO stock solutions. This mixture was then treated as a compound with total concentration of 10 mM which was diluted as described previously (0.1% final DMSO concentration). The assay plate was incubated for 72 h. Fluorescence intensities were read at 560 nm excitation/590 nm emission after 1.5-2 h incubation. Dose response curves for each drug combination were obtained using GraphPad prism-4 software.

Data analysis: GraphPad-Prism (version 4 and 5) was used to create IC_{50} curves (non-linear regression analysis), means, standard deviation of all replicates and graphs. Microsoft Excel was used to calculate mean, standard deviation, % coefficient variation and Z-factors. CompuSyn (version 1) was used to determine synergy; this is the third generation of computer software developed by Nick Martin of MIT, Cambridge MA in 2005. This program was used in this project to quantify synergy using the Chou-Talalay method.

RESULTS AND DISCUSSION

Confirmation of hit compounds in mda-mb-231: The selected compounds were identified from the Prestwick chemical library using the MDA-MB-231 cell line during previous studies in the Scott lab using High Throughput Screening (HTS) in a synthetic lethal screen. Compounds that were found to be more than 50% active, have unknown anti-cancer activities and FDA approved were selected for further screening with different TNBC cell lines. Table 1 showed set of FDA approved drug and their screen hits using MDA-MB-231 with their percentage inhibition values.

Table 1: Compounds identified in a primary screen in MDA-MB-231 cells

Compound	Inhibition (%)	N
Hydrochlorothiazide	56	1
Amlodipine besylate	55	2
Emetine dihydrochloride	74	2
Pyrviniumpamoate	53	2
Quinacrine	50	2
5-Azacytidine	60	2
Thiethylperazine malate	58	2

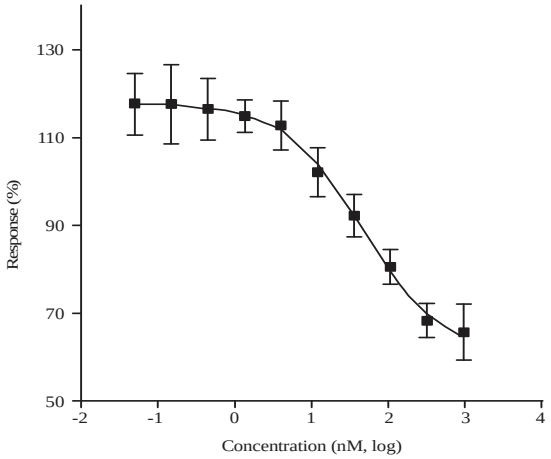

Fig. 2: The effect of dasatinib on MDA-MB-231 cell viability

In order to confirm activity of the compounds in the MDA-MB-231 cell line, this determined their IC50 values in a resazurin-based viability assay. The incubation time for cells treated with the test compounds was 48-72 h. Cell treatment was performed in a 96-well plate. In order to test the compounds in different TNBC cell lines and due to different growth rates between cell lines, this optimized cell number to be plated from previous studies done in the Scott lab. Optimal cell numbers were seeded onto assay plates with different cell lines and allowed to incubate for 48-72 h. For the MDA-MB-231 cell line, 10,000 cells/wells were used; for the HCC1806 cell line; 3000-5000 cells/wells were used. Cell viability was quantified using a commercial resazurin-based reagent (PrestoBlue® reagent). Previous studies have shown that dasatinib potently inhibits the growth of most TNBC cell lines[10]. Dasatinib (das) is used at the beginning of these studies as an assay method control compound. A dose response curve for dasatinib was generated (Fig. 2). The observed IC50 was 46 nM which was similar to that observed by Pichot *et al.*[11], in which they arrived at an IC50 of 37 nM when using the same cell line. IC50 of the dasatinib is a relative IC50, rather than absolute. The IC50 curve did not get to zero, but showed a maximal ~50% inhibition effect on the cells which suggested that dasatinib is not toxic to the cells but rather prevented cell growth. This data is consistent with the anti-proliferative activity that has been described for TNBC cell lines in the literature[12] and previously in the Scott lab[13].

For the purpose of this project the following compounds were selected from previous studies in the Scott lab and tested in MDA-MB-231 cell line to confirm the previous results. Table 2 summarized the compounds that showed activity against the MDA-MB-231 cells in this assay, while other compounds have not shown inhibitory effects on MDA-MB-233 and were excluded from being tested in HCC1806.

Table 2: IC50s of dasatinib, digoxin, emetine, pyrvinium, hydrochlorothiazide and quianacrine in MDA-MB-231

Compound	IC50 (nM) ±SD	N
Hydrochlorothiazide	55±33	2
Dasatinib	55±33	6
Emetine dihydrochloride	60±15	4
Digoxin	274±156	3
Pyrviniumpamoate	778±178	4
Quinacrine	1562±619	2

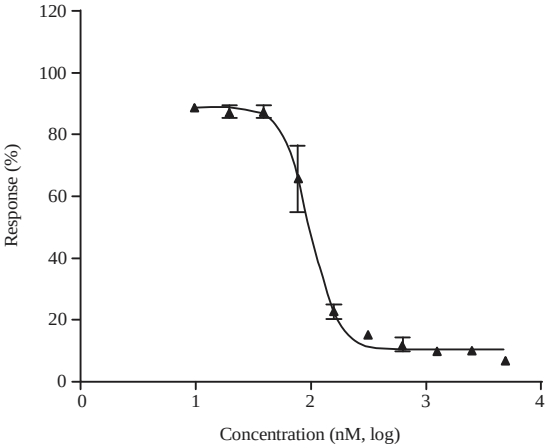

Fig. 3: The effect of digoxin on HCC1806 cell viability

Confirmation of selected compounds activities in HCC1806: Compounds tested in MDA-MB-231 cell line and found active against it were tested in the HCC1806 cell line to assess activity in abroad panel of triple negative breast cancer cell lines. IC50 determinations were performed with these compounds.

Digoxin: Digoxin is used to treat heart failure and abnormal heart rhythms (arrhythmias), it helps the heart work better by increasing its contractility and it helps control heart rate[14]. A representative dose response in HCC1806 cells is shown with a starting concentration of 10 µM. Using data normalized to in-plate controls, the average IC50 value from 11 experiments was determined to be 181±67 nM for digoxin (Fig. 3). This compound achieved >80% inhibition that may indicate a cytotoxic mechanism, rather than just anti-proliferative.

Emetine: Emetine is used to treat or control nematode infection[15]. Emetine was found to be active in HCC1806 with an average IC50 of 138±64 nM (Fig. 4). Since the observed activity at higher concentrations was well below 50% activity, it appeared that emetine was likely inducing cell death.

A representative dose response in HCC1806 cells is shown with a starting concentration of 10 µM. Using data normalized to in-plate controls, the average IC50 value from 10 experiments was determined to be 138±64 nM.

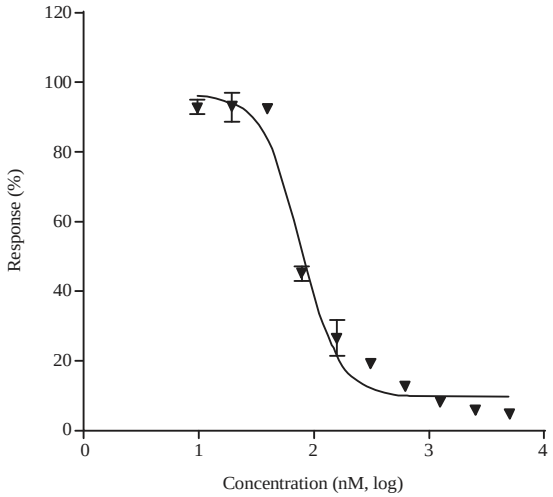

Fig. 4: The effect of emetine on HCC1806 cell viability

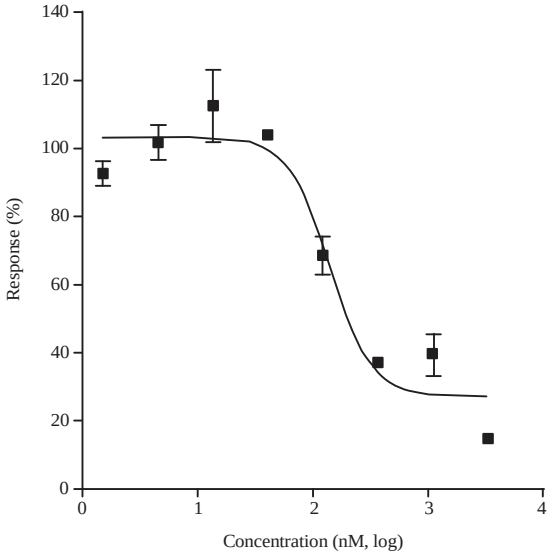

Fig. 5: The effect of pyrvinium on HCC1806 cell viability

Pyrvinium pamoate: Pyrvinium pamoate is used in the treatment of pinworm infection[16]. A dose response curve was generated and the compound was found to be active in HCC1806 cell line with an average IC50 of 238±144 nM (Fig. 5). This compound again achieved >90% inhibition, this is showing some cytotoxic effect, rather than just anti-proliferative.

A representative dose response in HCC1806 cells is shown with a starting concentration of 10 µM. Using data normalized to in-plate controls, the average IC50 value from 4 experiments was determined to be 4515±853 nM.

Disulfiram: Disulfiram is used as alcohol deterrent[17]. This compound was tested only in HCC1806 since it was a hit in a different high throughput screen performed in the Scott lab. A dose response curve was generated and the compound was found to be active in HCC1806 cell line with an average relative IC50 of 138±6 nM. This is partial curve with only ~ 40% inhibition, showing mild anti-proliferation effect (Fig. 6).

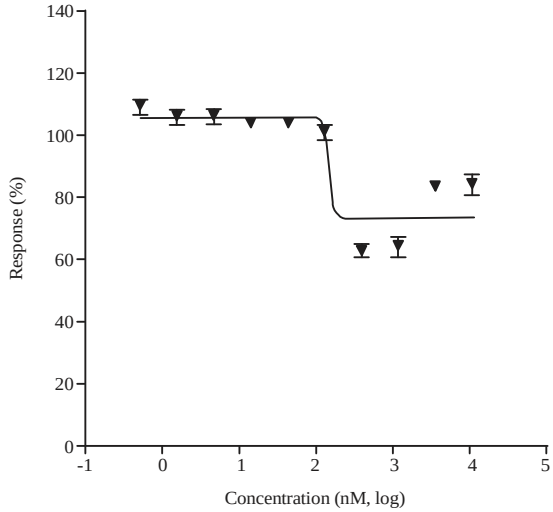

Fig. 6: The effect of disulfiram on HCC1806 cell anti-proliferation

Table 3: IC50s of disulfiram, emetine, digoxin and pyrvinium, in HCC1806

Compound	IC50 (nM) ±SD	N
Disulfiram	138±6	4
Emetine dihydrochloride	138±64	10
Digoxin	181±67	11
Pyrviniumpamoate	238±144	5

A representative dose response in HCC1806 cells is shown with a starting concentration of 10 µM. Using data normalized to in-plate controls, the average IC50 value from 4 experiments was determined to be 138±6 nM.

A combined representative dose response in cell lines tested is shown with a starting concentration of 10 µM. Using data normalized to in-plate controls, the average IC50 values from 3 experiments were determined to be 338 nM, 276 nM and 348 nM for MDA-MB-231, MDA-MB-468 and HCC1806, respectively. Table 3 summarized that disulfiram, emetine, digoxin and pyrvinium, showed possible activity against the HCC1806 cells in this assay.

IC_{50} determinations of combinations of compounds in HCC1806: The compounds that were confirmed actives in and MDA-MB-231 and HCC1806 were further tested in combination based on their IC_{50s} values generated from HCC1806. The compounds that were tested include: 5-Azacytidine, digoxin, emetine, pyrviniumpamoate, disulfiram and albendazole.

Combination tests:

Digoxin, emetine and their combination: HCC 1806 cells were seeded at 3×10^3 cells/well in 96-well plate. After 24 h, cells were treated in triplicate with 3-fold serial dilutions of individual compounds and their combination at approximately the same ratio as their IC50 ratios determined previously. Thus, the compounds were mixed at 1:1, 1:2, or 1:3 ratios depending on the IC50s of the tested individual compounds. The combined compound was formed by mixing volumes of equal concentration (10 mM) stock in 100% DMSO so the total compound concentration remained at 10 mM. After 3 days, cells were treated with PrestoBlue® reagent and read using PheraStar. Dose response curves were generated and IC50s calculated using GraphPad program. Combination indices (C.I.s) were obtained using the method of Chou and Talalay when the compounds appeared to potentially have a synergistic effect. HCC1806 cell line was treated with various concentrations of emetine, digoxin and their combination at a 1:1 ratio. The dose response curves were generated and the compounds were found to be active in HCC1806 cell line with IC50 of 190 nm, 104 nM and 64 nM for their combination, for this particular experiment (Fig. 7).

A representative dose response in HCC1806 cells is shown with a starting concentration of 10 µM. Using data normalized to in-plate controls, the average IC50 value from this experiment was determined to be 190 nm, 104 nM and 64 nM for digoxin, emetine their combination, respectively, for digoxin, emetine and their combination, respectively. According to the previous definition of synergism, the curve above, Fig. 6 showed some degree of synergism in some concentration points. Therefore, the synergistic effect was tested using Chou-Talalay Method (Fig. 8). The x-axis represent fraction affected or % inhibition, Y-axis represent the Combination Index (CI). The points shown in the graph below depict combination data points. The horizontal value was set equal 1 and represents additive effects as explained in the introduction. For this graph per se, the points below the horizontal line represent synergism, the points above the horizontal line represent antagonism, while the points lie along the horizontal line represent the additive.

Pyrvinium, emetine and their combination: HCC1806 cell line was treated with emetine, pyrvinium and their combination in 1:1 ratio, using 10 µM starting concentrations for both, the dose response curve was generated and the compound was found to be active in HCC 1806 cell line with IC50 of 363 nM for pyrvinium, 128 nM for emetine and 253 nM for their combination and there was no apparent synergy observed (Fig. 9).

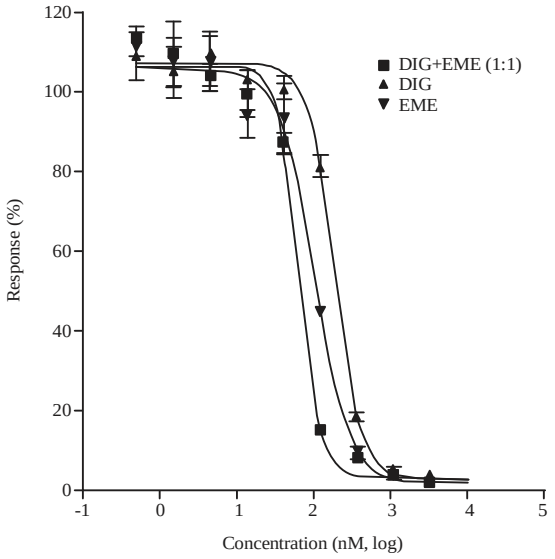

Fig. 7: The effects of digoxin, emetine and their combination in HCC1806

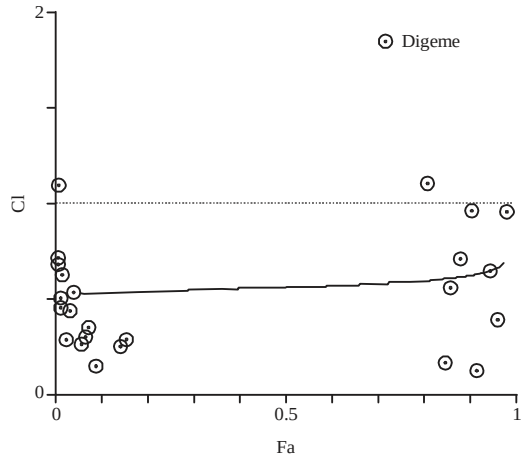

Fig. 8: Combination index plot using Chou-Talalay method

A representative dose response in HCC1806 cells is shown with a starting concentration of 10 µM. Using data normalized to in-plate controls, the average IC50 value from 2 experiments was determined to be 363 nM for pyrvinium, 128 nM for emetine and 253 nM for their combination. HCC1806 cell line was treated with emetine, disulfiram and their combination in 1:1 ratio. The dose response curve was generated and the compound was found to be active in HCC1806 cell line with IC50 of 134 nM for disulfiram, 88 nM for emetine and 154 nM for their combination. In the case of this combination, there was no apparent synergy observed (Fig. 10).

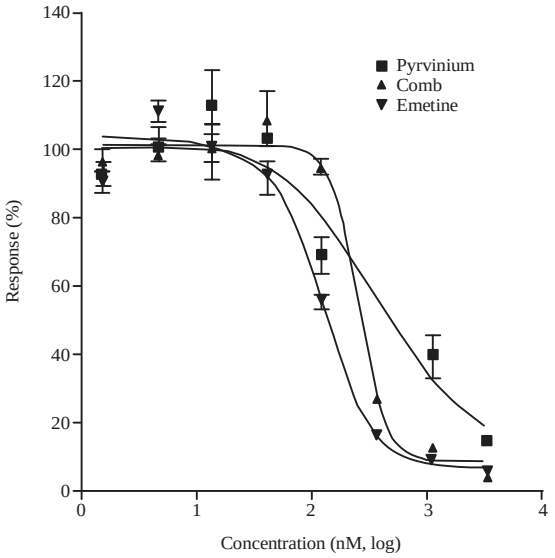

Fig. 9: The effects of pyrvinium, emetine and their combination in HCC1806

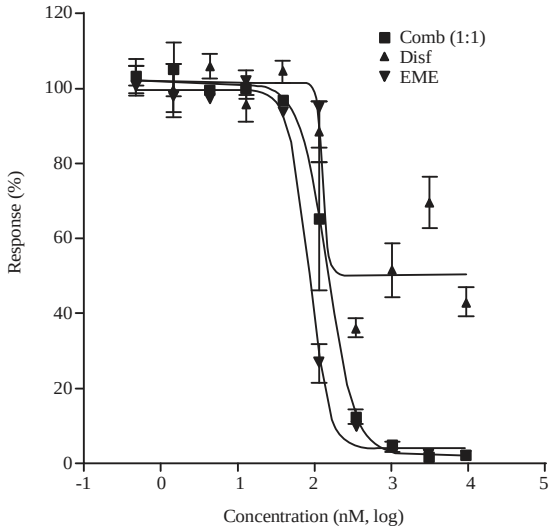

Fig. 10: The effects of disulfiram, emetine and their combination in HCC1806

A homogeneous cell-based assay was utilized towards re-purposing known drugs that have anti-cancer properties. It was sought that combinations of known drugs that would have synergistic activity against TNBC. Selective combinations of these drugs were tested for synergistic activity in killing or inhibiting proliferation of TNBC cell lines.

Digoxin is used to treat heart failure and abnormal heart rhythms (arrhythmias). It helps the heart work better and it helps control heart rate by its positive inotropic mechanism of increasing intracellular Ca^{2+} concentration leading to contractions of heart muscle[18]. However, it may hold greater potential than it is currently employed for TNBC treatment. To explore this possibility, this study set out testing the compound against two TNBC cell lines, MDA-MB-231 and HCC 1806. Results from inhibition studies clearly demonstrated that MDA-MB 231 and HCC 1806 are profoundly inhibited by digoxin. This was manifested by obtaining average IC50s of 274±156 nM (Table 2) and 181±67 nM (Table 3, Fig. 3) in MDA-MB-231 and HCC1806, respectively. This data indicates a potential novel use for digoxin for TNBC. Findings obtained by this study is consistent with a previous *in vitro* report that digoxin was active against prostate cancer cell lines both *in vitro* and in an *in vivo* animal study[19].

Emetine is used to treat or control nematode infection, as an anti-bacteria agent and to induce vomiting[15]. However, it may be re-purposed for other indications. The potential of this compound was investigated using the MDA-MB-231 and HCC1806 by conducting dose response experiments. The average IC50s in two TNBC cell lines were 60±15 nM (Table 2) and 138±64 nM (Table 2, Fig. 4) showing inhibitory activity. The observed activity of emetine at higher concentrations was well below 50% of controls and thus it appeared that emetine was likely inducing cell death. Any potential use of emetine in TNBC would need to be verified with animal studies. In addition, emetine has toxicity issues that would need to be resolved. Analogs of emetine may be discovered that have more potent activity with less toxicity, but would need to go through normal expensive and time-consuming drug development.

Pyrvinium Pamoate is used in the treatment of pinworm infection. This compound achieved >90% inhibition suggestive of a cytotoxic mechanism, as opposed to just anti-proliferative. This was obtained by dose response curves using MDA-MB-231 and HCC1806 and obtaining an average IC50 of 778±178 (Table 2) and 238±144 nM (Table 3, Fig. 5), respectively. This is showing possible inhibitory effect of pyrvinium pamoate in both cell lines being tested. The combinations of pyrvinium pamoate with emetine, digoxin, 5-azacytidine and disulfiram showed antagonistic effects rather than synergistic effects. The single agent activity was consistent with reports that showed pyrvinium has anti-cancer activity in some BC cell lines, mainly SUM149 and SUM 159 cell lines[20]. However, further testing of different TNBC cell lines as well as animal model studies would be needed for further investigations into re-purposing this drug for TNBC.

Disulfiram functions as an alcohol deterrent by interfering with ethyl alcohol metabolism, causing unpleasant side effects and consequently discouraging the use of alcohol[21]. This compound has been investigated in the Scott lab for its

inhibitory effect on TNBC cell lines. In addition, this activity was confirmed against TNBC cell lines as reported by Robinson *et al.*[22]. Here tested the compound mainly in HCC1806. This compound may hold possibility of being repurposed for treating TNBC. The results obtained showed inhibitory effect of disulfiram with an IC50 value of 138±6 nM (Table 3, Fig. 6) obtained in HCC1806. This is a new use for disulfiram that increases its potential as a TNBC drug. Unfortunately, no synergism was observed in combination with any of the other compounds. However, further investigations are necessary to identify drugs that are synergistic with disulfiram. Synergism is the interaction of two or more drugs when their combined effect is greater than the sum of the effects seen when each drug is given alone. All possible hit compounds have been tested to investigate possibility of synergistic effects. Only emetine and digoxin showed possible synergistic effect when combined, with IC50s of 190 nM for digoxin, 104 nM for emetine and 64 nM for their combination (Fig. 7). However, this effect was noticed only at some concentration points, not all, so synergy was not clearly demonstrated. More experiments would be needed to confirm synergy, possibly using two-fold serial dilutions instead of three and testing at shorter exposure times. Also, using other assay methods to measure cell death may help determine synergy. If confirmed, this would be a novel combination of already approved drugs. However, further testing of different TNBC cell lines as well as animal model investigations would be needed.

CONCLUSIONS AND RECOMMENDATIONS

The results revealed that some selected FDA approved non-oncology drugs have cytotoxic/anti-proliferative properties against two TNBC cell lines. The results have confirmed that emetine, digoxin, disulfiram and pyrvinium pamoate can inhibit proliferation and/or viability of TNBC cell lines MDA-MB-231 and HCC1806 with IC50 values ranging from 52 nM to 2077 nM. Emetine and digoxin have shown synergistic effect at certain concentrations when using Chou-Talalay method of analysis. Future studies should include the re-testing of digoxin and emetine combinations using smaller dilution steps and shorter incubation times. Moreover, the digoxin/emetine combination should be tested for efficacy in animal models of TNBC such as xenograft tumor models. Such tests would include various molar combinations as well as individual dose testing of these drugs.

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REFERENCES

1. Vogelstein, B., N. Papdopoulos and K.W. Kinzler, 2013. Cancer genome landscapes. Science, 339: 1546-1558.
2. Society, A.C., 2013. Breast cancer facts and figures 2013-2014, pp: 1-40.
3. Nowacka-Zawisza, M. and W.M. Krajewska, 2013. Triple-negative breast cancer: Molecular characteristics and potential therapeutic approaches. Postepy Hig. Med. Dosw (Online), 67: 1090-1097.
4. Chen, X., J. Li, W.H. Gray, B.D. Lehmann, J.A. Bauer, Y. Shyr and J.A. Pietenpol, 2012. TNBC type: A subtyping tool for triple-negative breast cancer. Cancer Inform., 11: 174-156.
5. Bosch, A., P. Eroles, R. Zaragoza, J.R. Vina and A. Lluch, 2010. Triple-negative breast cancer: Molecular features, pathogenesis, treatment and current lines of research. Cancer Treat. Rev., 36: 206-215.
6. Dent, R., M. Trudeau, K.I. Pritchard, W.M. Hanna, H.K. Kahn, C.A. Sawka, L.A. Lickley, E. Rawlinson, P. Sun and S.A. Narod, 2007. Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin. Cancer Res., 13: 4429-4434.
7. Lehmann, B.D., J.A. Bauer, X. Chen, M.E. Sanders, A.B. Chakravarthy, Y. Shyr and J.A. Pietenpol, 2011. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J. Clin. Invest., 121: 2750-2767.
8. Perou, C.M., T. Sørlie, M.B. Eisen, M. van de Rijn, S.S. Jeffrey and C.A. Rees, 2000. Molecular portraits of human breast tumours. Nature, 406: 747-752.
9. Chou, T.C., 2010. Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Res., 70: 440-446.
10. Tarpley, M., T.T. Abdissa, G.L. Johnson and J.E. Scott, 2014. Bosutinib reduces the efficacy of dasatinib in triple-negative breast cancer cell lines. Anticancer Res., 34: 1629-1635.
11. Pichot, C.S., S.M. Hartig, L. Xia, C. Arvanitis, D. Monisvais, F.Y. Lee, J.A. Frost and S.J. Corey, 2009. *Dasatinib synergizes* with doxorubicin to block growth, migration and invasion of breast cancer cells. Br. J. Cancer, 101: 38-47.
12. Finn, R.S., J. Dering, C. Ginther, C.A. Wilson, P. Glaspy and N. Tchekmedyian, 2007. Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/"triple-negative" breast cancer cell lines growing *in vitro*. Breast Cancer Res. Treat., 105: 319-326.
13. Neubig, R.R., M. Spedding, T. Kenakin and A. Christopoulos, 2003. International union of pharmacology committee on receptor nomenclature and drug classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. Pharmacol. Rev., 55: 597-606.
14. Adelstein, E., D. Schwartzman, S. Jain, R. Bazaz and S. Saba, 2014. Effect of digoxin on shocks in cardiac resynchronization therapy-defibrillator patients with coronary artery disease. Am. J. Cardiol., 113: 970-975.

15. Burhans, W.C., L.T. Vassilev, J. Wu, J.M. Sogo, F.S. Nallaseth and M.L. DePamphilis, 1991. Emetine allows identification of origins of mammalian DNA replication by imbalanced DNA synthesis, not through conservative nucleo some segregation. EMBO J., 10: 4351-4360.
16. Inoue, A., C. Kawakami, K. Takitani and H. Tamai, 2014. Azacitidine in the treatment of pediatric therapy-related myelodysplastic syndrome after allogeneic hematopoietic stem cell transplantation. J. Pediatr. Hematol. Oncol., 36: e322-324.
17. Skinner, M.D., P. Lahmek, H. Pham and H.J. Aubin, 2014. Disulfiram efficacy in the treatment of alcohol dependence: A meta-analysis. PLoS One, 9: e87366.
18. Adelstein, E., D. Schwartzman, S. Jain, R. Bazaz and S. Saba, 2014. Effect of digoxin on shocks in cardiac resynchronization therapy-defibrillator patients with coronary artery disease. Am. J. Cardiol., 113: 970-975.
19. Platz, E.A., S. Yegnasubramanian, J.O. Liu, C.R. Chong, J.S. Shim and S.A. Kenfield, 2011. A novel two-stage, transdisciplinary study identifies digoxin as a possible drug for prostate cancer treatment. Cancer Discov., 1: 68-77.
20. Xu, W., L. Lacerda, B.G. Debeb, R.L. Atkinson, T.N. Solley and O.D. LiL, 2013. The antihelmintic drug pyrvinium pamoate targets aggressive breast cancer. PLoS One, 8: e71508.
21. Skinner, M.D., P. Lahmek, H. Pham and H.J. Aubin, 2014. Disulfiram efficacy in the treatment of alcohol dependence: A meta-analysis. PLoS One, 9: e87366.
22. Robinson, T.J., M. Pai, J.C. Liu, F. Vizeacoumar, T. Sun, S.E. Egan and A. Datti, 2013. High-throughput screen identifies disulfiram as a potential therapeutic for triple-negative breast cancer cells: Interaction with IQ motif-containing factors. Cell Cycle, 12: 3013-3024.