Case Report: A Non-small Cell Lung Cancer Patient Treated with GcMAF, Sonodynamic Therapy and Tumor Treating Fields

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Abstract. *Background/Aim: Macrophage activating factor (MAF)-based immunotherapy has a wide application for use in treating many diseases via macrophage activation. Sonodynamic therapy (SDT) using low-intensity ultrasound and tumor treating field (TTF) therapy are novel therapeutic modalities. SDT is usually combined with ozone therapy to improve local hypoxia within the tumor environment. Case Report: We treated a 77-year-old male diagnosed with non-small cell lung cancer ((NSCLC) stage 3B) using second-generation serum GcMAF and oral colostrum MAF-based immunotherapy combined with SDT, TTF and ozone therapies. Results: This case report demonstrates that GcMAF, oral colostrum MAF, SDT, TTF and ozone therapy can be used for NSCLC without adverse effects. Conclusion: This case report suggests a new concept of cancer treatment using local destruction of cancer tissue, in this case conducted with SDT and TTF therapy, to be used in combination with serum GcMAF and colostrum MAF immunotherapy as a systemic treatment.*

Immunotherapy has become an attractive new strategy in the treatment of cancer (1). Gc protein-derived macrophage activating factor (GcMAF), also known as vitamin D binding protein-macrophage activating factor (DBP-MAF), is a potent endogenous macrophage activator found naturally in the blood (2). GcMAF has many biological activities; it activates macrophages *via* superoxide radical generation (3) and phagocytic activation (4) and has been demonstrated to have anti-angiogenic (5, 6) and anti-tumor (7-9) activity *in vivo*. First-generation GcMAF is produced from Gc protein isolated from human serum using an affinity column modified with 25-hydroxy-vitamin D3 (10). GcMAF, that is isolated from serum, is much more unstable in the presence of oxygen at room temperature and in the absence of antioxidants, such as albumin and uric acid, that are abundantly present in blood (11). Second-generation GcMAF is prepared from degalactosylated/desialylated human serum without isolation of Gc protein using vitamin D affinity chromatography. It enhanced the phagocytic activity of mouse peritoneal macrophages in activity assays and extended the survival time of mice bearing Ehrlich ascites tumors (12). In 2014, Saisei Mirai clinics (cell processing center, clinic in Kobe, Osaka and Tokyo) developed a new form of MAF made from bovine colostrum instead of human serum in collaboration with Tokushima University (13). Colostrum MAF has the advantage that it can be orally administered, namely, in an acid-resistant enteric capsule to activate macrophages in the gut-associated lymphoid tissue (GALT) (14). This is considered to be the largest macrophage pool in the body playing a very important role in maintaining and regulating mucosal immunity (14). Additionally, colostrum MAF is administered as a powder in the mouth to activate macrophages in the lymphoid tissue of the mouth and throat, known as the Waldeyer's tonsillar ring (14).

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Key Words: Immunotherapy, macrophage activating factor (MAF), GcMAF, colostrum MAF, sonodynamic therapy (SDT), tumor treating field (TTF) therapy, ozone therapy, non-small cell lung cancer.

Sonodynamic therapy (SDT), which uses the principle of ultrasound-dependent enhancement of cytotoxic activities of a sonosensitizer, can be used to produce free radical oxygen (15) to selectively destroy cancer cells (16-18). The concept of SDT consists of introducing a sonosensitizer into the body that preferentially accumulates in cancer cells (16). Since ultrasound is capable of passing completely through the body, it becomes possible to destroy cancer cells without using damaging invasive procedures. It also has the ability to destroy metastases in most places in the body, making it a very versatile and important therapy. SDT is considered a promising new modality for cancer treatment, without causing serious side-effects.

Tumor treating fields (TTF) therapy is a novel treatment modality delivered *via* continuous non-invasive application of low-intensity, intermediate-frequency alternating electric fields to the region of the tumor (16). TTF have demonstrated effectiveness in the treatment of solid tumors *in vitro* and *in vivo* (16, 17). Several pilot clinical trials and larger randomized studies in patients with solid tumors, including glioblastoma, have demonstrated the feasibility, safety and effectiveness of continuous TTF application in patients (18).

Tumor hypoxia, in which the tumor is deprived of an adequate oxygen supply, is a well-recognized factor in cancer treatment resistance to chemotherapy and radiotherapy, as well as SDT, which requires the production of oxygen-free radicals in order to be effective (19). Therefore, any method of increasing oxygen supply within the tumor environment should increase the efficacy of SDT (19). Ozone therapy is a medical treatment that is used to increase the amount of oxygen in the blood. It is achieved by ozonating the patient's own blood outside the body and injecting it back into the body within a relatively short amount of time. In clinical situations, SDT is usually combined with ozone therapy to improve local hypoxia within the tumor environment (19).

Lung cancer is a leading cause of cancer death worldwide, causing 1.4 million deaths annually (20). The majority of lung cancers are histologically grouped as non-small cell lung cancer (NSCLC) (approximately 87%), with many NSCLC patients presenting with advanced stage III or IV at initial diagnosis (21). Second-line treatments offer only modest survival benefit, often with significant toxicity for patients with advanced NSCLC who progress after receiving a platinum-based chemotherapy regimen (22). Recently, we reported that serum GcMAF-based immunotherapy combined with several other therapies, such as SDT and ozone therapy, was effective in the treatment of cancer patients (19, 23). In this case, we treated a 77-year-old male diagnosed with NSCLC (adenocarcinoma, stage 3B) with serum GcMAF and oral colostrum MAF-based immunotherapy combined with SDT, TTF and ozone therapies.

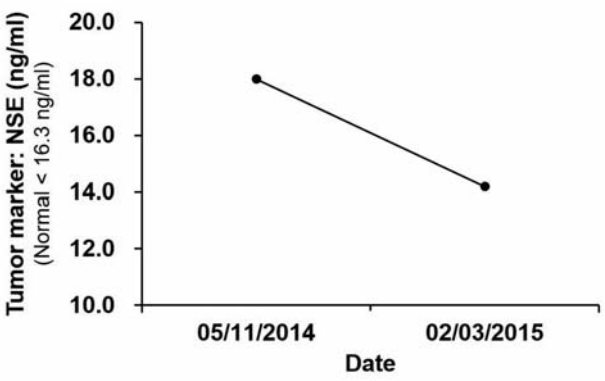

Figure 1. *Tumor marker, neuron-specific enolase (NSE), data before and after treatment.*

Case Report

A 77-year-old male was diagnosed with NSCLC (adenocarcinoma, stage 3B) in January 2014. Surgery could not be offered because it was considered too late. Nine cycles of chemotherapy (gemcitabine/carboplatin/pemetrexed) as palliative chemotherapy was conducted. However, chemotherapy caused kidney damage and was discontinued. In July 2014, the patient began high-dose second-generation GcMAF, which was administered at 0.5 ml, two times a week intramuscularly. In November 2014, he received SDT, TTF therapy and ozone therapy, three times a week, for a total of six times. In March 2015, tumor marker neuron-specific enolase (NSE) decreased to within the normal range (Figure 1). In the same month, he received SDT and Ozone therapy, three times a week, for a total of six times. The patient also started taking daily colostrum MAF, both orally in an acid-resistant capsule and sublingually as a powder in the mouth. He reported better sleep quality, more energy and reduced frequency of nighttime urination (nocturia) after taking oral colostrum MAF. Figure 2 shows chest contrast-enhanced computed tomography (CT) image data before and after treatment. A low-density area inside a right intrapleural nodular tumor, shown in a chest contrast-enhanced CT on 20th April 2015 (Figure 2B), indicates necrotic tissue and the effect of these therapies. The radiologist report indicates that there was no change in the size of the tumor. In other words, the tumor had not increased in size over a 15-month period.

Discussion

Cancer is a broad group of diseases involving unregulated cell growth, forming malignant tumors that invade nearby parts of the body and can metastasize and spread to more distant parts. For effective treatment, it is important to destroy local cancer tissue using therapies with minimal side-effects,

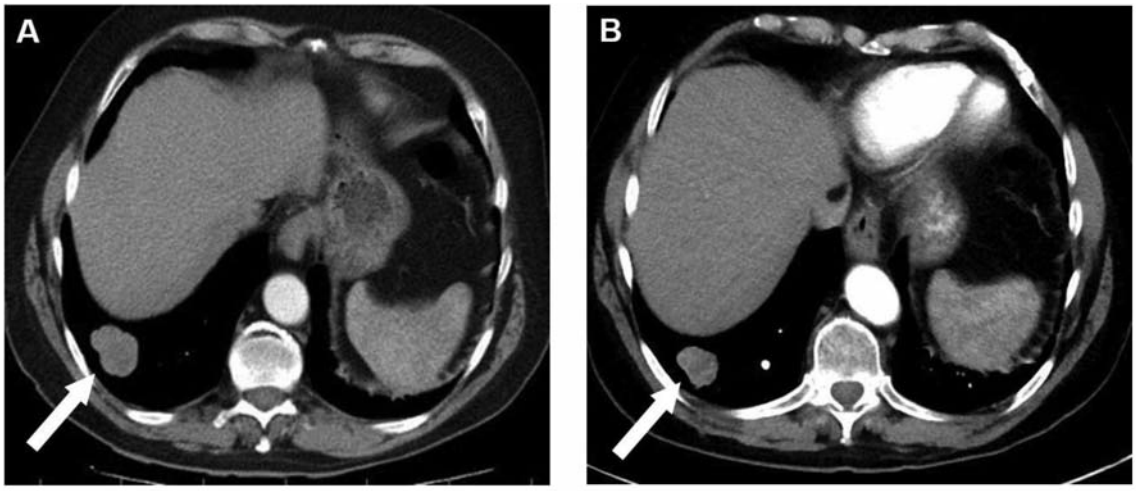

Figure 2. *Chest contrast-enhanced computed tomography (CT) image data before and after treatment. A: Chest contrast-enhanced computed tomography (CT) horizontal plane of a 77-year-old male patient on 13th January 2014 showing a right intrapleural nodular tumor before starting treatment with GcMAF, STD/PDT, ozone therapy and TTF. B: Chest contrast-enhanced CT horizontal plane on 20th April 2015 showing a low-density area inside the tumor.*

while, at the same time, stimulating the immune system. In patients with advanced-stage NSCLC, the prognosis is poor with a median survival of eight months when treated with platinum-based chemotherapy (24). In this case, using a combination of GcMAF immunotherapy, SDT, TTF and ozone therapies, the tumor had not enlarged for 15 months and several of the patient's symptoms improved. We highlight this case of a patient with terminal NSCLC having had good effects by using serum GcMAF and oral colostrum MAF-based immunotherapy combined with SDT, TTF and ozone therapies. It suggests that this combined therapy can be used together with standard treatments, in particular targeted-therapies, with minimal toxicity and without negative effects on the immune system, to achieve better outcomes for patients with cancer. Furthermore, the combined therapy may be capable of controlling tumor progression by inducing direct inflammatory necrosis inside tumors, producing antitumor immunity *via* antigen-presenting cells to prevent immune escape in a variety of deep and superficial tumors. We are planning to further refine and improve our protocols with this combined therapy.

Conclusion

The combination of MAF-based immunotherapy and local cancer destruction therapy can play a central role in future treatments against certain human cancers.

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Received April 5, 2016
Revised May 10, 2016
Accepted May 11, 2016