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

Vishveswaran Jothi, Vishak Raman, Rajnish Sharma*, Ignacio Camarillo,

and Raji Sundararajan
Purdue University,
*Chitkara University, India

e-mail: raji@purdue.edu

***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. It is of practical interest to evaluate the effect of Veliparib alone as an anti-cancer drug. In addition, to enhance its uptake, electrical pulses could be used. This phenomenon, known electroporation has shown to enhance up to 1000x the drug effectiveness. Towards this we studied the effects of Veliparib on triple negative human breast cancer cells, MDA-MB-231 using electroporation. A dosage of 330μM was used and both high intensity, short duration pulses of 1200V/cm, 100μs and low intensity, long duration pulses of 500V/cm, 20ms were studied. These pulses could kill the cells up to 30% and 24% respectively at 330μM dosage of Veliparib. The drug alone could kill only 18% cells.**

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 [1]. 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 [2].

At the time of diagnosis to find type of breast cancer, usually a patient is screened for an expression of estrogen receptor (ER), progesterone receptor (PR) and evaluated for the amplification of HER-2/Neu. The type of breast cancers which do not display any of these expressions are more difficult to be treated and are categorized as Triple Negative Breast Cancer (TNBC) [3]. The probability of overall survival rate of the TNBC patients is a poor 2.1%, as per the study conducted by Bhumsuk keam et. al. on 145 patients [4], while the relapse survival rate of TNBC patient is 0.1%. Fig. 1 shows the probability of survival of both TNBC and non-TNBC patients [4].

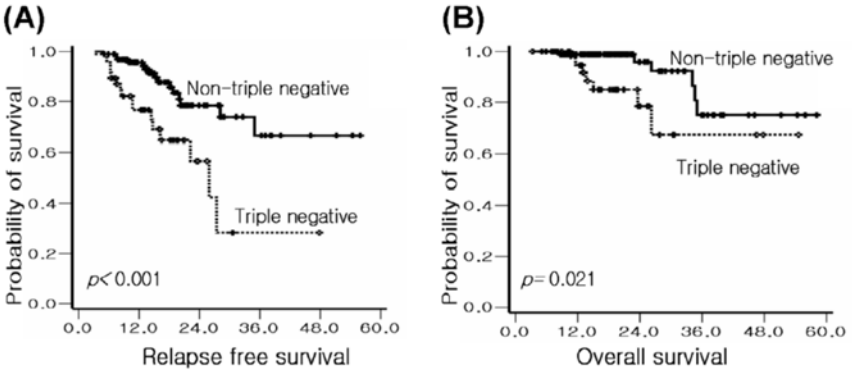


Fig. 1. Probability of relapse free survival of TNBC and non-TNBC patients (A) and Probability of overall survival of TNBC and non TNBC patients (B) [4].

Due to lack of the triple receptors, it is difficult to manage or control or treat the aggressive TNBC cancers, as most of the breast cancer drugs aim one or other of these three receptors. Hence novel, especially physical techniques, such as electroporation is tried to treat the TNBC cells. Since our cells, cancer or normal ones, consist of ions, polar or charged molecules, membranes, and organelles, they respond to external electrical fields. Thus, we can elicit both therapeutic and diagnostic response using electrical voltages, as basically all matter, living or non-living are governed by electromagnetic phenomenon. Cancer being an electrostatic phenomenon, it only makes sense to use electrical pulses to treat it.

Towards this, we chose MDA-MB-231, triple negative cell line. It is a spindle shaped invasive adherent type epithelial cell [5]. This cell line is derived from the metastatic site of pleural effusion from mammary gland of a Caucasian woman at M.D. Anderson Cancer Centre in 1973 [5]. Among the five types of molecular classification in breast carcinoma, MDA-MB-231 is a classified as Claudin-low type. It has intermediate response to chemotherapy [6].

In addition, we also chose Veliparib, a Poly (ADP Ribose) Polymerase inhibitor, as the anti-cancer drug, as these are useful in the treatments of several types of cancers with poor prognoses [7].

PARPs are a family of enzymes implicated in a host of key cellular processes, including chromosome stability, regulation of apoptosis, cell division, and transcriptional regulation and differentiation [7]. They perform the important role of repairing DNA damage that

results from very day environmental stresses and DNA replication errors. Inhibiting PARP should increase the tumor cell-killing potential of chemotherapy. Hence, typically it is utilized along with chemotherapeutic drugs, such as carboplatin to treat TNBC.

The role of the PARP inhibitors to be a potential antitumor drug is first introduced by Masahiko et al [8]. Fig. 2 shows the mechanism of PARP inhibitor in aiding cell death [9].

In order to increase the efficiency of the Veliparib, even after reaching its saturation efficiency, electroporation was used in combination with the drug. Also, the study conducted by Viktoria et al. concludes that electroporation reduces the cell adhesion and cell replication [10]. Since cell adhesion is an important process in cancer insemination, thus electroporation prevents the growth of tumor cells.

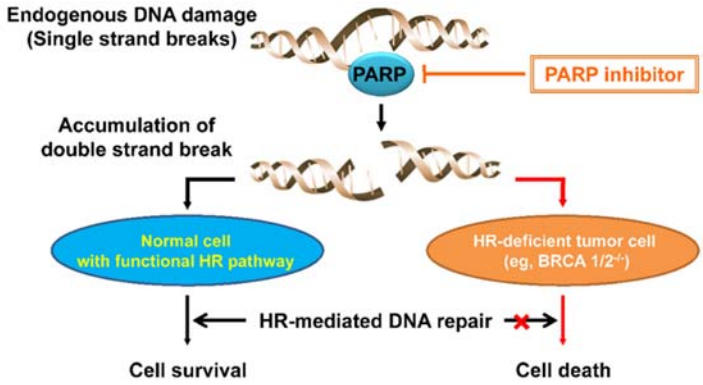


Fig. 2. Mechanism of PARP inhibitors to cause cell death [9].

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. It is also proven to be the most effective treatment than other conventional methods [11]. This method utilizes high intensity, short duration pulses to create temporary pores in the hydrophilic and hydrophobic membrane in the cells. It does not create any shock wave or Joule effect on the cells and tissues [12, 13]. Due to its transient and physical nature, it does not increase the toxicity in the human body unlike chemotherapy.

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. This cell line is negative to ER, PR, and HER2 receptors. The other characteristics of this cell line are low in Ki67, E-cadherin, claudin-3, claudinin-4 and claudinin-7 [6].

B. The Drugs

Veliparib di-hydrochloride (ABT-888, Medchemexpress LLC, NJ) was used for this study. It is a 2-[(R)–2-methylpyrrolidin-2-yl]-1H-benzimidazole-4-carboxamide di-hydrochloride and the chemical formula for this drug is $C_{13}H_{18}Cl_2N_4O$ with the molecular weight

of 244.29234 g/mol. Its structure is shown in Fig. 3 [14]. It potentially inhibits both PARP-1 and PARP-2 with K_is (inhibitory constants) of 5.2 and 2.9nmol/L, respectively [15].

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. It is used to treat ovarian cancer, oral cancer, basal like breast cancer, pancreatic cancer, prostate cancer [15, 16].

The various side effects include gastrointestinal toxicity, nausea, vomiting, secondary leukemia, myelodysplastic syndrome, diarrhea, constipation, stomach pain, fatigue [15]. These side effects can be effectively reduced if the concentration of Veliparib used in the treatment is reduced.

Veliparib, solubilized in DMSO at 10mM/mL was used at concentrations of 330, 500, and 830µM for this study.

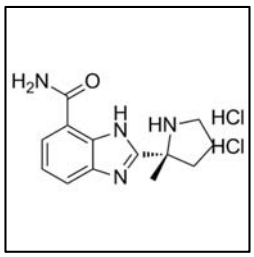


Fig. 3. Chemical Structure of Veliparib (di-hydrochloride) [14].

C. The Electroporation Technique

Unipolar square wave pulses, generated using BTX ECM 830 electroporator (Genetronics Inc., San Diego, CA) were used in this study. Fig. 4 shows the protocol used in this study. Table 1 shows the applied pulse parameters. The frequency of the pulses is 1Hz. The pulse parameters are based on previous research on these cell lines [17-19].

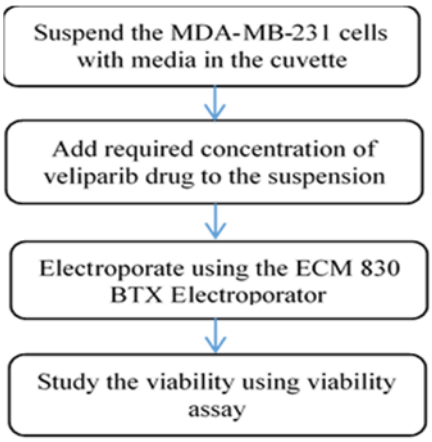


Fig. 4. Procedure for in-vitro Electroporation.

TABLE 1: PULSE PARAMETERS STUDIED

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

D.The Viability Assay

20μL of treated samples and 20μL of trypan blue were mixed together. From this mixture, 20μL was used to count both live and dead cells using the Nexcelom Bioscience Cellometer. The percentage viability was also directly measured using the cello meter.

III. RESULTS AND DISCUSSION

Fig. 5 shows the dose curve of triple negative MDA-MB-231 cells treated with Veliparib. The different dosage used are 330μM, 500μM, and 830μM respectively. 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 Fig. 5, it can be observed that the effect of Veliparib on triple negative MDA-MB-231 cell line is high at 330μM than at 500μM and 830μM. The viability of the MDA-MB-231 cells at 330μM concentration of Veliparib is 82.1%, while the viability of the same cells at 500μM and 830μM are 91.8% and 94.3% respectively. This shows an increase in cell viability with an increase in the dosage. This could be due to the saturation of the drug. This is in correlation with the results obtained by Jung-Min Lee et al. [20], where they have reported that single agent treatment using Veliparib alone killed 11% of cells at 50μM. While in this study, even though the concentration level of Veliparib was increased to 6 times than that of the study by Jung-Min Lee, the percentage effectiveness was not proportionately high. This shows the saturated effectiveness and limited efficiency of using only the drug to treat the triple negative cancer cells.

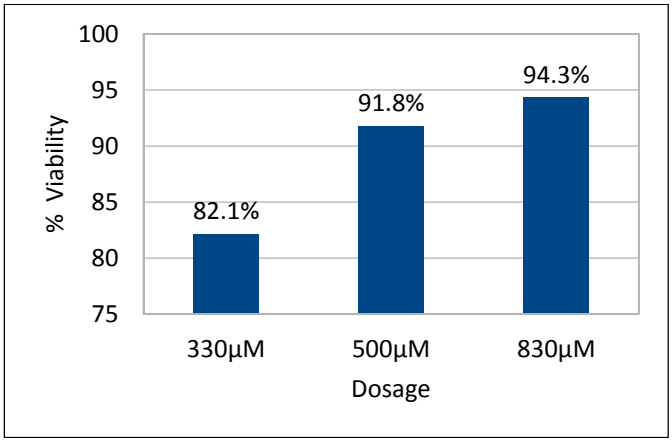


Fig. 5. Dose curve of Veliparib on MDA-MB-231 cells without Electroporation.

Fig. 6 shows the viabilities of the cell line without any treatment (control), drug only, and then the combination of drug and electric pulses at (1200V/cm, 100 µs, 8pulses) and (500V/cm, 20ms, 2pulses). The control has a viability of about 90%, while the drug only has a viability of 82.1%, indicating only a cell-kill of 18%. This shows the aggressiveness of the TNBC cell line.

To enhance the cell-kill, we used the synergy of the electrical pulses and the PARP inhibitor. In this case, with the high intensity, short duration electric pulses of 1200V /cm, 100µs, 8pulses, the cell-kill is 30%, with a viability of 70%. This is 67% higher than the drug only.

Using the low intensity, long duration pulses of the 500V/cm, 20ms, 2pulses, there is a cell-kill of 24%, which is 33% higher than the drug only.

These indicate the potent effect of electroporation in cell-kill using the PARP inhibitor. The synergy of using electric pulses and Veliparib increase the cell death from 18% to 30%. By optimizing the pulse parameters and the dosages, it is possible to obtain the desired cell-kill or viability.

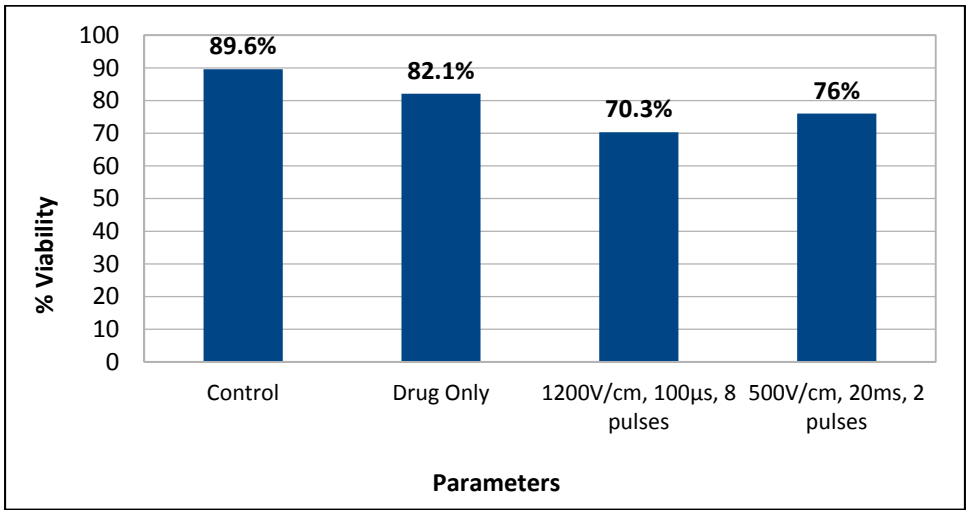
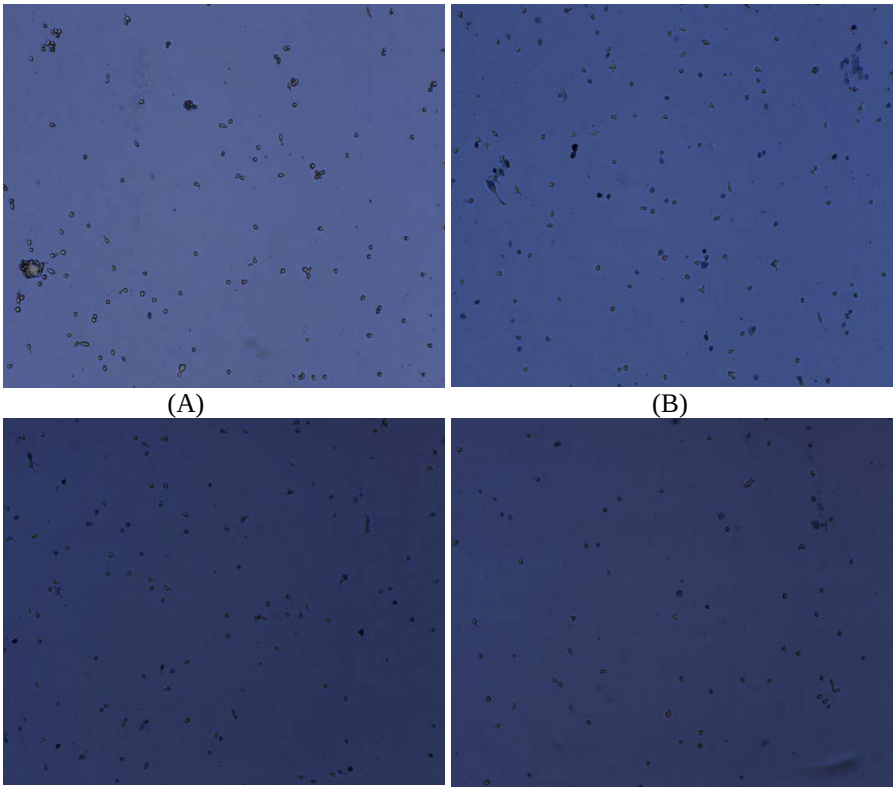


Fig. 6. Viabilities of MDA-MB-231 cells without any treatment, with Veliparib alone (330µM) and combination of Veliparib (330µM) and electroporation at 1200V/cm, 100µs, 8pulses and 500V/cm, 20ms, 2pulses.

Fig. 7 shows microscopic views of the cells at various conditions. Fig. 7A shows the control sample. It can be seen that there are more number of live cells compared to other three conditions. Fig. 7B shows the microscopic image of the drug only sample. This has less number of cells than the control, but more number of cells that the electroporated

samples. Both the electroporated samples show less number of cells than either the control or the drug only samples.



(A) (B)

(C) (D)

Fig. 7A. MDA-MB-231 sample without any treatment. Fig. 7B. MDA-MB-231 sample treated with Veliparib (330μM) drug alone. Fig. 7C. MDA-MB-231 sample treated with combination of Veliparib (330μM) and Electroporation at 1200V/cm, 100μs, 8 pulses. Fig. 7D. MDA-MB-231 sample treated with combination of Veliparib and Electroporation at 500V/cm, 20ms, 2pulses.

IV. Conclusions

Human body could be considered as a complex electrostatic component which has different tiny electrostatic sub components as cells and tissues that are spatially distributed within. Use of electrical pulses is an attractive option to treat cancer. Towards this:

- In this study, enzyme Veliparib is used in combination with electroporation, at a concentration of 330μM to study its effect on typically difficult to treat TNBC cell line, MDA-MB-231 cells.
- High intensity, short duration (1200V/cm, 100μs, 8 pulses) and low intensity, long duration (500V/cm, 20ms, 2 pulses) pulses were utilized to enhance the uptake.

- The combination of Veliparib and electroporation yields viabilities of 70% and 76%, compared to 82% with Veliparib only, indicating the effectiveness of electrical pulses in enhancing the cell-kill.
- This technique is transferable to clinical practice as an alternate treatment for triple negative cancers.

REFERENCES

[1] P.L. Welcsh et al., "BRCA1 and BRCA2 and the genetics of breast and ovarian cancer", *Human Molecular Genetics*, vol. 10(7), 705-13, 2001.

[2] http://www.cancer.gov/about-cancer/causes-prevention/genetics/brca-fact-sheet.

[3] K.J. Chavez et al., "Triple Negative Breast Cancer Cell Lines: One tool in the Search for Better treatment of Triple Negative Breast Cancer", *Breast Dis*, vol. 32(1), 35-48, 2010.

[4] K. Bhumsuk et al., "Prognostic impact of clinicopathologic parameters in stage II/III breast cancer treated with neoadjuvant docetaxel and doxorubicin chemotherapy: paradoxical features of the triple negative breast cancer", *BMC cancer*, vol. 7(1), 2007.

[5] Product catalog, Available: http://www.cellbiolabs.com/mda-mb-231-reporter-cells. May 2016.

[6] L.H. Deborah and S. Valerie, "Choosing the right cell line for breast cancer research", *Breast Cancer Research*, vol. 13(4), 2011.

[7] The PARP Inhibitors: down but not out. www.onclive.com. May 2016.

[8] S. S. Masahiko and L. Tomas, "Role of poly (ADP-ribose) formation in DNA repair", *Letters to Nature*, vol. 356, 1992.

[9] S. Seiya and I. Hiroaki, "DNA Repair and Chemotherapy", in *Advances in DNA Repair*, C.C. Chen, Ed. InTech, 2015, 359–80.

[10] N.P. Viktoria et al., "Multiple effects of electroporation on the adhesive behaviour of breast cancer cells and fibroblasts", *Cancer cell Int.*, vol. 12(1), 2012.

[11] L. G. Campana, et al., "Bleomycin-based electrochemotherapy: clinical outcome from a single institution's experience with 52 patients", *Ann Surg Oncol*, vol. 16, 191-9, 2009.

[12] J. Teissie and T. Y. Tsong, "Electric field induced transient pores in phospholipid bilayer vesicles", *Biochemistry*, vol. 20, pp. 1548-54, 1981.

[13] J. C. Weaver, "Electroporation of Cells and Tissues", *IEEE Transactions On Plasma Science*, vol 28, 24-33, 2000.

[14] http://www.medchemexpress.com/DataSheet/Veliparib-dihydrochloride.html 2016.

[15] L.M. Wagner, "Profile of veliparib and its potential in the treatment of solid tumors", *OncoTargets and therapy*, vol. 8, 1931-39, 2015.

[16] S. Pahuja et al., "A phase I study of veliparib (ABT-888) in combination with weekly carboplatin and paclitaxel in advanced solid malignancies and enriched for triple-negative breast cancer (TNBC)", *Journal of Clinical Oncology*, Vol. 33, 2015.

[17] S. Raji, et al., "Effective proliferation control of human cancer cells using electrical pulses", *IEEE Transactions on Dielectrics and Electrical Insulation*, vol. 19(6), 2225-36, 2012.

[18] J. Gehl and P. F. Geertsen, "Efficient palliation of hemorrhaging malignant melanoma skin metastases by electrochemotherapy", *Melanoma Research*, vol. 10(6), 585-89, 2000.

[19] J. Zhang et al., "Cisplatin and gemcitabine as the first line therapy in metastatic triple negative breast cancer", *International Journal of Cancer*, vol. 136(1), 204–11, 2015.

[20] J.M. Lee et al., "Navitoclax and Veliparib yield cytotoxicity with lower doses than used for single agents in women's cancers", *Molecular Cancer Therapeutics*, vol.12, 2013.