Rationales of GcMAF as a therapeutic target for SARS-CoV-2

The fact that around 96% of patients recover from the infection despite there are no antiviral therapeutics that specifically target human coronaviruses saying that the human immune system has mechanisms to combat SARS-CoV2.

What is GcMAF?

GcMAF is activating macrophages lymphokines. GcMAF or Gc protein-derived macrophage activating factor is a protein that resulted in sequential deglycosylation of his precursor - the vitamin D-binding proteins (the Gc protein). This process requires beta-galactosidase and sialidase secreted on surfaces of activated B and T lymphocytes respectively. The GcMAF is can produce by exposing isolated Gc proteins or biological liquid (containing Gc proteins cow's milk, human serum) to immobilized beta-galactosidase and sialidase.

What is macrophage subtype targeting by GcMAF?

Due to differences in GcMAF clinical effects in depends on the pathological condition type and stage, suggesting that its mode of action is different from other macrophage activation cytokines as interferon-γ (IFN-γ), which specifically targeting M1 macrophages. GcMAF interactions are not restricted by some specific phenotype only. Macrophages are highly plastic cells, their transcriptional profiles supposed to be highly responsive to the GcMAF stimuli, all range of phenotypes that are often grouped as M1, M2a, M2b and M2c types could in different level respond to make an activated phenotypes combination better adapted to a range of specialized functions. On our clinical observation, the follow GcMAf effects were seen. Regression of inflammatory clinical and biochemical markers observed on patients with rheumatoid arthritis. The stable improvement neurocognitive functions, attention and behavior under GcMAF treatment on autism spectrum disorders which likely are results of turn to the relatively anti-inflammatory macrophages phenotype associated with these conditions the proinflammatory state of microglia, as macrophages are plastic cells and may transition between different states. GcMAF is also shown efficiency in the prevention of clinical symptoms onset when taken on the prodromal period of influenza.

Targeting alveolar macrophages

In murine models of pulmonary infection alveolar macrophages clear pathogens up to a defined threshold without overt features of pneumonia, but when alveolar macrophages fail to control these subclinical infections recruitment of inflammatory cells, predominantly neutrophils, is required to control infection [Dockrell DH et al. 2003]. It was also demonstrated that mice with decreased numbers of alveolar macrophages, or with alveolar macrophages of reduced microbicidal capacity, are more susceptible to development of

pneumonia and that the threshold inoculum required to generate pneumonia is reduced significantly [Dockrell DH et al. 2003].

However, alveolar macrophages are very long-lived cells that may live for prolonged periods steady state [Murphy J *et al.*2008]. GcMAF's could be prophylactic/preventive therapeutic for pre-activation of macrophages and making them polarize from steady to functional alert stage at high risk of exposure to COVID19.

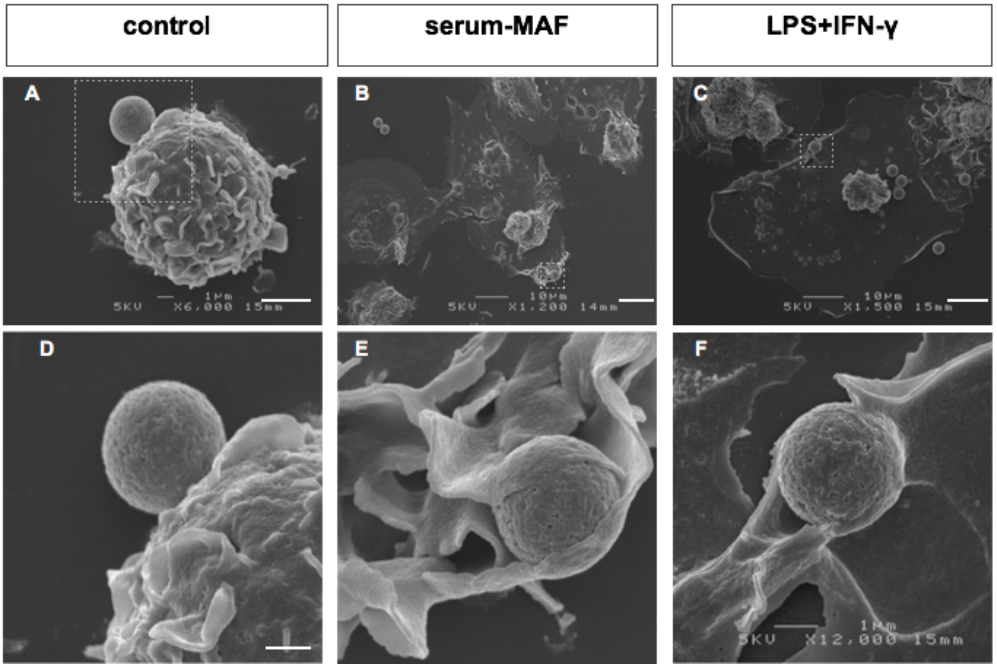
Alveolar macrophages may demonstrate simultaneously both M1 and M2 characteristics during acute inflammation in the lungs, their ability to generate inflammatory responses is regulated tightly to ensure that lung injury is controlled. M2 activated alveolar macrophages are ensuring tissue remodeling and repair, they characterized by high levels of IL-10, and scavenger receptor,s expression and exert anti-inflammatory regulation. Both phenotypes are important for GcMAF's targets for controlling systemic and lung inflammation and prevention of acute respiratory distress syndrome in COVID 19 pneumonia.

Reconstitution of gaps in the innate immune response to SARS-CoV

In recent studies, the very rapid phagocytic activation under Sai Sei Mirai's serum GcMAF treatment was shown. It also had shown to increase antigens internalization in 3-fold over compare to LPS+IFN-γ on the macrophage cell line. There is a link to a short video of serum Gc-MAF induced macrophages membrane restructuring happens within 5 min treatment with GcMAF, the filmed phagocytosis also looks higher compared to the control: https://lt.saisei-eu.com/lhp/wp-content/uploads/vid/mv-ssi-2020.mp4
(please, just copy and paste it on the browser).
In the video and figures 1, 2, 3, 4 presented data from the following publications:

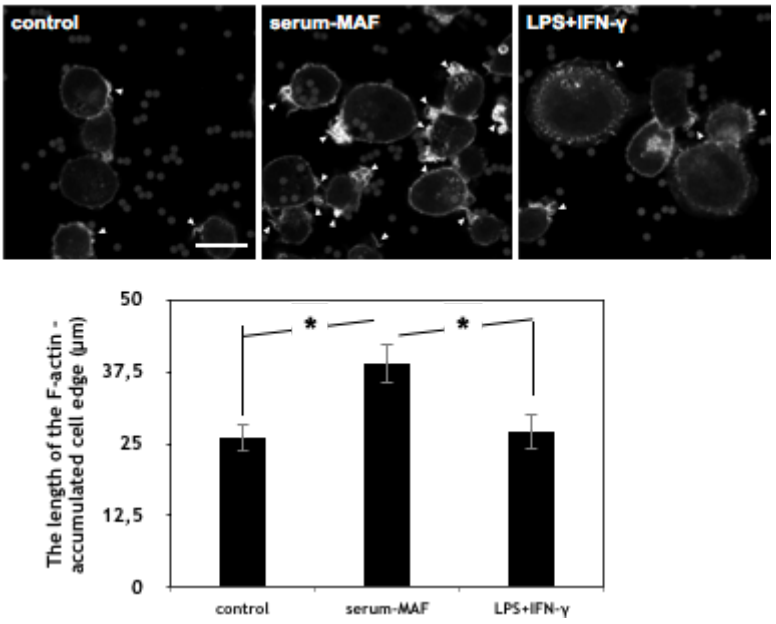
1. Kawakatsu K, Nishikata T *et al.* Characteristic Morphological Changes and Rapid Actin Accumulation in Serum-MAF-treated Macrophages. *Anticancer Res*. 2019.
2. Mami Ishikawa *et al.* A high-throughput quantitative assay system for macrophage phagocytic activity. Macrophage 2018.

Figure 1



Morphological changes of THP-1-derived macrophages following MAF treatments. After a 4-h treatment with 8.17 µg/ml serum-MAF (B, E), 100 pg/ml LPS + 20 ng/ml IFN- γ (C, F), and control (A, D), beads were added to macrophages and fixed for SEM observation. Low magnification, whole cell images (A-C) and enlargements of the engulfing region (white rectangle) are shown (D-F). Scale bars; 10 µm in A-C, 1 µm in D-F.

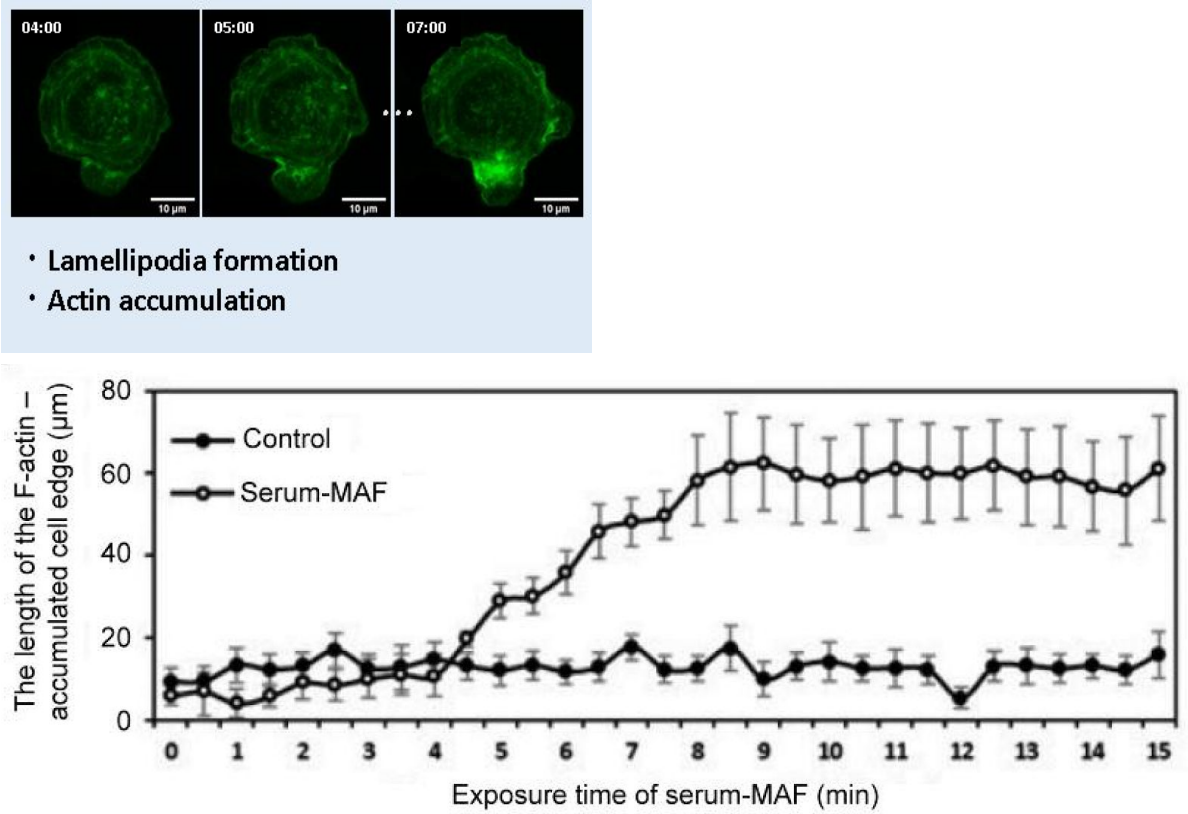
Figure 2



Differences in actin accumulation at the edge of the cells. Confocal images of Lifeact-THP-1 derived macrophages, treated with 8.17 µg/ml serum-MAF (B), 100 pg/ml LPS + 20 ng/ml IFN- γ (C), or without MAF (A) are shown. White arrowheads indicate actin accumulations in lamellipodia tips. Three-dimensional analysis of serum-MAF activated macrophage (D)

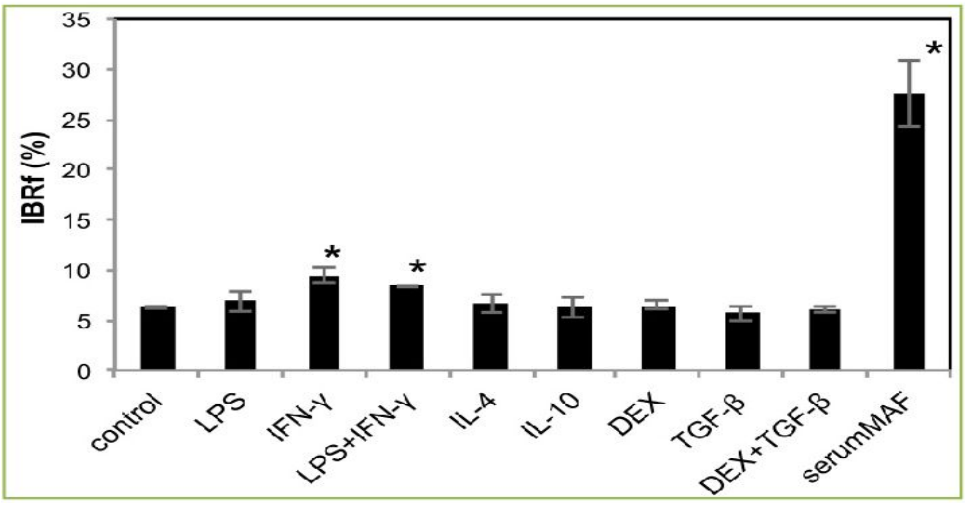
represents intricate membrane ruffling at the site of actin accumulation. Scale bar; 10 µm. Actin accumulation was quantitatively analyzed using these images (E).

Figure 3



Time lapse images of Lifeact-THP-1 derived macrophages after the start of treatment with serum MAF compare to control are indicated in the graf. Actin accumulation was quantitatively analyzed.

Figure 4. The MAFs activated macrophages phagocytic activity comparison [Mami Ishikawa *et al.* 2018]



The virus replication cycle of SARS-CoV is completed in approximately 6 h [Ng *et al.*, 2003], the virus simply appears to replicate to high titres well before type I IFNs are induced. In a microarray analysis of the cellular gene expression of SARS-CoV-infected human macrophages, SARS-CoV failed to induce significant IFN-α/β gene expression [Cheung CY, *et al.,*2005]. SARS-CoV has developed mechanisms to induce a delayed response of the innate immune system in both 293 fibroblast cells and DCs, which allows the production of infectious progeny virus in both cell types [Spiegel M *et al.*2006].

Classical activation of M1 macrophages polarization requires in IFN-γ in combined with TLR4 signaling and characterized by increased expression of pro-inflammatory mediators and effectors enabling phagocytosis and killing of pathogens. However, Gc-MAF itself shown to induce sustained activation on Lifeact-THP-1 derived macrophages seen after 5 min of treatment (Figure 3). Such a rapid macrophage activation followed by the fast phagocytic membrane's structure remodeling (Figure 1, 2) and 3 fold over antigens internalization compare to the most potent known macrophage activators combination LPS+IFN-γ (Figure 4) are first time reported.

Expected that administration of GcMAF in humans during the very early stage of SARS-CoV-2 infection induces the similar to observed on *in-vitro* pre-activation of mucosal alveolar macrophages, which initially have to accomplish with type I IFNs synthesis upregulation.
This could be the way to overcome the SARS-CoV mechanism of replication via suppressing the IFN-α/β response in host macrophages with innate immune response delay.

SARS-CoV infections of macrophages lead to the initiation of viral gene transcription and viral protein synthesis, but no infectious virus produced, and hence SARS-CoV infections of human macrophages appeared to be abortive [Chung Y. Cheung, et al. 2005]. In this connection, GcMAF's ability to strongly enhance the **phagocytosis** of SARS-CoV viral particles by macrophages on the background of early sufficient IFN-α/β response is a chance to increase the probability of the SARS-CoV abortive infection course. Even the viral replication restriction is crucial, as uncontrolled by innate immunity, it leads to progressive increases in the viral load, which in turn driving the systemic hyperactive proinflammatory response, - the dramatic point on SARS-CoV disease responsible for the clinical severity and mortality.

Impaired GcMAF synthesis

GcMAF activation macrophages pathway is required of membranous glycosidases of inflammation-primed lymphocytes which rapidly convert serum Gc protein to the potent macrophage-activating factor. This inflammation-primed macrophage-activation process appears to be the major macrophage-activation cascade. Defect in the inducible β-galactosidase of B lymphocytes in the macrophage-activation cascade produces dysfunctional macrophages (Encyclopedia of Immunology, 1998).

It was shown that SARS-CoV could not only infect lymphocytes but also replicate in them. The lymphopenia detected in 63% among hospitalized patients with pneumonia. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood are reported [Zheng, H *et al.*2020]. All the above affecting the conversion of Gc protein to GcMAF due to the deficiency membranous glycosidases secreted by T and B lymphocytes.

The exogenous GcMAF therapy can bypass the defective lymphocyte function and act directly on phagocytes, and most important that it allows initiating the driven by adaptive immunity Gc MAF's macrophages activation pathway whenever earlier before of the needed lymphocytes activation appears.

Conclusion

The induced by GcMAF respiratory tract macrophages activation can be a key to the more rapid and efficient innate immune response against COVID19, which is pivotal in the condition of no pre-existing immunity in humans. Absolute safety and high functionality in other unmet therapeutic needs of COVID 19 infection made GcMAF an urgent candidate for clinical trial implementation.

SaiSei Mirai Pharma, Japan GcMAF formulations

SaiSei Mirai Pharma, GcMAF producing technologies patented in US and EU. Currently two genetically engineering GcMAF proteins are under development and below the list already available products:

1. Systemic delivery injectable forms formulated as 2,5 ml vials for subcutaneous injections
   - GcMAF from autologous (patient own) serum
   - GcMAF from donors plasma
2. Intrapulmonary delivery: GcMAF powder and spray.
3. Oral delivery: GcMAF capsules, powder and spray

GcMAF capsules, powder and spray are registered in EU, US and Ukraine as a supplement.

Planned activities

1, Conduct fast running non randomized clinical study in the countries most affected by **COVID-19**.

The study title: **''GcMAF therapy of non-severe cases of COVID-19 and prophylaxis of close contacts as prevention strategy"**

Brief Summary: We plan to evaluate GcMAF efficacy in treatment of recently infected COVID-19 patients and on prophylactic treatment contacts. The strategy entails starting GcMAF treatment immediately after COVID-19 . The induced respiratory tract macrophages

activation be a key to the more rapid and efficient innate immune response against COVID19 in COVID-19 infected individuals and close contact. therapy expected to boost innate immunity viral replication mechanisms thus g COVID19 transmission and progression.
Study Type: Interventional Clinical Trial
Estimated Enrollment : 350 participants
Allocation:
Masking: label

rimary Purpose: Prevention therapy

Comparison of outcomes of GcMAF therapy versus conventional treatment in similar age and comorbid status patients diagnosed with COVID19 at same time and base of retrospective data analysis.
Comparison of outcomes of GcMAF prophylaxis versus a self isolation of close contacts with confirmed COVID-19 cases d s database.
Primary Outcome Measures:
1.Effectiveness of GcMAF immunoprophylaxis assessed by incidence of secondary COVID-19 cases among contacts [Time Frame: Up to 21 days after start of treatment]

Secondary outcome measures:
1. COVID19 disease-severity worsening ratios during treatment (from mild to moderate, from moderate to severe), case-hospitalization ratio and case-fatality ratio.[Time Frame: 28 days]
2. Rate and time of disease remission :
Rate of no fever [Time Frame: 28 days]
Rate of respiratory symptom remission [Time Frame: 28 days]
Rate of lung imaging recovery [Time Frame: 28 days]
Rate of C-reactive protein (CRP) recovery [Time Frame: 28 days]
Rate of undetectable viral RNA (continuous twice) [Time Frame: 28 days]
Time for hospitalization [Time Frame: 21 days]

3. The virological clearance rate of throat swabs, sputum days 4 [Time Frame: 4 days after start of treatment]

Eligibility Criteria
1.Ages Eligible : 18 Years to 75 Years
2.Sexes Eligible: All
3.Accepts Healthy Volunteers:

Inclusion Criteria for COVID19 case:
Patients who meet the requirements of the New Coronavirus Infection Diagnosis (Acute respiratory infection symptoms or acute cough alone and positive PCR)

Aged ≥18 years male or female;
Willing to comply with all study procedures,
Able to provide oral and written informed consent

Inclusion Criteria for a contact:
Patients who been in contact with confirm COVID-19 cases and meet the WHO's contact definition
Willing to take study supplement;
Willing to comply with all study procedures;
Able to provide oral, informed consent and/or assent.

Arm1. Experimental : Subjects exhibiting ARI symptoms at a participating hospital complete a survey collecting demographic and clinical data and provide a swab to be tested on-site with a molecular assay.Case GcMAF capsules.
Arm2. Experimental prophylactic: Contact subjects will GcMAF capsules for prophylaxis.

Treatment regimen: the dietary supplement - GcMAF acid resistant coated capsules 148 mg, oral administration 2 capsules 3 times per day for mild severity cases and 2 capsules 6 day for moderate severity cases. The treatment duration is 20 days.
Contacts will be offered a prophylactic regimen of 2 capsules 3 times per day for 20 s.

We plan to conducting clinical trial on a international platform to meet patient recruitment goals quickly and efficiently, at times with very limited resources. In best case scenario we want to recruiting patients from multiple countries and multiple ethnicities. But for now we have a two clinical sites in Ukraine. We will also apply for potential EU partners as soon as possible. It wasn't done in time as we only found the information about the project 8 days before the deadline, but we are working on it.

Regulations: this clinical study will met current EU Clinical Trials Directive - Good Clinical Practice Guidelines from ICH, addresses the good clinical practice, an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. We will apply for the Fast-Track clinical trial approval procedure. Research Electronic Data Capture (REDCap) is planned to be used for patients electronic medical records recording and keeping.

Dear reviewer, considering the current situation with COVID-19 I understand that any trials organization procedure is time consuming, and now it is a luxury to pass through all this the steps for its approval when thousands are dying daily. I will be happy if there will be the simplest and shortest way for the trial to proceed to give the GcMAF capsules to people in the epicenters of the problem for those considered as contact, already sick and isolated, and for patients in hospital with moderate COVID-19 symptoms. If there will be obvious positive clinical dynamics in the first 50-100 patients (which will evaluate under

Methodological guidelines - e.g. statistical principles for clinical trials (CPMP/ICH/363/96) or clinical trials in small populations (CHMP/EWP/83561/2005), for the next set of patients we will take throat swabs and sputum for PCR test daily in the first four days of treatment, blood plasma samples for IL6, IL10, INFa cytokine profile levels measuring each three days, isolate and freeze in livable condition PBMC. The PBMC could be tested for the cells functionality in baseline, and after first and second weeks of GcMAF treatment. The GcMAF effects on M1 and M2 macrophage polarization will be evaluated on M1 Polarization Assay (M1 polarization measuring by TNF-α secretion via the MSD platform, imaged via high content imaging (HCI) and M2 Polarization Assay (M2 polarization of monocytes measure by CCL18 secretion via ELISA using high content imaging).
The clinical study protocol is under development and it will be done in around two weeks.

Just a small remark, I have been working for two years in the human retroviruses lab of NIH/US and have 12 years working in infectious diseases in the Ukraine. For me, as an experienced doctor, I have seen much faster than usual resolution of clinical signs of acute respiratory tract infections with GcMAF treatment saying that there is underline some of the potent mechanism activity. Just two months ago I had three Japanese patients with extreme fatigue, dry cough, high CRP level and leukocytosis in the blood and 37.2 to 38.2 C body temperature. They showed very good dynamics by the next day on GcMAF treatment. I ask them to be tested for SARS-CoV-2 IgG antibodies, they will provide results soon. The similar trend was seen in other patients with clinic c, but test just appeared in Ukraine

Safety

The Japanese company SaiSei Pharma has a patented technology extracting vitamin D binding protein from dairy products and converting it to GcMAF. The organic basis of dietary supplement - GcMAF capsules provides the safety and digestibility comparable to the product's sources bovine colostrum. There were no adverse effects reported during 8 years its marketing.
The clinical study will fall under Directive 2001/20/EC (Clinical Trials Directive), Regulation EU No 536/2014 (Clinical Trials Regulation), This study ethical requirements will met this document regulations

Short GcMAF's history overview

Based on experimental researches and the clinical observation Gc-MAF is one most promising candidate to immune boosting drug and at the same time it is the most difficult issue to have a deal with due to long painful story of controversy behind it. This story is started from the fundamental discovery of the mechanism macrophage activation drived by the Macrophages Activator Factor. It was shown that GcMAF results from sequential deglycosylation of the vitamin-D binding protein (the Gc protein), via enzymes secreted by stimulated lymphocytes. This work was done by *N Yamamoto* and team in 1991 and since that time till now his name is highly cited on Gc-MAF experimental publications. His work about GcMAF antiangiogenic activity on cancer formation been published most-cited original-research cancer journal [1]. Anticancer activity of GcMAF was independently supported by other authors [2,3,5].

For the last decade other favorable Gc-MAF's biological activities were discovered. This lymphokine was shown to be an important player on connective tissue metabolism [4], apart of macrophage activator Gc-MAF was found as a potent osteoclast-activating factor [7,9]. GcMAF reported to normalize the observed in autism dysregulation on gene expression of the endocannabinoid system, and to down-regulate the over-activation of bone marrow-derived macrophage from autistic children [6]. The deficiency of endogenous Gc-MAF considered to associate with high nagalase concentrations macrophage impairment in alcohol abuse persons [8].

Despite of the diversity reported biological activities and safety Gc-MAF seems to be a less lucky then other cytokines as interleukin-2, IFN-α , IFNγ. These cytokines passed through the clinical trials, even in if some cases their efficiency was evaluated as mild for indicated conditions, they have been registered as a drugs [10].

In the case with Gc-MAF the first few clinical cases records published by Yamamoto, were retracted by journals due to the ethical approval issues and methodological errors in the studies. Another unfairly affected Gc-MAF reputation story was related with David Noakes who manufacturing Gc-MAF as a medicinal product without a manufacturer's licence (see.wikipedia.information for more details).

Saisei Mirai company has a different from those been used by Yamamoto and others technology GcMAF production, it is calls the second and third generation of GcMAF, which shown excellent macrophage activation properties [11,12]. We had planning to resolve the contradictions about the GcMAF therapeutic efficiency by conducting clinical trial under Good Clinical Practise standards and provide statistically reliable efficacy results. But today's is uncontrolled situation with COVID 19 requires its testing in urgent regime.

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