Abstract
Metastatic cancer is often a fatal disease with low survival rate that in a course of its progression implies a pathogenic cascade involving inflammation, overexpression of reactive oxygen species, loss of DNA repair, genome instability, neoangiogenesis, epithelial infiltration, collagen destruction, and immunosuppression and apoptosis evasion by cancer cells. Understanding the cross-interaction mechanism between the immune co-stimulatory and inhibitory molecules on one side and tumors cells is a key point in the development of a successful immunotherapeutic strategy to fight aggressive cancers. The Active Specific Immunotherapy (ASI) and the GC protein Macrophage Activating Factor (GcMAF) are two immunotherapies capable of modulating the innate and adaptive immunity against cancer. The aim of this work is to present a case of an invasive metastatic carcinoma treated with ASI and GcMAF Forte and discuss the potentials of the individualized immunotherapy in advanced forms of cancer. Immunotherapy improves status of immune system and as a result it increases patient’s life span (and probably survival rate). In conclusion, the ASI and GcMAF Forte may offer a promising prospective immunological biomedical approach to boost immunity and enhance life expectancy in patients with metastatic cancer.

Keywords: Active Specific Immunotherapy, Cancer, Gcmaf Forte, Immunity, Metastasis, Immunotherapy.

Abbreviations
- APC: Antigen Presenting Cells
- ASI: Active Specific Immunotherapy
- DBP: Vitamin D3-binding Protein
- DC: Dendritic Cells
- GcMAF: GC protein Macrophage Activating Factor
- HA: Hyaluronic Acid
- MAF: Macrophage Activating Factor
- MHC: Major Histocompatibility Complex
- NK: Natural Killers
- TAA: Tumors Associated Antigens

Introduction
Tumor progression via metastasizing is one of the leading causes of death in cancer patients. There are different approaches to explain the development of cancer and metastasis cascade events, but recently new insights on molecular and cell immunology reached a common sense in which the immune instability, due to chronic inflammation and oxidative stress, may impair the cellular DNA repairing system and, by doing so, trigger the carcinogenic process of chromosomal and epigenetic mutagenesis, apoptosis evasion, angiogenesis, immune escape, signaling changes and bizarre releasing of inflammatory cytokines [1]. During the metastasis expansion, cancer cells interact with the immune system to stimulate each phase of the pathological cascade and activate the invasion of the surrounding tissues and lymphatic vessels. This immunological interplay was already defined in the 19th century by Rudolf Virchow, who hypothesized that cancer cells were originated from chronic inflamed micro-environment [2]. One of the key problems in cancer biology is the immunosuppressive environment created by tumors, both locally and systematically, that inhibits the variability, plasticity and diversity of the immune cells population and, therefore, their ability to recognize antigens and place an efficient anti-tumor reaction. To initiate the cancer-immunity cycle, cancer antigens will be exposed by an antigen presenting cell (APC) such as the dendritic cells (DC), macrophages and B cells through the Major Histocompatibility Complex (MHC) molecules to the T-cells that will migrate to the tumor site and, due to the epitope-antigen recognition target, induce cancer death by the releasing the pro-inflammatory cytokines and stimulating parallel apoptosis mechanisms. The proper identification of the tumor’s antigens and the specificity of the T-cells against it proved that the adaptive immune system can detect cancer and impair its capability to grow and develop [3]. Other immune cells, such as macrophages, neutrophils, mast cells, eosinophils and the Natural Killers (NK) also have anti-metastatic potential through activation of tumors phagocytosis, reactive oxygen species formation and indirect recruitment of T-cells with anti-cancer chemokines production [4-6]. Suggestions on immunotherapy as an important anti-cancer tool is becoming clearer everyday with the fact that the tumors’ fate depends on the immune system recognition of cancer cells to modulate inflammation and immunosuppression and setting up a quick and efficient anti-tumor action. In fact, the inhibition of the anti-tumor immune response through the systematic immunosuppressive
microenvironment contributes to cancer progression and metastasis [7]. Back in 1893, Willian Coley treated patients with inoperable sarcomas with a pool of toxins derived from killed bacteria [8]. In 2013, after the discovery of several compelling molecules being able to re-activate immunity, Science magazine highlighted cancer immunotherapy as the “2013 Breakthrough of the Year” [9]. Currently there are several prpitiuous immunotherapy strategies evaluated in clinical trials or used as an experimental treatment. For instance, one approach engages to target the T-cell inhibitory molecules such as PD-1 and CTLA-4, suppressors molecules, which, once blocked, will unleash the immune reaction against cancer [7]. Another perspective to fight metastatic cancers is the isolation of the cancer specific pre-activated T-cells from the patient and, after an ex vivo culture and expansion, use the cells as an autologous vaccine in order to recognize the cancer antigens derived from somatic mutations. Such approach has been proved efficient in metastatic melanoma, breast, colorectal and biliary cancers [10-12]. It is possible to activate the direct anti-tumor properties in vivo by the use of DC’s immune role, which can be isolated, maturated and activated in vitro to be subsequently infused into the cancer patient [13]. DCs can also target the cancer derived peptides, DNA or mRNA as a personalized vaccination [14, 15]. Moreover, the same mechanisms of immunotherapy are pertinent to NK cells, neutrophils and macrophages, however its efficacy is yet to been proven [16]. In the past few years the concept of Active Specific Immunotherapy (ASI) has emerged. ASI employs the advanced immunological technologies to use the patient’s unique individual tumor cells’ antigenic diversity in order to instigate the powerful cell-mediated adaptive immune response against his ‘own’ cancer [17]. The basic concept of ASI is to identify the tumors associated antigens (TAA) in order to develop an individualized vaccine, targeting the exact anti-idiotypic antibodies formed against the tumor’s variable regions, in which is expected to form the ligand complex of tumor-antigen-binding-antibodies and therefore, release a systemic innate and adaptive immune chain reaction against the cancer cells. In a previous experience, Bucaea et. al. co-cultured white blood cells from the hepatocellular carcinoma of guinea pigs who have been immunized and cured. That resulted in spontaneously activation and migration of macrophages and other lymphocytes into the tumor surface with the internalization of toxic proteinases by tumor cells leading to a dramatic cytotoxic event and tumor destruction [18]. Based on these studies, it’s understandable that with the proper stimuli and modulation, the immune system already possesses the specific anti-tumor recognition factors that may allow an improvement of cancer patients’ health status.

In our previous works we discussed efficacy of GcMAF Forte in management of advanced metastatic colorectal carcinoma, efficacy of ASI and Super Transfer Factors in management of autoimmune conditions and pathogenic mechanisms of complementary immunotherapy in biological medicine [19-21].

Gcmaf Immunotherapy

Gc-globulin regulates the immunity, osteoclastic activity and act as a primary defense against infectious factors such as immunodeficiency virus and sepsis. Gc-globulin affects the activation and fortification of immune cells exhibiting anticancer activity [22]. Alpha-N-acetylgalactosaminidase a.k.a. Nagalase is a matrix-degrading enzyme secreted by cancer cells during tumor invasion and a component of the envelope protein of several virus, such as HIV and HSV-1 and 2 [23-25]. Nagalase deglycosylates the vitamin D3-binding protein DBP (also known as Gc-protein). Gc-protein contains three sugars and is the precursor for the major macrophage-activating factor (MAF). By complete deglycosylation, Gc-protein can no longer be converted to MAF, which is produced from the Gc-protein by the sequential removal of the galactose and sialic acid without touching the remaining sugar N-acetylgalactosamine. The macrophage main phagocytic activation and antigen presentation is the checkpoint of the immune development cascade and its inhibition leads to immune suppression and possible cancer or virus progression. Increased nagalase activity has been associated with a wide variety of cancers such as breast, prostate, ovary, leukemias, etc. Depending on the size, malignancy and invasiveness of the individual’s tumor, the nagalase may increase [26, 27]. The GcMAF Forte is a preparation of the Gc-protein-derived Macrophage Activating Factor, which is naturally synthesized in the liver from the vitamin D3-binding protein (DBP) combined with a High Molecular Weight Hyaluronic Acid. HA is one of the structural components of the extracellular matrix and it contributes significantly to cell proliferation and migration. The High Molecular Weight HA, when injected subcutaneously into human has a long-lasting very strong stimulus in granulocyte activity, making it a potential treatment for infectious disease and cancer [28, 29]. Administration of GcMAF stimulates the mitogenesis of progenitors immune cells, activates macrophages and produces antibodies, becoming either a powerful immunotherapeutic tool. GcMAF does not activate other immune cells such as DCs, but process the tumor antigens via MHC-II complex-mediation of T-cells and, afterwards B-cells [26]. Finally, GcMAF supports humoral immunity by producing, developing and releasing large amounts of antibodies against cancer [30]. With the understanding of the severity and complexity of the metastatic cancer biology and its resistance to treatments, the aim of this article is to present a case study with the possible benefits of using GcMAF Forte combined with the ASI therapy and a balanced biomedical nutritional regimen of diet and life style.

Case Report

In February 2018, a female patient, 44 years old, elementary school teacher, presented herself to our biomedical center with the following clinical history: diagnosed with right breast cancer (BRAC-2 positive) in 2003, treated with mastectomy and follow up for 5 years with tamoxifen. In 2013, she was diagnosed with left breast cancer, with the further left mastectomy and one year of tamoxifen. In 2015 she was diagnosed with multiple metastases in liver, lungs and bones. She underwent radiotherapy and chemotherapy with Docetaxel and Carboplatin for six months. In 2016, she was prescribed with Letrozole therapy with Zolendronic acid. Parallel to the conventional oncological treatment she performed 6 sessions with systemic hyperthermia. The following PET scan showed reduction of the metastatic lesions in size and quantity. The recent PET scan done in January 2018 showed worsening of lymphadenopathy in lungs, five new liver lesions and overall spreading of metastases to spine and ieliac bones. The CEA and CA-15-3 markers in January 2018 were 8.17 U/ml and 120.8 U/ml respectively. Patient was started on Capecitabine 3000 mg per day, which resulted the immune phenotyping assays becoming severely immunosuppressive, CEA increasing to 9.71 U/ml, CA 15-3 increasing to 290.5 U/ml and patient developing anemia.

She started the treatment protocol with vitamin D3 10.000 IU daily, Artemisia Annua 1200 mg a day, Liposomal Curcumin 9g, Melatonin 3 mg, Fermented Amino complex and a nutrient infusion, 10 sessions,
two times a week, with Vitamin C 25g, Alpha Lipoic Acid 600 mg, Taurine 500 mg, Magnesium 500 mg, Zn 20 mg, Selenium 900 mcg, Mn 2, 18 mg, Glutathione 600 mg, ATP 1mg, B Complex, Bicarbonate and Ferrum Homaccord. Her nutrition was changed to a dairy free, low sugar, Mediterranean-like, enriched with fermented natural probiotics, polyphenols and polyunsaturated fatty acids. Although there wasn’t any change in the tumor metastatic status during the treatment time the patient had dramatically improved quality of life and performance status. Throughout the modified treatment regime patient was not hospitalized, was pain-free, did not develop anemia, had good quality sleep, had good appetite and maintained bodyweight, could enjoy her daily life activities, including doing some light exercises with a personal trainer. In April 2018, after a detailed health evaluation was agreed to advance with a protocol combining the autologous Active Specific Immunotherapy (ASI) with GcMAF Forte.

ASI Preparation
After three days clotting, 30 ml of theuffy-coat of the patient’s peripheral blood was isolated under the GMP laminar-flow-technique and the immune molecular elements separated by patented different biochemical and physical steps. Afterwards a culture of immune activating additives was inserted to the buffy-coat and a preparation of 30 vials of vaccine 1.1 ml each for subcutaneous injections three times per week. The GcMAF Forte was given subcutaneously 1 vial weekly for 10 weeks. The intravenous nutrients were stopped due to the poor condition of the veins; however other oral supplements was continued to be given.

Results
In May 2018, seven days after finished the ASI and GcMAF Forte injections, the measures of the activity of the α-N-acetylglactosaminidase (nagalase) was 1.05 nmol, slightly over the normal reference (<0.95 nmol); which could indicate a good outcome in controlling metastasis progression by the action of the Activated Macrophage Factors (MAF) in inhibiting the nagalase and exposing tumors to the immune system recognition and attack. The immune phenotype analysis showed maintenance of the Dendritic, B, NK and T-cells population and there was no sign of severe anemia. The PET scan and the cancer markers (CEA and CA 15-3) showed stable normal reference (<0.95 nmol); which could indicate a good outcome in controlling metastasis progression by the action of the Activated Macrophage Factors (MAF) in inhibiting the nagalase and exposing tumors to the immune system recognition and attack. The immune phenotype analysis showed maintenance of the Dendritic, B, NK and T-cells population and there was no sign of severe anemia. The PET scan and the cancer markers (CEA and CA 15-3) showed stable disease. Patient continued receiving regular systemic hyperthermia. PET scan and the cancer markers (CEA and CA 15-3) showed stable disease. Patient continued receiving regular systemic hyperthermia. Quality of life was relatively good; patient continued regular light physical exercises, social activities and had a good pain control with small dosage of pain killers on the pro re nata basis. Until the present day patient stays at home with the same biomedical integrative regimen protocols and is also been regularly under the hospital palliative care department’s follow up.

Discussion
In this article we presented a short case study of a patient suffering from metastatic invasive breast carcinoma, resistant to chemotherapy and radiotherapy, with no further options of conventional medical treatment left and a poor life expectancy. Given the multiple hurdles of the case, especially when dealing with aggressive tumor with accelerated metastasizing, most importantly we succeeded in efficiently modulation of immunity, provided strong support in maintaining homeostasis, good quality of life and adequate pain management. As it is known members of the PD1/PD-L1 axis, which are tumor-infiltrating molecules, inhibit the immune responses [31]. This action essentially blocks the cytotoxic T-cells activity against cancer cells that, even with a systemically boosted immunocompetent mechanism, may contribute to the tumor cells survival and escaping the immune recognition. Another problem in cancer vaccines development is the heterogeneity of tumors and the idiosyncrasy of cells and molecular features of the patient’s cancer type. In order to establish the perfect anti-idiotypic antibodies the immune complex must be extremely variable and tuned individually. It is not conceivable to treat an extreme genetically heterogeneous disease with homogeneic tools. The best idea would be to focus on the individuality of one’s immune system and the appropriate adjuvants capable of reducing mortality and preventing the recurrence by adapting to the system. When the ASI is combined with GcMAF Forte and a good nutritional regimen, low in refined sugars, rich in polyphenol and other anti-inflammatory substances, with high dose of vitamins, amino acids and minerals, plus light exercises to retrieve the antioxidant capacity, the patient experienced an obvious increase of life expectancy with improved well-being, better immunity, remaining mentaly well and physically active. With no illusions of complete cure from her condition, but to manage well the time that has been left and keep the best possible immunological status.

Conclusion
Complementary immunotherapy with Active Specific Immunotherapy and GcMAF Forte offers a very promising strategy for cancer management, may support patient’s health status and improve survival rate and life expectancy. Overall, even if considering that every immune boosting vaccines with a possible anti-metastatic potential, when applied as monotherapy, might be rather ineffective or limited to a few tumor types, fighting the oxidative stress, chronic inflammation and immunosupression with combination of ASI and GcMAF Forte may be a usefull biomedical resource for advanced metastatic cancers.

Disclaimer
Authors claim no potential conflict of interests.

References


