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► **To cite this version:**

Jaak Ph. Janssens, José Russo, Irma Russo, Luc Michiels, Gilbert Donders, et al.. Human Chorionic Gonadotropin (hCG) and prevention of breast cancer. *Molecular and Cellular Endocrinology*, 2007, 269 (1-2), pp.93. 10.1016/j.mce.2006.06.015 . hal-00531897

**HAL Id: hal-00531897**

**<https://hal.science/hal-00531897>**

Submitted on 4 Nov 2010

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## Accepted Manuscript

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PII: S0303-7207(07)00041-X  
DOI: doi:10.1016/j.mce.2006.06.015  
Reference: MCE 6607

To appear in: *Molecular and Cellular Endocrinology*

Received date: 28-4-2006  
Revised date: 12-6-2006  
Accepted date: 12-6-2006

Please cite this article as: Janssens, J.P., Russo, J., Russo, I., Michiels, L., Donders, G., Verjans, M., Riphagen, I., Van den Bossche, T., Deleu, M., Sieprath, P., Human Chorionic Gonadotropin (hCG) and prevention of breast cancer, *Molecular and Cellular Endocrinology* (2007), doi:10.1016/j.mce.2006.06.015

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## Human Chorionic Gonadotropin (hCG) and prevention of breast cancer

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### Acknowledgement:

The authors acknowledge Magda Buttiens, Medinvents, for preparing the manuscript and the hospital staff in conducting the clinical studies. The study is sponsored by the Hormone Group of the European Cancer Prevention Organization.

**Abstract**

Animal and 'in vitro' experiences learned that hCG (human Chorionic Gonadotropin) is capable to protect from breast cancer. Receptors for hCG/LH (luteinizing hormone) are present on human female and male breast cancer cells. hCG decreases proliferation and invasion of breast cancer MCF-7 cells by inhibiting NF-kappa B, AP-1 activation and other genes. Doxorubicin toxicity is enhanced by conjugation with beta-hCG in MCF-7 cells. All these pieces of evidence suggest that hCG is active in human breast cancer. Direct proof however is missing. We performed a pilot study phase I trial for testing the inhibitory effects of recombinant hCG (rhCG) on primary breast cancer. 25 postmenopausal women with newly diagnosed breast cancers of more than 1,5 cm were biopsied before randomization to receive either 500 µg rhCG (n=20) or placebo. After 2 weeks, surgery was done and tissues were analysed with regard to morphological, immunohistochemical and biochemical changes in tissues and plasma. rhCG reduces significantly the proliferative index and the expression of both the oestrogen receptor and progesterone receptor. rhCG does not modify the hormonal level of estradiol, progesterone, inhibin and follicle stimulating hormone but increases significantly the level of LH. In a second pilot study we tested the clinical efficacy through an open-label single centre study in 13 postmenopausal women with metastatic breast cancer. 500 µg rhCG once every two days shows activity in postmenopausal metastatic breast cancer. The time to progression is relatively short. Response to previous hormonal treatment is indicative for rhCG activity. Given the data in primary and

metastatic breast cancer rhCG further large scale investigation is highly warranted.

rhCG can be an realistic option in (chemo-) prevention trials.

Accepted Manuscript

## ***Introduction***

Breast cancer is the most common malignancy of women in the Western world, affecting approximately 1 in 9. In spite of advances in screening, diagnosis and treatment, approximately one fifth of affected women will die of their disease<sup>1, 2, 3, 4, 5, 6, 7</sup>.

Among the epidemiological factors identified to reduce breast cancer risk is pregnancy, particularly pregnancy at an early reproductive age. Both direct and indirect mechanisms have been postulated for this effect, and of the role of human Chorionic Gonadotropin (hCG)<sup>8</sup>. Further work has identified the hCG receptor in normal human breast tissue and more recently in breast cancer tissue. Human Chorionic Gonadotropin has also demonstrated an antiproliferative effect on breast cancer cells in vitro. This inhibitory effect has been shown to be independent of ovarian function. Based on this evidence, and on the pre-clinical and human safety information generated on this well-characterized hormone, studies were designed on efficacy and safety to evaluate the potential benefit of recombinant-hCG in patients with metastasized breast cancer.

Human chorionic gonadotropin is produced by placental trophoblasts as early as 6 days post-conception and stimulates both corpus luteum and early fetoplacental endocrine functions. To maintain early pregnancy, hCG stimulates secretion of estrogen and progesterone by the corpus luteum organ until this function is assumed by the placenta itself<sup>9</sup>.

Placental hCG is a member of the same family of glycoprotein hormones as the pituitary gonadotropins: human follicle stimulating hormone (hFSH), human luteinizing hormone (hLH), and human thyroid stimulating hormone (hTSH). Human FSH and hLH have similar molecular weights of about 30 kDa. Human CG is a larger molecule of 38 kDa and is the most heavily glycosylated of the glycoprotein hormones, resulting in a longer circulating half-life<sup>10</sup>. Like the other members of this hormone family, hCG is composed of a single alpha-subunit of 92 amino acids (common to the glycoprotein hormones) and a target - specific beta-subunit of 145 amino acids<sup>11, 12, 13</sup>. The first 114 amino acids of hCG share 80% homology with the first 114 amino acids of hLH with which it shares a common receptor the LH/hCG receptor.

At the cellular level, hLH and hCG initiate their activity by first binding to a specific glycoprotein component of the cell plasma membrane, the LH/hCG receptor. Both LH and hCG stimulate adenylate cyclase on the internal membrane converting adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). Cyclic AMP stimulates the activation of an inactive protein kinase which, among the other actions, stimulates steroidogenesis in the mitochondria of the target cell by transforming cholesterol into pregnenolone. Other actions of LH/hCG include the induction of proteolytic enzymes, prostaglandin synthesis, inhibin production, induction of 17beta-hydroxysteroid dehydrogenase and changes in gene metabolism<sup>14</sup>.

Evidence suggests<sup>15</sup> that 72 per cent of the human breast cancers contain a combined hCG/LH receptor on the cell membrane<sup>16</sup>. The presence of the receptor is correlated with premenopausal status, well differentiated tumors, the presence of ER-alpha receptors and the lobular adenocarcinoma subtype. It is presumed that the

presence of hCG/LH receptors predict for hCG activity i.e. inhibition of tumor growth as is the case in vitro situations<sup>17</sup>. Also the progression of preneoplastic lesion such as intraductal proliferations and carcinoma in situ has been noted<sup>18,19</sup>. However, despite the awareness of this useful knowledge, we are not able to analyse the receptors at the time of the study.

The potential benefit of hCG in treatment of breast cancer comes from the demonstration of a direct effect of hCG in the mammary epithelium in vitro using MCF-10 and MCF-7 cells, a normal and a neoplastic human breast epithelial cell line, respectively<sup>20,21</sup>. In these cells, hCG inhibits growth, depresses cell proliferation, lengthens the G1 phase of the cell cycle, and induces the synthesis of alpha and beta inhibin, non-steroidal glycoproteins belonging to the TGF-beta family with demonstrated tumor suppressor activity. There is also evidence that LH/hCG receptors are expressed in these cell lines<sup>22,23</sup>.

Due its molecular similarity to hLH and its ready availability by extraction from urine, hCG has been used pharmacologically for use in men, women and children. In men, hCG induces testosterone production; in women, hCG induces final follicular maturation and triggers ovulation in patients receiving infertility therapy; in male children, hCG induces descent of the cryptorchid testis. Currently, hCG of recombinant origin is at the scene to replace the pharmaceutical product extracted from urine.

Human CG administered to virgin rats induces maturation of the breast, formation of lobules and milk secretion, mimicking the effect of pregnancy<sup>24,25</sup>. The differentiated gland has a lower proliferative activity and a more efficient mechanism of DNA repair,



both acting synergistically to inhibit the initiation of DMBA (Dimethylbenzanthracene)-induced tumors<sup>26</sup>.

A direct role for hCG in the differentiation of breast tissue has been evidenced by the presence of the LH/hCG receptor in normal breast tissue. Other hypotheses suggest an indirect effect of hCG through indirect effects on estrogen or progesterone secretion, or via the paracrine product inhibin. Inhibin is expressed in breast tissue and is induced by hCG<sup>27, 28, 29</sup>. This up-regulation of inhibin occurs in the absence of ovarian function and has been confirmed in in vitro studies.

The discovery that hCG induces the synthesis of inhibin in the mammary gland led to the postulation that hCG acts through stimulation of an autocrine/paracrine loop for inducing gland differentiation. This hypothesis is supported by the fact that the maximal peak of inhibin expression coincides with the peak of lobular formation, an effect that is evident in the absence of ovaries and in human breast epithelial cells in vitro. These data suggest that hCG has a direct role in the induction of final breast maturation and differentiation, and may have an important indirect role in inhibiting further cell proliferation in the breast.

In summary, outstanding laboratory work indicates that hCG is involved in the etiology of breast cancer. Epidemiological evidence supports this work, indicating that timing of the first full term pregnancy is a major risk indicator. But what do we know about the clinical activity of hCG? Does hCG have clinical efficacy in advanced and early breast cancer? If we could consider hCG for prevention of breast cancer, is it a safe product? What is necessary to embark on breast cancer prevention with hCG?

For studying these questions, we examined the therapeutic usefulness of hCG in primary and metastatic postmenopausal breast cancer patients. Postmenopausal women were elected because we did not want to interfere in these clinical pilot projects with fertility aspects of hCG in premenopausal patients. Although this consideration could be of minor importance the question was an issue with regard to the ethical aspects of these new studies.

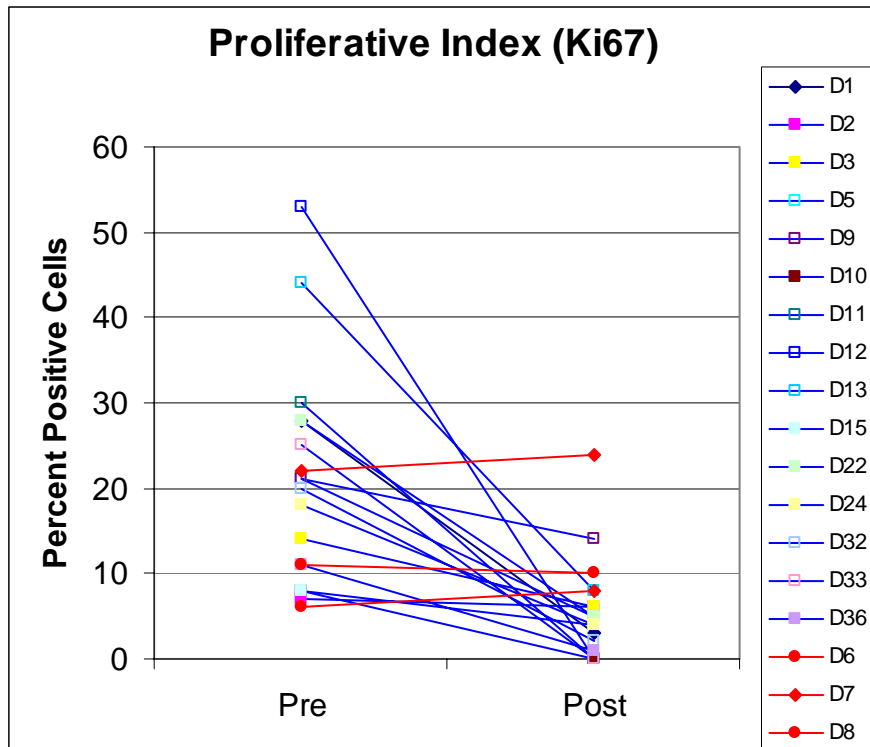
Recombinant-hCG has been widely studied in over 600 patients in a number of clinical indications. Its safety profile has been well characterized and is comparable to that of the marketed preparation of hCG. Clinical evidence of rhCG activity in breast cancer had been studied now in two clinical trials. The first 'proof of principle' study was launched in '98. It was a pilot study phase I trial for testing the inhibitory effect of recombinant human chorionic gonadotropin on advanced primary breast cancer. The second study started in '99. It was an open-label, single center study to test the inhibitory effect of recombinant human chorionic gonadotropin on metastatic breast cancer in postmenopausal women.

***'Proof of Principle'***

Post-menopausal women (25 in total) with newly diagnosed breast cancers of larger than 1,5 cm in diameter were invited to the study. They received multiple tru-cut needle biopsy of the primary tumor for morphological, immunohistochemical and biochemical analyses. Large core biopsy methods were not available at this time. A blood sample was taken as well. 20 patients were given rhCG and 5 patients received placebo. Randomization was done immediately after histological confirmation of malignancy in a 4 to 1 ratio. Then 500 microgram of rhCG or saline was injected IM every other day. Each patient received 7 injections. Tumor response and side effects were evaluated according to contemporary standards<sup>30,31</sup>. Two weeks after the start of the treatment, surgery was performed with either lumpectomy or mastectomy according to the clinical presentation and choice of the patient. Each patient had axillary dissection. The study was terminated after surgery. None of the patients had metastatic disease. Post-surgical treatment was given according to the institutional guidelines with adjuvant radiotherapy, chemotherapy or/and hormone therapy.

Seven intermediate end points of hCG effects on breast cancer were considered meaningful. A decrease in cell proliferation (inhibition) was expected with downregulation of the alpha oestrogen receptor and progesterone receptor. Activation of programmed cell death genes, p53 and p21 tumor suppressor genes, c-myc oncogene and inhibin growth factor was hypothesised as well.

All patients, treated with rhCG, showed a decrease in proliferative index (Ki67) between the start and the end of treatment. No effect was seen in the placebo treated patients.



*Figure 1: Treatment of primary breast cancer with 500  $\mu$ g rhCG decreases the proliferation index, as measured with Ki67 antibodies.*

The proliferation index dropped from an average of 20% of labelled cells up to 5, meaning a 75 per cent overall reduction. The placebo's had an average of 10 per cent and remained at this level after treatment.

Both progesterone (PgR) and oestrogen (ER) receptors decreased from an average of 34 to 10 per cent labelled cells to give an overall reduction of 74 per cent.

During rhCG treatment the oestradiol level in the plasma remained about 8 ng/dl and was identical for the placebo and rhCG group. This observation excludes the possible interference of endogenous oestrogen change as an explanation of rhCG activity. No change was also observed for Inhibin, progesterone, and follicle stimulating hormone (FSH) concentration during the treatment period. In contrast,

(luteotrope hormone) LH concentrations were increased. Probably rhCG interferes with LH measurement.

From this study we could conclude that rhCG reduces significantly the proliferative index in primary breast cancers. rhCG reduces also the expression of both ER and PgR but does not modify the hormonal level of estradiol, progesterone, inhibin nor FSH. The levels of LH seemed significantly increased but here an interference with rhCG can be expected. The subclinical effects of rhCG stimulated the research for clinical antitumor activity.

### ***Metastatic breast cancer in postmenopausal women***

The open-label, single centre study tested the inhibitory effect of recombinant human chorionic gonadotropin (rhCG) on metastatic breast cancers in postmenopausal women. The primary objective was to assess the effect of rhCG on the tumor response rate. Secondary objectives were to assess the effect on symptoms of the tumors, to assess the adverse systemic effects, to measure time to tumor progression and to assess the effects of endocrinology and tumor markers. All 13 postmenopausal breast cancer patients with metastatic breast cancer were treated every other day with 500 µg rhCG IM. Every 60 days, a clinical evaluation was performed with study of the tumor parameters. Tumor response was on radiological or clinical measurable tumor lesions according to the WHO-criteria.

### **Cases**

#### ***Patient GRF: Bone scintigraphy***

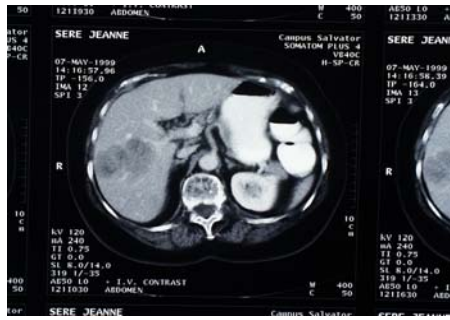
A patient (Figure 2) with bone metastasis had bone scintigraphy at the start of the



*Figure 2: Bone scintigraphy before and 24 days after treatment with rhCG.*

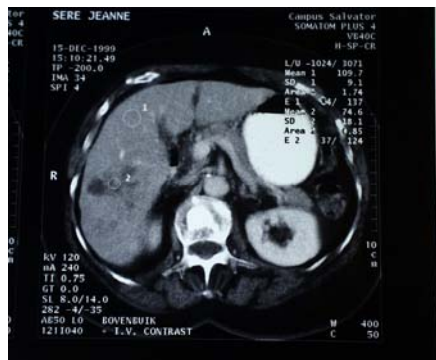
treatment and a CA 15.3 concentration of 568 U/ml. After 240 days the bone scintigraphy

showed no new lesions and the CA 15.3 was 439 U/ml. The patient was considered as stable disease under rhCG treatment.

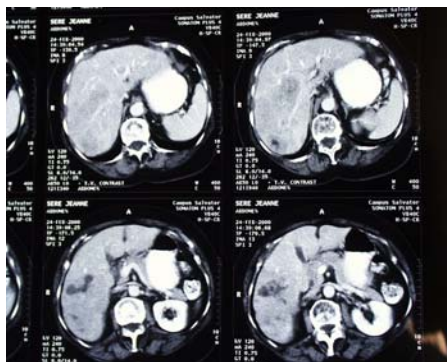


*Patient JS: CT scan liver*

A patient with a liver metastasis of 7,4 x 7,4 cm accepted to undergo rhCG treatment. At that time her tumor marker was 143 IU/ml, after 60 days the

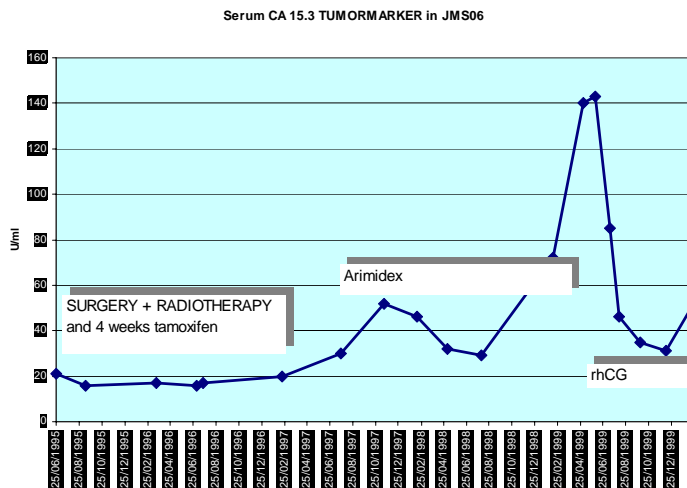


tumor measurements were 4,9 x 4,9 cm and CA 15.3 was 46 IU/ml; at day D120 the measurements were 4,4 x 4,4 cm with a CA 15.3 of 35 IU/ml, at Day 180 the measurements were 5,3 x 4,2 cm with a CA 15.3 of 31 U/ml. After 240 days the patient was progressive because of new lesions. The original liver metastasis measured 5,3 x 4,4 and the CA 15.3 was 52 U/ml.



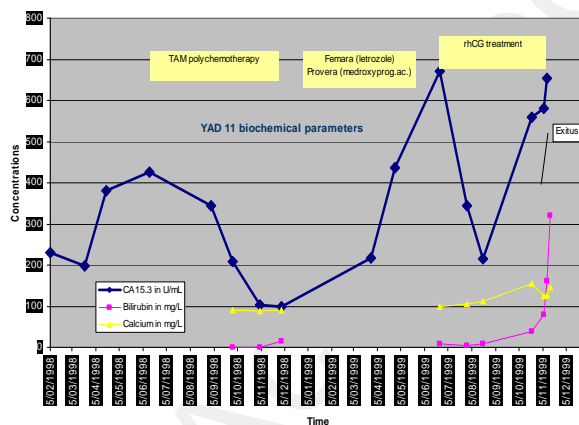
*Figure 3: Patient with liver metastasis prior to rhCG treatment, D60 and D240.*

*Patients JMS and YAD: CA 15.3 tumor marker*



*Figure 4: CA 15.3 chart of a patient treated with steroid hormonal therapy and rhCG.*

chemotherapy was initiated.



*Figure 5: CA 15.3 chart of a patient treated with chemotherapy, hormonal therapy and rhCG.*

the patient progressed and died due to liver function failure.

Two years after surgery and radiotherapy and 4 weeks of tamoxifen (that the patient didn't support) she had recurrent disease that was successfully treated with an aromatase inhibitor for a short duration. The liver metastases were rapidly progressive. rhCG was active during almost one year on the liver metastases. Then

A second patient (YAD) had metastatic disease in bone and liver. By the first sign of metastatic disease she received chemotherapy with paclitaxel, adriamycin and mitomycin C. The metastatic activity regressed. Some months later she progressed and aromatase inhibitors showed no effect. rhCG was successful to reduce the tumor burden for about 2 months then



**Overall results in metastatic breast cancer.**

Thirteen patients accepted to enter the study. They received 500 µg rhCG IM every other day for at least 60 days. Then tumor response was evaluated. The evaluation was repeated every 60 days. 4 patients had progressive disease after 60 days, 7 patients had stable disease and 2 patients had a decrease of the soft tissue localizations for more than 50 per cent of the initial diameters. No complete remission was seen partly as a result of the high percentage of patients with bone lesions. The longest remission was 240 days in a patient with partial remission.

The response in the liver was evaluable in 9 patients: with 2 progressive disease, 2 partial remissions and 5 stable diseases. The longest remission was seen in a partial regression patient and lasted more than 240 days. The response in the bone could be evaluated in 7 patients with 2 progressive diseases and 5 stable diseases. The longest stable disease was 240 days.

Overall the response to rhCG treatments was correlated to the response on previous hormonal therapy. Patients with earlier progressive disease on hormonal treatment (n=2) one had a progressive disease on rhCG. 7 patients with stable disease on previous hormonal treatment showed SD on rhCG and 2 progressive diseased. Of two patients with prior partial remission on hormonal treatment, one has partial remission on rhCG.

**Conclusion**

In conclusion, rhCG is active in the treatment of postmenopausal metastatic breast cancer. The response duration is relatively short but most of the patients had

extensive prior treatments. A response to previous hormonal treatment is indicative for response to rhCG.

### ***General Conclusion and future research***

From the evidence that rhCG has activity on breast cancer cells 'in vitro' and the clinical responses in both early and metastasized breast cancer there is reason to believe that rhCG might be active in cancer prevention trials. Before preventive studies can be undertaken, the clinical results should be confirmed in a large multicentre study. In addition, an activity has to be seen in premenopausal women as prevention should be undertaken long before the disease can emerge. The dose that was chosen was rather arbitrary and based upon physiological levels in pregnant women. Further tuning on the dose of rhCG is a matter of comparative research. More important is the LH/hCG receptor data that could be used to predict activity. These data were not available at the time of the above described clinical studies. Probably the most important need is the identification of intermediary risk factors for breast cancer. These can be on the gene level, in the proteomics area or even clinical. Research in these fields is intense and combination of different parameters (matrix analysis) seems the way to go. Unless there is a consensus on these combination of risk factors, preventive studies tend to be unacceptable costly and long. Nevertheless, when these pilot observations can be confirmed, and considering the abundant epidemiological, animal and preclinical evidence, hCG seems a fine candidate in the chemoprevention of breast cancer.

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