Clinical Plasma Medicine 19–20 (2020) 100109

Contents lists available at ScienceDirect

Clinical Plasma Medicine

journal homepage: www.elsevier.com/locate/cpme

Canady cold plasma conversion system treatment: An effective inhibitor of cell viability in breast cancer molecular subtypes

Lawan Ly[a], Xiaoqian Cheng[a], Saravana R K Murthy[a], Taisen Zhuang (Supervision)[b], Olivia Z Jones (Writing – review and editing)[a], Giacomo Basadonna (Supervision)[a,c], Michael Keidar (Supervision)[a,d], Jerome Canady (Conceptualization; Funding acquisition; Methodology; Supervision; Writing – review and editing)[a,d,*]

[a] Jerome Canady Research Institute for Advanced Biological and Technological Sciences, Takoma Park, MD, United States
[b] Plasma Medicine Life Sciences, Takoma Park, MD, United States
[c] University of Massachusetts School of Medicine, Worcester, MA, United States
[d] The George Washington University, Washington DC, United States

ARTICLE INFO

Keywords:
Breast cancer
Breast cancer molecular subtypes
Cold atmospheric plasma
Cold plasma device

ABSTRACT

Breast cancer is a heterogenous disease which can be classified into subtypes by the presence or absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 receptor (HER2). Cold atmospheric plasma (CAP) has been shown to be a potential treatment for cancer. In this report, a 92–99% reduction of viability by CAP treatment was achieved across all seven tested breast cancer cell lines ($p \leq 0.05$). Increasing treatment duration and power significantly reduced breast cancer cell viability (** $f_{(2,2)} \leq 0.0176$, *** $f_{(5,14)} \leq 0.0033$). The authors are the first to report that breast cancer sensitivity to CAP is based on receptor status. Cells with identical receptor status showed the least difference in CAP sensitivity ($p \leq 0.05$), the difference being 33% between the two ER+/PR+/HER2- cell lines ($p \leq 0.05$) and 22–44% between the three TNBC cell lines ($p \leq 0.05$). HER2-negative cell lines, irrespective of ER/PR status, also showed ≤ 50% difference in CAP sensitivity ($p \leq 0.05$). Moreover, demonstration of ER-/PR-/HER2+ CAP susceptibility and ER+/PR+/HER2+ CAP resistance suggests that ER/PR status is a significant factor in determining CAP sensitivity in HER2-positive cells. Our novel findings on CAP sensitivity will provide insight on how to optimize CAP treatment to better overcome CAP resistance and thus prevent tumor recurrence.

1. Introduction

Among women worldwide, breast cancer is the most frequently diagnosed cancer and the most common cause of cancer death [1]. The major breast cancer molecular subtypes are based on estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) expression [2]. With a total of eight combinations of ER, PR and HER2 expression [3], breast cancer is acknowledged as a highly complex disease. Molecular profiling, however, can provide information on disease prognosis and therapeutic approach [4,5].

Approximately 75% of breast cancers are ER-positive (ER+) [6–8] while 55–65% are PR-positive (PR+) [7,8]. Survival rates of patients are highest with ER+/PR+ tumors, intermediate with either ER+/PR- or ER-/PR+ tumors, and lowest with ER-/PR- tumors [9]. Several studies have reported changes in hormone receptor status between primary and metastatic breast cancer with discordance rates estimated to be 20% for ER and 40% for PR (both of which are higher than HER2 discordance rate) [10–13]. Patients with discordant receptor status have lower rates of survival than patients with consistent receptor status possibly due ineffective therapeutic interventions compared to patients with consistent receptor status [13]. Tamoxifen, a selective estrogen-receptor modulator, reduces risk of disease recurrence by 47% after 5 years and mortality by 26% after 10 years in ER+ patients [14] but increases the risk for thromboembolic events significantly [15]. Additionally, the absence of ER expression is associated with *de novo* resistance to tamoxifen [16]. In comparison, patients treated with letrozole, an aromatase inhibitor, had a lower chance of relapse over a 5 year period but reported increased incidences of adverse events [17].

HER2 amplification (HER2+) occurs in approximately 25–30% of primary human breast cancers and is the most significant prognostic

* Corresponding author.
E-mail address: drjcanady@canadysurgicalgroup.com (J. Canady).

https://doi.org/10.1016/j.cpme.2020.100109
Received 19 March 2020; Received in revised form 16 June 2020; Accepted 31 August 2020

factor compared to other factors such as ER, PR, tumor size, and age [18,19]. HER2, in addition to other human epidermal growth factor (hEGF) receptors, is involved in a complex network of pathways that are responsible for signaling normal cellular processes such as cell growth, migration, differentiation, and death [20]. An overexpression of HER2, therefore, promotes aggressive tumor behavior which is characterized by significantly decreased rates of disease-free and overall survival [18,19]. Trastuzumab (Herceptin), a humanized monoclonal antibody developed to target and inhibit the function of HER2 [21], is a generally well-tolerated monotherapy for metastatic HER2-positive breast cancers [22,23]. Randomized trials have reported adjuvant and neoadjuvant trastuzumab improved chance of overall survival in HER2+ breast cancer patients than those who received only chemotherapy [24–26. However, risk of cardiac dysfunction was significantly raised in combination with anthracycline and cyclophosphamide according to Slamon et al. [26]. Moreover, a 25–35% chance of central nervous system metastasis 6–12 months after the start of trastuzumab-based therapies due to the inability of trastuzumab to cross the blood-brain barrier have been reported [27–29]. FDA-approved dual anti-HER2 regimen, pertuzumab in combination with trastuzumab and docetaxel, significantly improved progression-free survival but with > 30% of patients exhibiting side effects such as diarrhea, neutropenia, nausea, fatigue, and peripheral neuropathy [30].

The overexpression of ER, PR, and HER2 is classified as triple positive breast cancer (TPBC) [31]. It is estimated that 10% of all breast cancer tumors are ER+/PR+/HER2+ [32,33]. Since HR and HER2 receptors are expressed, TPBCs can be treated with hormonal and HER2-targeted therapies. Overall and disease-free survival in ER+/PR+/HER2+ patients significantly improves in response to a combination of endocrine therapy, trastuzumab, and chemotherapy [34]. However, endocrine therapy resistance has been linked to crosstalk between ER and HER2 signaling pathways [35–37].

Triple negative breast cancer (TNBC), characterized by the lack of expression of ER, PR, and HER2, accounts for approximately 10 to 20% of all breast cancers [38–41. Compared to all other breast cancer phenotypes, TNBC has a significantly higher the rate of recurrence and risk of metastatic spread to the lungs, liver, and brain despite adjuvant chemotherapy [40,41]. While neoadjuvant chemotherapies have achieved higher rates of pathologic complete response in TNBC patients than in non-TNBC patients, TNBC patients with residual disease have higher recurrence and death rates in the first 3 years than non-TNBC patients with residual disease [42]. TNBC patients do not respond to endocrine therapies or HER2-targeted therapies, such as trastuzumab [43,44]. Recently there has been a growing interest in TRAIL (TNF (tumor necrosis factor)-related apoptosis-inducing ligand) which activates Death Receptors (DR.) 4 and 5 to induce apoptosis [45]. Potential TRAIL-targeting therapies have demonstrated the ability to induce apoptosis in TNBC cell lines with a mesenchymal phenotype [46] and suppresses tumor growth and metastasis in mice models [47,48]. Another target of interest for TNBC are cyclin dependent kinases (CDK) or cell cycle regulators [49]. Several FDA-approved CDK4/6 inhibitors (palbociclib, ribociclib and abemaciclib) have been shown to improve survivability, although accompanied by neutropenia, fatigue, nausea and diarrhea [49]. Up until recently, TNBC treatment options were limited to surgery, chemotherapy, and radiotherapy [44], however, the development of therapies non-dependent on receptor status is promising for TNBC patients.

Cold atmospheric plasma (CAP) technology utilizes ionized gas for various applications such as wound healing [50], HIV treatment [51], and cancer treatment [52]. Since the introduction of the first plasma-producing electrosurgical device by Morrison 40 years ago [53], there have been many advancements in plasma technology. The Canady Cold Plasma Conversion System (CCPCS), composed of the Canady Helios Cold Plasma Scalpel with the USMI Canady Cold Plasma Conversion Unit, is a novel electrosurgical system that produces CAP for the treatment of surgical margins upon tumor resection (U.S. Patent No. 9999,462) [54]. One of the advantages of CCPCS is that the CAP temperature remains between 26 and 30 °C during the duration of the treatment [55] and does not cause any thermal or physical damage to normal tissue [56]. Our previous studies have demonstrated the ability of the system to significantly reduce the viability of various malignant solid tumor cell lines (including pancreatic adenocarcinoma, renal adenocarcinoma, esophageal adenocarcinoma, colorectal carcinoma, and ovarian adenocarcinoma) by 80–99% 48 h post-CAP treatment [57]. For breast adenocarcinoma, TNBC in particular, an 80% reduction of cell viability was achieved 48 h after treatment with the CCPCS [55].

In this study we evaluated the efficacy of the CCPCS on various breast cancer cell lines based on ER, PR, and HER2 status. The human breast cancer cell lines that were studied include MCF-7, T-47D, SK-BR-3, BT-474, MDA-MB-231, Hs578T, and HCC1806. Receptor status of these cell lines are shown in Table 1.

Table 1
A list of the seven human breast cancer cell lines tested in this study along with receptor status.

Cell Line	ER (+/-)	PR (+/-)	HER2 (+/-)	References
MCF-7	+	+	–	[58,59,60,61]
T-47D	+	+	–	[60,61]
SK-BR-3	–	–	+	[58,59,60,61]
BT-474	+	+	+	[59,60,61]
MDA-MB-231	–	–	–	[58,59,60,60]
Hs578T	–	–	–	[59,60]
HCC1806	–	–	–	[59,61]

2. Methods

2.1. Cold plasma device

All experiments were performed at Jerome Canady Research Institute for Advanced Biological and Technological Sciences, Takoma Park, MD, USA, using the Canady Cold Plasma Conversion System. Our electrosurgical device, consists of the USMI SS-601 MCa high-frequency electrosurgical generator (USMI, Takoma Park, MD, USA) integrated with a USMI Canady Cold Plasma Conversion Unit and connected to a Canady Helios Cold Plasma ™ Scalpel. The conversion unit has three connectors: a gas connector (to a helium tank), and electrical connector (to the generator), and an electro-gas connector (to the scalpel). The conversion unit also features a high voltage transformer that up-converts voltage up to 4 kV, down-converts frequency to less than 300 kHz, and down-converts power less than 40 W. Additional details and schematics on plasma generation by CCPCS can be found in our previous study [55]. The helium flow rate was set to a constant 3 L/min and the power was set to 80, 100 and 120 P The plasma scalpel tip was placed 1.5 cm above the surface of the cell media and remained unmoved for the duration of the treatment. The CAP treatment was performed in a laminar flow tissue culture hood, Purifier Logic+ Class II, Type A2 Biosafety Cabinet (Labconco, Kansas City, MO, USA) at room temperature.

2.2. Cell culture

Human breast cancer cell lines T-47D, SK-BR-3, and BT-474, were purchased from ATCC (Manassas, VA, USA). MCF-7, MDA-MB-231, Hs578T, and HCC1806 were generously donated by Professor Kanaan's laboratory at Howard University. All cell lines except SK-BR-3 were cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA) and 1% Pen Strep (Thermo Fisher Scientific, Waltham, MA, USA) in a 37 °C and 5% CO_2 humidified incubator (Thermo Fisher Scientific, Waltham, MA, USA). The exceptions for culture conditions include T-47D, which was additionally supplemented with 0.5 mg/mL

insulin, and SK-BR-3, which was cultured in McCoy's 5A Medium. When cells reached approximately 80% confluence, cells were seeded at a concentration of 10^5 cells/well into 12-well plates (USA Scientific, Ocala, FL, USA) with a 1 mL media volume per well for cell viability assays.

2.3. Cell viability assay

Thiazolyl blue tetrazolium bromide (MTT) assay was performed on the cells 48 h after plasma treatment following the manufacturer's protocol with all MTT assay reagents purchased from Sigma-Aldrich (St. Louis, MO, USA). The absorbance of the dissolved compound was measured by BioTek Synergy HTX (Winooski, VT, USA) microplate reader at 570 nm.

2.4. Statistics

All viability assays were repeated 3 times with 2 replicates each. Data was plotted by Microsoft Excel 2016 as the mean ± standard error of the mean. A student *t*-test or a one-way analysis of variance (ANOVA) was used to check statistical significance where applicable. The differences were considered statistically significant for * $p \leq 0.05$. A one-way multivariate analysis of variance (MANOVA) followed by a Post-Hoc test was used to check statistical significance where applicable. The differences were considered statistically significant for ** $f \leq 0.0176$ and for *** $f \leq 0.0033$.

3. Results

3.1. Reduction of viability by cap in breast cancer cell lines

Various operational parameters of the CCPCS were tested to determine the optimal CAP dosage for each breast cancer cell line. A flow rate of 3 L/min was selected for all experimental conditions with power settings of 80, 100, and 120 P Treatment duration ranged from 1 to 6 min to reduce viability by at least 90%. MTT assays were performed to assess viability 48 h post-CAP treatment.

Fig. 1A-C demonstrates that CAP produced by the CCPCS had a clear time and dose dependent effect on the reduction of viability across all seven breast cancer cell lines that were tested. Viability data categorized by cell line subtypes can be found in Suppl. Figs. 1-4. Helium (0p), for the maximum treatment duration, minimally reduced cell viability (Suppl. Figure 4) with no significant effect on MDA-MB-231 and HCC1806 viability (Suppl. Figure 4). Increasing power and treatment duration from 40 to 80 P for 1–5 mins (from our previous study) to 80–120 P for 1–6 min (in our current study) yielded a greater reduction of viability in MDA-MB-231 (Suppl. Figure 5). Treatment duration and power variability significantly reduced breast cancer cell viability, as shown in Suppl. Figures 6–12 (** $f_{(2,2)} \leq 0.0176$, *** $f_{(5,14)} \leq 0.0033$). Ultimately, a 92–99% decrease in breast cancer viability was achievable across all breast cancer cell lines 48 h after 120 P at 5 or 6 min of CAP treatment ($p \leq 0.05$) (Fig. 1A-C).

The viabilities of both the ER+/PR+/HER2- cell lines, MCF-7 and T-47D were reduced to approximately 1% ($p \leq 0.0001$) and 5% ($p \leq 0.0001$), respectively, after given the highest CAP dose of 120 P for 6 min (Fig. 1A-C). For SK-BR-3, it was unnecessary to extend CAP treatment duration past 5 min since viability was already reduced by 99% after 5 min of CAP treatment at all tested powers ($p \leq 0.0001$) (Fig. 1A-C). Even with 80 P 1 min of CAP treatment, the most minimal dose, SK-BR-3 viability was nearly halved ($p \leq 0.015$) in contrast to all other cell lines (Fig. 1A). Unlike ER-/PR-/HER2+ cells, TPBC cells, required stronger doses and longer treatment to reduce viability (Fig. 1A-C). Treatment of 5 min at 100 P was the minimum CAP dosage to halve BT-474 viability ($p \leq 0.01$) (Fig. 1A-C). The only dosage to decrease BT-474 viability by > 80% was 120 P for 6 min, by which viability was reduced to 5% ($p \leq 0.0001$) (Fig. 1A-C). Viabilities were reduced by 92–98% ($p \leq 0.0001$) in all three TNBC cell lines with HCC1806 showing the greatest overall CAP resistance (Fig. 1A-C). Amongst all seven tested breast cancer cell lines varying in receptor status, our data demonstrated ER-/PR-/HER2+ cells to be the most CAP susceptible and TPBC cells to be the most CAP resistant (Fig. 1A-C).

To evaluate whether receptor status was also significant factor in the reduction of viability by CAP treatment, the statistical significance of viability data was considered between cell lines across all treatment condition, displayed in Fig. 2A. We found there was a 33% difference between the two ER+/PR+/HER2- cell lines ($p \leq 0.05$) and a 22–44% difference between the three TNBC cell lines ($p \leq 0.05$) (Fig. 2B), suggesting breast cancer cells with identical receptor status have similar susceptibility to CAP. This is also further supported by the 17–50% difference between HER2-negative cell lines ($p \leq 0.05$). Compared to all other cell lines, the ER-/PR-/HER+ cell line was the most significantly different by 73–93% ($p \leq 0.05$) (Fig. 2B), indicating ER-/PR-/HER2+ as a significant factor contributing to CAP susceptibility.

4. Discussion

The purpose of this study was to determine the sensitivity of breast cancer cell lines to CAP treatment based on the receptor status. Identical receptor status showed the least difference in CAP sensitivity within the two ER+/PR+/HER2- cell lines (33%) and the three TNBC cell lines (22–44%) ($p \leq 0.05$) (Fig. 2B). HER2-negative status, irrespective of ER/PR status, also showed ≤ 50% difference in susceptibility when cell lines MCF-7 (ER+/PR+/HER2-), T-47D (ER+/PR+/HER2-), MDA-MB-231 (ER-/PR-/HER2-), Hs578T (ER-/PR-/HER2-), and HCC1806 (ER-/PR-/HER2-) are compared ($p \leq 0.05$) (Fig. 2B). Moreover, in the presence of HER2+ status, ER/PR status is significant in determining CAP sensitivity when Sk-Br-3 (ER-/PR-/HER2+) and BT-474 (ER+/PR+/HER2+) are compared (Fig. 2). While 1 min of CAP treatment, regardless of power, nearly halved CAP-sensitive Sk-Br-3 viability ($p \leq 0.015$), it required 5 min 100 P of CAP to produce the same result in CAP-resistant BT-474 cells ($p \leq 0.01$) (Fig. 1). Although significant resistance of TPBC to CAP treatment was demonstrated, we were able to significantly reduce the viability of TPBC cell viability by 95% ($p \leq 0.0001$).

Our data suggests that ER/PR status is most important determining factor in CAP susceptibility for HER2+ breast cancer cells. Testing additional TPBC and ER-/PR-/HER2+ cell lines in future studies could further supplement our findings on CAP sensitivity. Nonetheless, our current data suggests molecular profile should be considered when determining the optimal CAP dosage for the treatment of breast cancer, especially in HER2+ breast cancer. Potentially, adjuvant and neoadjuvant trastuzumab or hormonal therapy alongside CAP treatment could improve the chance of overall survival in HER2+ breast cancer patients. The molecular pathway that determines the susceptibility of HER2+ cells to CAP treatment warrants further investigation. An insight on CAP mechanism will give us a better understanding on how to optimize CAP treatment to better overcome CAP resistance.

We hypothesize that differential regulation of apoptotic genes is the major contributor to CAP selectivity. The importance of apoptotic malfunction in the TNBC prognosis is well documented in several studies [62]. Poor prognosis in TNBC is attributed to pro-survival factors, such as B-cell lymphoma 2 (Bcl-2) [63] and myeloid cell leukemia 1 (Mcl-1). Additionally, TRAIL receptors also contribute to apoptosis dysregulation, however targeted therapies against TRAIL and several DRs have failed to improve patient outcomes [64]. By identifying and targeting molecular markers responsible for CAP resistance, we expect to see a greater reduction of viability by smaller CAP dosages.

The authors acknowledge that there is the question to whether different cell media influences CAP sensitivity. In this study, SK-BR-3 was the only cell line to be cultured in McCoy 5A media as opposed to RPMI 1640 (all of which was in accordance to ATCC recommendations). A study done jointly with The George Washington University,

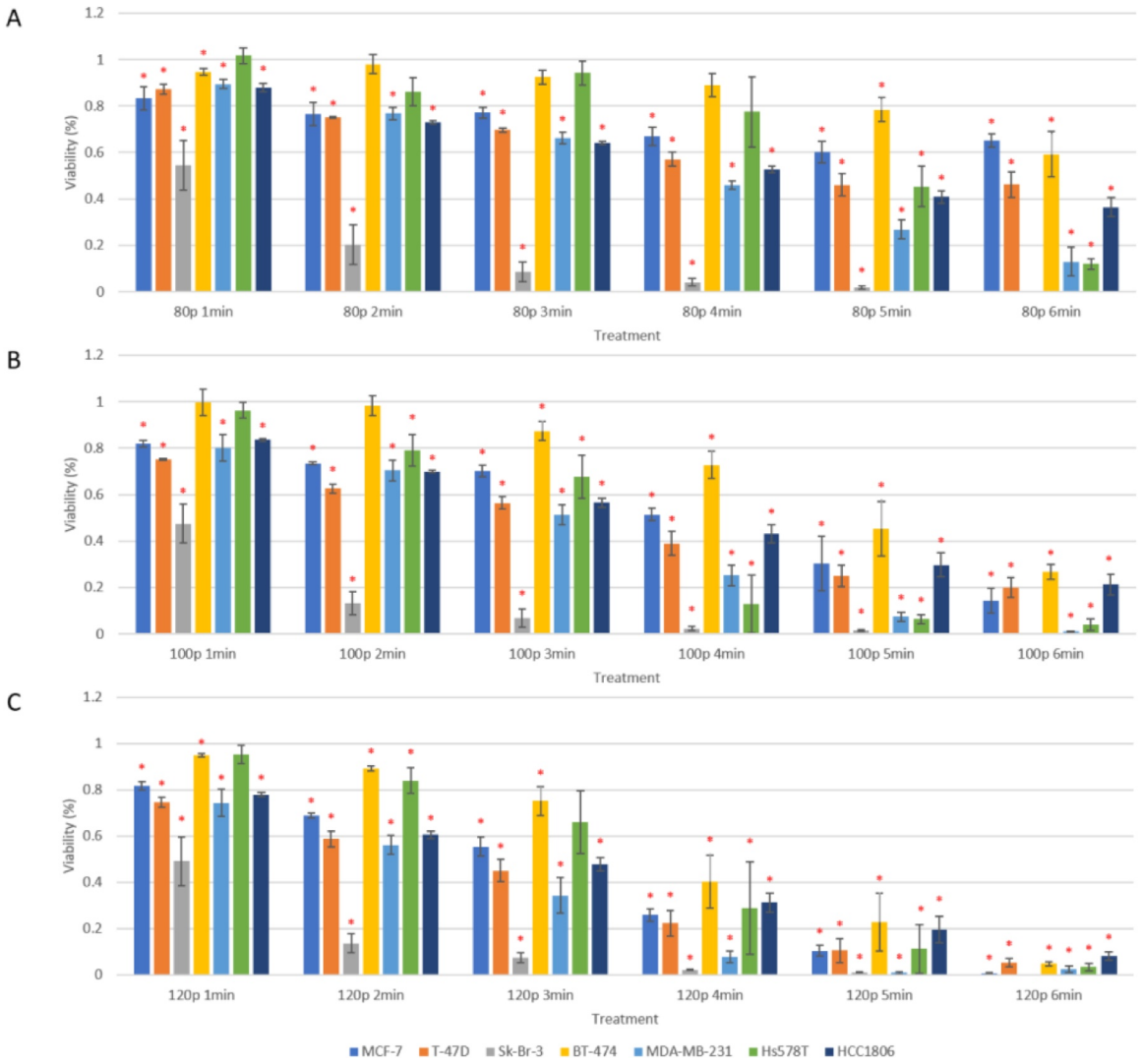


Fig. 1. Bar graph showing the viability of CAP treated cells in relative percentage to no treatment controls among all 7 tested breast cancer cell lines at 80 P (A), 100 P (B), and 120 P (C) after 48 h. CAP treatment significantly reduces viability of all tested breast cancer cell lines. * $p \leq 0.05$.

investigated the interaction between CAP-generated effective species and amino acids present in the media. It was concluded that cysteine and tryptophan consumed the most CAP-generated effective species, thus weakening the anti-tumor ability of CAP on cells [65]. However, when glioblastoma and breast cancer cells were cultured in the same type of media, glioblastoma cells consumed CAP-generated effective species at a faster rate than breast cancer cells, suggesting distinct expression of extracellular proteins dependent on cell line [65]. Gene profiling in our subsequent study will reveal that oxidative stress-related genes cause preferential uptake of reactive oxygen and nitrogen species by CAP susceptible cells.

Thus far, we have demonstrated the promising cancer therapeutic potential of the CCPCS. A phase I FDA Investigation Device Exemption Approval clinical trial is currently undergoing in the United States and Israel. As an electrosurgical device, CCPCS is used to apply CAP to the surgical margins after tumor resection. We expect that the CCPCS will prevent tumor recurrence thereby improve patient outcome. In addition, CAP treatment alongside chemotherapy can further improve breast cancer management. Furthermore, our findings on CAP sensitivity will be the foundation in the development of customized CAP-based therapy regiments for various breast cancer subtypes.

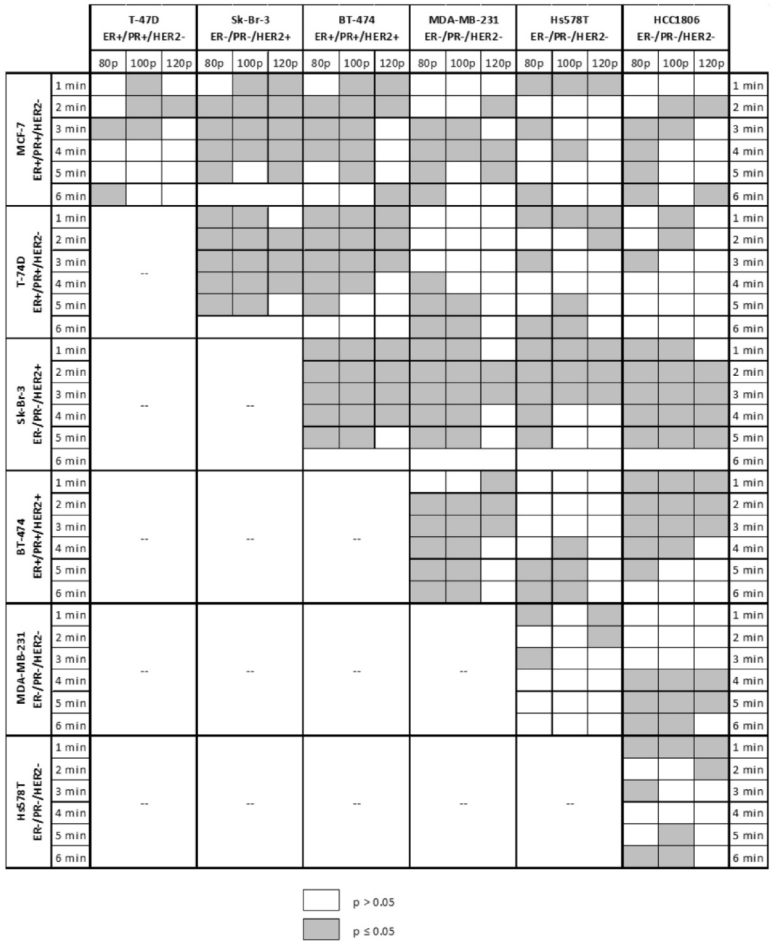
5. Conclusion

There is a present demand for a therapy that effectively treats all breast cancer subtypes regardless of receptor status since effectiveness of current endocrine and HER2-targeted therapies are dependent on receptor status [43,44]. To this end, we offer CCPCS as a solution after demonstrating its ability to reduce breast cancer viability by 92–99% ($p \leq 0.05$) regardless of receptor status. Our study revealed that molecular profiling is necessary when selecting appropriate CAP dosages especially in HER2-positive breast cancers in which ER/PR status is a significant factor in determining CAP sensitivity. Future studies on cell cycle change and apoptosis initiation, are necessary to confirm CAP-induced cell death by CCPCS. Identification of molecular signaling pathways are also warranted. Understanding CAP mechanism behind CAP sensitivity is key to increasing CAP effectiveness thereby better preventing tumor recurrence.

CRediT authorship contribution statement

Lawan Ly: Conceptualization, Data curation, Investigation, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing. **Xiaoqian Cheng:** Conceptualization, Data curation, Formal analysis,

A



B

	T-47D ER+/PR+/HER2-	Sk-Br-3 ER-/PR-/HER2+	BT-474 ER+/PR+/HER2+	MDA-MB-231 ER-/PR-/HER2-	Hs578T ER-/PR-/HER2-	HCC1806 ER-/PR-/HER2-
MCF-7 ER+/PR+/HER2-	33%	87%	56%	50%	33%	44%
T-47D ER+/PR+/HER2-	--	87%	67%	28%	50%	17%
Sk-Br-3 ER-/PR-/HER2+	--	--	93%	80%	73%	93%
BT-474 ER+/PR+/HER2+	--	--	--	72%	28%	67%
MDA-MB-231 ER-/PR-/HER2-	--	--	--	--	22%	44%
Hs578T ER-/PR-/HER2-	--	--	--	--	--	44%

Fig. 2. The comparison of the reduction of viability between cell lines. (A) Chart showing whether there is statistical difference between two cell lines under specific CAP treatments ($p \leq 0.05$). (B) Chart showing percentage of CAP conditions in which there is a statistical difference between two cell lines ($p \leq 0.05$).

Investigation, Methodology, Project administration, Validation, Writing - review & editing. **Saravana R K Murthy:** Funding acquisition, Writing - review & editing. **Taisen Zhuang:** . **Olivia Z Jones:** . **Giacomo Basadonna:** . **Michael Keidar:** . **Jerome Canady:** .

Acknowledgements

The authors would like to thank Professor Yasmine Kanaan's laboratory at Howard University for their generous donation of breast cancer cell lines (MDA-MB-231, Hs578T, and HCC1806) and the engineering team at Plasma Medicine Life Sciences for technical support of the plasma unit. This work was supported by the Jerome Canady Research Institute for Advanced Biological and Technological Sciences.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cpme.2020.100109.

References

[1] F. Bray, et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin 68 (6) (2018) 394–424.
[2] N. Howlader, et al., Differences in Breast Cancer Survival by Molecular Subtypes in the United States. Cancer Epidemiology, Biomarkers Prevention 27 (6) (2018) 619–626.
[3] K. Bauer, C. Parise, V. Caggiano, Use of ER/PR/HER2 subtypes in conjunction with the 2007 St Gallen Consensus Statement for early breast cancer, BMC Cancer 10

(2010).
[4] M. Kittaneh, A.J. Montero, S. Gluck, Molecular profiling for breast cancer: a comprehensive review, Biomark Cancer 5 (2013) 61–70.
[5] M.J. Duffy, et al., Clinical use of biomarkers in breast cancer: updated guidelines from the European Group on Tumor Markers (EGTM), Eur J Cancer 75 (2017) 284–298.
[6] W.F. Anderson, et al., Estrogen receptor breast cancer phenotypes in the Surveillance, Epidemiology, and End Results database, Breast Cancer Res. Treat. 76 (1) (2002) 27–36.
[7] G.A. Colditz, et al., Risk factors for breast cancer according to estrogen and progesterone receptor status, J Natl Cancer Inst 96 (3) (2004) 218–228.
[8] M. Nadji, et al., Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers, Am J Clin Pathol 123 (1) (2005) 21–27.
[9] A. Alanko, et al., Significance of Estrogen and Progesterone Receptors, Disease-Free Interval, and Site of First Metastasis on Survival of Breast Cancer Patients, Cancer 56 (7) (1985) 1696–1700.
[10] E. Curtit, et al., Discordances in estrogen receptor status, progesterone receptor status, and HER2 status between primary breast cancer and metastasis, Oncologist 18 (6) (2013) 667–674.
[11] B. RJ, et al., Changes in Estrogen Receptor, Progesterone Receptor and Her-2/neu Status with Time: discordance Rates Between Primary and Metastatic Breast Cancer, Anticancer Res. 29 (5) (2009) 1557–1562.
[12] D. Sighoko, et al., Discordance in hormone receptor status among primary, metastatic, and second primary breast cancers: biological difference or misclassification? Oncologist 19 (6) (2014) 592–601.
[13] C. Liedtke, et al., Prognostic impact of discordance between triple-receptor measurements in primary and recurrent breast cancer, Ann Oncol 20 (12) (2009) 1953–1958.
[14] B.L. Riggs, L.C. Hartmann, Selective estrogen-receptor modulators – mechanisms of action and application to clinical practice, N Engl J Med 348 (7) (2003) 618–629.
[15] K.I. Pritchard, et al., Increased Thromboembolic Complications With Concurrent Tamoxifen and Chemotherapy in a Randomized Trial of Adjuvant Therapy for Women With Breast Cancer, J. Clinical Oncology 14 (10) (1996) 2731–2737.
[16] S.R.D. Johnston, et al., Changes in Estrogen Receptor, Progesterone Receptor, and pS2 Expression in Tamoxifen-resistant Human Breast Cancer, Cancer Res. 55 (15) (1995) 3331–3338.
[17] A.S. Coates, et al., Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98, J Clin Oncol 25 (5) (2007) 486–492.
[18] D.J. Slamon, et al., Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene, Science 235 (4785) (1987) 177–182.
[19] D.J. Slamon, et al., Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer, Science 244 (4905) (1989) 707–712.
[20] Y. Yarden, M.X. Sliwkowski, Untangling the ErbB signalling network, Nature Reviews Molecular Cell Biology 2 (2) (2001) 127–137 p. pages.
[21] M.A. Molina, et al., Trastuzumab (Herceptin), a Humanized Anti-HER2 Receptor Monoclonal Antibody, Inhibits Basal and Activated HER2 Ectodomain Cleavage in Breast Cancer Cells, Cancer Res. 61 (12) (2001) 4744–4749.
[22] C.L. Vogel, et al., Efficacy and Safety of Trastuzumab as a Single Agent in First-Line Treatment of HER2-Overexpressing Metastatic Breast Cancer, J. Clinical Oncology 20 (3) (2002) 719–726.
[23] J. Baselga, et al., Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule, J Clin Oncol 23 (10) (2005) 2162–2171.
[24] I. Smith, et al., 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial, The Lancet 369 (9555) (2007) 29–36.
[25] L. Gianni, et al., Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort, The Lancet Oncology 15 (6) (2014) 640–647.
[26] D.J. Slamon, et al., Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2, New England Journal of Medicine 344 (11) (2001) 783–792.
[27] A. Clayton, et al., Incidence of cerebral metastases in patients treated with trastuzumab for metastatic breast cancer, Br. J. Cancer 91 (2004) 639–643.
[28] J.C. Bendell, et al., Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma, Cancer 97 (12) (2003) 2972–2977.
[29] S. Gori, et al., Central nervous system metastases in HER-2 positive metastatic breast cancer patients treated with trastuzumab: incidence, survival, and risk factors, Oncologist 12 (7) (2007) 766–773.
[30] G.M. Blumenthal, et al., First FDA approval of dual anti-HER2 regimen: pertuzumab in combination with trastuzumab and docetaxel for HER2-positive metastatic breast cancer, Clin Cancer Res 19 (18) (2013) 4911–4916.
[31] P. Vici, et al., Triple positive breast cancer: a distinct subtype? Cancer Treat Rev 41 (2) (2015) 69–76.
[32] N. Howlader, et al., US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status, J Natl Cancer Inst 106 (5) (2014).
[33] A.A. Onitilo, et al., Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival, Clin Med Res 7 (1–2) (2009) 4–13.
[34] N. Hayashi, et al., Adding hormonal therapy to chemotherapy and trastuzumab improves prognosis in patients with hormone receptor-positive and human epidermal growth factor receptor 2-positive primary breast cancer, Breast Cancer Res Treat 137 (2) (2013) 523–531.
[35] A. AlFakeeh, C. Brezden-Masley, Overcoming endocrine resistance in hormone receptor-positive breast cancer, Curr Oncol 25 (Suppl 1) (2018) S18–S27.
[36] C.K. Osborne, R. Schiff, Mechanisms of endocrine resistance in breast cancer, Annu Rev Med 62 (2011) 233–247.
[37] M. Giuliano, M.V. Trivedi, R. Schiff, Bidirectional Crosstalk between the Estrogen Receptor and Human Epidermal Growth Factor Receptor 2 Signaling Pathways in Breast Cancer: molecular Basis and Clinical Implications, Breast Care (Basel) 8 (4) (2013) 256–262.
[38] K.F. Trivers, et al., The epidemiology of triple-negative breast cancer, including race, Cancer Causes Control 20 (7) (2009) 1071–1082.
[39] E.A. Rakha, et al., Prognostic markers in triple-negative breast cancer, Cancer 109 (1) (2007) 25–32.
[40] J. Qiu, et al., Comparison of Clinicopathological Features and Prognosis in Triple-Negative and Non-Triple Negative Breast Cancer, J Cancer 7 (2) (2016) 167–173.
[41] R. Dent, et al., Triple-negative breast cancer: clinical features and patterns of recurrence, Clin Cancer Res 13 (15 Pt 1) (2007) 4429–4434.
[42] C. Liedtke, et al., Response to Neoadjuvant Therapy and Long-Term Survival in Patients With Triple-Negative Breast Cancer, J. Clinical Oncology 26 (8) (2008) 1275–1281.
[43] C. Liedtke, et al., Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer, J. Clinical Oncology 26 (8) (2008) 1275–1281.
[44] H.A. Wahba, H.A. El-Hadaad, Current approaches in treatment of triple-negative breast cancer, Cancer Biol Med 12 (2) (2015) 106–116.
[45] C. Oakman, G. Viale, A. Di Leo, Management of triple negative breast cancer, Breast 19 (5) (2010) 312–321.
[46] M. Rahman, et al., TRAIL induces apoptosis in triple-negative breast cancer cells with a mesenchymal phenotype, Breast Cancer Res Treat 113 (2) (2009) 217–230.
[47] N. Jyotsana, et al., Minimal dosing of leukocyte targeting TRAIL decreases triple-negative breast cancer metastasis following tumor resection, Sci Adv 5 (7) (2019).
[48] Y.E. Greer, et al., MEDI3039, a novel highly potent tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor 2 agonist, causes regression of orthotopic tumors and inhibits outgrowth of metastatic triple-negative breast cancer, Breast Cancer Res 21 (1) (2019) 27.
[49] F. Lynce, A.N. Shajahan-Haq, S.M. Swain, CDK4/6 inhibitors in breast cancer therapy: current practice and future opportunities, Pharmacol Ther 191 (2018) 65–73.
[50] Z. Shahbazi Rad, F. Abbasi, Davani, Non-thermal atmospheric pressure dielectric barrier discharge plasma source construction and investigation on the effect of grid on wound healing application, Clin Plasma Med 4 (2) (2016) 56–64.
[51] O. Volotskova, et al., Cold Atmospheric Plasma Inhibits HIV-1 Replication in Macrophages by Targeting Both the Virus and the Cells, PLoS ONE 11 (10) (2016) p. e0165322.
[52] M. Keidar, et al., Cold atmospheric plasma in cancer therapy, Phys Plasmas 20 (5) (2013).
[53] Morrison C.F., J., Electrosurgical Method and Apparatus for initiating an electrical discharge in an inert gas flow.
[54] J. Canady, et al., Integrated cold plasma and high frequency plasma electrosurgical system and method, U.S. Patent, Editor, U.S. Patent Innovations, LLC: United States (2018).
[55] X. Cheng, et al., Treatment of Triple-Negative Breast Cancer Cells with the Canady Cold Plasma Conversion System: preliminary Results, Plasma 1 (1) (2018) 218–228.
[56] L. Ly, et al., A New Cold Plasma Jet: performance Evaluation of Cold Plasma, Hybrid Plasma and Argon Plasma Coagulation. Plasma 1 (1) (2018) 189–200.
[57] W. Rowe, et al., The Canady Helios Cold Plasma Scalpel Significantly Decreases Viability in Malignant Solid Tumor Cells in a Dose-Dependent Manner, Plasma 1 (1) (2018) 177–188.
[58] K. Subik, et al., The Expression Patterns of ER, PR, HER2, CK5/6, EGFR, Ki-67 and AR by Immunohistochemical Analysis in Breast Cancer Cell Lines, Breast Cancer: Basic and Clinical Research 4 (2010) 35–41.
[59] M. Riaz, et al., miRNA expression profiling of 51 human breast cancer cell lines reveals subtype and driver mutation-specific miRNAs, Breast Cancer Research 15 (2) (2013).
[60] R.M. Neve, et al., A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes, Cancer Cell 10 (6) (2006) 515–527.
[61] J. Kao, et al., Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery, PLoS ONE 4 (7) (2009) e6146.
[62] M. Nedeljkovic, A. Damjanovic, Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer-How We Can Rise to the Challenge, Cells 8 (9) (2019).
[63] T. Inao, et al., Bcl-2 inhibition sensitizes triple-negative human breast cancer cells to doxorubicin, Oncotarget 9 (39) (2018) 25545–25556.
[64] J. Lemke, et al., Getting TRAIL back on track for cancer therapy, Cell Death Differ 21 (9) (2014) 1350–1364.
[65] D. Yan, et al., Principles of using Cold Atmospheric Plasma Stimulated Media for Cancer Treatment, Sci Rep 5 (2015) 18339.