

# Electroporation-based Enhanced Anti-Cancer Effect of Veliparib on Triple Negative Breast Cancer Cells

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**Abstract**—Veliparib is a poly (ADP-ribose) polymerase (PARP) inhibitor with antitumor activities. It is used along with carboplatin, the platinum chemo drug for treating triple negative breast cancer. They have shown promising efficacy and safety results in Phase I and II clinical trials in patients with triple negative breast cancer. PARP is involved with the base-excision repair of single strand DNA breaks while BRCA proteins help to restore double-strand breaks. Breast cancer with BRCA mutation leave the cells susceptible such that PARP inhibition combined with this genetic defect cannot repair DNA breaks resulting in cell death. Since many women with a BRCA mutation have a triple negative phenotype, it is shown that PARP inhibitor when combined with chemotherapy, such as carboplatin significantly extend the overall survival of metastatic triple negative breast cancer. To reduce the toxicity and side effects, it is possible to enhance the uptake of chemo drugs using electrical pulses. This phenomenon, known electroporation has shown to enhance up to 1000x. Towards this we studied the effects of veliparib on triple negative human breast cancer cells, MDA-MB-231 using electroporation. With Veliparib at 330 $\mu$ M concentration and using 1200V /cm, 100 $\mu$ s, 8 pulses and 500V /cm, 20ms, 2 pulses, we could kill the cells up to 30% and 24% respectively. The drug only sample had only 18% cell kill. Considering that it is difficult to treat triple negative cells due to their lack of the estrogen receptor, progesterone receptor, and Her2 expression, any assistance from other methods, especially physical methods like electroporation is a potential method to treat triple negative breast cancers.

## I. INTRODUCTION

Out of many types of genes which human beings possess, BRCA 1 and BRCA 2 are the ones which produce proteins, those that help in the suppression of any kind of tumor. In case of alteration or mutation of any of these genes, they may fail to produce protein leading to hampering the process of repair of DNA. This may lead to some serious kind of additional genetic alterations leading to development of cancer. Mutation of either of these genes i.e. BRCA 1 and BRCA 2 remain a phenomenon which may be inherited from one's either of the parents with almost 50 % probability of being passed on to the kids. Women

inheriting any of these genes are at a much higher risk of developing a breast cancer at some stage of their lives [1]. As per the study conducted by Bhumsuk keam et. al, the probability of survival rate of the TNBC patients are less than 3 percent. Figure 1 shows the probability of survival of both TNBC and non TNBC patients [2].

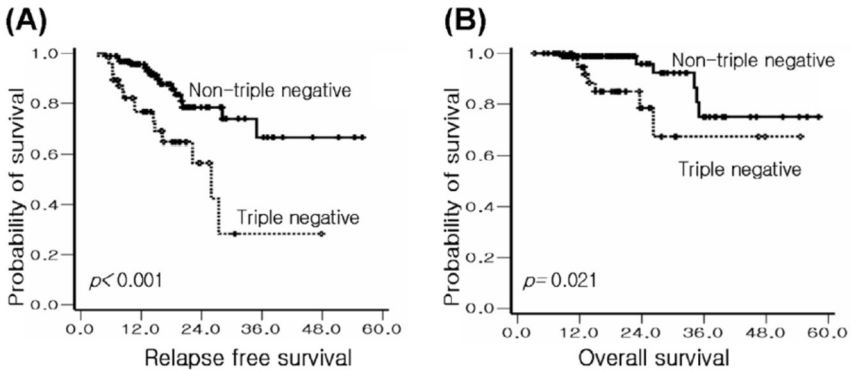


Fig. 1A. Probability of relapse free survival of TNBC and non TNBC patients

Fig. 1B. Probability of overall survival of TNBC and non TNBC patients

At the time of diagnosis of type of breast cancer, usually patient is screened for an expression of estrogen receptor (ER), progesterone receptor (PR) and amplification of HER-2/Neu evaluated. Type of breast cancer tumors which do not display any of these expressions are more difficult to be treated and are categorized as Triple Negative Breast Cancer (TNBC). As of now, the number of TNBC cell lines stands to be 27, however there may be other cell lines which may be derived from these [3].

As a part of the present research effort, we have made our studies on one particular variety of TNBC cell line viz. MDA-MB-231. Effect of potent drugs such as combination of Gemcitabine and Cisplatin has been studied earlier while controlling the pulse and dose parameters in Combination Electrochemotherapy [4].

This work utilizes Veliparib, a potential anticancer drug for many cell lines. Veliparib is a PARP (Poly ADP Ribose Polymerase) inhibitor. Figure 2 Shows the mechanism of PARP inhibitor in aiding cell death. The role of the PARP inhibitors to be a potential antitumor drug is first introduced by Masahiko et al. The basic mechanism when PARP inhibitor combined with  $\gamma$ -irradiated mammalian cells is to prevent the repair of broken DNA strands by combining with PARP that helps to repair the DNA strands. Thus the lesions do not replicate before treated by chemo drugs [5,6].

Electroporation is a novel and a robust local treatment technique in treating cancer that could not be treated by conventional therapies such as chemotherapy, radiotherapy, surgery. Due to its robust and physical nature, it does not increase the toxicity in the human body in contrast to the chemotherapy. The adverse effects of electroporation are confined to slight burns in the skin and contraction of muscles [7]. This method utilizes high voltage short duration pulses to create temporary pores in the hydrophilic and hydrophobic membrane in the cells.

Electroporation uses the electrical properties of the cell. A cell could be represented as a minute battery with a potential varying from  $-40\text{mV}$  to  $-95\text{mV}$  [8]. When electric pulses of higher voltages are applied across the cells the membrane breaks down and creates pores

through which the chemo drugs are delivered to the cells in a high efficient manner. Since the pulses are of short duration the cell membrane reseals itself and reaches its resting potential. The drug that enter into the cell through the pores creates apoptosis.

In this study, the PARP inhibitor is used as the chemo drug without any combination of other conventional chemo drugs. By using optimized dosage of Veliparib along with right selection of parameters for Electroporation there is an increase in cell death.

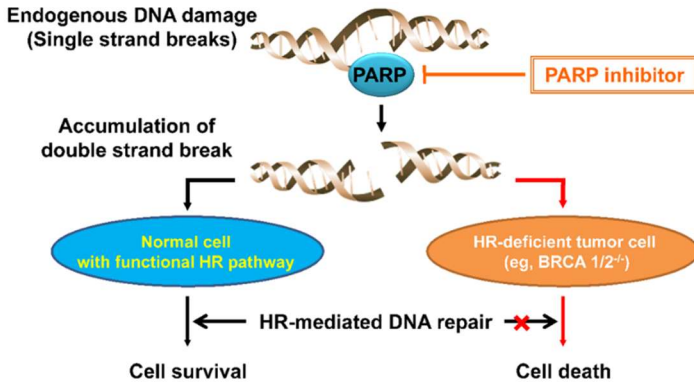


Fig. 2. Mechanism of PARP inhibitors to cause cell death

## II. MATERIALS AND METHODS

### A. The Cells

Triple negative MDA-MB-231 basal type human adenocarcinoma epithelial breast cancer cells are used for this study. As mentioned above, this cell line is negative to ER, PR, and HER2 receptors.

Tumor cells have higher conductivity and permittivity than the healthy cells. These electrostatic properties of the tumor cells are exploited in this study to increase the efficiency of the drug delivery thus to increase the percentage of cell death.

### B. The Drugs

Veliparib di-hydrochloride (ABT-888) was used for this study. Veliparib was solubilized in DMSO at 10mM/mL and concentration of 330  $\mu\text{M}$ , 500  $\mu\text{M}$  and 830  $\mu\text{M}$  were used for this study.

Veliparib is a 2-[(R)-2-methylpyrrolidin-2-yl]-1H-benzimidazole-4-carboxamide di-hydrochloride and the chemical formula for this drug is  $\text{C}_{13}\text{H}_{18}\text{Cl}_2\text{N}_4\text{O}$  with the molecular weight of 244.29234 g/mol. Its structure is shown in Figure 3. It potentially inhibits both PARP-1 and PARP-2 with  $K_{is}$  (inhibitory constants) of 5.2 and 2.9 nmol/L, respectively [9]. As seen with many PARP inhibitors, this activity is generally selective and Veliparib does not appear to have substantial effects on other receptors or ion channels at pharmacologically relevant concentrations. Veliparib is used to treat ovarian cancer, oral cancer, basal like breast cancer, pancreatic cancer, prostate cancer [9,10].

However, the side effects of Veliparib are not limited to gastrointestinal toxicity, nausea,

vomiting, secondary leukemia and myelodysplastic syndrome [9]. But also, Diarrhea, Constipation, stomach pain, fatigue. These side effects can be effectively reduced if the concentration of Veliparib used in the treatment is reduced.

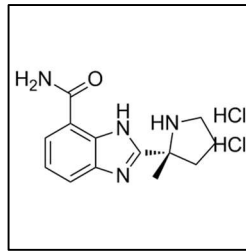


Fig. 3. Chemical Structure of Veliparib (di-hydrochloride)

### C. The Electroporation Technique

Unipolar square wave pulses used in the study are generated using BTX ECM 830 electroporator (Genetronics Inc., San Diego, CA). Figure 4 shows the complete procedure for electroporation used in this study. The applied pulse parameters for this study are shown in Table-1. The frequency of the pulses is 1Hz. The pulse parameters are based on previous research on these cell lines [11-13]. The electroporation technique is explained in the flowchart presented in the Figure 4.

TABLE 1: PULSE PARAMETERS STUDIED

S. No	Electric Field Intensity V /cm	Pulse Duration	No. of Pulses
1	1200	100 $\mu$ s	8
2	500	20ms	2

### D. The Viability Assay

20 $\mu$ L of treated samples and 20 $\mu$ L of typhen blue were mixed together. From this mixture, 20 $\mu$ L was used to count both live and dead cells using the Nexelcom Bioscience Cellometer. The percentage viability was also directly measured using the cello meter.

## III. RESULTS AND DISCUSSION

Figure 5 shows the dose curve of triple negative MDA-MB-231 cells treated with veliparib. The different dosage used are 330  $\mu$ M, 500  $\mu$ M, 830  $\mu$ M. This dose curve gives the effect of veliparib alone as an anti-tumor drug without any combination of other chemotherapeutic drugs such as bleomycin, cisplatin and carboplatin.

From Figure 5, it can be clearly seen that the effect of veliparib on triple negative MDA-MB-231 cell line is high at 330  $\mu$ M than other 500  $\mu$ M and 830  $\mu$ M. So this study deals with 330  $\mu$ M of veliparib drug in combination with electroporation in order to maximize the cell death. The study conducted by Jung-Min Lee et. al confirms that single agent treatment using veliparib alone killed 11% of cells at 50  $\mu$ M. In this study, even though the concentration level of veliparib was increased to 6 times than of the study by Jung-

Min Lee the percentage effectiveness was not proportionately high. This shows the limited efficiency of using only the drug to treat the triple negative cancer cells.

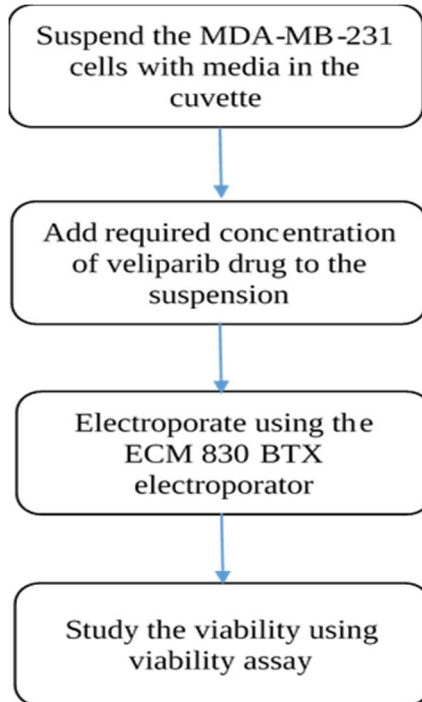


Fig. 4. Procedure for in-vitro Electroporation.

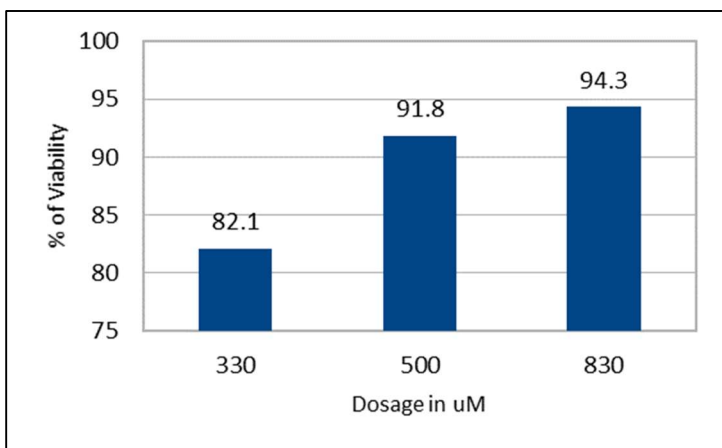


Fig. 5. Dose curve of Veliparib on MDA-MB-231 cells without Electroporation.

Figure 6 shows the viabilities of the cell line without any drug or electroporation (control), drug only, and then the combination of drug and electric pulses at (1200V/cm, 100

$\mu\text{s}$ , 8 pulses) and (500V/cm, 20ms, 2 pulses). From Figure 6, the electric pulses of (1200V/cm, 100  $\mu\text{s}$ , 8 pulses) kill 30% of the cells while (500V/cm, 20 ms, 2 pulses) kills 24% cells. This is due to the fact that higher potential increases the cell death than the low potential electric pulses. Even though the energy applied is higher in case of the second parameter than the first parameter, the cell death is higher at 1200V/cm than the 500V/cm. The combined effect of using electric pulses and veliparib increase the cell death from 18% to 30%. With the increase in electric potential and energy, higher cell death could be achieved by using veliparib as the anti-tumor drug.

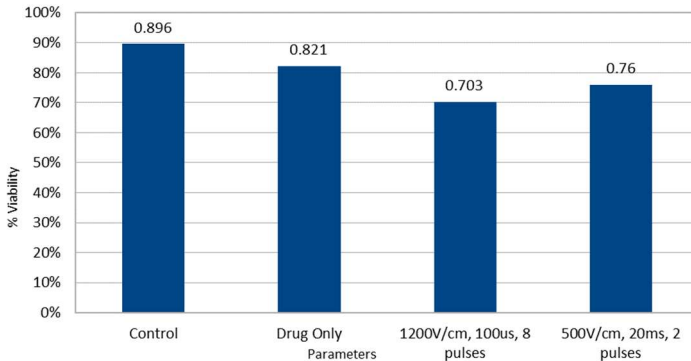


Fig. 6. Viabilities without any drug, with veliparib alone and combination of veliparib and electroporation at 1200V/cm, 100  $\mu\text{s}$ , 8 pulses and 500V/cm, 20ms, 2 pulses.

#### IV. CONCLUSION

The combination of veliparib (200  $\mu\text{M}$ ) with ECT leads to notable increase in cell death which is not achieved using the chemo drug alone. This study suggests that optimizing the electric pulse parameters will reduce the cell viability. Also this study suggests the use of electroporation in addition to the combination of chemo drug such as veliparib with carboplatin, cisplatin and gemcitabine to reduce their drug concentration to achieve higher cell death. Thus it reduces the side effects due to the toxicity of the chemo drug.

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