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Utilization of Intravenous (IV) Curcumin, Genistein, and Trastuzumab to Reduce HER2 receptors in Breast Cancer Patients

Shreya Bhandari[1] and Hasmitha Kamineni[2]
[1]11th Grade Independence High School Frisco, Texas 75035, UNITED STATES.
[2]11th Grade Independence High School Frisco, Texas 75035, UNITED STATES.

[1]Corresponding Author: shreya.bhandari0220@gmail.com

ABSTRACT

In recent decades, due to the increased growth, spread, and onset of cancer, interest has arisen in studying the methods that could be used to combat it. Breast cancer is the most prevalent form of cancer in the world. Due to the growing pervasiveness of this issue, a larger body of research is forming in the area of breast cancer with the intent to contain the spread of the potentially ravaging disease or diagnose it earlier. This research is intended to propose an alternative, possibly more efficient method to inhibit the chances of metastasis and continued prevalence of breast cancer in a patient. The related work discusses similar research being done and builds on it to incorporate the novel method being proposed while explaining the components of the proposed treatment in question. The proposed method aims to deplete HER2 protein receptors in breast cancer patients through the intravenous (IV) administration of the tyrosine kinase inhibitors (TKIs) curcumin and genistein, as well as trastuzumab, which is more commonly known under the brand name Herceptin. Depleting HER2 protein receptors can potentially cause the severity of HER2-positive breast cancer to decrease substantially, as well as reduce the probability of metastasis and recurrence, the rate of which is considerably higher in HER2-positive cases when compared to that of HER2-negative cases. The future work deals with alternate methods that could be explored with a similar intent as this research study and describes the potential implications of the study.

Keywords- Breast cancer, HER2 receptors, tyrosine kinase inhibitors (TKIs), curcumin, genistein, trastuzumab (Herceptin), HER2-positive, HER2-negative

I. INTRODUCTION

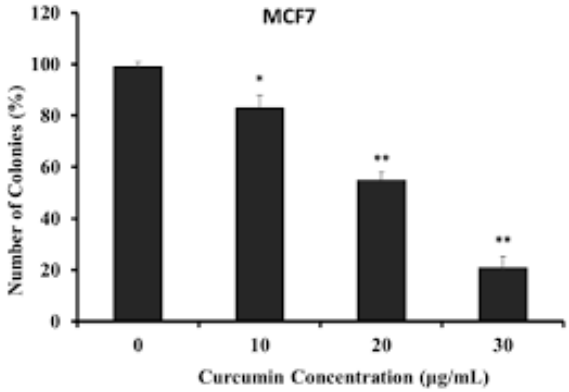
Breast cancer can present itself in the form of either of two categories: HER2-positive or HER2-negative. Every year, 20-25% of breast cancer cases are HER2-positive. The HER2 receptors in breast cells, encoded for by the HER2 gene in chromosome 17, ensure that the breast cell is maturing appropriately throughout its life cycle. However, overamplification of the gene can lead to overproduction of copies of the gene, thereby resulting in an excess of the HER2 protein receptors, which are RTKs (receptor tyrosine kinases). The two categories mentioned have their subsets: HR+/HER2-, HR-/HER2-, HR-/HER2+, OR HR+/HER2+. HR+ connotes the presence of receptors for estrogen and progesterone, which can further promote growth of HER2-positive breast cancer. HER2+ indicates that the tumor is producing high amounts of the protein HER2/neu, which has been associated with certain types of aggressive breast cancer. A promising treatment is the usage of TKIs, or tyrosine kinase inhibitors, to compete with ATP in order to bind reversibly or irreversibly at the cytoplasmic catalytic kinase domain and block autophosphorylation and activation of HER2, which can inhibit downstream proliferation and survival signals [1]. Synthetic TKIs can be utilized, but the plethora of side effects are not ideal. Curcuminoids, which make up 2.5% to 6% of the rhizome of turmeric, consist of certain compounds produced by specific genes that allow curcumin, a curcuminoid, to serve as a TKI. If the turmeric were to be incorporated into the human body intravenously, then it could serve as a possible treatment since the curcumin molecules produced can serve as TKIs in the affected cells. Genistein, a phytoestrogen, is found in various plants, such as soybeans and is an isoflavone in the flavonoids compound group. Research conducted recently has indicated that the compound, which is also a TKI, can inhibit HER2 protein expression in the BT-474 breast cell line and has been proven to be generally effective [2]. Genistein has been found to considerably impede the proliferation, invasion, and metastasis of breast cancer cells while also being able to promote apoptosis. Regardless of the combined efficacy of curcumin and genistein, trastuzumab, when used in conjunction with the two aforementioned elements, can have a more significant impact on the breast cancer cells, as demonstrated by recent and current research. Trastuzumab, which is an antineoplastic agent, prevents the HER2-positive tumorous cells from receiving growth signals and thereby slowing or stopping their growth through the process of attaching to them. Trastuzumab's function involves attaching to the HER2/neu protein and thereby preventing the HER2-overexpressing or tumor cells from continuing to grow and divide. The three components' possible effect on HER2 protein expression will be explored extensively in the upcoming sections.

II. OBJECTIVES

- To develop a relatively new method used to reduce HER2 receptors in breast cancer patients.
- To analyze the relationship between curcumin, genistein, and trastuzumab.
- To explore the relationship between HER2 and the spread of breast cancer.

III. RELATED WORKS

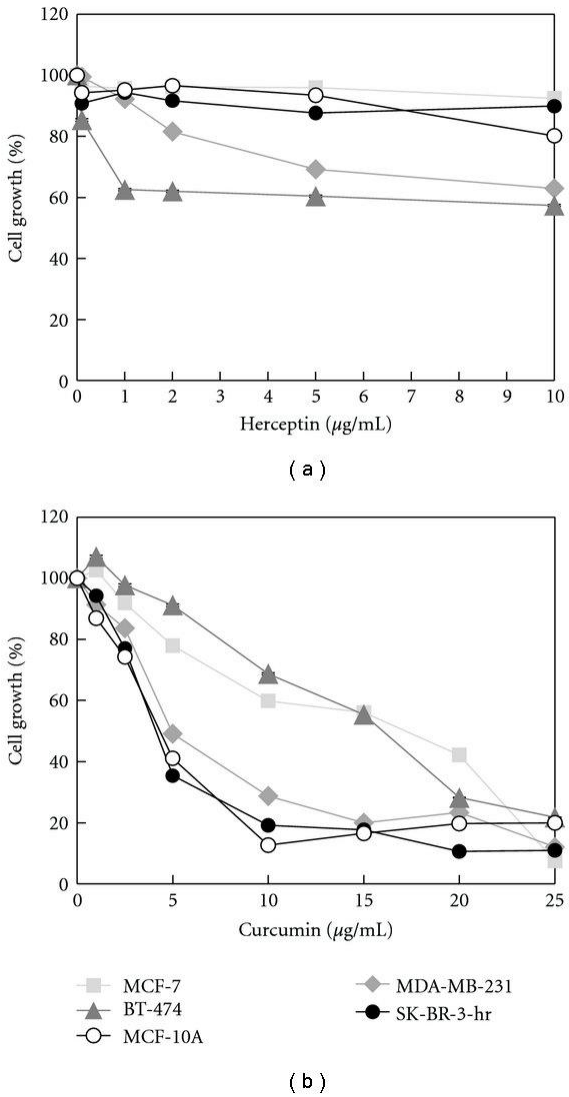
HER2 is a proto-oncogene on chromosome 17 that controls cell-based growth and repair. It functions as a receptor tyrosine kinase that is overexpressed in 15-30% of breast cancer cases, leading to increased cancerous growth; these types of cancers are labelled HER2-positive [3]. The overamplification of the gene can result in 25-50 copies of the HER2 gene and a 40-100 fold increase in the HER2 protein, resulting in 2 million receptors at the tumor's surface [4]. Recent research has demonstrated that HER2 overamplification is an early precedent in human breast tumorigenesis. Treatment, as mentioned, can consist of various types of competitive tyrosine kinase inhibitors (TKIs), including reversible and irreversible ones. Some have been introduced into the chemotherapy process, such as neratinib, which is quite coveted in rigorous chemotherapy treatments since it binds irreversibly to the intracellular tyrosine kinase domain, while lapatinib and tucatinib bind reversibly. Trastuzumab is a monoclonal antibody treatment, and its major mechanism is to have demonstrated that HER2 overamplification is an early precedent in human breast tumorigenesis. The drug can be utilized after surgery or radiotherapy and perhaps chemotherapy during the early stages of HER2-positive breast cancer in order to reduce chances of resurgence and metastasis. Additionally, the drug can be used in the advanced stage to increase chances of survival and slow the metastasis. Administration can occur through a subcutaneous injection to the patient's thigh or by infusion through a drip. The possibility of the usage of trastuzumab in conjunction with various TKIs has been explored increasingly in the past few years, but all the TKI substitutes have been chemically derived. In a study exploring the conjunction aforementioned, a group of researchers utilized various TKIs with trastuzumab and compared the efficacy of each combination. Lapatinib, neratinib, and afatinib were tested with trastuzumab and sometimes with pertuzumab. In two cell lines that were trastuzumab-resistant, the TKIs, most notably afatinib, which was previously shown to formulate a synergistic inhibition of growth in eight cell lines, were able to inhibit cancerous growth. The following table illustrates the percentage of normalized growth in terms of natural TKIs, curcumin is the primary option. Curcumin is a hydrophobic polyphenol (superoxide superoxide dismutase 1) from turmeric and has been shown to prevent cancerous growths and reduce metastatic potential in various types of cancers. According to a study, curcumin was found to decrease the cell growth of certain breast cancer lines, including MCF-7, MDA-MB-231, MCF-10A, BT-474, and SK-BR-3-hr [5]. In the NFkB, HER-2AP-1, RANKL, MCF-7, and COX-2 cell lines, curcumin has reduced tumor growth and angiogenesis while also inhibiting metastasis [6]. The inception of the c-Jun N-terminal Kinase (JNK) and ROS pathway is meant to intercept the formation of ROS (reactive oxygen species) by triggering apoptosis with the same amount of efficiency as the commonly utilized chemotherapeutic drugs. The following table illustrates the effect of various curcumin concentrations on the number of colonies (%).



[Fig 1: Effect of various curcumin concentrations on colony formations. Image from Hossein Abbaspour, *Research Gate*]

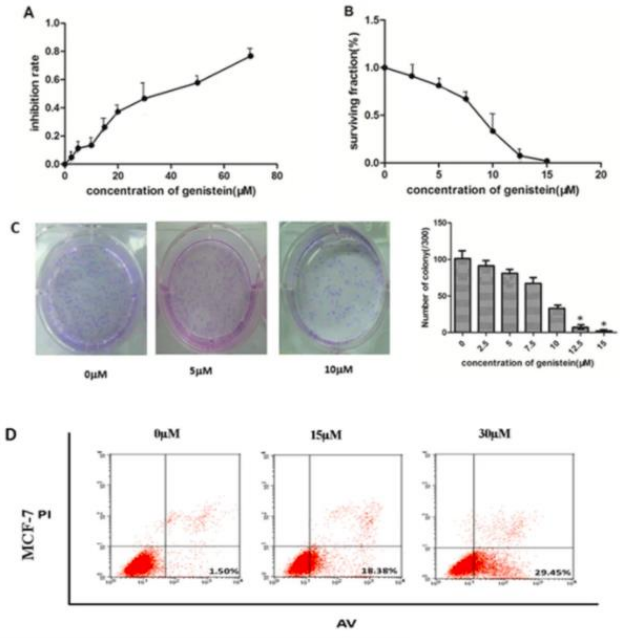
As illustrated by figure 1, curcumin, in terms of MCF-7 cells, can reduce the size of the clustered tumor colonies, which are supplied by angiogenesis (the blood vessel network established by cancerous growths) by targeting them and occasionally triggering paraptosis, which significantly decreases breast cancer tumors [7]. Curcumin can also pause the replication of the tumorous cells and can instead aid in inducing a resting state. Additionally, anticancer doses of curcumin have been shown to arrest non-malignant cells in the G0 phase in a reversible manner but do not effectuate apoptosis in them [8]. On the other hand, curcumin can protect the patient's T cells from being affected by metastasis and apoptosis induced by chemotherapy. T cells are essential for immune system health, as the patients can be especially prone to common diseases that can prove to be fatal for them due to chemotherapy. Though the optimal route for curcumin has been a debated topic between researchers, the oral route creates less systematic availability, and glucuronidation and sulfation in terms of intestinal metabolism have been shown to play a role. In terms of colon, breast, bladder, and other such cancers, curcumin can induce p53-dependent apoptosis. Curcumin also participates in the promotion of caspase-3-mediated cleavage of β-catenin

and reduces β-catenin/Tcf-Lef transactivation capacity for c-Myc and cyclin D1 [9]. Recent research has found curcumin to be capable of down regulating EGFR, as well as influence the PI3K/AKt and MAPK pathways, which trastuzumab interfered with. Taxol (brand name being Paclitaxel) used in combination with trastuzumab is the primary treatment currently utilized for HER2-positive breast cancer whether in the early or late stages (including metastization). The table below illustrates the effect of curcumin and trastuzumab on cell growth (%).



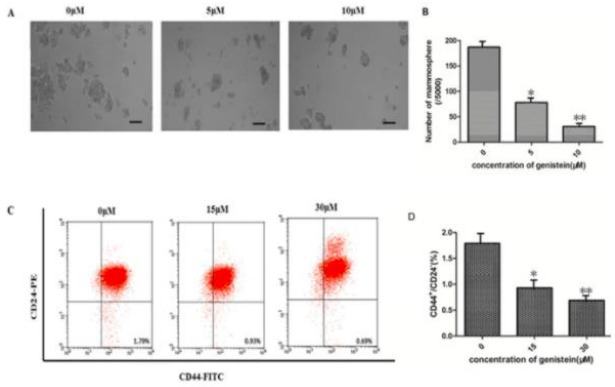
[Fig 2: Effect of various curcumin and herceptin concentrations on cell growth (%) of various breast cancer cell lines. Image from Hung-Wen Lai et al., *Research Gate*]

According to the data in figure 2, while trastuzumab might menially decrease the cell growth percentage, perhaps as a result of resistance, curcumin can stimulate decreased cell growth in all cell lines, especially MCF-7, which illustrates the importance of utilizing both components, as each can influence different pathways and mechanisms in varying methods. In terms of the chemoresistance of the aforementioned treatment, the activation of NFkB is important in terms of anti apoptosis and proliferation of the overexpressing cells. In terms of genistein, a study conducted by researchers Panhong Fan et al., dived into the techniques that genistein utilizes to suppress the breast cancer stem cells from MCF-7 human breast cancer cells. The study involved examining the formation of the mammosphere, tumor growth, and the pathway Hedgehog. The researchers seed MCF-7 cells in 96-well plates (density: 3 × 104 cells/well). Then, the cells were introduced to genistein through incubation for 48 hours with concentrations of 0, 2.5, 5, 10, 15, 20, 30, 50, and 70 μM. Finally, Sigma-Aldrich was added and the cells were incubated for an additional 2 hours. This experiment helped assay cell proliferation. The researchers also conducted a colony formation assay in which MCF-7 cells were treated with 0 to 15 μM genistein for 48 hours and then the colony formation rates were calculated and compared. The cell apoptosis analysis involved 1 × 10^6 cells being treated with various concentrations of genistein, washed with phosphate-buffered saline, and examined by flow cytometry. Through these methods, the researchers examined the inhibitory effect of genistein in cancer cell spread. This effect was measured by calculating the number of inactive cells compared to the control at increasing concentrations of genistein. It was found that adding more genistein increased the number of inactive cancer cells, decreased colony numbers, and accumulated more apoptosis of the cancer cells (figure 3).



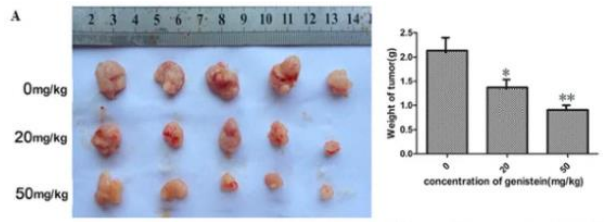
[Fig 3: Genistein's impact on the number of inactive cancer cells, colony numbers, and apoptosis. Image from Panhong Fan et al., *BMC*]

In addition, many breast cancer stem cells have clusters of cells resembling spheres called mammospheres. These can increase the spread of breast cancer through secondary structure formation and lineage formation. The researchers varied concentrations of genistein and studied the effect of the mammospheres. Figure 4 shows a decrease in the number and size of the mammospheres as genistein concentrations were increased.



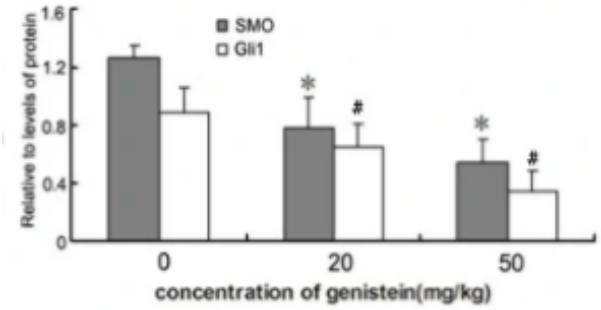
[Fig 4: Genistein's impact on the number and size of mammospheres. Image from Fan et al., *BMC*]

Next, in the tumor growth analysis (in vivo), 16 mice were injected with MCF-7 cells that were in 100 µl phosphate-buffered saline and materiel in a 1 to 1 ratio. After about 2 weeks, the mice were injected with either 0.1% DMSO solution or 20-50 mg/kg genstein dissolved in 0.1% DMSO solution. Finally the tumors were measured in terms of weight. It was found that the experimental mice's tumors were between 46% and 68% the weight of the control mice's tumors. Figure 5 shows how increasing the concentration of genistein decreases the weight of the tumors.



[Fig 5: Genistein's impact on the weight of the tumors. Image from Fan et al., *BMC*]

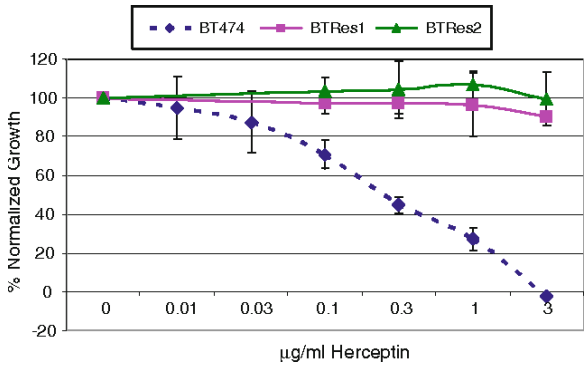
Deregulating some biological pathways, including cancer biosignaling pathways inhibits the formation of tumors. One such pathway is the Hedgehog-Gli1 pathway which controls embryonic development. To investigate whether this pathway was inhibited mRNA levels and protein expression of Smo and Gli1 from the tumors (from both control mice and mice treated with genistein) were examined. Results show that after the treatment involving genistein, Smo and Gli1 also had a decrease in their mRNA levels and protein levels, showing that genistein decreases the number of breast cancer stem cells by attacking the Hedgehog-Gli1 pathway (figure 6) [10].



[Fig 6: Genistein's impact on SMO and Gli1 relative protein levels. Image from Fan et al., *BMC*]

IV. PROPOSED WORK

While IV curcumin, genistein, and trastuzumab have individually been found to have positive effects on limiting the spread of breast cancer by reducing HER2 receptors, we propose that a combination therapy might be more effective, which will be dealt with in depth in this section. Genistein has been shown to inhibit the spread of HER2 receptors. In terms of trastuzumab, the following table illustrates the percentage of normal growth in correlation with the amount of trastuzumab administered.



[Fig 7: Effect of herceptin on normalized cell growth (%). Image from Shawn P. Fessler et al., *PubMed*]

According to the data displayed, breast cancer cells in the BT-474 line can demonstrate resistance against trastuzumab, leading to a lower rate of normalized growth. This finding validates the usage of curcumin and genistein as complementary additions. Genistein has had a significant effect on down regulating HER2 receptors and suppressing AKT activity of HER2 positive breast cancer. The researchers confirmed this by observing the protein levels in SKOV-3 cells that were treated with genistein. The result was that endocytosis was increased in the HER2 receptors and the receptors were unable to express themselves. Genistein was also able to induce apoptosis of HER2 cell lines. When 10

micrograms per millimeters of genistein was given for 12 hours to BT-474 cells, cell apoptosis was induced. When compared to 48 hours of 10 micrograms per millimeters of tamoxifen treatment, the first line of treatment for patients with various types of cancer diseases, cell apoptosis witnessed was quite small compared to the results with genistein. Genstein has seen to have a more potent efficacy on MCF-7, whereas tamoxifen was lagging behind. Genistein has also been shown to have no influence on normal cell morphology and development at 10 micrograms per millimeters for 48 hours. Another key element involved in breast cancer is p27, a cdk inhibitor. p27 regulates cell proliferation and reduced p27 can result in uncontrolled cell growth, followed by the onset of cancer. When used alone, Genistein can have tremendous impacts on p27. Genistein downregulates p27 in MCF. Researchers Ganji Purnachandra Nagaraju et al. examined the mechanisms by which this occurred. They examined mRNA and protein levels of MCF-7/erbB-2 cells at varying concentrations of genistein, and found evidence of downregulation. However, they hypothesized utilizing trastuzumab, an anti-erbB-2 agent, would reduce/inhibit this downregulation. The researchers found that trastuzumab does in fact inhibit p27 down regulation when ER+/erbB-2+ BT-474 cells are treated with genistein [11]. In terms of the interactions between curcumin and genistein, it can be said that both have been seen to have inhibitory effects on a multitude of cancer types and are both products found in turmeric and soybeans. In a study in which both curcumin and genistein were utilized and added to MCF-7 cells, complete inhibition of evocation of the cells was exhibited, positively impacting breast cancer patients. On the other hand, curcumin can work with trastuzumab without negative effects. The results of a recent study performed on mice are shown in the data table below.

Herceptin (μg/mL)	Curcumin (μg/mL)	Fa	CI	Effect
0.1	5	0.34	0.669	Synergistic
0.1	10	0.45	0.836	Synergistic
0.1	15	0.54	0.900	Synergistic
1	5	0.52	0.393	Synergistic
1	10	0.52	0.713	Synergistic
1	15	0.54	0.953	Synergistic
2	5	0.52	0.467	Synergistic
2	10	0.53	0.750	Synergistic
2	15	0.54	1.013	Additive
5	5	0.5	0.794	Synergistic
5	10	0.52	1.007	Additive
5	15	0.56	1.075	Additive
10	5	0.49	1.354	Antagonistic
10	10	0.54	1.195	Antagonistic
10	15	0.57	1.241	Antagonistic

A value of CI <1 represents a case where synergism of herceptin and curcumin was present. CI values of 1 and >1 represent additive and antagonistic effects, respectively. Fa, fraction affected; CI, combination index.

[Fig 8: Indicated effect of various curcumin and herceptin concentrations in terms of Fa and CI. Image from Hung-Wen Lai et al., *Research Gate*]

According to the data, in terms of the BT-474 cell line, at a concentration of approximately 5 µg/mL of trastuzumab and 5 µg/mL of curcumin was found to have a CI value of 0.794 and attested to the claim that curcumin and trastuzumab can work in a synergistic manner. Furthermore, a biphasic interaction can be found between trastuzumab and curcumin; as explained by the data table, an antagonistic effect was observed when a high dose of curcumin was administered (>10 µg/mL) with herceptin (5-15 µg/mL). On the other hand, a synergistic effect was present when a low dose of trastuzumab was administered (0.1-1 µg/mL) along with curcumin (5-15 µg/mL). Trastuzumab was able to inhibit the phosphorylation of MAPK and AKT in the cells, while even at higher concentrations (≥10 µg/mL), it was not able to decrease the amount of HER2 oncoprotein. Curcumin, however, was able to reduce the phosphorylation of MAPK and AKt while depleting HER2. Curcumin can significantly deplete ODC (cellular ornithine decarboxylase), as well as the polyamines it produces; overt polyamine synthesis is associated with cancerous growths [12]. Additionally, p27 can be induced by curcumin, which has been proven to reduce the overexpressing cells. Trastuzumab can impact HER2 by binding to the juxtamembrane domain of HER2, resulting in the antibody downregulating HER2 expression [13]. Additionally, it has been demonstrated that a positive correlation exists between the dosage of curcumin and the cell cycle arrest of MCF-7 cells in either the G2M phase or late S phase. Recent

research has also demonstrated that curcumin can serve as a chemosensitizer by repressing NFkB and reducing the effect of TS, making it crucial to use trastuzumab as a complementary component. While herceptin can only be given by IV, we propose utilizing IV curcumin and genistein as well due to the efficacy associated with IV medications. IV administration is typically associated with stronger, faster, and better results compared to oral administration.

V. CONCLUSION

This paper proposes a synergistic therapy in which IV curcumin, genistein, and trastuzumab is utilized to enhance the recovery process of HER2 positive breast cancer, which is one of the most prevalent forms of cancer, specifically in women. The proposed method was identified, explained, and specified, as required for research and future implementation purposes. The therapy will involve IV curcumin which reduces tumor growth and angiogenesis while also inhibiting metastasis, IV genistein which will significantly down regulate HER2 receptors and suppress AKT activity, and IV trastuzumab which inhibits the downregulation of p27 when genistein is utilized.

CONFLICTS OF INTEREST

As per the researchers, there is no conflict of interest.

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