Clinical Experience of Integrative Cancer Immunotherapy with GcMAF

(GcMAF 含有ヒト血清を用いたがん、AIDS など感染症における臨床効果の検討、及び有効性の高い総合治療プロトコルの開発)

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Inui Toshio

(乾　利夫)

Chapter 1 : Introductory chapter

Cancer is a complex disease characterized by uncontrollable growth and extension of cancer cells. More than 200 types of cancer are known. Because the pathological causes and clinical status of each cancer vary significantly, multimodality therapy that combines surgery, chemotherapy, radiation therapy and immunotherapy is thought to be more effective.

Cancer immunotherapy is the use of the immune system to eradicate cancer. In particular, it involves stimulating the patient's immune system to locate and eliminate the cells that are cancerous. The mechanisms of protection fall into two broad categories – innate immunity and adaptive immunity. There are several types of cells in the innate immune system: phagocytic neutrophils, macrophages, dendritic cells, mast cells and natural killer (NK) cells. The adaptive immune system is comprised of lymphocytes, T-cells and B-cells. These are able to recognize and remember specific pathogens, and their products, including antibodies.

Immunotherapy has become an attractive new strategy in the treatment of cancer (1). The laboratory and clinical study of cancer immunotherapy – such as dendritic cells, autologous lymphocyte-activated killer (LAK) cells, autologous NK cells, monoclonal antibodies, cancer peptide vaccines, cytokines, and biological response modifiers (BRM) – is rapidly advancing. However, in the clinical setting, the results of cancer immunotherapy are mixed.

We therefore contend that cancer immunotherapy should be multimodal and customized to each individual patient. We propose integrative cancer immunotherapy based on second-generation group-specific component macrophage activating factor (GcMAF) as a promising candidate for a patient-friendly cancer immunotherapy.

< Gc protein-derived macrophage-activating factor (GcMAF)>

Macrophages are known to have a critical role in antitumor immunity, can infiltrate into tumors, and are found in most tumor sites. GcMAF was first developed by Dr. Nobuto Yamamoto in 1991 (2), suggesting that it can be used as an important immunotherapy for cancer treatment (3-8).

In previous studies, Gc protein was isolated from human serum using an affinity column modified with 25-hydroxyvitamin D3. GcMAF was prepared from the isolated Gc protein by an artificial enzymatic method, and originally only from 1f1f subtype Gc protein (9). The process of separating Gc protein from serum leads to the much lower stability, concentration and activity of the GcMAF that is produced. Additionally, reuse of the affinity column must be avoided because of cross-contamination between different serum samples. To overcome the problems associated with purification of Gc protein, we prepared degalactosylated/desialylated from

human serum, which we coined serum GcMAF or second generation GcMAF.

Second Generation GcMAF was developed in 2011 by Saisei Mirai in collaboration with Dr. Hitoshi Hori and Dr. Yoshihiro Uto in Tokushima University (10). It has several unique properties, such as increasing phagocytic activity of macrophages (11), superoxide radical generation (12), anti-angiogenesis effect (13), anti-tumor effect, and increases in the number of monocytes in the blood have been observed in many patients (14). In addition, it has been shown to increase the maturation of dendritic cells *in vitro*.

Saisei Mirai will have treated more than 1,000 patients with GcMAF, both with and without conventional therapies (15).

<Colostrum MAF>

In the GcMAF development timeline, there have been two major types of GcMAF until now -- purified GcMAF and serum GcMAF . In 2014, Saisei Mirai developed a new form of macrophage-activating factor (MAF) made from colostrum in collaboration with Tokushima University. This new form, referred to as colostrum MAF, is manufactured using bovine colostrum instead of human serum (16). It is administered orally in an acid-resistant enteric capsule to activate macrophages in the gut-associated lymphoid tissue (GALT), and 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 or pharyngeal lymphoid ring. In addition to these two areas of the body, colostrum MAF may be administered in other areas where macrophages reside. In terms of practical clinical use, colostrum MAF has certain advantages over a MAF produced from serum, because it is derived from bovine colostrum, a food, instead of human serum, and it is administered orally and sublingually instead of by injection. These factors favor its widespread use in the near future.

The mucosa-associated lymphoid tissue (MALT) is scattered along mucosal surfaces in our body and makes up the majority of the human lymphoid tissue. The main function of MALT is to produce and secrete immunoglobulin A (IgA) (17, 18). MALT is populated by lymphocytes such as T cells and B cells, as well as macrophages and plasma cells, where each cell is well situated and prepared for encountering antigens, pathogens through the mucosal epithelium. The components of MALT are subdivided into several groups, such as gut-associated lymphoid tissue (GALT), and Waldeyer's tonsillar ring or nasal-associated lymphoid tissue (NALT) which is made up of the palatine tonsils, nasopharyngeal tonsil (adenoid), lingual tonsil and the less prominent tubal tonsils. Because the tonsils are the first site of contact with inhaled and ingested microorganisms, they are considered the first line of defense against exogenous invaders (19). GALT is considered to be the largest macrophage pool in the body, playing a very important role

in maintaining and regulating mucosal immunity (17, 18). Oral colostrum MAF is directly aimed at activating the huge number of macrophages in these parts of the body to stimulate the immune system.

The general goals of GcMAF and oral colostrum MAF immunotherapy are to improve well-being and quality of life (QOL), return the patient to good health so that they are able to participate in regular life style activities, achieve long term survival, enhance the effect of other therapies, repair the immune system, increase the number of monocytes and activate them to destroy cancer cells, viruses, bacteria, and other pathogens in the body, and increase the rate of maturation of dendritic cells.

<*Sonodynamic therapy*>

There is an ever-increasing amount of data showing that sonodynamic therapy (SDT), which refers to the use of low-intensity ultrasound with a sonosensitizer, can be used to produce free radical oxygen to selectively destroy cancer cells. The concept of *sonodynamic therapy* consists of introducing a substance into the body that preferentially accumulates in cancer cells. This substance is then activated by ultrasound vibration instead of light stimulation, as with traditional photodynamic therapy using a light activated sensitizer. Since ultrasound is capable of passing

completely through the body, the concept of destroying cancer cells without using damaging invasive procedures becomes possible. It is also the ability to destroy possible or known metastasis that is so exciting to users of this therapy. SDT is considered to be a promising new modality for cancer treatment, without causing serious side effects. Recently new sonosensitizers which only respond to ultrasound have been developed in Japan. This allows for the treatment of cancer patients without any light toxicity which can be sustained during exposure to natural sunlight.

<Ozone autohaemotherapy and hyperbaric oxygen therapy>

Tumor hypoxia is a well-recognized factor in cancer treatment resistance to chemotherapy and radiotherapy as well as SDT, which requires free radical oxygen production to be effective. Therefore any method of improving local hypoxia within the tumor environment should increase the efficacy of Sonodynamic Therapy. Ozone therapy is a ~~form of alternative~~ medicinal treatment that ~~purports~~ is used to increase the amount of oxygen by the introduction of ozone into the body via ozonated blood. Hyperbaric oxygen therapy is the medical use of oxygen at levels higher than atmospheric pressure. The equipment used for hyperbaric oxygen therapy consists of a pressure chamber and a means of delivering pure oxygen. In clinical situations,

sonodynamic therapy is usually combined with ozone autohaemotherapy to improve local hypoxia within the tumor environment.

<Hyper T/NK cell therapy>

In a previous study, tumor-infiltrating lymphocytes (TIL) were reported to be effective in experimental and clinical research of advanced cancer (20, 21). TILs recognize specific antigens expressed by autologous tumor cells. Sekine and colleagues developed a feasible method to obtain large numbers of activated or effective TILs (22). Administration of these expanded TILs demonstrates clinical activity in some patients with several types of cancer, making it a useful adoptive immunotherapy.

We developed a cultivation method with autologous plasma from patients with specific antibodies for membrane antigens of NK cells based on Sekine et al's techniques and our own clinical results. This cultivation method is used not only to obtain activated T-lymphocytes, but also 'hyper T-cells' and NK cells. Hyper T-cells, a name we coined, are unique immature multipotent T-cells with various capabilities. Hyper T-cells have a broader specificity for antigens expressed by autologous tumor cells, are able to proliferate, and maintain their activity for long periods *in vivo* (23). Therefore, expansion of hyper T-cells has the potential for being a

suitable and important factor in adoptive immunotherapy against cancer. NK cells are a unique subset of lymphocytes, distinct from T-lymphocytes. They contribute to essential immune systems, such as host antimicrobial and antitumor immunity, without requirement for prior immune sensitization of the host (24). NK cells are promising effector cells for immunotherapy against cancer. For immunotherapy, three cell types—T-lymphocytes, hyper T-cells, and NK cells—are cultured simultaneously. We developed a simple method of simultaneously culturing T-lymphocytes, hyper T-cells, and NK cells, which together seem to be important for effective immune therapy. Using these cells in combination allows the advantage of one cell type to compensate for the disadvantage of using the other alone – their synergistic actions contribute to the eradication of tumor cells.

<High-dose vitamin C therapy>

The recommended dietary allowance (RDA) of vitamin C for women is 75 mg/day and for men is 90 mg/day. While the regular RDA dose of vitamin C is employed as a nutritional supplement, high-dose vitamin C (50-100 g) is advocated as an anticancer agent. Concentrations of 1,000-5,000 μmol/l are selectively cytotoxic to tumor cells *in vitro* (25, 26). Plasma concentration of vitamin C after intravenous infusion of 50-100 g of vitamin C reaches about

3,000-4,000 μg/ml or 17,000-22,700 μmol/l. Tumoricidal ascorbate concentrations can be achievable in the human body without significant side-effects.

<*Alpha lipoic acid*>

Alpha-lipoic acid is approved in Germany as a drug for the treatment of polyneuropathies, such as diabetic and alcoholic polyneuropathies, and liver disease. Alpha-lipoic acid has antioxidant effects. It is reduced intracellularly to dihydrolipoic acid, which in cell culture regenerates by reduction of antioxidant radicals, such as vitamin C and vitamin E (27). Alpha-lipoic acid enhances the antitumor efficacy of ascorbate to the point where significant tumor cell killing can occur at concentrations achievable by intravenous infusion (28).

Chapter 2 : Case report

Case Report 1.

55-year-old female was diagnosed with breast cancer (left side, with skin invasion) in August 2009. She was treated by lumpectomy, with no chemotherapy or radiotherapy. She refused any further standard treatment after the operation. The tumor was estrogen, progesterone, and Herceptin receptor positive. In October 2011, she noticed a right axillary tumor. At that time there were no treatments being undertaken. The tumor kept growing and tumor markers were increasing. In July 2012 a needle aspiration biopsy was done to confirm the recurrence of the tumor. She still refused standard treatment and started receiving Hyperthermia (total 24 times with Thermotron RF-8) and i.v. high dose vitamin C (total 10 times). She presented in my clinic in January 2013. Her symptoms at presentation were a cough, back pain and severe swelling of the right arm (edema), and her pathological findings were invasive ductal carcinoma (IDC), N0 (no nodes were involved), margin (–), grade 3, ER+, PR+, Her2+. Chest PET CT on 6-Jun-2013 (Figure 1) showed (A) rt. axillary tumor (B) backbone metastases (C) intrapleural nodular tumor and rt. pleural effusion.

Second generation high dose GcMAF was conducted 0.5 ml, 2 times weekly intramuscularly,

total 21 times. Sensitizers for SDT were modified Tin Chlorin e6, 25 mg i.v., and 5-aminolevulinic acid (5-ALA), 10mg/kg BW orally. A total of 19 treatments of SDT were conducted from June to September 2013. Exemestane (Aromasin) aromatase inhibitor was also given to the patient at a dose of 25 mg/day orally.

By the beginning of October 2013 the patient showed dramatic improvement of symptoms such as cough, back pain and rt. hand edema from the combination therapy with SDT, GcMAF and hormone therapy. Her axillary tumor (Figure 1A) decreased in size and disappeared completely.

Chest PET CT on 6-Jun-2013 (Figure 2A) showed lung pleural effusion and intrapleural nodular tumor before treatment with SDT. A later chest CT on 9-Sep-2013 (Figure 2B) showed the complete disappearance of the pleural effusion and intrapleural nodular tumor in the right lung after treatment with SDT, GcMAF and hormone therapy.

There was a significant increase in monocyte percentage and monocyte number (Figure 3) and a rapid decrease in tumor markers (Figure 4) during the treatment period from January 2013 to October 2013. There were no serious side effects from the treatments except slight joint pain from the hormonal therapy with Exemestane aromatase inhibitor.

Case Report 2.

74 year old female with pancreatic cancer, multiple liver metastasis and rheumatoid arthritis was admitted to hospice for terminal care. On the 4th October 2014, while in the hospice, she developed a high fever. After developing the high fever she was treated with antibiotics but the high fever continued. She had loss of consciousness on 8th October 2014. Two days later, she was temporarily transferred to another hospital for emergency tests and treatments. No evidence of pneumonia was found so on the 14th October 2014, she returned to the hospice and received intravenous hyperalimentation (IVH) feeding. Patient continued to remain in deep coma with continuing high fever and antibiotics not working, but causing renal function disorder. On 18th October 2014, she was diagnosed to have several hours to several days to live, so family members gathered to farewell her in the hospice. On that night, the patient's son began to apply colostrum MAF powder dissolved in a small amount of water to the lymphoid tissue areas in the mouth, 2 times per day. The next day the patient showed a reduction in the fever and in 3 days the fever had almost disappeared. On 29th October 2014, after 3 weeks in a coma, and 11 days after starting oral colostrum MAF, patient opened her eyes and was able to follow movements with her eyes. On 4th November 2014, patient started to talk and wanted to eat, being surprised the month was already November. At this point eating rehabilitation was started while at the same time continuing IVH. The doctor in the hospice said that this result was a miracle. It was the first case observed out of 2000 patients who died in the same situation, saying oral colostrum

MAF was an amazing medicine.

Case Report 3.

71 year old female with chronic fatigue syndrome (CFS). She had been suffering from symptoms such as dizziness, chronic fatigue, palpitation, tachycardia, depression, unrefreshing sleep, stomach pain, regular sore throats and so on for around 40 years. She was diagnosed with autonomic neuropathy, climacteric disorder, depressive state, Meniere's disease, chronic gastritis, cardiac neurosis, chronic cystitis, post hepatitis B infection, colon polyps and so on by physicians and psychotherapists. Her blood tests showed no abnormality except cholesterol which was slightly elevated, with sometimes borderline high transaminase. Brain MRI scan, cardiac catheter examination and gastroscopy showed no abnormality within the last 2 years. Colonoscopy showed colon polyps which were removed during the colonoscopy examination and an abdominal CT scan showed only a liver cyst. On the 5th November 2014, she started to take daily colostrum MAF powder in the mouth, exposing the lymphoid tissue with the resident macrophages, and also colostrum MAF orally in an acid-resistant enteric coated capsule. Within a few days, she noticed that her malaise, dizziness, tachycardia, insomnia, nocturnal urination and stomach pain were improving and she was feeling better with improved quality of life. Over

a one month period she also noticed that her skin became smooth, silky skin and that the blotches on her face and arms became lighter, disappearing in some places on the skin. She was very happy, being able to do her usual work with more energy like most other people do. Over a four month period, she also noticed improvements in hair growth on her head.

Case Report 4.

45 year old female with chronic fatigue syndrome (CFS). She is the daughter of the previous case report patient. She had stomach pain, severe fatigue especially after work, unrefreshing sleep, headaches, pain in the joints and so on since she was 20 years old. When she was younger, her body weight was 43 kg which declined over the years to 37-38 kg. This fatigue could not be improved by bed rest or taking medications and got worse from working. Her blood tests showed no abnormality and a brain MRI scan, abdominal MRI scan and an ultrasound scan were also normal. Gastroscopy showed gastritis and the presence of H. pylori bacteria in her stomach, for which she was treated with antibiotics. After getting rid of the bacteria, her severe stomach pain still continued and she was not able to control the symptoms even with proton pump inhibitors (PPIs). On the 12th November, she started to take colostrum MAF powder in the mouth, and one capsule orally a day, as described in the previous case report. In a couple of

days, she felt much better with reduced malaise in the morning and reduced stomach pain. She was able to move her body easily without the usual joint pain, muscle pain and fatigue, and began forgetting to take her stomach pills. Within a one month period, she noticed that the freckles on her face became lighter and her skin became smooth and silky. She was very happy because she didn't have symptoms of malaise anymore, had good sleep, significantly decreased stomach pain and decreased menstrual cramps. Within a 4 month period, she noticed her hair was regrowing all over her head. Due to occupational use of paint thinner she had lost hair on her forehead which was now coming back.

Case Report 5.

A 71-year-old man was diagnosed with thymic carcinoma with lung metastasis. The patient received 24 weeks of the integrative immunotherapy. No progression of the cancer was found 12 months after completion of the therapy.

Case Report 6.

A 74-year-old man was diagnosed with prostate cancer with multiple bone metastases. He received 12 weeks of the integrative immunotherapy combined with hyperthermia therapy. Bone scintigram results nine months after initiation of the therapy were normal and metastatic tumors had disappeared.

Case Report 7.

A 72-year-old woman was diagnosed with metastatic liver cancer after sigmoidectomy and bilateral oophorectomy. She received 24 weeks of the integrative immunotherapy combined with 55 Gy of radiation. There was no evidence of local recurrence or metastatic disease on Positron Emission Tomography (PET) and Computed Tomography (CT) scans 12 months after initiation of the therapy.

Case Report 8.

20 year old female was diagnosed with atopic dermatitis. Blood test showed high IgE. She started taking oral GcMAF, 2 capsules daily. At first her skin became sensitive and reddish,

feeling itchy. After one month, her skin became stable (Figure 5). After 2 months, her skin became very smooth, silky and soft.

Case Report 9.

13 year old female was diagnosed with atopic dermatitis. Blood tests showed high IgE. She started taking oral GcMAF, 2 capsules daily. Reduced skin redness, reduced scrattching and improvement in skin condition are remarkable (Figure 6).

Case Report 10.

12 year old male was diagnosed with alopecia totalis. He is diagnosed when he was 10 years old. His mother suffered from alopecia 20 years ago. He took steroid lotion, anti-histamine drugs and light therapy – none of the treatments worked. He started taking oral GcMAF, 2 capsules daily. After 2 months there was visible evidence of new hair growth over the whole head (Figure 7).

Case Report 11.

50 year old male was diagnosed with colon cancer stage 2. This patient suffered from severe

pollinosis (hay fever) for 20 years during the peak hay fever period (from February to May) every year. Every year he took anti-histamine, and sometimes laser treatment. He began taking only oral GcMAF, 2 capsules a day. In 3 days, his symptoms improved and he no longer required anti-histamine medicine. There was a significant decrease in tumor markers CEA as 6.2 μg/l to 5.6μg/l during the treatment period from July 2014 to October 2014. Flexible sigmoidoscopy was examined remarkable shrinkage of tumor (Figure 8).

Case Report 12.

We had an amazing case of 45 year old male with multiple sclerosis (MS), end stage progressive for 24 years. Diagnosis was made in 1989, confirmed by lumbar puncture and MRI scan of the brain. He was wheelchair bound for 4 years. Initially pulse steroid therapy worked. Second generation high dose GcMAF was conducted 0.5 ml, 2 times weekly intramuscularly and later he started taking 4 capsule of oral GcMAF daily. Responded is very well to GcMAF from the start. He has gone off all medications for pain and bladder control and is off antibiotics. After 6 weeks treatment, patient is able to walk for the first time after 4 years being wheelchair bound. He has complete bladder control – without medication. Brain fog is much better.

Chapter 3: Discussion and Conclusion

We have used this multimodality integrative immunotherapy based approach in nearly more than 1,000 patients with cancer. The results of this integrative immunotherapy look hopeful. We also plan to conduct a comparative clinical study to clarify its efficacy to that of several integrative immunotherapies using different Gc protein subtypes, different concentrations of GcMAF, and different macrophage status to find the relationship between each therapy and the curative effect of GcMAF-containing human serum. We aim to determine the optimal combination of the integrative immunotherapy from the results of these clinical and analytical studies.

This type of integrative immunotherapy can be of benefit to patients and is a promising treatment. We expect that the described immunotherapy using second-generation GcMAF will play a central role in future treatments against human cancer, both alone and in combination with other therapies, such as sonodynamic and photodynamic therapy.

We showed the case report of a terminal breast cancer patient having had good effects from SDT, GcMAF and hormonal therapy (case report 1). It suggests SDT and GcMAF can be used with standard treatments to get better outcomes for cancer patients. SDT, GcMAF and hormonal therapies are non-invasive, well tolerated treatments that may be capable of controlling tumor progression by working synergistically. Furthermore, SDT and GcMAF may be capable of

controlling tumor progression by inducing direct inflammatory necrosis inside tumors, producing anti-tumor 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 SDT and GcMAF.

At some point, patients with a terminal illness tend to be placed in palliative care, which provides pain relief and other measures designed to make the end stages of terminal illness as comfortable as possible (19). It is common that once a patient reaches this point, curative treatment efforts are discontinued or scaled back. By this stage the treatment for the disease may no longer be effective and can be as painful and uncomfortable as the disease itself. So naturally the focus turns from treatment to palliation with one of the most important goals of medical practitioners being to ease suffering and improve quality of life (19). With this new goal in mind, we need to consider the factors which cause discomfort and reduced quality of life. In patients with cancer, fatigue and anorexia rank as the top two reasons for emotional and physical distress, with pain ranked third. Nausea, constipation, altered mental state, such as delirium, and dyspnea have been described as the next most common symptoms. It is not uncommon for family members to interpret fatigue to mean that the patient is "giving up," when in actual fact the symptom of fatigue is beyond the patient's control (30).

Infection is responsible for substantial patient distress and an important contributing factor in

many of these adverse symptoms (29). Mohammed (29) goes on to suggest that although infection in cancer patients has been widely reported, very few studies have focused on infection and its management in palliative care patients. Antibiotic use is common practice in palliative care in patients with advanced cancer as one of the supportive treatments near the end of life as a means of controlling infections (29). A big dilemma for physicians caring for terminal cancer patients is deciding whether antibiotics are effective and providing benefit or not. And it must be considered that antibiotics are not necessarily safe medications, have a long list of potential adverse effects and increase the risk of antimicrobial resistance. In terms of colostrum MAF, we don't have this dilemma since there are no side effects and there is no risk of resistance to the treatment. Therefore the benefit-risk ratio is very high with this option. By comparison, the benefit-risk ratio use of antibiotics is more difficult to determine. As we saw in our case study of infection in a terminally ill cancer patient, GcMAF based immunotherapy has demonstrated that it's quite effective and could play a critical role in combination with, or even without antibiotics in patients with cancer and infections. The treatment with colostrum MAF has been shown to be non-toxic, improving quality of life (QOL), prolonging life and curing the infection, which addresses the major goals of palliative care.

With regards to CFS, we highlighted case reports of two patients with CFS who had very good effects from oral colostrum MAF. It suggests oral colostrum MAF can be used to achieve

much better outcomes for patients with CFS, including additional benefits such as skin repair, decreased freckles, blotches, and hair regrowth. Even though the cause of CFS has as yet not been identified, research suggests that infections and immune dysfunction are thought to play a critical role in the development of the disease. Key findings point to increased gut permeability and an anti-LPS immune response to gram-negative enterobacteria with subsequent gut-derived inflammation, causing systemic inflammation and oxidative and nitrosative stress. Research by our group finds that macrophage activation with GcMAF-based immunotherapy, unlike that of LPS does not result in nitric oxide (NO) and tumor necrosis factor (TNF-α) and interleukin-1 beta (IL-1β) cytokine production (4, 31). According to research by Uto *et al*. (4), 10 ng colostrum MAF has a significantly higher macrophage phagocytic activity than 1 μg LPS, and at a much lower concentration. Therefore it suggests that macrophages have a much higher affinity for activation by GcMAF than for LPS. Administering exogenous GcMAF will result in suppression of the LPS related macrophage activation and thus GcMAF will induce a good phagocytosis without the IL-1β and TNF-α release. These experimental results are interesting considering the findings that some cytokines are implicated in fatigue symptoms, correlated with our clinical findings of reduced fatigue in CFS patients with colostrum MAF therapy.

There are two ways colostrum MAF is able to act. By administration orally in the enteric capsule allows it to reach the gut where it can activate macrophages in the Payer's patches and

from there enter the blood stream. There is also the possibility that colostrum MAF can act via sublingual absorption into the bloodstream where it can reach many places in the body. Our clinical results suggest the possibility that the colostrum MAF molecule is able to be absorbed via either of these routes allowing it to have similar effects to injected GcMAF.

Some researchers suggest that symptoms of CFS and fatigue in patients generally can be caused by chronic cytokine production due to bacteria when macrophages are activated by LPS. Our work and that of others finds that macrophage activation by purified GcMAF, serum GcMAF and colostrum MAF does not result in production of cytokines while still increasing phagocytosis (4).

In conclusion, it is clear that infectious diseases are important factors in both chronic fatigue syndrome and cancer with both diseases sharing the common symptom of fatigue. Considering our case studies of a terminal cancer patient with serious infection and patients with CFS, it is clear that oral colostrum MAF addresses the possible cause(s) as well as the symptoms of fatigue in these very different and seemingly unrelated diseases. We propose further clinical and experimental work to elucidate the mechanisms by which MAF has beneficial effects on fatigue in cancer, chronic fatigue syndrome and other chronic diseases. Importantly, colostrum MAF shows promising clinical results in patients with infectious diseases and for symptoms of fatigue which is common in many chronic diseases.

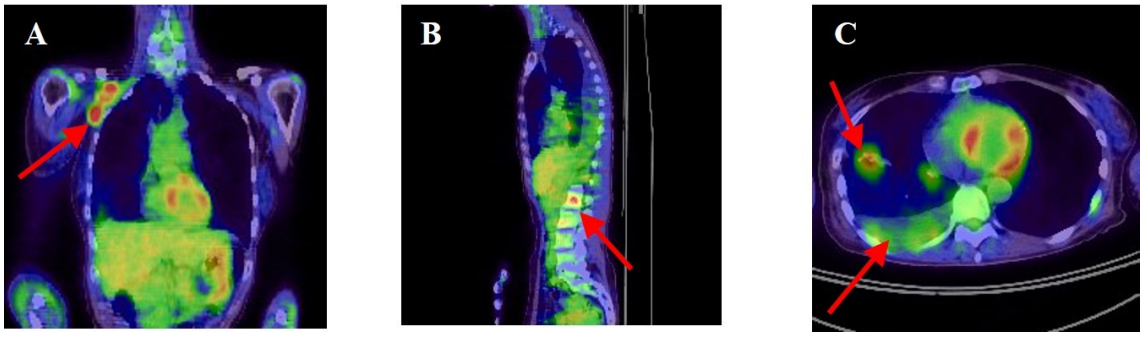


Figure 1. Chest PET CT of 55-year old female on 6-Jun-2013 shows (A) rt. axillary tumor (B) backbone metastases (C) intrapleural nodular tumor and rt. pleural effusion.

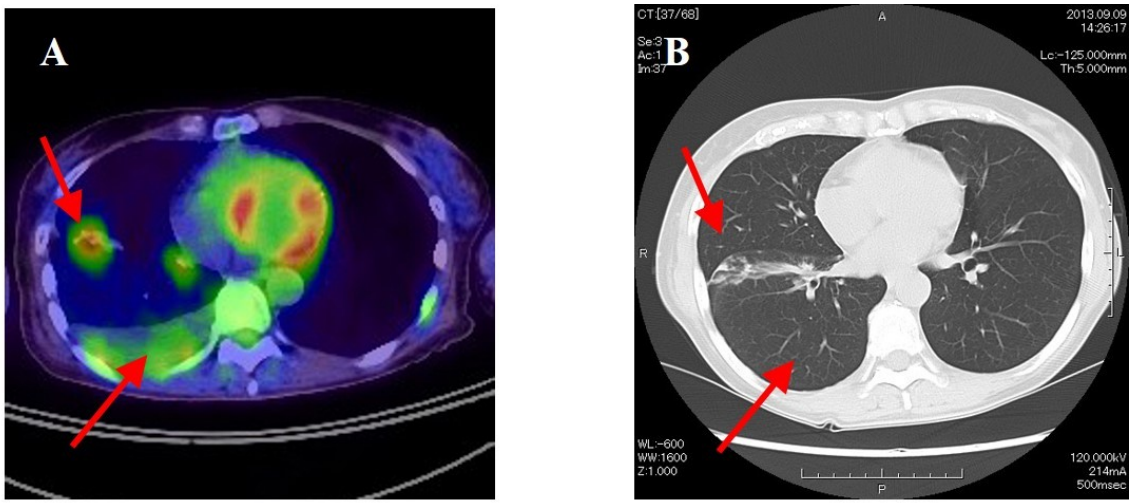


Figure 2. (A) Chest PET CT horizontal plane of 55-year old female on 6-Jun-2013 shows lung pleural effusion and intrapleural nodular tumor before treatment with SDT. (B) Chest CT horizontal plane on 9-Sep-2013 shows the complete disappearance of the lung pleural effusion and nodular shadow in the right lung after treatment with SDT, GcMAF and hormone therapy.

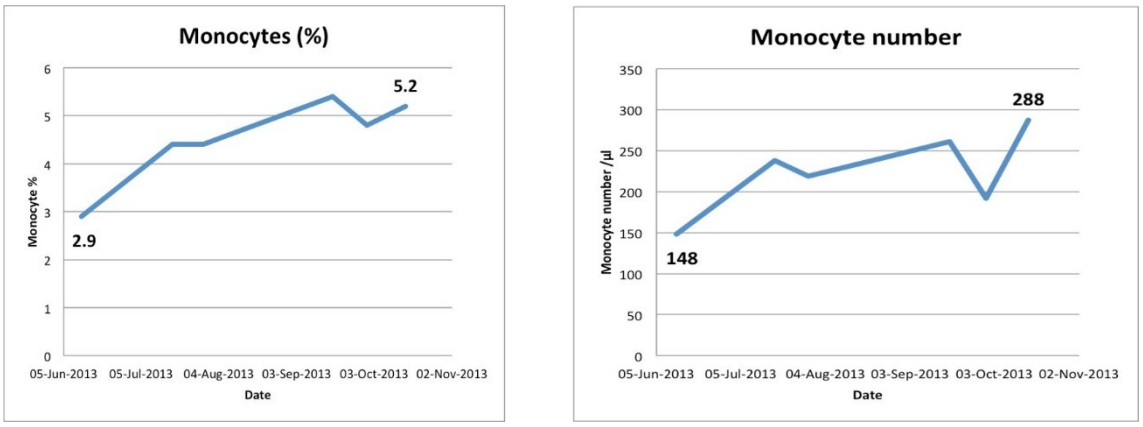


Figure 3. Change in blood monocyte percentage and monocyte number of 55-year old female during GcMAF therapy. The patient's monocyte number rose during high dose GcMAF treatment and indicated a good response to the therapy.

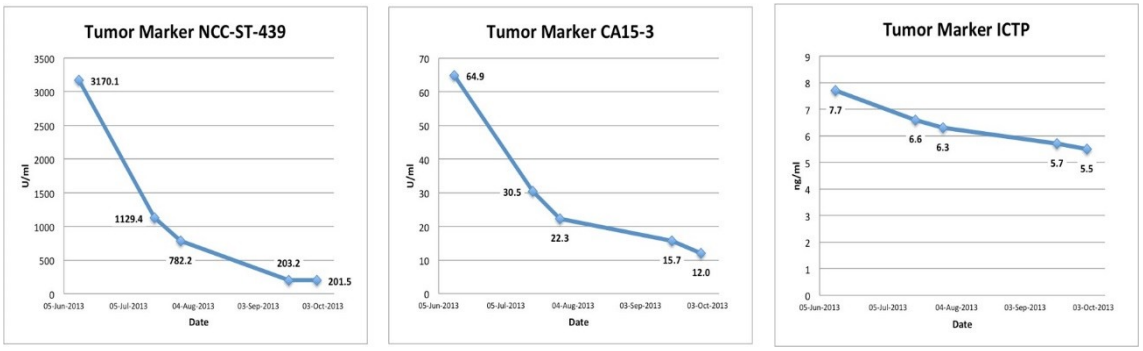
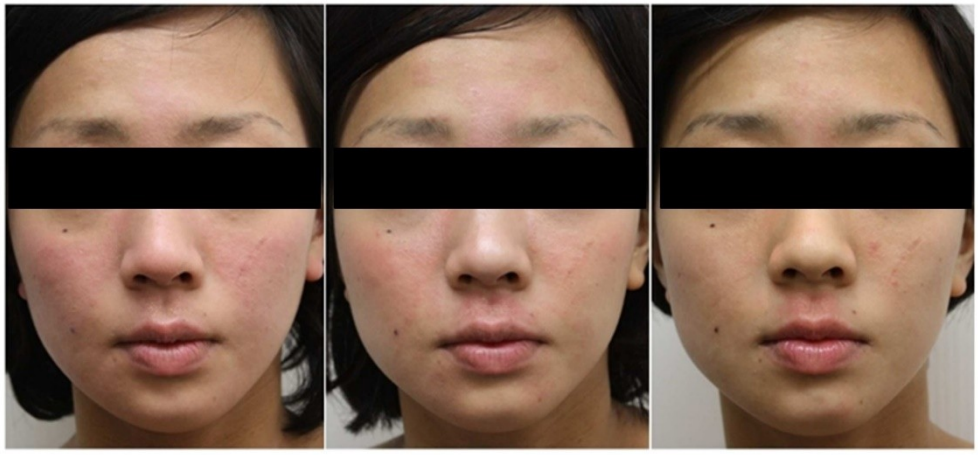


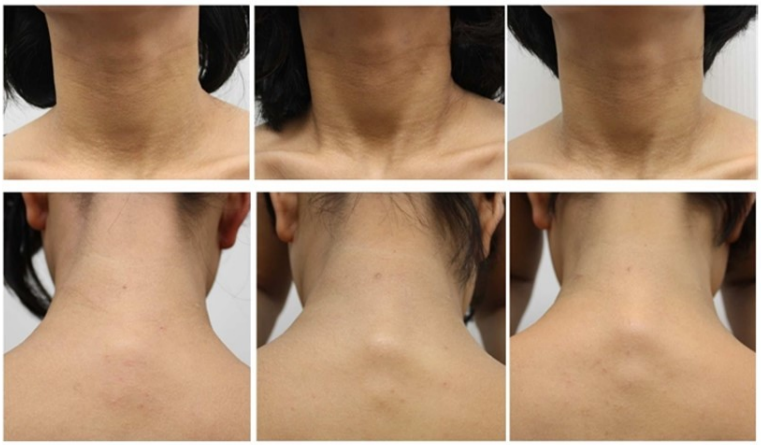
Figure 4. The patient's tumor markers rapidly decreased during the treatment period.

Face

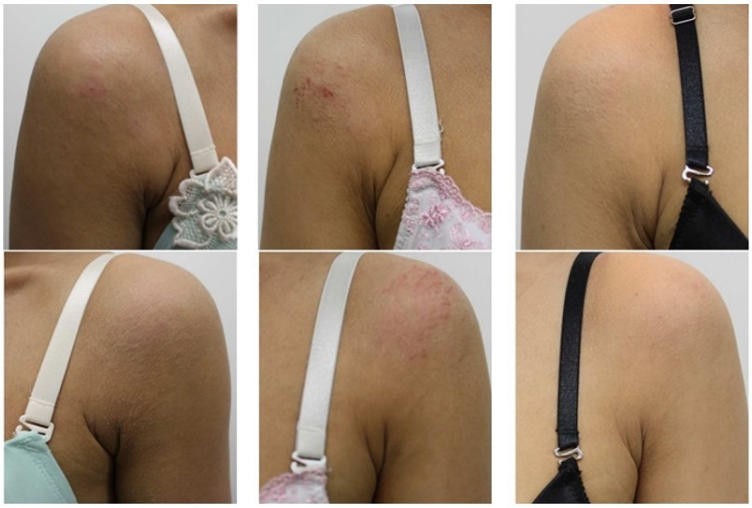


2014.12.18	2015.1.22	2015.2.26
Before	**1 month**	**2 months**

Neck



Shoulder



2014.12.18 **Before**

2015.1.22 **1 month**

2015.2.26 **2 months**

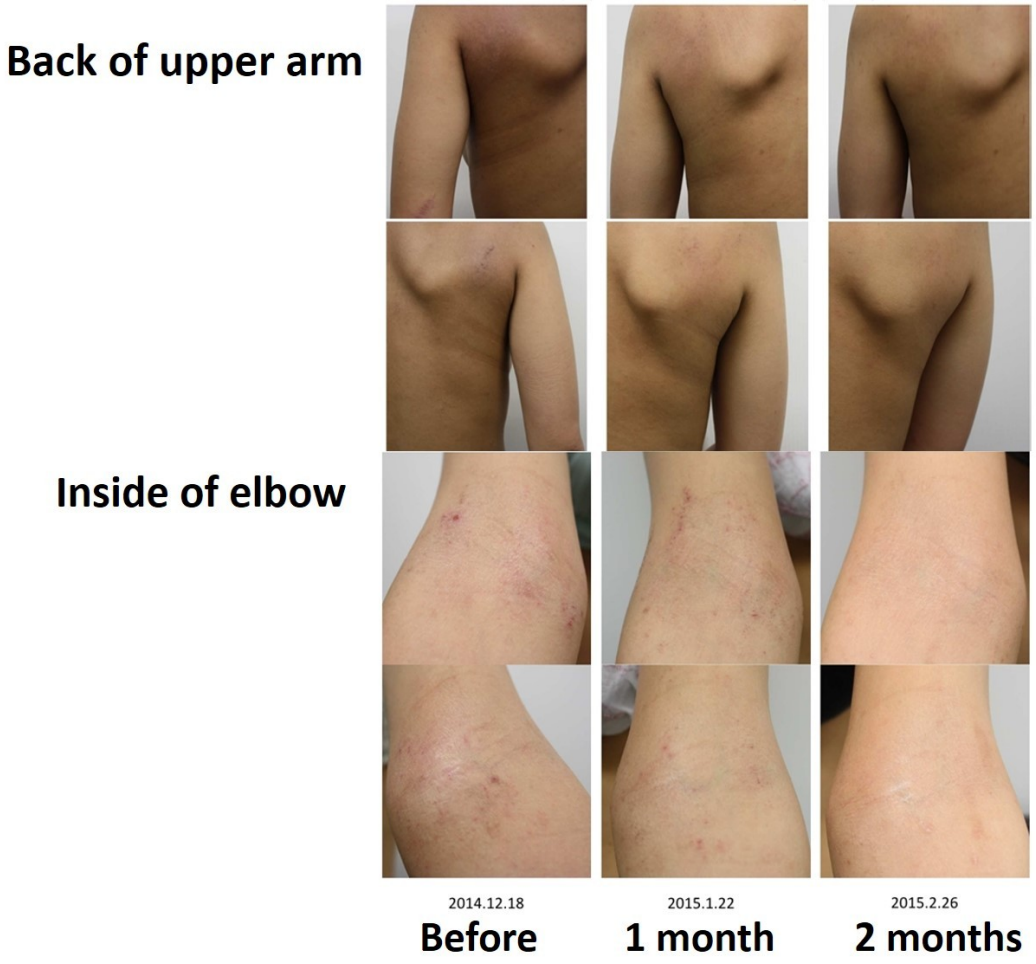
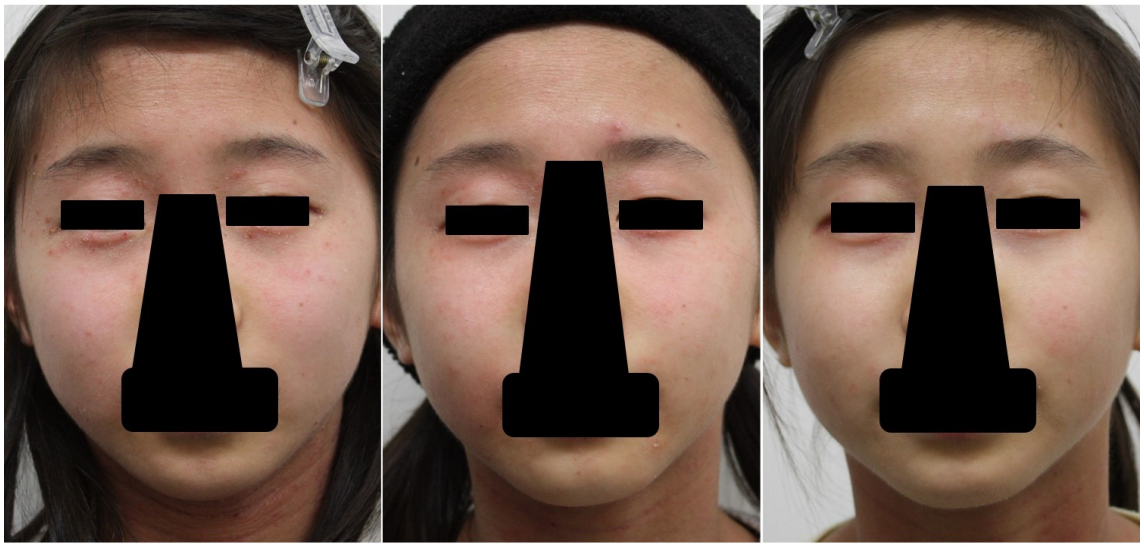


Figure 5. The pictures of 20 years old female was diagnosed with atopic dermatitis, before treatment, after 1 month and after 2 months treatment. Reduced skin redness, reduced scratching and improvement in skin condition are remarkable.

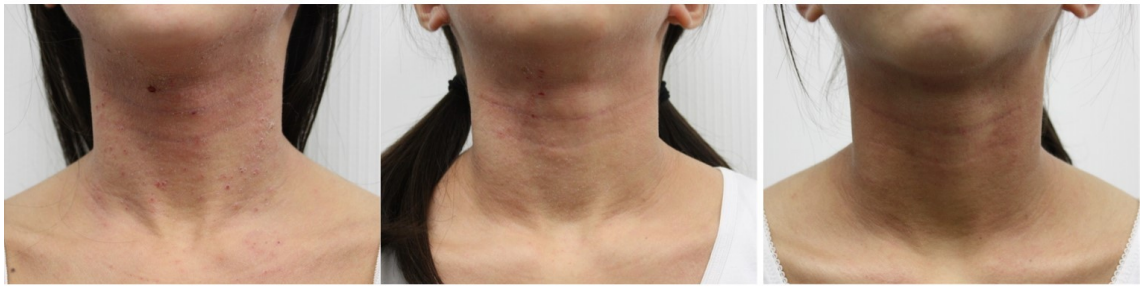
Face

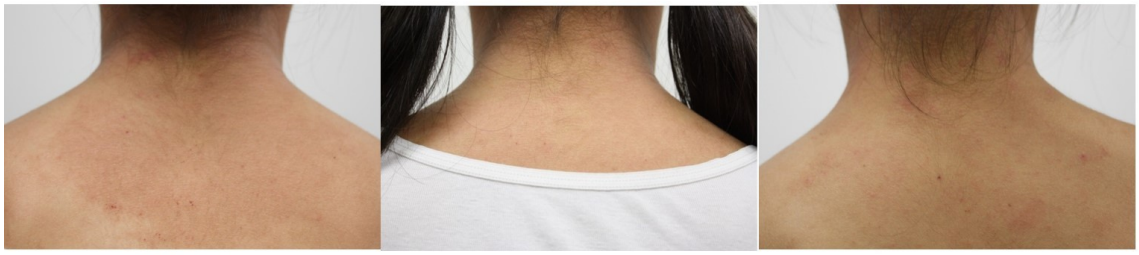


2015.3.17 | 2015.4.11 | 2015.5.23

Before | **1 month** | **2 months**

Neck





2015.3.17 | 2015.4.11 | 2015.5.23

Before | **1 month** | **2 months**

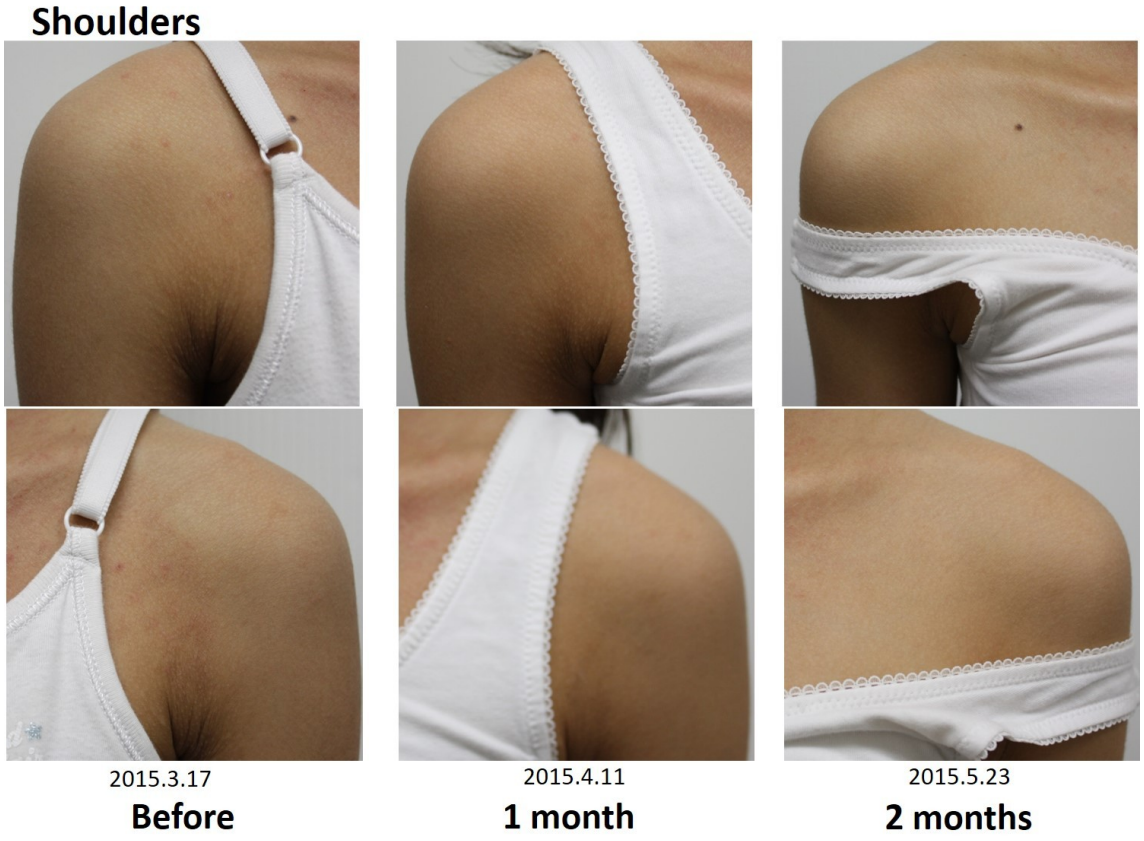
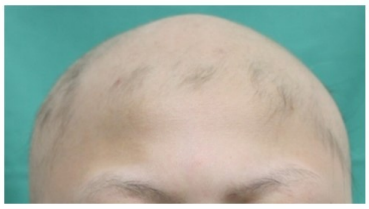
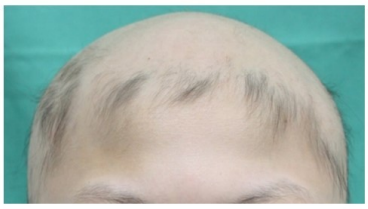


Figure 6. The pictures of 13 years old female was diagnosed with atopic dermatitis, before treatment, after 1 month and after 2 months treatment. Reduced skin redness, reduced scratching and improvement in skin condition are remarkable.

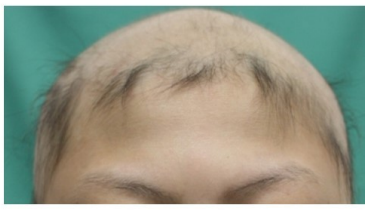
Front, top of the head



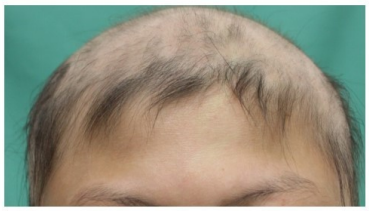
2015.3.27
Before



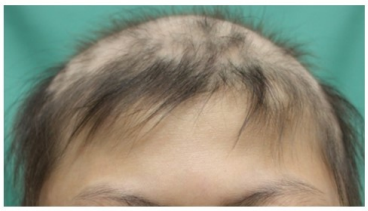
2015.4.25
1 month



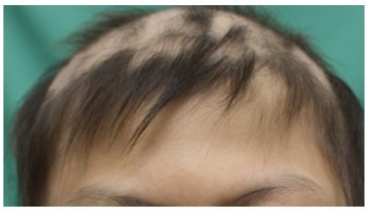
2015.5.23
2 months



2015.6.20
3 months

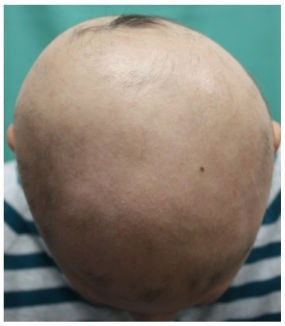


2015.7.24
4 months



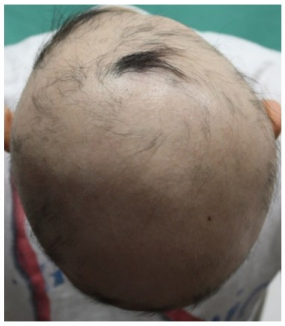
2015.8.25
5 months

Back of the head



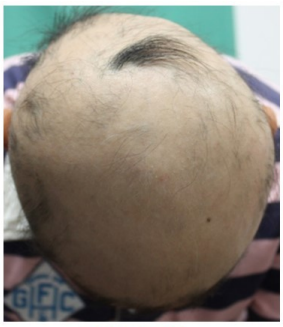
2015.3.27

Before



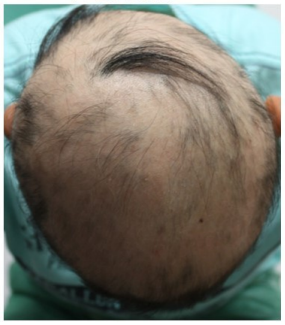
2015.4.25

1 month



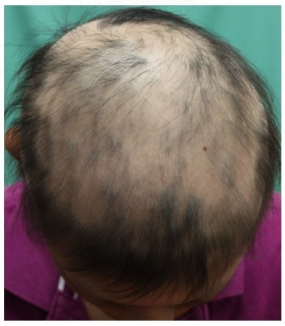
2015.5.23

2 months



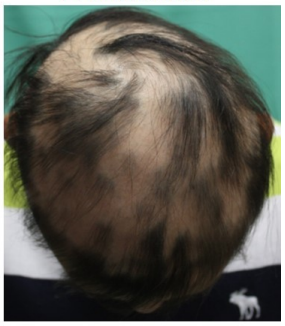
2015.6.20

3 months



2015.7.24

4 months



2015.8.25

5 months

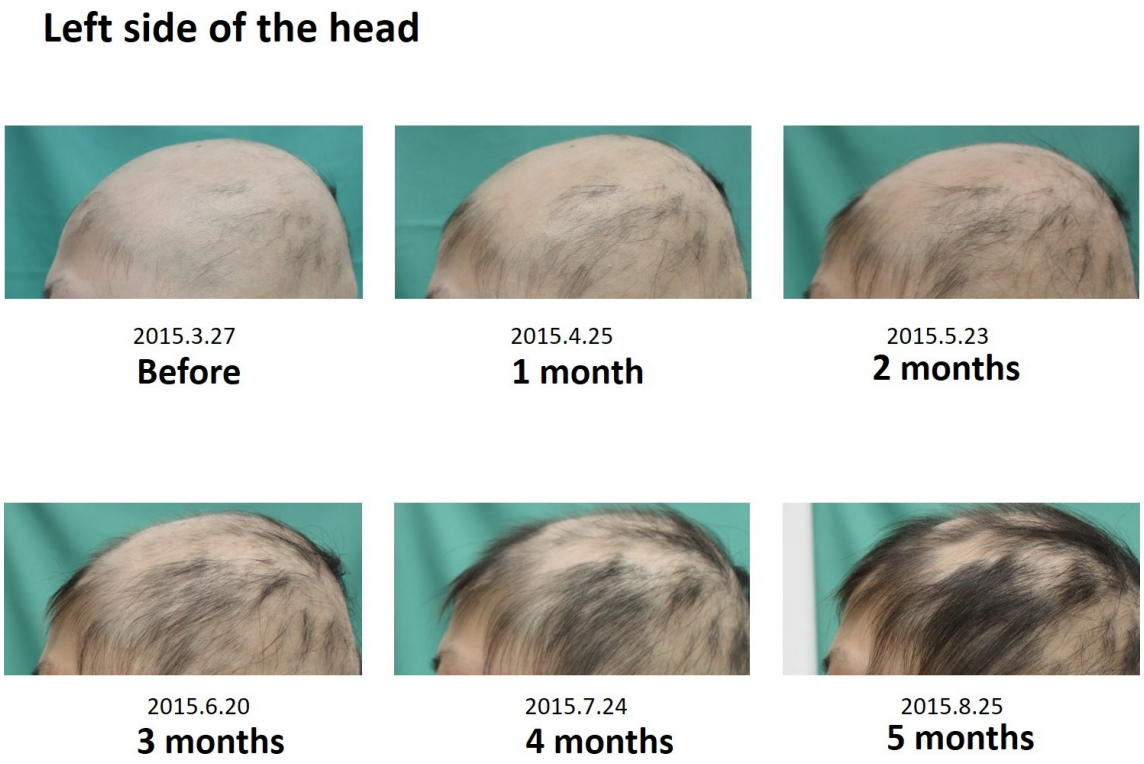


Figure 7. The pictures of 12 years old female was diagnosed with alopecia totalis, before treatment, after 1 to 5 months treatment. Visible evidence of new hair growth over the whole head, the side of head, and the back of head are showed.

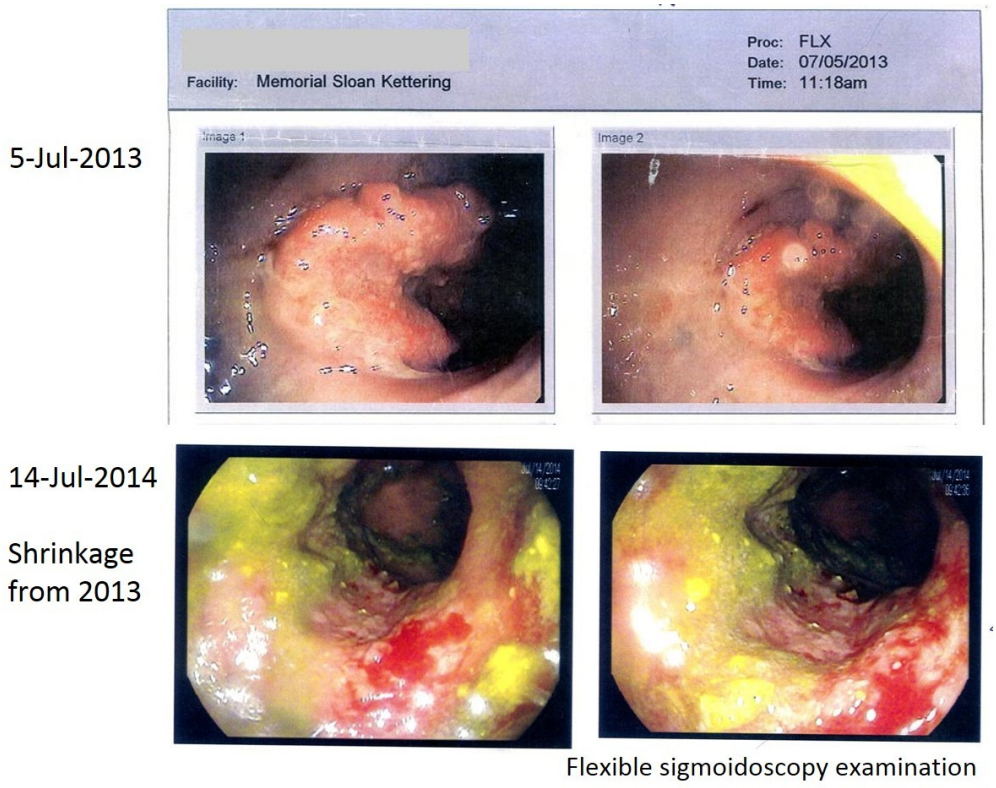


Figure 8. The pictures of 50 years old male was diagnosed with colon cabcer, before treatment, after 1 year treatment. Visible evidence of remarkable shrinkage of tumor is showed.

17-Sep-2014

31-Oct-2014
After 6 weeks of treatment

31-Oct-2014

After 6 weeks of treatment

12-Dec-2014

After 3 months of treatment

Figure 9. The pictures of 45 years old male was diagnosed with multiple sclerosis (MS), end stage progressive for 24 years. The first picture was taken before GcMAF therapy and it shows the patient's poor quality of life. He couldn't do anything, including changing clothes, going to the bath room. His wife helped him do everything. After 6 weeks of GcMAF therapy, he started walking again. You can see he has thin, weak leg muscles. He could also climb stairs. After 3

months of GcMAF therapy, he was in the process of rehabilitation. Later he started taking 4 capsule of oral GcMAF dailly. His wife was astonished looking at what he was able to do. Patient is now able to peddle on an exercise bike

References

1 Mellman, I. *et al.* Cancer immunotherapy comes of age. *Nature* 480*:* 480-489 (2011).

2 Yamamoto, N. *et al.* Vitamin D_3 binding protein (group-specific component) is a precursor for the macrophage-activating signal factor from lysophosphatidylcholine-treated lymphocytes. *Proc. Natl. Acad. Sci. USA* 88, 8539-8543 (1991).

3 Yamamoto, N. *et al.* Immunotherapy of metastatic breast cancer patients with vitamin D-binding protein-derived macrophage activating factor (GcMAF). *Int. J. Cancer* 122, 461-467 (2008).

4 Pacini, S. *et al.* Effects of vitamin D-binding protein-derived macrophage-activating factor on human breast cancer cells. *Anticancer Res.* 32, 45-52 (2012).

5 Yamamoto, N. *et al.* Immunotherapy for prostate cancer with Gc Protein-derived macrophage-activating factor, GcMAF. *Transl. Oncol.* 1, 65-72 (2008).

6 Yamamoto, N. *et al.* Immunotherapy of metastatic colorectal cancer with vitamin D-binding protein-derived macrophage activating factor, GcMAF. *Cancer Immunol. Immunother.* 57, 1007-1016 (2008).

7 Yamamoto, N. *et al.* Immunotherapy of HIV-infected patients with Gc protein-derived macrophage activating factor (GcMAF). *J. Med. Virol.* 81, 16-21 (2009).

8 Yamamoto, N. *et al.* Structural modification of serum vitamin D_3-binding protein and

immunosuppression in AIDS patients. *AIDS Res. Hum. Retroviruses* 11,1373-1378 (1995).

9 Yamamoto, N. *et al.* Conversion of vitamin D_3 binding protein (group-specific component) to a macrophage-activating factor by the stepwise action of beta-galactosidase of B-cells and sialidase of T-cells. *J. Immunol.* 151, 2794-2802 (1993).

10 Uto.Y. *.et al.* GcMAF: our next-generation immunotherapy. *Nature*, 485, (2012).

11 Kuchiike,D. *et al.* Degalactosylated/Desialylated Human Serum Containing GcMAF Induces Macrophage Phagocytic Activity and In Vivo Antitumor Activity *Anticancer Res* 33, 2881-2885 (2013).

12 Mohamad, S.B. *et al.* Preparation of Gc protein-derived macrophage activating factor (GcMAF) and its structural characterization and biological activities. *Anticancer Res.* 22, 4297-4300 (2002).

13 Kisker, O. *et al.* Vitamin D binding protein-macrophage-activating factor (DBP-*maf*) inhibits angiogenesis and tumor growth in mice. *Neoplasia* 5, 32-40 (2003).

14 Inui T. *et al.* Clinical Experience of Integrative Cancer Immunotherapy with GcMAF. *Anticancer Res* 33, 2917-2919 (2013)

15 Inui T. *et al.* Case report: A breast cancer patient treated with GcMAF, sonodynamic therapy and hormone therapy. *Anticancer Res 34*: 4589-4594 (2014).

16 Uto Y. *et al.* Degalactosylated/Desialylated Bovine Colostrum Induces Macrophage

Phagocytic Activity Independently of Inflammatory Cytokine Production. *Anticancer Res* 35: 4487-4492 (2015).

17 Cesta M: Normal Structure, Function, and Histology of Mucosa-Associated Lymphoid Tissue. *Toxicologic Pathology* 34: 599-608 (2006).

18 Brandtzaeg P. *et al.* Terminology: nomenclature of mucosa-associated lymphoid tissue. *Mucosal Immunol* 1: 31-37 (2008).

19 Hellings P. *et al.* The Waldeyer's ring. *Acta Oto-rhino-laryngologica Belgica* 54: 237-241 (2000).

20 Rosenberg SA. *et al.* A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science* 233: 1318-1321 (1986).

21 Rosenberg SA. *et al.* A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. *N Engl J Med* 316: 889-897 (1987).

22 Rosenberg SA. *et al.* A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science* 233: 1318-1321 (1986).

23 Utsuyama M. *et al.* Differential age-change in the numbers of $CD4^+CD45RA^+$ and $CD4^+CD29^+$ T-cell subsets in human peripheral blood. *Mech Ageing Dev* 63: 57-68 (1992).

24 Ljunggren HG. *et al.* Prospects for the use of NK cells in immunotherapy of human cancer.

Immunology 7: 329-339 (2007).

25 Padayatty SJ. *et al.* Intravenously administered vitamin C as cancer therapy: Three cases. *CMAJ* 174: 937-942 (2006).

26 Chen Q. *et al.* Pharmacologic ascorbic acid concentrations selectively kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc Natl Acad Sci USA* 102: 13604-13609 (2005).

27 Packer L. *et al.* α-Lipoic acid as a biological antioxidant. *Free Rad Biol Med* 19: 227-250 (1995).

28 Casciari JJ. *et al.* Cytotoxicity of ascorbate, lipoic acid, and other antioxidants in hollow fibre i*n vitro* tumours. *Br J Cancer* 84: 1544-1550 (2001).

29 Mohammed AA. *et al.* The pattern of infection and antibiotics use in terminal cancer patients. *J Egypt Natl Canc Inst* 26: 147-152 (2014).

30 Ross DD. *et al.* Management of common symptoms in terminally ill patients: Part I. Fatigue, anorexia, cachexia, nausea and vomiting. *Am Fam Physician* 64: 807-814 (2001).

31 Mohamad SB. *et al.* Gc protein-derived macrophage activating factor (GcMAF): isoelectric focusing pattern and tumoricidal activity. *Anticancer Res* 23: 4451-4457 (2003).