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*Review*

# Early-stage *CCNG1*+ HR+ HER2+ Invasive Breast Carcinoma in Older Women: Current Treatment and Future Perspectives for DeltaRex-G, a *CCNG1* Inhibitor

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**Abstract.** *Women with HR+HER2+ early-stage breast cancer are disadvantaged by the lack of clinical trials focused on women ≥70 years of age. In the past years, there has been increasing controversy on the use of toxic chemotherapy as standard of care treatment for early- stage HR+ HER2+ breast carcinoma in older women. With precision medicine coming of age, molecular profiling of tumors and circulating tumor DNA has identified target oncogenes that could be used in designing an optimal treatment for this group of women. This article reviews the current treatment of early-stage triple receptor positive breast cancer, the risks of chemotherapy in older women, and CCNG1, a novel biomarker in development for the use of DeltaRex-G, a CCNG1 inhibitor. Further, future perspectives for DeltaRex-G in older women with early stage CCNG1+ HR+ HER2+ breast cancer are discussed.*

HR+HER2+ breast cancer is the second most common subtype of invasive breast cancer (IBC) with an age-adjusted rate of 13.4 new cases per 100,000 women, according to the 2014-2018 SEER Cancer registry (1).

Current treatment for early-stage triple receptor positive invasive breast cancer (IBC) includes partial or total mastectomy, neoadjuvant/adjuvant chemotherapy, and radiation therapy. While there are definite differences in the biology and treatment responses of HER2+/HR+ and HER2+/HR– breast cancers, these IBC groups are treated basically the same (2). In terms of chemotherapy for both early-stage HER2+/HR+ and HER2+/HR– breast cancers, the preferred neoadjuvant/adjuvant regimens include doxorubicin and cyclophosphamide first, then a combination of paclitaxel and trastuzumab, and optionally, endocrine therapy depending on HR positivity (3). According to NCCN guidelines, there is insufficient data for these chemotherapy recommendations for women ≥70 years of age. Further, the prognosis of patients with early- stage triple receptor positive IBC is uncertain even when HER2 is amplified or overexpressed, since this population was not studied in the available randomized clinical trials (3). In this regard, there is increasing controversy regarding the use of toxic chemotherapy as standard of care treatment for older women with early-stage triple receptor positive IBC (4). With precision medicine coming of age, molecular profiling of tumors and circulating tumor DNA have identified target oncogenes that could be used in designing an optimal treatment for this group of women. In fact, Dieci and Guarnieri reported that HER2+/HR+ breast cancer patients do not respond well to neoadjuvant chemotherapy and that these patients need a more personalized treatment program

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Table I. *Clinical trial NCT#, site, principal investigator/s, phase of trial, cancer type and treatment outcome using DeltaRex-G for solid malignancies.*

| Clinical trial NCT # | Phase of trial | Clinical site: Principal investigator/s phase of trial | Cancer type | # Patients | Overall survival (OS) |
|---|---|---|---|---|---|
| NCT00504998* Dose level 1-3 | I/II | Santa Monica, CA, USA: SP Chawla Manhattan, NY, USA: HW Bruckner (Duke) Durham, NC, USA: MA Morse Phase 1/2 | Pancreatic adenocarcinoma, gemcitabine-resistant | 20 | 28.6% One year 21.4% 1.5-years |
| NCT00505713* Dose level 1-4 | I/II | Santa Monica, CA, USA: SP Chawla, PI Phase 1/2 | Bone and soft tissue sarcoma, chemotherapy-resistant | 36 | 38.5% One-year 31% 2-years |
| NCT00505271* Dose level 1-4 | I/II | Santa Monica, CA, USA: SP Chawla, PI Manhattan, NY: HW Bruckner, PI Phase ½ | Breast cancer, chemotherapy resistant | 20 | 60% One-year OS |
| NCT00572130* Dose level 1-2 | II | Santa Monica, CA, USA: SP Chawla, PI Phase 2 | Osteosarcoma, chemotherapy resistant | 22 | 27.3% One year 22.7% 2-years |
| NCT 04091295* Dose level 2 | Expanded access for intermediate size population | Santa Monica, CA, USA: SP Chawla, PI Expanded access for intermediate size population | Pancreatic cancer, sarcoma, breast cancer, basal cell CA | 12 | 41.7% 2-years |

*Dose Level 1=1×10e11 cfu, 2-3 times a week; Dose Level 2=2×10e11 cfu, 3 times a week; Dose Level 3=3×10e11 cfu, 3 times a week; Dose Level 4=4 × 10e11 cfu, 3 times a week; cfu: Colony forming units; OS: overall survival; CR: complete remission; PR: partial response; SD: stable disease.

(2). Because HER2+/HR+ breast cancer has shown a diminished response to chemotherapy, there is a growing interest for the design of alternative therapeutic regimens that do not rely on chemotherapy. These strategies aim to target the estrogen receptor, HER2 receptor pathways, and the individual patient's distinct molecular profile in order to achieve better treatment outcomes (2). Sophisticated molecular profiling of tumors and detection/quantification of circulating tumor DNA are now available to assist in treatment design and tumor surveillance (5-7).

The results of the phase III ExteNET Trial showed that the addition of neratinib after completion of chemotherapy, and trastuzumab therapy, significantly prolonged disease-free survival, albeit minimally, compared to placebo; this regimen gained United States Food and Drug Administration (USFDA) approval. However, in Europe, approval was limited to HER2+/HR+ patients only since by subgroup analysis, the benefit of neratinib was mostly confined to HER2+/HR+ patients (8). Other clinical trials using drugs aimed at personalized medicine involved previously treated patients with advanced disease (4, 9-10).

The St. Gallen Panel suggested that postmenopausal women should consider taking bisphosphonates to prevent breast cancer recurrence (11). The Panel acknowledged that their recommendations do not apply to all patients and personalized adjuvant therapy should take into consideration the tumor types and features, co-morbidities, patient inclinations as well as treatment cost constraints (11). Despite multiple trials showing significant improvements in treatment outcome parameters with the addition of lapatinib to trastuzumab-based neoadjuvant chemotherapy, the extended results from the ALTTO study do not indicate a decrease in recurrence risk with adjuvant lapatinib (12). Trastuzumab reduced risk in small, sub-centimeter, node-negative breast cancers (13) while paclitaxel and trastuzumab were shown to be an effective regimen for stage I breast cancers with low rates of recurrence (14). Further, dual blockade with pertuzumab and trastuzumab improved outcome in individuals who have an elevated risk of experiencing a recurrence, due to lymph-node involvement or hormone-receptor negativity (15).

Regarding gene expression profiling for early-stage breast cancer, the MINDACT trial evaluated a 70-gene signature with clinical risk criteria and predicted breast cancer patients who would not benefit from adjuvant chemotherapy (16). Both the TAILORx and West German Plan B trials used a very low 21-gene recurrence score and found a cohort of patients with HR positive breast cancer who benefited with

endocrine therapy alone (17, 18). Concerning adjuvant therapy – endocrine therapy, in postmenopausal women, multiple trials have provided evidence that prolonged use of an aromatase inhibitor reduced rates of breast cancer recurrence although the absolute benefit was modest (19, 20). Randomized trials showed no difference in patient outcome between anastrozole and letrozole as adjuvant treatment (21). Most recently, interim results of the SABC 2022 NCT02344472 - Detect V/CHEVENDO (Chemo *vs*. Endo) trial showed that the omission of chemotherapy in the treatment of HR+HER2+ metastatic breast cancer might be an effective and well tolerated option (22).

## Risks of Therapy in Older Women

For older women, cardiovascular disease (CVD) is associated with a greater mortality risk than breast cancer itself, according to the American Heart Association (23). Although cardiology and oncology are distinct fields in medicine, they are frequently interconnected when it comes to cancer therapy. The risk of heart failure, myocardial ischemia, and hypertension increase with age, and CVD risk factors such as obesity and dyslipidemia are higher in older breast cancer survivors than the risk of tumor recurrence. In addition, survivors could develop late cardiac events as a result of cancer treatment, including chemotherapy, radiotherapy, and targeted therapy with anti-HER2 agents (24-26). The administration of cancer treatment may lead to early or delayed onset of cardiotoxicity, which can manifest as left ventricular dysfunction, heart failure, hypertension, arrhythmias, myocardial ischemia, valvular disease, thromboembolic disease, pulmonary hypertension, and pericarditis (27). Studies have shown that doxorubicin-based adjuvant chemotherapy for breast cancer treatment can cause arrhythmias, conduction abnormalities and cardiomyopathy in doxorubicin-treated patients versus patients who did not receive doxorubicin (28). Alkylating agents, including cisplatin and cyclophosphamide, can also damage DNA, resulting in cytotoxicity and myocyte death. Bradycardia, supraventricular tachycardia, and atrial fibrillation have all been reported in patients receiving systemic alkylating agents (27, 29). In the BIG I-98 trial (30) conducted by the Breast International Group, it was found that anastrozole and letrozole resulted in a higher occurrence of hypercholesterolemia compared to tamoxifen. However, the MA.17 trial did not observe any significant differences in hypercholesterolemia rates with letrozole (31). Aromatase inhibitors (AIs) work by preventing the activity of the aromatase enzyme and depleting estrogen levels in postmenopausal women (32). Because AIs deplete endogenous estrogen production, patients receiving AIs have been demonstrated to have a notably elevated risk of CVD (dysrhythmia, valvular dysfunction, pericarditis, heart failure, or cardiomyopathy) (33). Therefore, given the increased risk therapy-related CVD in older patients with IBC, the use of toxic chemotherapy *vs*. non-toxic targeted therapies as adjuvant treatment for early-stage HR+ HER2+ IBC in older women requires serious consideration.

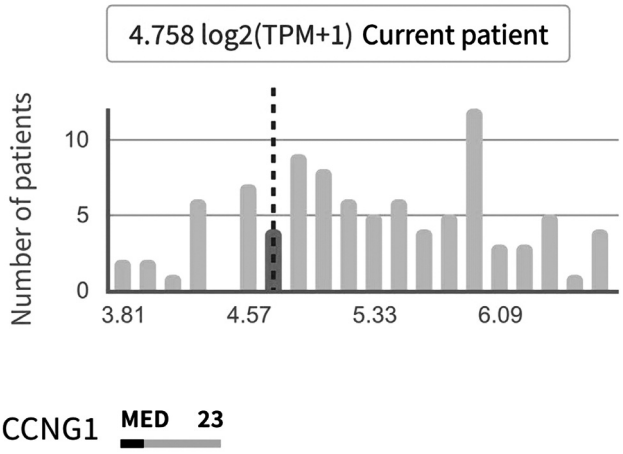


Figure 1. *Enhanced CCNG1 gene expression in a patient with early-stage HR+ HER2+ IBC. CCNG1 gene expression level (TPM) was calculated based on the patient's RNA-seq results and was compared to the RNA-seq data from BostonGene's internal diagnosis-matched patient reference cohort. The gene expression value is indicated as a percentile representing the proportion of patients from this cohort with lower expression levels.*

## Future Perspectives

*CCNG1: A novel biomarker in development for cancer therapy/gene therapy.* DeltaRex-G, a tumor-targeted retrovector encoding a *CCNG1* inhibitor gene, has resulted in extended survival rates of over 10 years for patients with chemo-resistant metastatic pancreatic adenocarcinoma, malignant peripheral nerve sheath tumor, osteosarcoma, B-cell lymphoma, and breast carcinoma with minimal, if any, systemic toxicity (34, 35). Recently, we evaluated the level of *CCNG1* expression in tumors as a potential biomarker for *CCNG1* (Cyclin G1-blocking) inhibitor therapy (36). RNA expression levels of CCNG1 that were previously assessed as part of whole-genome molecular profiling of tumors (TCGA, *N*=9161), neighboring "tissues" (TCGA, *N*=678), and GTEx normal tissues (*N*=7187) across 22 organ sites were analyzed. Increased levels of *CCNG1* RNA and Cyclin G1 protein were noted in tumors compared to normal tissue counterparts. By immunohistochemical staining, normal breast tissue expressed 5% *CCNG1* (nuclear staining percentage) while ductal carcinomas of breast expressed between 35-95% *CCNG1*. Taken together, these data support the use of *CCNG1* as a novel biomarker for identification of patients who may benefit from *CCNG1* inhibitor therapy.

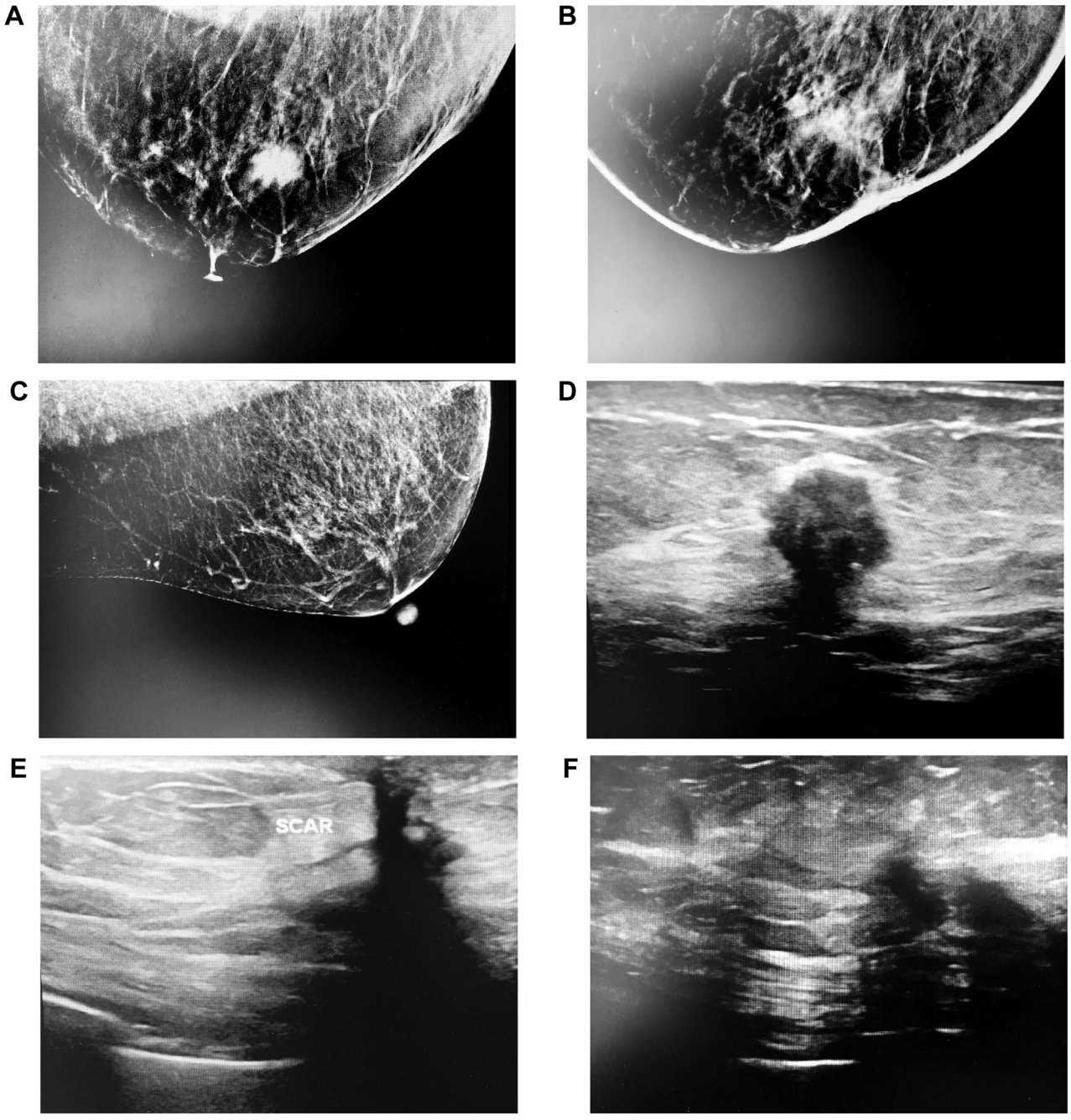


Figure 2. *No recurrence of breast cancer was identified, after adjuvant therapy with DeltaRex-G, Trastuzumab and Letrozole in a patient with triple receptor positive invasive breast cancer. A, B, C: Mammogram at diagnosis, 6 months, and 1 year after treatment initiation; D, E, F: Ultrasound at diagnosis, 6 months and 1 year after treatment initiation.*

*Targeting the tumor microenvironment and oncogenic drivers*. Strategically targeting the tumor microenvironment (TME) with a drug that has a navigational system for identifying abnormal signature (SIG) proteins in the TME would augment biodistribution and drug concentration in the TME near the target cancer cells. Cancer cells and its associated fibroblasts make stroma that encapsulates the tumor and shields the tumor from recognition by the innate immune system. Stroma is a barrier for entry of therapeutic drugs in the TME. Therefore, we can deduce that agents that destroy stroma producing cells would reduce extracellular matrix production and favor entry of therapeutic agents in the TME.

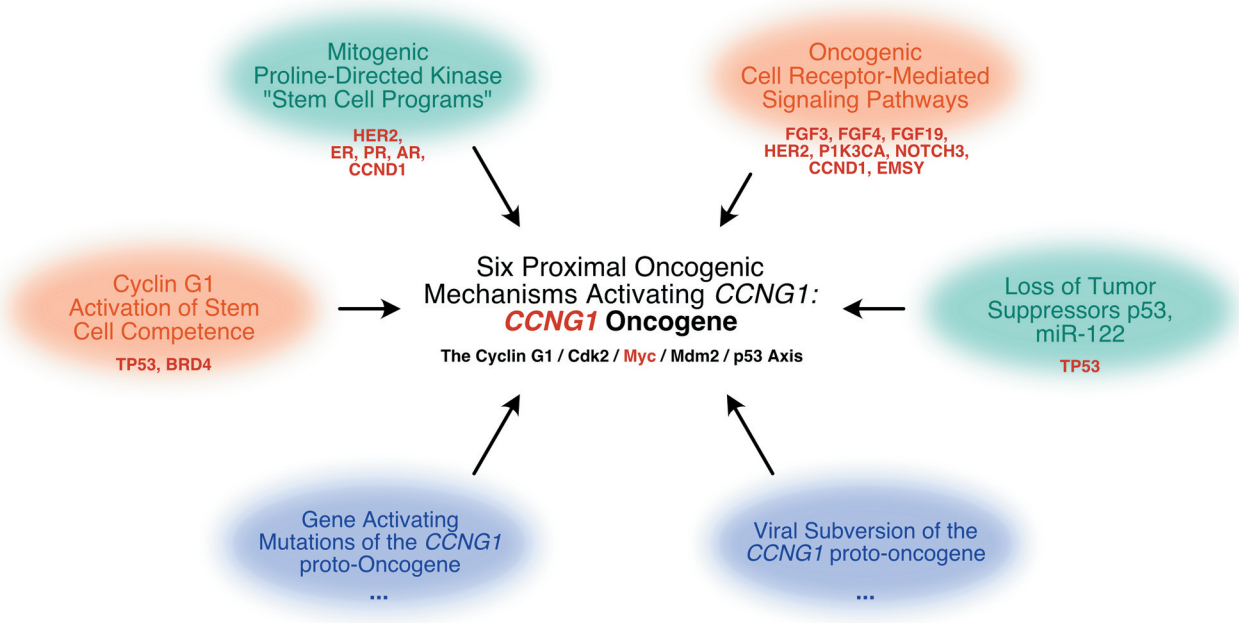


Figure 3. *Six proximal oncogenic mechanisms activating the CCNG1 oncogene. The oncogenic drivers amplified in this case study are marked RED.*

Further, the development of inhibitors of various oncogenes along the Cyclin G1 (*CCNG1*)/Cdk2/Myc/MDM2/p53 axis, are concepts which have been proven safe and effective in clinical trials worldwide (34). Specifically, DeltaRex-G – the first and, so far, only tumor-targeted gene expression vector of its kind – has a navigational system, a collagen-binding decapeptide that binds to stroma collagen exposed by the invading tumor and delivers a genetic payload, a CCNG1 inhibitor gene (34, 37-41). Based on demonstrations of its unique safety and efficacy in hard-to-treat Stage 4 cancers, DeltaRex-G has gained USFDA approval for the 'Blessed Protocol: Expanded access for DeltaRex-G for advanced pancreatic cancer and sarcoma for an intermediate size population (NCT04091295) (42). Table I shows clinical trial NCT#, site, principal investigator/s, phase of trial, cancer type and treatment outcome using DeltaRex-G for solid malignancies. The outcomes of a Phase 1/2 clinical trial that employed DeltaRex-G for chemotherapy resistant Stage 4 breast cancer provided further evidence on the safety and anti-tumor activity of DeltaRex-G in IBC (43). Further, long-term survivors have been reported in women with refractory metastatic triple negative and triple receptor positive breast carcinoma with DeltaRex-G gene therapy (35).

*A case study of an older woman with early- stage CCNG1+ HR+ HER2+IBC*. A 75-year-old female incidentally noticed a mass over the upper quadrant of her left breast. The patient has been on hormone replacement therapy since going through menopause 20+ years ago. She underwent a screening mammogram followed by ultrasound confirming a left upper quadrant breast mass at the 2 o'clock position suspicious for malignancy. Histopathological examination of five core biopsies showed ER+PR+, HER 2 amplified (3+) poorly differentiated invasive ductal carcinoma, with Ki-67 of 15% and up to 30% in some spots. Breast MRI showed a solitary 1.6 cm spiculated left breast mass corresponding to biopsy-proven malignancy. The patient underwent left breast partial mastectomy with sentinel lymph node resection. Surgical pathology report confirmed the diagnosis of invasive ductal carcinoma, poorly differentiated, tumor size of 1.7×1.6×1.5 cm, ER+PR+, AR+, HER2 amplified with sentinel lymph node positive for isolated tumor cells.

*Enhanced CCNG1 gene expression in archived tumor of patient with HR+ HER2+ IBC*. Molecular profiling of the patient's tumor showed enhanced expression of *CCNG1* (Figure 1). *CCNG1* gene expression level (transcripts per million; TPM) was calculated based on the patient's RNA-seq data and was compared to BostonGene's internal reference cohort of diagnosis-matched patients. The patient's CCNG1 expression was in the 23$^{rd}$ percentile indicating that 23% of the cohort had a lower CCNG1 expression than observed in this patient. Molecular profiling also revealed amplification of the following genes – CCND1, ERBB2, FGF4, FGF19, BRD4, FGF3, EMSY, NOTCH3 with TP53 and PIK3CA mutations, indicating chemotherapy resistance and a poor prognosis (36, 44-57).

Based on her molecular profile, the patient opted to receive DeltaRex-G instead of chemotherapy. Post- surgery, the patient received DeltaRex-G (1.2-3-6×10e11 cfu/dose) intravenously three times a week for 4 weeks (one treatment cycle) for a total of 4 treatment cycles, letrozole 2.5 mg orally daily and trastuzumab 2 mg/kg every week up to 18 doses, and then every 3 weeks for 1 ½ years. Treatment with letrozole is ongoing. To date, the patient has received 48 doses of DeltaRex-G, 37 doses of trastuzumab and 710 doses of letrozole with no treatment related adverse reactions and no evidence of recurrence 24 months from diagnosis (Figure 2), with a persistently negative Signatera MRD, an assay for the detection of molecular residual disease (MRD) or circulating tumor DNA (ctDNA) (7). In previous breast cancer studies, a negative Signatera result predicted less chance of recurrence or a favorable response to an ongoing treatment (7).

The oncogenic drivers found in this patient's molecular profile represented proximal oncogenic mechanisms that activate the *CCNG1* pathway (Figure 3), suggesting that inhibition of the *CCNG1* pathway could potentially be a viable adjuvant/first line treatment option for this patient. By inhibiting the *CCNG1* axis, DeltaRex-G restores the function of the lost or disabled *TP53* and *miRNA-122* tumor suppressor genes (58). Moreover, the targeted gene delivery platform of "pathotropic" targeting, which enhances biodistribution of DeltaRex-G into the tumor microenvironment is generally applicable for "pathotropic" delivery of immunotherapy agents as well as for *in situ* vaccination (59).

## Conclusion

In summary, data to support the use of toxic chemotherapy as adjuvant treatment for early-stage triple receptor positive IBC in older women is inadequate. Secondly, *CCNG1* is a novel biomarker that can potentially identify patients who will benefit from DeltaRex-G (a *CCNG1* inhibitor) gene therapy. Thirdly, this is the first FDA authorized treatment protocol using DeltaRex-G, letrozole and trastuzumab as adjuvant therapy for a patient with *CCNG1*+ HR+ HER2+ early- stage IBC and no recurrence of breast cancer was noted two years after DeltaRex-G treatment initiation. Conceivably, a regimen with DeltaRex-G, letrozole and trastuzumab would be a viable adjuvant/first line treatment option for *CCNG1*+ HR+ HER2+ early- stage IBC. Randomized Phase 2/3 clinical trials are needed to confirm this promising concept.

## Conflicts of Interest

SPC, OO, GI, DAB, NO, LF and ST have no competing interest. KS is an employee of Boston Gene and has stock options in Boston Gene. EMG and FLH are co-inventors of the targeted gene delivery system represented by DeltaRex-G.

## Authors' Contributions

SPC, OO, GI, LF, NO and EMG are clinical and collaborating investigators of the FDA approved clinical protocol, and contributed to the design of the protocol, data collection, interpretation, literature review and manuscript drafting. ST and DAB contributed to the literature review and manuscript drafting. KS contributed to molecular and immune profiling studies and manuscript drafting. FLH contributed to the design of the protocol, literature review, data analysis, and manuscript drafting. The final manuscript was approved by all Authors.

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