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Journal of Stem Cell Research & Therapeutics

Review Article
Open Access

FloraStilbene: immunotherapy adjuvant for breast cancer

Abstract

Despite significant advancements in therapeutic approaches to triple-negative breast cancer, treatments remain relatively ineffective once metastasis occurs. The introduction of immunotherapy has revolutionized oncological therapies, yet significant hurdles remain before its full potential can be realized. In this review, we examine immune escape mechanisms shared between pregnancy (the 'fetal allograft') and cancer. We discuss the use of abortion-inducing agents in the context of cancer immunotherapy, and we also provide rationale and preliminary data on FloraStilbene™, a combination of the polyphenol antioxidant pterostilbene and the glucocorticoid receptor antagonist mifepristone, for the stimulation of anticancer immunity.

Keywords: triple-negative breast cancer, metastasis, cancer immunotherapy, immune escape mechanisms, fetal allograft, pregnancy and cancer parallels, abortion-inducing agents, florastilbene™, immune modulation.

Volume 9 Issue 1 - 2024

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Received: November 25, 2024 | **Published:** December 20, 2024

Introduction

Breast cancer is the most prevalent oncological condition in females and represents a significant medical problem.[1] According to a 2022 estimate by the American Cancer Society, there were 287,850 newly diagnosed cases of breast cancer with invasive properties. From 1989 to 2019, the mortality rate from breast cancer was reported to be 41% higher in black women compared to white women, presumably due to later diagnoses.[2] It is widely accepted that about 10% of breast cancers are associated with mutations, such as alterations in BRCA1 and BRCA2.[3] Breast cancer is typically categorized based on the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), with cells lacking all three—referred to as 'triple-negative'- considered the most invasive.[4]

The treatment of early-stage breast cancer typically involves a combination of surgery,[5] radiation therapy,[6] and systemic therapy (such as chemotherapy, hormonal therapy, or targeted therapy).[7] The specific treatment plan depends on various factors, including the size and location of the tumor, its spread to the lymph nodes, and the patient's overall health condition and preferences. The most common surgical options are lumpectomy or mastectomy. Radiation therapy is often recommended after surgery to help eradicate any remaining cancer cells and reduce the risk of recurrence, using high-energy radiation to target the affected area of the breast. Systemic therapy may also be recommended for some patients with early breast cancer, particularly if the cancer is hormone receptor-positive or HER2-positive.

As the disease progresses, the primary goal of treating advanced breast cancer shifts towards controlling the spread of the cancer, managing symptoms, and improving the patient's quality of life.[8] Common treatments for advanced breast cancer include: systemic therapy- such as chemotherapy, hormone therapy, targeted therapy, and immunotherapy-designed to attack cancer cells throughout the body; surgery to remove or debulk the primary tumor or large metastases, or to alleviate symptoms such as pain or dyspnea; radiation therapy, which may be used to shrink tumors and relieve symptoms like pain, neurological symptoms, or dyspnea; and palliative care, focusing on providing support to patients and families by managing symptoms, maintaining quality of life, engaging in goals-of-care discussions, and addressing the emotional and psychological effects of the disease. Treatment for advanced breast cancer is typically focused on managing the disease rather than curing it, and thus may be ongoing and change over time depending on the cancer's response to therapy.

Historically, cancer immunotherapy has focused on melanoma and renal cancers, with the possibility of immunotherapy for breast cancer being relatively understudied.[9] Due to the higher incidences

of immunologically mediated spontaneous remissions in these two cancers, they have traditionally been viewed as 'immunogenic'.[10–15] This review will discuss more recent findings suggesting that immune responses in breast cancer may exist and can be therapeutically leveraged.[16]

The immune system fights breast cancer

Immunotherapy offers the ability to specifically target and kill tumor cells without some of the toxicity associated with traditional oncological therapies such as radiation and chemotherapy. Despite initial controversies regarding the natural role of the immune system in controlling cancer development, a theory known as 'immunosurveillance',[17] it is now widely accepted that the immune system not only keeps cancer at bay but also that that proper immune stimulation can be used as a therapy for cancer.[18] Initial clinical approvals of immunotherapy began with immunogenic tumors such as melanoma and kidney cancer.[19] but it eventually expanded to other cancers, including breast cancer.[20]

This expansion is underscored by the recognition of tumor immunogenicity, which is determined partly by whether immune cells infiltrate the cancer and whether this correlates with a better or worse prognosis. Melanoma, for instance, was identified as immunogenic through studies demonstrating that 'tumor-infiltrating lymphocytes' (TILs) were associated with enhanced survival.[21] The active role of TILs in suppressing the tumor was demonstrated in reports where TILs were extracted from patients, expanded outside the body—free from the tumor's immunosuppressive pressures—and then re-infused into the same patients.[22] In three consecutive clinical trials using TILs, objective response rates between 49% and 72% were observed in advanced melanoma.[23] Furthermore, it was shown that the re-infused cells homed back to the tumor,[24,25] leading to the discovery of the first tumor-specific antigens, the melanoma-associated antigen (MAGE) family.[26]

Similarly, in breast cancer, numerous studies have demonstrated a correlation between TILs and improved survival. Ren et al. examined 68 patients with triple-negative breast cancer and found a strong correlation between tumor infiltration by T cells, specifically CD3 and CD8, and longer progression-free survival.[27] Similar findings have been reported by other independent researchers.[28,29] An increased number of TILs is associated with a better response to therapy.[30] It is believed that TILs control the tumor through direct killing, primarily by CD8 cytotoxic lymphocytes,[31] as well as by suppressing tumor growth and angiogenesis, mediated by the production of cytokines such as interferon-gamma.[32] The secretion of perforin and granzyme B by CD8 T cells, known mechanisms of cellular destruction, logically correlates with improved survival, a finding that has indeed been demonstrated.[33]

Building on the role of specific immune cells, T cells are known to eradicate cancer by recognizing molecular signatures on tumors known as "tumor-associated antigens." Cancerous cells, to gain an advantage over normal cells, often start producing new proteins that are not typically found in the adult body, usually mutated versions of existing proteins. When cancer begins producing these proteins, T cells recognize and attempt to eliminate the tumor.

T cells are considered part of the adaptive immune system, because of their ability to create immunological memory. Innate immune system cells, such as natural killer (NK) cells, are another mechanism of protection from neoplasia. Unlike T cells, which recognize peptides presented on HLA molecules, NK cells eliminate cells that lack HLA molecules. HLA molecules serve as a 'negative signal' that prevents NK cell activation; a mechanism central to the 'missing self' hypothesis.[34] Originally thought of as the immune system's 'backup' when cancer cells down regulate HLA to evade T cell-mediated killing, NK cells also target cells expressing proteins indicative of 'cellular stress.' Given that cancer cells produce proteins at a much faster rate than normal cells, they express these 'danger proteins,' which activate NK cells.[35] Studies have demonstrated that breast cancer patients with higher levels of NK cell activity tend to live longer than those with lower levels.[36]

Breast cancer fights the immune system

If the body has such potent means of protecting itself against cancer, why do tumors arise and progress? One of the main reasons is that cancer effectively leverages components of the immune system that down regulate immunity after immune recognition. One such immune regulatory mechanism involves T regulatory (Treg) cells. These cells have been demonstrated to protect the body against autoimmunity,[37] transplant rejection,[38] and immunologically mediated miscarriages.[39] Importantly, this mechanism is co-opted by cancer cells to escape immune destruction.

One example of this is a study by Bates et al. who assessed the numbers of Treg cells (identified by FOXP3 expression) in tissue microarray cores from pure ductal carcinoma in situ (DCIS), invasive breast cancer, or from comparable areas of normal terminal duct lobular breast tissue. Treg cell numbers were significantly higher in samples from in situ and invasive breast carcinomas than in normal breast tissue. Importantly, high numbers of FOXP3-positive Treg cells identified patients with DCIS at increased risk of relapse and patients with invasive tumors with both shorter relapse-free and overall survival. Another important finding in the same study was that high numbers of FOXP3-positive Treg cells can identify patients at risk of relapse after 5 years.[40] The correlation between Treg numbers and poor prognosis has been reported by other studies.[41–43] Interestingly, some drugs used in breast cancer, such as estrogen blockers, may reduce Treg numbers.[44]

Besides being associated with poor prognosis Tregs also play a role in response to therapy. In a clinical study, 93 patients with breast cancer diagnosed by core-needle biopsy (CNB) and treated with primary systemic chemotherapy (PSC) were examined. CNB and surgically resected specimens were stained with a FOXP3 mouse monoclonal antibody to compare the numbers of FOXP3- positive cells in the tumors before and after PSC. A median cut-off value of >16.3/high power field (HPF) and >6.6/HPF defined high numbers of Tregs in CNB and in surgical specimens, respectively. The patients were assigned into four groups (HH, high number of FOXP3-positive cells in both CNB and surgical specimen; LL, low number in both specimens; HL, high in CNB and low in the surgical specimen; LH, low in CNB and high in surgical specimen). Lymph vessel invasion-positive, clinically non-responder and ER-negative tumors contained significantly more FOXP3- positive cells after PSC. Prognosis was better among patients with low numbers than high numbers of FOXP3-positive cells both in CNB and in surgically resected specimens. In multivariate analysis, the LL group demonstrated significantly better recurrence-free survival than the non-LL group (LH, HL, and HH). These findings suggest that the number of FOXP3-positive cells identified during PSC represents a promising predictive factor that might also be an important therapeutic target for breast cancer.[42]

In another study, it was shown that pathologic complete responses (pCR) to chemotherapy in breast cancer patients were associated with decreases of intratumor Tregs. High CD8 infiltration and no

Foxp3 infiltration on final histologic specimens were independently associated with pCR. This study suggests that Treg cells may be suppressing the activity of effector cells that otherwise could be capable of killing the tumor.[45]

Cancer suppressing effects of RU-486

In the search novel immune stimulators researchers have exampled biological situations that may resemble the growth and progression of neoplasia. Correlations between cancer and pregnancy have previously been made based on shared characteristics such as angiogenesis, cellular trafficking, and immune modulation.[46] The process of immunologically mediated miscarriage is associated with similar types of immune responses as those seen in tumors regressing from immunotherapy, specifically, activation and infiltration of NK cells,[47–51] macrophages,[52–54] and CD8 cytotoxic T cells.[55] Given these similarities, the authors questioned whether agents that terminate pregnancy might also have effects against cancer. Abortogenic agents have been shown to possess anticancer properties; these include agents that suppress indoleamine 2,3-dioxygenase,[56–61] myeloid-derived suppressor cells,[62–67] and Treg,[68–71] as well as checkpoint inhibitors.[72] Furthermore, cancer and pregnancy share numerous means of immune evasion, including over-expression of Fas ligand,[73–76] hCG,[77–83] HLA-G,[84] PD-L1,[85–93] TIM-3,[72,94,95] arginase,[96–99] and VISTA.[100–102]

RU-486, now known as mifepristone, was originally synthesized by Georges Teutsch based on experiments aimed at developing artificial steroids. The name originates from the name of the company that developed it, Roussel-Uclaf (RU), and it was compound number 38486, shortened from RU-38486 to RU-486. This compound was first noted for its potent binding to the glucocorticoid receptor and for blocking glucocorticoid biological activities in tissue culture. Subsequently, it was found to block progesterone receptor activity only in the presence of progesterone. This finding led to studies on its abortifacient effects, which are associated with erosion of the endometrium, detachment of the chorion from the decidua basalis, atrophy of the corpus luteum, enhanced uterine contractibility, cervical softening and dilatation, and eventual expulsion of the embryo and endometrium.[103] Although the abortifacient effects have been ascribed to progesterone receptor antagonism, there is increasing evidence that immunological mechanisms such as suppression of Treg cell activity play a significant role in its action.[104] Given the fundamental role of Treg cell activity in cancer's escape from the immune response, if RU486 can reduce the number or activity of these cells, it may have a potential role as a cancer therapeutic.

One suggestion that RU-486 may induce abortion in part through immune modulation came from Mao et al., who showed that progesterone increases the numbers of Treg cells as well as augmenting their immune suppressive activity. Blocking progesterone signaling with RU-486 resulted in a loss of Treg number and activity, which correlated with immunological cell infiltration, inflammatory cytokine secretion, and eventual fetal loss.[105] A direct cause-and-effect relationship between Treg loss and fetal death was illustrated in a publication in which RU-486 was administered to pregnant mice, resulting in impaired Treg functional competence, increased cytotoxic CD8 T cells, and fetal loss. Importantly, adoptive transfer of Treg cells- but not conventional T cells- alleviated fetal loss.[104] Essentially, this shows that depletion of Treg cells is a mechanism of action, as the transfer of healthy Treg cells prevented abortion.

Besides inducing immunity by reducing Treg cells, RU-486 also augments the maturation of dendritic cells. These cells, classically known as 'professional antigen-presenting cells' for their unique ability to activate naïve T cells,[106] promote the generation of Treg cells and a healthy pregnancy when in an immature state.[107,108] Conversely, when dendritic cells are mature, they no longer induce the generation of Treg cells but instead lead to immune activation.[109] Just as Treg cells allow for cancers to escape immune killing, immature dendritic cells have been shown to provide means of tumor immune evasion through the induction of energy in tumor-reactive T cells or conversion to Treg cells.[110,111]

Yinghua et al., conducted a series of experiments to assess the ability of RU-486 to alter immunity by DC manipulation.[112] They showed that the drug promoted the expression of the DC maturation markers CD80, CD86, and ICAM-1 while decreasing the cancer-associated immune suppressive molecules indoleamine 2,3-dioxygenase[113–115] and TGF-beta.[116] Importantly, when Tregs were cultured with RU486-cultured DC, the Tregs lost suppressive activity. These experiments suggest that RU486 possesses a direct maturation-inducing effect on DC, which blocks Treg generation through modulating the upstream cytokine TGF-beta.

It appears that some parallels may exist between the process of RU-486 induction of immunological reactions against the 'fetal allograft' and immune-mediated tumor rejection. If this is the case, then it is important to provide an overview of existing work evaluating this abortogen in the context of oncology.

Immune stimulatory effects of RU-486

The mechanisms of cancer immunity induced by RU-486 could involve the reduction of Tregs, which is associated with immune suppression in numerous cancers, inhibition of glucocorticoid signaling, and suppression of transforming growth factor beta (TGF-beta) activity. In one series of experiments, BALB/c-green fluorescent protein (GFP)+ bone marrow (BM) cells were transplanted into immune deficient NSG mice to generate an immune competent NSG/BM-GFP+ (NSG-R) mouse model. Treatment with RU-486 inhibited the growth of 59-2-HI tumors and caused alterations in the tumor microenvironment similar to those observed in fetal loss. Tumors in RU-486-treated immune competent mice showed increased infiltration of F4/80+ macrophages, natural killer, and CD8 T cells, displaying a central memory phenotype. Mechanistically, RU-486 induced immunogenic cell death both in vitro and in vivo, as depicted by the expression and subcellular localization of the alarmins calreticulin and HMGB-1, and the induction of a gene program characteristic of immunogenic cell death. Moreover, RU-486-treated tumor cells efficiently activated immature DC, evidenced by enhanced expression of MHC-II and CD86, and induced a memory T-cell response, attenuating tumor onset and growth after re-challenge. Of relevance to current clinical oncology, RU-486 treatment increased the sensitivity of tumors to inhibition of the PD-L1 checkpoint.[117]

To demonstrate that RU-486 induces anticancer immunity through immune stimulation and not necessarily progesterone inhibition, a series of experiments were conducted using several human prostate cancer cell lines in murine immune deficient and immune competent hosts. The experiments assessed effects of RU-486 alone or in combination with IL-12 adenoviral gene therapy. Treatment of human PC3 prostate xenograft (androgen independent) or TRAMP-C1 tumors (androgen receptor positive) with the combination Ad5IL-12 vector and RU-486 produced significantly better therapeutic efficacy compared to controls. Additionally, combination therapy increased the capacity of tumor sentinel lymph node lymphocytes to produce Granzyme B in response to tumor cell targets. Finally, combination therapy tended towards a decrease in CD4+/FoxP3+ T regulatory cell

populations in the draining lymph nodes. These experiments provide in vivo support for the hypothesis that RU-486 directly acts as an immune stimulator.[118]

In addition to its immune modulating anticancer effects, RU-486 has been reported to directly suppress neoplasia in several animal models. For example, in one study, sixty-one mice developing spontaneous leukemia were treated with RU-486 and 33 controls with olive oil. Quality of life was determined by body conditioning score (BCS). Treatment was initiated when the mice were 6 months old. Within 2 weeks of therapy, 11.4% of the RU-486 treated mice died compared to a 50% mortality in the control group. The BCS was 5 (highest quality) in 82% of treated mice vs. 11% of controls after 2 weeks of therapy.[119] Similar therapeutic responses were seen in a lung cancer model where mice received RU-486 at 0.3 mg three times weekly from 8 weeks compared to olive oil in the controls. The survival at one year for mice treated with mifepristone was 57.6% vs. 26.6% for controls.[120]

Use of RU-486 in cancer patients

Clinical signals exist suggesting the potential utility of RU-486 in oncology. In one report, RU-486 was administered at 200 mg per day orally to two patients with stage IV colon cancer suffering from extensive metastases. This regimen was well-tolerated, and both patients not only survived far longer than expected but also experienced marked improvements in quality of life and increased energy upon initiating RU-486. Though the metastatic lesions did not disappear, no new ones appeared for a substantial time, and the existing ones did not grow.[121]

In contrast to many therapeutic interventions, RU-486 appears not to be limited by the blood-brain barrier. A 43-year-old male with end-stage stage IV glioblastoma multiforme was treated exclusively with RU-486 at 200 mg orally daily. The patient exhibited definite palliative effects for several weeks and lived significantly longer than predicted before treatment.[122]

Additionally, two case reports describe the administration of mifepristone monotherapy daily at 200 mg to a moribund woman with never-treated metastatic lung cancer and a male with bilateral renal cell carcinoma who had undergone only a unilateral hemi nephrectomy. Both patients experienced long-term high-quality survival- 5 years for the patient with lung cancer, with complete remission of all lung lesions, and 12 years for the male patient with kidney cancer. Neither patient reported any treatment-associated adverse effects.[123]

Interestingly, RU-486 may also show activity in patients where other treatments have failed. A 68-year-old woman suffering from metastatic non-small cell lung cancer progressed despite treatment with a checkpoint inhibitor (nivolumab) and three rounds of multi-agent chemotherapy. After 1.5 years of treatment with single-agent mifepristone, her cancer remained stable, with some tumor regression reported.[124] Similar therapeutic outcomes were published for cases of pancreatic cancer,[125] leukemia, and osteosarcoma.[126]

The therapeutic potential of RU-486 has not been restricted to solid tumors alone. In one report, an 81-year-old woman with chronic lymphocytic leukemia, which had progressed to an acute rapidly progressing stage, was treated with only 200 mg of mifepristone daily. The patient showed dramatic improvement upon initiation of therapy and maintained remission until the time of publication, which was 12 months.[127]

Optimizing anticancer efficacy of RU486: formulating with Pterostilbene

Res Nova Biologics Inc has screened various compounds for augmentation of RU-486's immune modulatory efficacy. The naturally derived compound pterostilbene was identified as possessing the strongest ability to enhance multiple cancer inhibitory biological activities of RU-486. This compound is a naturally derived analogue of resveratrol[128] and has been shown to possess therapeutic activity in a wide variety of conditions, including diabetes, aging, depression, and brain injury.[129]

Mechanistically, pterostilbene exhibits several interesting biological functions, including activation of NRF2, which mediates numerous anti-apoptotic activities,[130,131] suppression of NF-kappa,[132] and inhibition of p38 MAP kinase,[133] both potent mediators of inflammation. Interestingly, the effects of pterostilbene on cancer can be considered paradoxical; it appears to have anti-apoptotic effects in non-malignant cells,[134] while inducing death in transformed cells both in vitro[135,136] and in vivo.[137]

In the context of immunotherapy, pterostilbene possesses numerous interesting properties. According to United States Patent #9682047B2, administration of pterostilbene was capable of enhancing the therapeutic effects of interleukin-2 in a murine model of melanoma. The immune stimulatory activities of pterostilbene appear to function through the suppression of feedback inhibition loops. For example, immune activation using agents such as interleukin-2 or toll-like receptor activators stimulates the immune suppressive enzyme cyclo-oxygenase 2 (COX-2),[138,139] which produces prostaglandin E2, a known promoter of Treg cell generation dependent on COX-2 activity.[140]

Another mechanism by which pterostilbene stimulates antitumor immunity is by down regulating oxidative stress and neutrophil activity, which are associated with numerous tumors and the suppression of T cell immunity through cleavage of the TCR-zeta chain.[141] Pterostilbene has been shown to suppress neutrophil activation in various systems, including a melanoma model of metastasis,[142] a cardiac ischemic reperfusion model,[143] and an arthritis model.[144] Mechanistically, pterostilbene induces accelerated apoptosis of activated neutrophils through a caspase-3 dependent mechanism and suppresses the production of oxidative radicals at a pre-apoptotic stage.[145]

Additionally, augmentation of tumor sensitivity to NK cell-mediated killing by pterostilbene has been reported. Yulin et al reported that pterostilbene treatment enhanced the expression of NK group 2 member D (NKG2D) ligands- major histocompatibility complex class I chain-related proteins A and B (MICA/B) on prostate cancer cells. These molecules are typically seen as activators of NK natural cytotoxicity towards cancer cells. The authors found that inhibition of miR-20a by pterostilbene was occurring, which normally silences expression of the 3' untranslated region (UTR) of MICA/B. Blocking expression of miR-20a by pterostilbene results in up regulation of MICA/B, making prostate cancer cells significantly more sensitive to NK-mediated killing.[146] Given that RU-486 enhances NK activity, a potent synergy is anticipated between these two agents. Accordingly, we initiated a series of experiments to assess this hypothesis.

FloraStilbene preclinical data

In order to assess whether FloraStilbene possesses ability to suppress tumor growth, the classical triple negative 4T1 model

was utilized. 4T1 cells were grown in RPMI 1640 media in a fully humidified atmosphere with 5% carbon dioxide. 4T1 triple-negative murine breast cancer cells were administered to female BALB/c mice at a concentration of 500,000 cells per mouse in the mammary pad. Mice were treated with either a) saline; b) pterostilbene at 2 mg/kg; c) RU-486 at 3 mg/kg; or d) a combination of pterostilbene and RU-486. Each treatment group consisted of 10 mice. As shown in Figure 1 below, significant synergy was observed in the regression of this model of breast cancer.

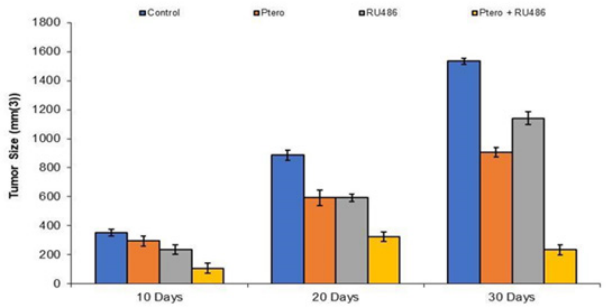

Figure 1 Suppression of 4T1 growth by FloraStilbene.

FloraStilbene™ increases NK cell activity. Tumor-bearing mice treated as described above were sacrificed at the indicated times, and NK activity was assessed using the MTT cytotoxicity assay against labeled 4T1 cells. It is known that MTT readings correspond to the number of cells present in the exponential growth phase. To utilize the MTT assay, MTT liquid was made at (10µl MTT solution in each 100µl media) added to each well and the plates were then incubated at 37°C for 5 hours. Subsequently, the remaining MTT solution was discarded DMSO was added to each well to dissolve the formazan crystals. The plates were shaken for 5 minutes on a plate shaker to ensure adequate solubility. Absorbance readings of each well was performed at 540 nm (single wavelength) using a multi scan plate reader.

NK cells were extracted from spleens using Magnetic Activated Cell Sorting (MACS) by Miltenyi Biotec, according to the manufacturer's instructions. NK cells were plated at a 10 to 1 ratio. As seen in Figure 2 below, an increase in NK cell activity was observed with FloraStilbene™ treatment.

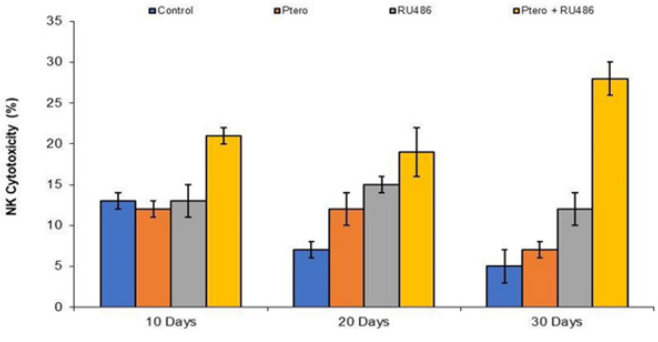

Figure 2 FloraStilbene increases NK activity.

In order to mechanistically assess the immuno modulatory activity of FloraStilbene, the expression of the TCR zeta chain was assessed. Erythrocyte depleted samples were examined by flow cytometry using intracellular cytokine staining. Briefly, 2 mm monensin was added to T cells for 4 h. Cells were fixed with 2% PFA and permeabilized with FACS buffer (PBS supplemented with 5% FBS and 0.1% sodium azide) containing 0.1% saponin. An anti-TCR zeta chain antibody, was used for indirect staining prior to a secondary goat antimouse *R*-phycoerythrin-conjugated antibody. Protection from loss of TCR-zeta was observed, suggesting a possible mechanism of immune-preservation/immune stimulation by FloraStilbene figure 3.

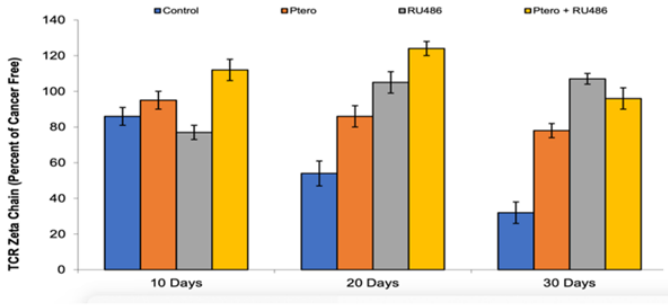

Figure 3 FloraStilbene protects TCR zeta.

Conclusion

Clinical uses of FloraStilbene

Stimulation of immunity to cancer is major unrealized goal. Preliminary data presented supports the possible use of combinations of pterostilbene and RU486. These studies have limitations, however early clinical responses have been observed which will be the subject of future publications.

We believe that increasing the activity of NK cells could enhance the efficacy of drugs already on the market for the treatment of breast cancer. For example, trastuzumab (Herceptin) represents a significant therapeutic modality whose efficacy is influenced by NK activity. In one study, immunological responses were assessed in 26 patients receiving trastuzumab monotherapy as maintenance management after chemotherapy (8 mg/kg load and then standard doses of 6 mg/kg every 3 weeks). Cytotoxic activity against the MHC class I-negative standard NK target K562 cell line and HER2-specific ADCC against a trastuzumab-coated HER2-positive SKBR3 cell line were assessed in peripheral blood mononuclear cells (PBMC) harvested after the first standard dose. After six months, seventeen patients were scored as responders and nine as non-responders according to the RECIST criteria, while progression-free survival (PFS) was calculated during a 12-month follow-up. It was shown that the responders had significantly higher levels of both NK and ADCC activities that were not different from those of eleven normal controls. The NK activity of the non-responders was significantly lower than that of the normal controls. At twelve months, there was a marked correlation between PFS and NK activity only. PFS was significantly longer in patients with high levels of NK activity, whereas its pattern was unrelated to high or low ADCC activity.[147]

Based on the direct and indirect cancer inhibiting properties of RU486 and pterostilbene, as well as our pilot data, we conclude that the FloraStilbene product being developed by Res Nova Biologics possesses promising potential as a monotherapy or as an adjuvant to existing immunotherapies.

Acknowledgments

None.

Conflicts of interest

The authors declare that there is no conflicts of interest.

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Citation: Ramos FS, Kesari S, Delgado G, et al. Thomas SA, Mankan N. FloraStilbene: immunotherapy adjuvant for breast cancer. *J Stem Cell Res Ther.* 2024;9(1):45–53. DOI: 10.15406/jsrt.2024.09.00172

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