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 evaluating 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 Islands. Common reagents were from Sigma Aldrich (Milan, Italy) and Gc-protein was used as control.
- Human breast cancer cells (cell line MCF-7) were obtained from the Istituto Zootecnico Impruneta 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 clinician. Data were reviewed for confirmation of test results, serum sample collection time, and test results. The oncolologic diagnosis was confirmed by other treating physicians.
- Administration of GcMAF to individual patients was performed exclusively by their treating physicians (Robert Eisinger, MD, Reno Integrative Medical Center; Reno, NV, USA; and Steven Hoffman, MD, CMC-Capile ad IJssel, The Netherlands). The informed consent was obtained in accordance with the rules and regulations for the 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 a chromogenic substrate. ELN Laboratories established a range of 0.32–0.95 mmol/mg/min 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 mmol/min.
- Further studies on higher numbers of subjects will establish which reference range is more appropriate. In any case, all determinations were performed in the same laboratory.
- A relative decrease of nagalase following GcMAF treatment was therefore used as an index of its therapeutic efficacy.

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 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-like GcMAF tumor cells.
- The plates were photographed under a microscope at low magnification to appreciate the formation of the typical cancer-like GcMAF tumor cells.
- In some cases, the formation of these tumor cells was observed in cultures of MCF-7 cells treated with control sera, which were used as controls.

Clinical Cases # 1

Female, born 1947. Carcinoma of left breast (found on survey), operated and excisional biopsy in 2000, chemotherapy for 4 cycles, no specific complaints left. Still some malaise, fatigue and sleep-disturbance.
- 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 was uneventfully intravenously administered 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 after 16 months since the beginning of the treatment. According to the literature, normalization of nagalase level in breast cancer patients is an index of an improvement of the tumour burden.

Clinical Cases # 2

Female, born 1950. Carcinoma of left breast, specific complaints, metastases probable. A few local operations, irradiation of thorax, combined with chemotherapy, Hereceptin-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 intravenous, later intravenous GcMAF, and a few acupuncture-treatments. No further complaints (subbed 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.

DISCUSSION

- The results presented here on breast cancer are consistent with the results obtained in a series of patients advanced cancer treated with GcMAF.

- 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.

References