VITAMIN D BINDING PROTEIN-DERIVED MACROPHAGE ACTIVATING FACTOR (GcMAF) INHIBITS HUMAN BREAST CANCER CELL PROLIFERATION AND DECREASES ALPHA-N-ACETYL GALACTOSAMINIDASE IN BREAST CANCER PATIENTS

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INTRODUCTION

- Alpha-N-acetylgalactosaminidase (nagalase) accumulates in serum of cancer patients and is responsible for deglycosylation of vitamin D binding protein (Gc-protein), which is the precursor of vitamin D binding protein-derived macrophage activating factor (GcMAF).
- Deglycosylated vitamin D binding protein cannot be converted into GcMAF and decreased endogenous GcMAF production contributes to immunodeficiency in advanced cancer patients.
- The increase in nagalase activity in cancer patients is due to the fact that cancer cells release nagalase and, therefore, nagalase activity reflects tumor burden, aggressiveness and progression of the disease.
- Determination of nagalase activity is currently proposed as a reliable way of evaluation of cancer severity.

- In serum, nagalase acts as endo-nagalase and it is unable to deglycosylate a monosaccharide, N-acetylgalactosamine (GalNAc), of GcMAF and, therefore, it is unable to degrade exogenously administered GcMAF.
- This led to the proposal of administering GcMAF to patients with elevated nagalase activity.
- It was observed that GcMAF exerts multiple anti-cancer effects *in vivo* and *in vitro*, both in experimental and in spontaneous tumours. The anti-cancer effects of GcMAF are often referred to as "immunotherapy".
- In the clinical cases presented here, we report examples of the results that have been obtained administering GcMAF to breast cancer patients with particular focus on the effects of GcMAF on serum nagalase activity.
- In addition, we report the direct effects of GcMAF on human breast cancer cells in culture.

MATERIALS and METHODS

- Highly purified, activity-tested GcMAF was obtained from Immuno Biotech Ltd, Guernsey, Channel Isles. Common reagents were from Sigma Aldrich (Milan, Italy). Gc-protein was used as control.
- Human breast cancer cells (cell line MCF-7) were obtained from the Istituto Zooprofilattico Sperimentale della Lombardia e dell' Emilia-Romagna, Brescia, Italy.

- A retrospective chart review for analysis of nagalase testing was accomplished on the initial cohort of patients tested by the treating clinicians All records were reviewed by physicians for confirmation of test results, confirmed diagnoses, the time intervals between testing, the dosing of subsequent GcMAF used and the observed clinical responses. The oncologic diagnosis was confirmed by other treating physicians.

- Administration of GcMAF to individual patients was performed exclusively by their treating physicians (Robert Eslinger, MD, Reno Integrative Medical Center; Reno, NV, USA, and Steven Hofman, MD, CMC-Capelle a/d Ijssel, The Netherlands) according to the rules and regulations of each respective Country. The original clinical records are conserved by the physicians in their respective locations as indicated.

- Nagalase testing. Although nagalase is not specific for any particular histological type of cancer, nevertheless, its decrease following GcMAF treatment is considered an index of the therapeutic efficacy of GcMAF since nagalase activity is proportional to tumour burden. Nagalase testing was performed at ELN Laboratories (Bunnik, The Netherlands) following the procedure published by Yamamoto et al. Nagalase activity was determined by using an endpoint enzymatic assay using a chromogenic substrate. ELN Laboratories established a reference range of 0.32–0.95 nM/min/mg of substrate based on serum collected from healthy volunteers, a range slightly higher than that previously reported which was between 0.35 and 0.65 nM/min/mg. Further studies on higher numbers of subjects will establish which reference range is more appropriate. In any case, since all determinations were performed in the same laboratory, a relative decrease of nagalase following GcMAF administration was therefore used as an index of its therapeutic efficacy.

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Potential Conflicts of Interest. DN is the CEO of Immuno Biotech Ltd (the company isolating and purifying the GcMAF protein). However, DN had no knowledge of the therapies being used nor of the names of any patients whose data were being analyzed. Neither he, nor any employee of Immuno Biotech Ltd, had any knowledge of the nagalase or other test results or the patient names used in this study.

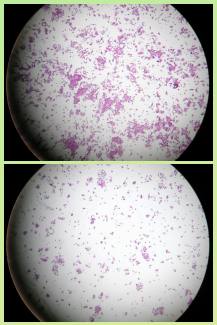
RESULTS

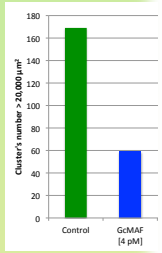
Direct effects of GcMAF on human breast cancer cells.

- MCF-7 cells were starved in serum-free medium for 24 h and incubated with GcMAF for further 24 h. At the end of the incubation period, cells were fixed and stained and the plates were photographed under a microscope at low magnification to appreciate the formation of the typical cancer cell clusters. GcMAF was dissolved in a solvent designed to fit its molecular structure; in particular, the solvent was designed to fit the hydrophobic domains binding vitamin D and fatty acids as well as the hydrophilic domain where GalNAc is attached to threonine at position 420.

↑ ***Upper panel***: Control. Human breast cancer cells form several clusters. Each cluster is formed by about 40 cells.

↓ ***Lower panel***: GcMAF (0.2 ng/ml; 4 pM). The dramatic reduction in clusters is clearly evident. Once dissolved in this particular solvent adapted to its molecular structure, GcMAF exerted a powerful anti-cancer effect at extremely low concentration.





The number of human breast cancer cell clusters with an area larger than 20.000 square microns was significantly reduced after incubation with 4 pM GcMAF.

The size of each individual cell, however, was not changed and in control plates as well as in GcMAF treated plates, the average size of human breast cancer cells was about 530 square microns.

in vitro

Clinical Cases # 1

Female, born 1947. Carcinoma of left breast (found on survey), operated with sentinel nodes in 2010, chemotherapy 4 of 6 series, no specific complaints left. Still some malaise, fatigue and sleep-disorder. Nagalase level at presentation on August 9, 2011: 1.70. January 16, 2012: 1.00. March 12, 2012: 0.72. December 11, 2012: 0.60. GcMAF-treatment (predominantly intravenous route) combined with acupuncture. GcMAF discontinued in April 2012. Aspecific complaints diminished. Patient still seen every few months. A significant decrease of nagalase level can be observed after 5 months of treatment. Such a decrease continued after interruption of GcMAF treatment, reaching normal values about 16 months since the beginning of the treatment. According to the literature, normalization of nagalase level in breast cancer patients is considered an index of eradication of the tumour burden.

Clinical Cases # 2

Female, born 1950. Carcinoma of left breast, specific complaints, metastases probable. After local operation, irradiation of thorax, combined with chemotherapy, Herceptin-therapy. Partly complaints in association with treatments. Nagalase level at presentation on May 11, 2011: 5.60. October 6, 2011: 2.90. February 21, 2012: 1.80. October 18, 2012: 1.10. Treated with intramuscular, later intravenous GcMAF, and a few acupuncture-treatments. No further complaints (subsided in 3-6 weeks), still in intravenous GcMAF-regime. A significant decrease of nagalase level can be observed after 5 months. After about 17 months of GcMAF treatment, nagalase levels are approaching normal values.


GC protein-derived macrophage-activating factor decreases α-*N*-acetylgalactosaminidase levels in advanced cancer patients

in vivo

DISCUSSION

- The observation reported here confirm and extend the results presented in (*Int J Cancer.* 2008 Jan 15;122(2):461-7; *Cancer Immunol Immunother.* 2008 Jul;57(7):1007-16; *Transl Oncol.* 2008 Jul;1(2):65-72; *J Med Virol.* 2009 Jan;81(1):16-26; *Autism Insights* 2012:4 31–38; *Anticancer Res.* 2013 Jul;33 (7):2917-9), and further stress the role of GcMAF in the immunotherapy of cancer and other chronic diseases.
- The results presented here on breast cancer are consistent with the results obtained in a series of patients with advanced cancer treated with GcMAF.


THERAPEUTIC EFFECTS OF HIGHLY PURIFIED DE-GLYCOSYLATED GCMAF IN THE IMMUNOTHERAPY OF PATIENTS WITH CHRONIC DISEASES

- The interest in the effects of GcMAF on human breast cancer cells is further demonstrated by the fact that a recent paper on this topic has been ranked in the top 5% of all scientific articles ever tracked by Altmetric.
- In conclusion, the results presented here support and reinforce the hypothesis that GcMAF treatment could become part of an integrated immunotherapy of breast cancer.