

Second Generation GcMAF immunotherapy



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1 Saisei Mirai Clinics. 2 The University of Tokushima. 3 Konan University.
4 Kobe University.

Introduction to Japan



GEISHA, KYOTO



TODAI-JI TEMPLE, NARA



MOUNT KOYA



KOBE



ARASHIYAMA, KYOTO



UMEDA, OSAKA



KIYOMIZU TEMPLE, KYOTO



HIMEJI CASTLE, HIMEJI

Introduction to Saisei Mirai

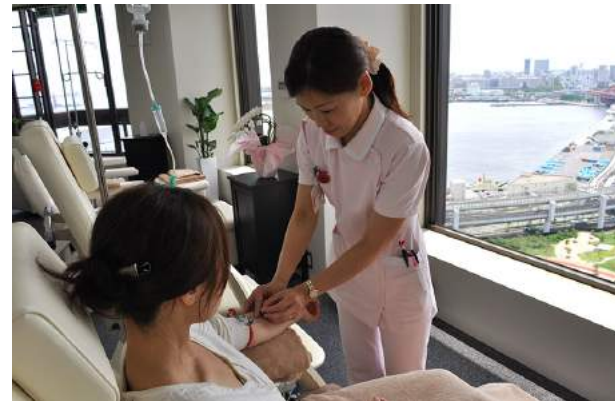
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Kobe Clinic,
Kobe, Japan

Saisei Mirai Cell Processing Center (CPC)



Cell Processing Center (CPC)



Hyper T/NK Cell Therapy



Skin rejuvenation

History of GcMAF development



Dr Nobuto Yamamoto



Dr Hitoshi Hori



Dr Yoshihiro Uto



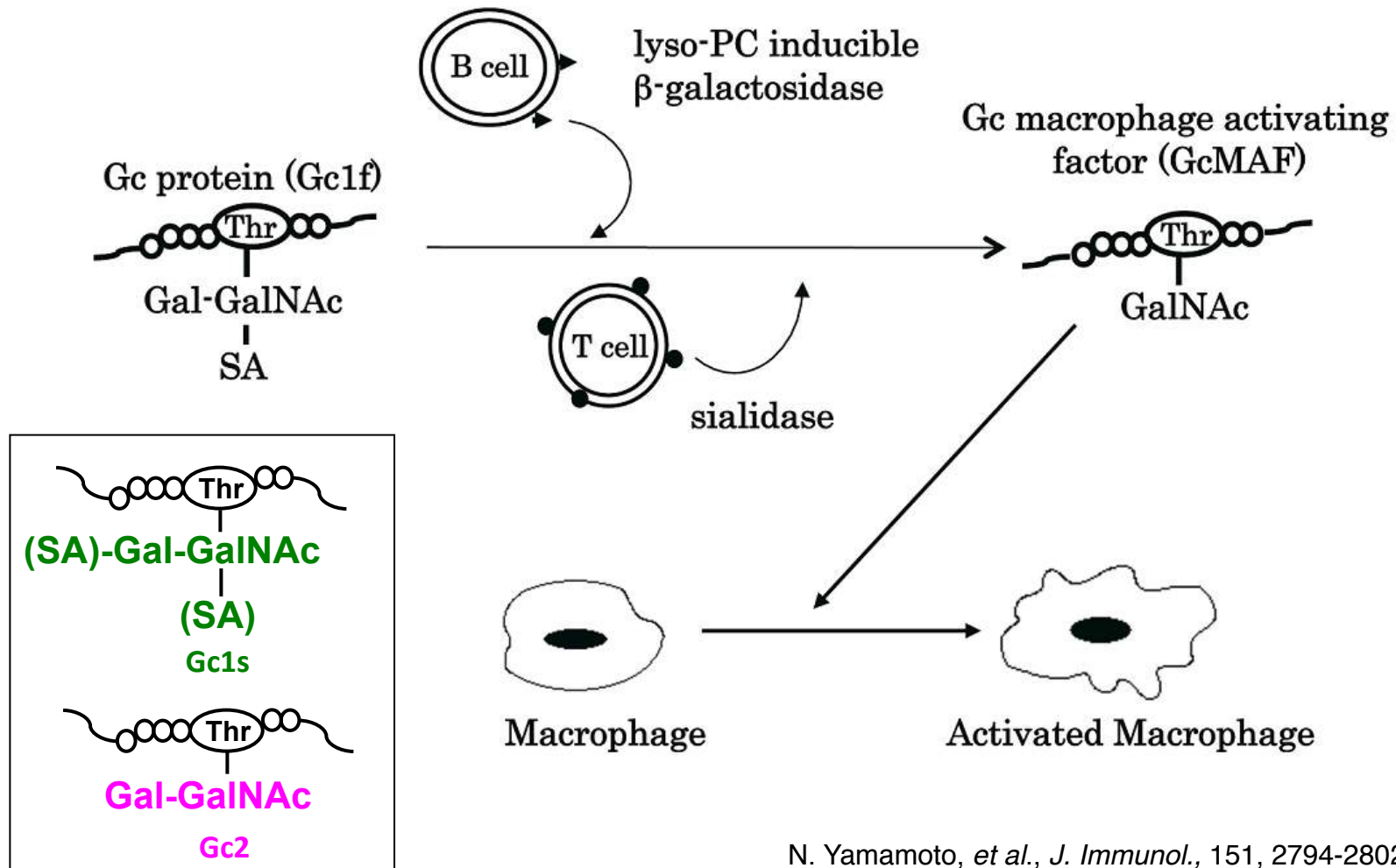
Working together...



GcMAF development timeline

1991	Dr Yamamoto developed GcMAF
1992	Dr Yamamoto visited Dr Hitoshi Hori at Tokushima University. GcMAF research started at Tokushima University
1998	Dr Yoshihiro Uto joined Dr Hori's GcMAF research team
2002	First research papers published on GcMAF
2010	Tokushima University began collaborating with Saisei Mirai to develop Second Generation High Dose GcMAF
2011	Second Generation GcMAF produced in our Cell Processing Center (CPC) for patients. Start of clinical use
2012	20 years since research on GcMAF began at Tokushima university
2013	Two research papers published in Anticancer Research by Saisei Mirai & Tokushima University
2013	Over 1000 patients treated with Saisei Mirai 2nd Generation GcMAF

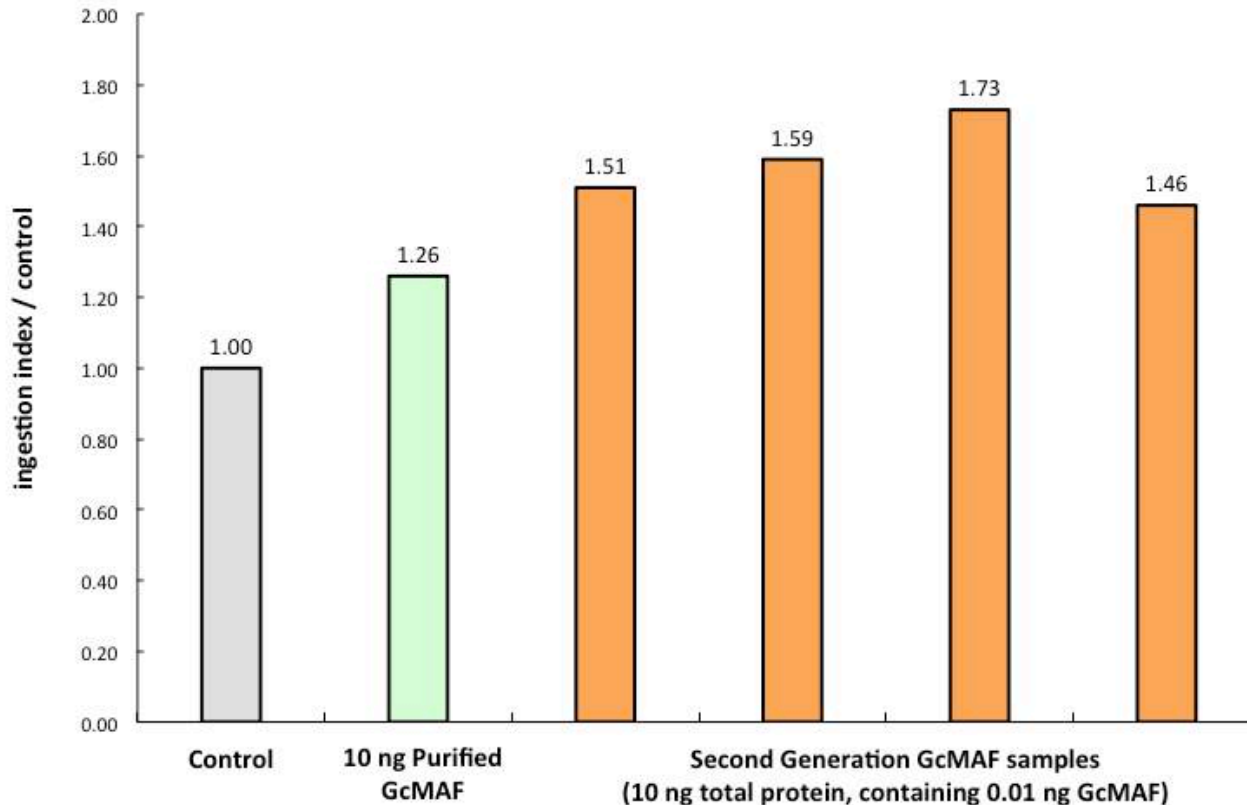
Proposal scheme: conversion of Gc_{1f} protein to GcMAF



N. Yamamoto, *et al.*, *J. Immunol.*, 151, 2794-2802, 1993.

- Our phagocytic activity data shows that GcMAF produced from all subtypes have high activity

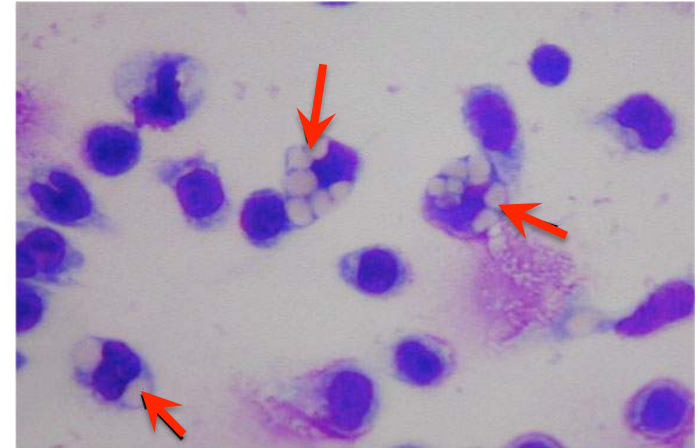
GcMAF phagocytic activity



- GcMAF is tested for macrophage phagocytic activity using mouse macrophages and sheep red blood cells at the University of Tokushima
- 2nd Generation GcMAF produced using our patented production process has very high activity

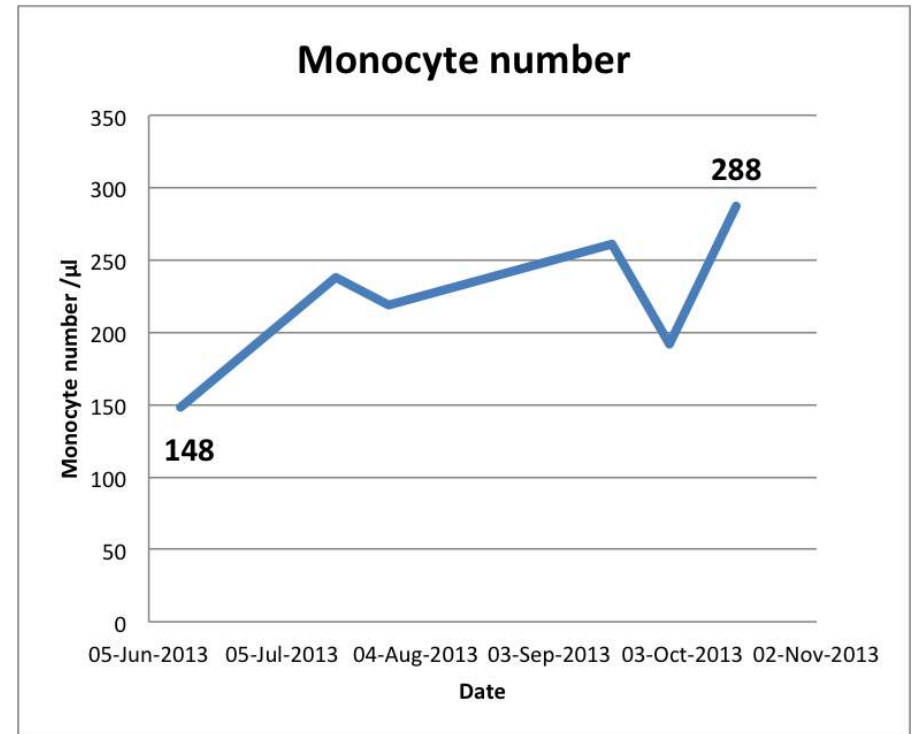
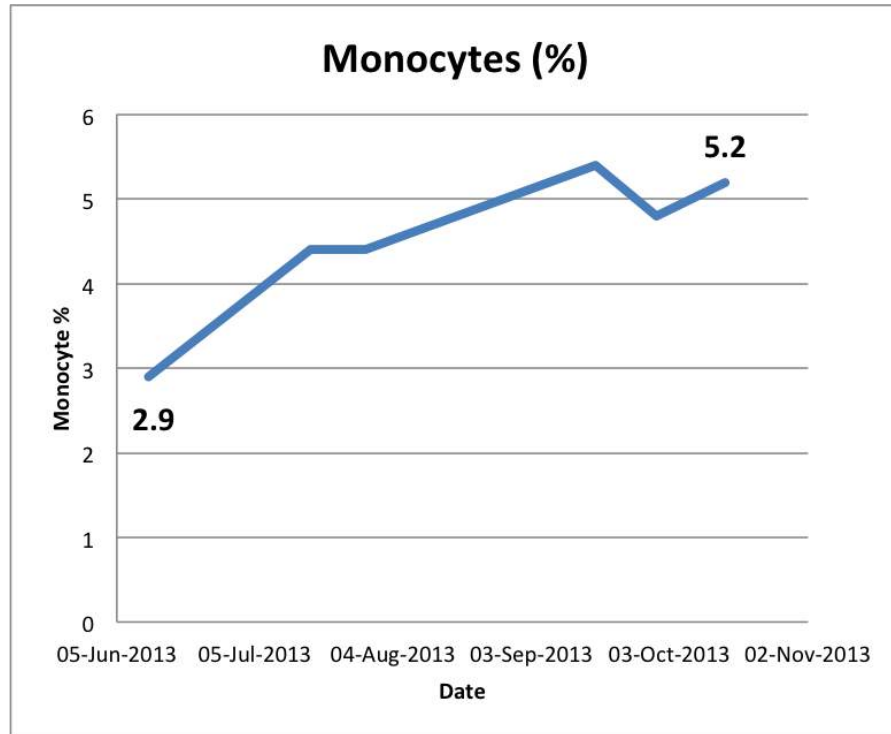
Biological activity of GcMAF

- Increased phagocytic activity
- Superoxide radical generation
- Anti-angiogenic effect
- Anti-tumor effect
- Increased number of monocytes in the blood
- Increased rate of maturation of dendritic cells (DCs) *in vitro*



Phagocytosis assay with Second Generation GcMAF. Photo, University of Tokushima.

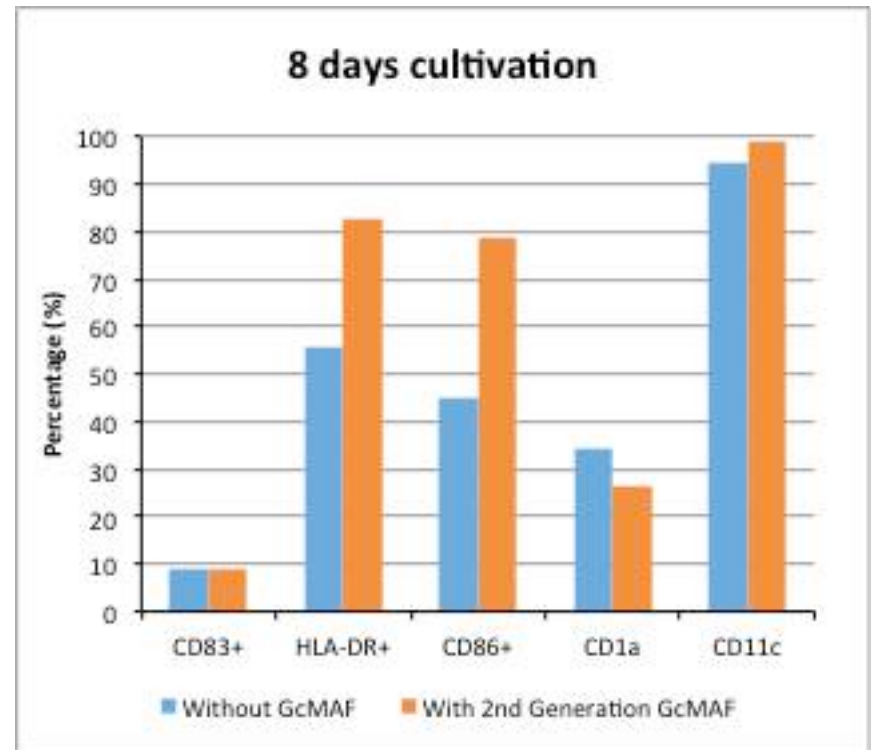
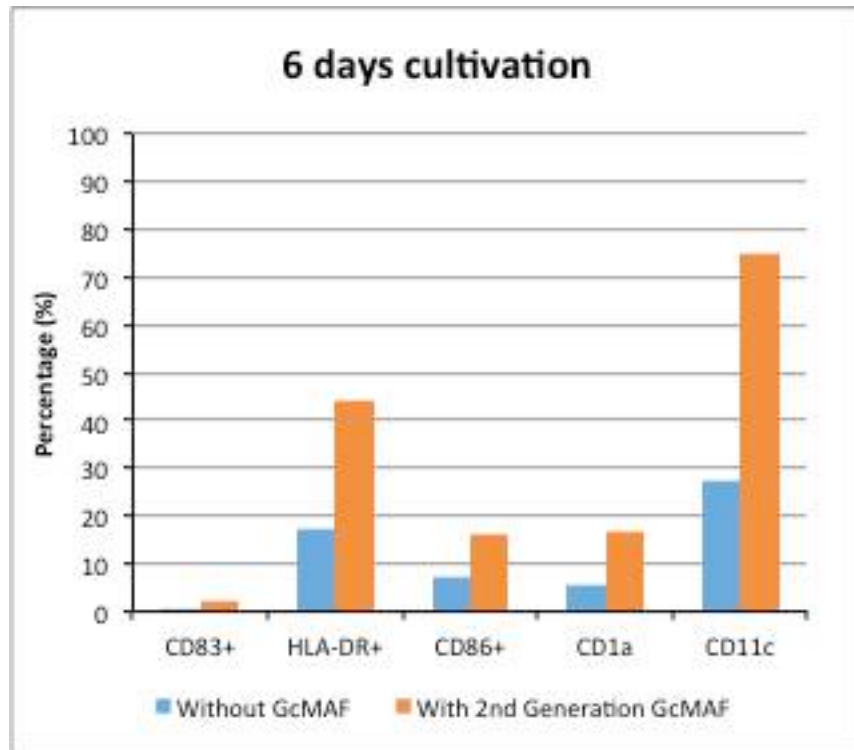
Change in monocyte percentage and monocyte number during GcMAF therapy



- A patient's monocytes will generally rise in the early stages of GcMAF therapy and indicates a good response to treatment.

Dendritic cells (DCs) + 2nd Generation GcMAF

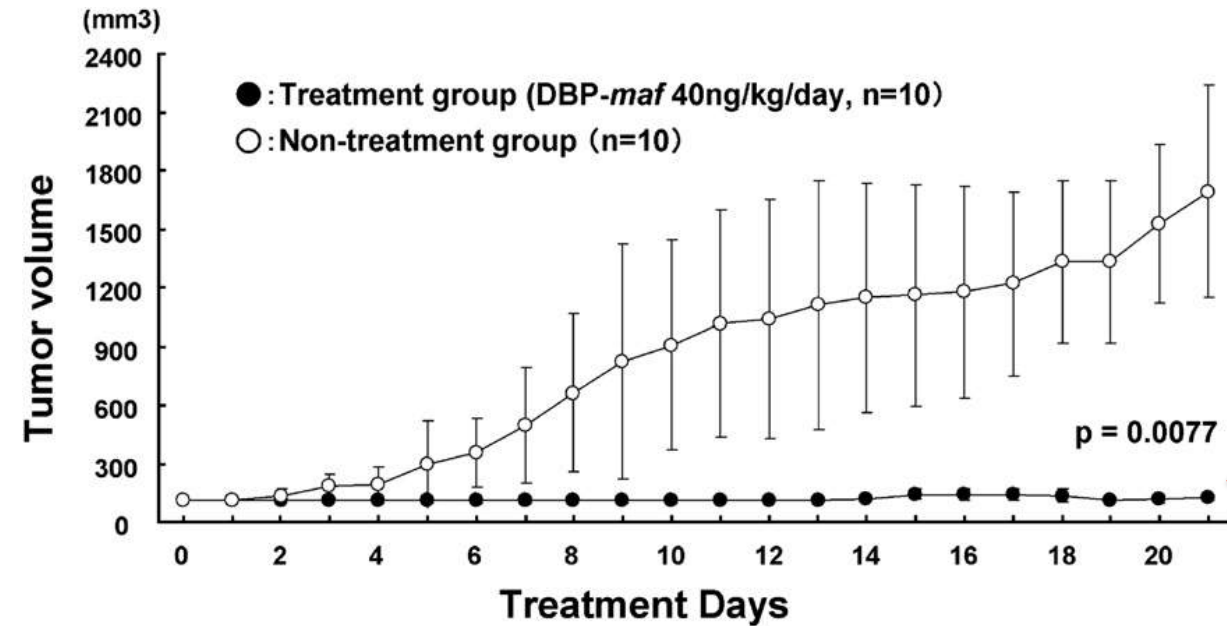
- Increased rate of maturation *in vitro*



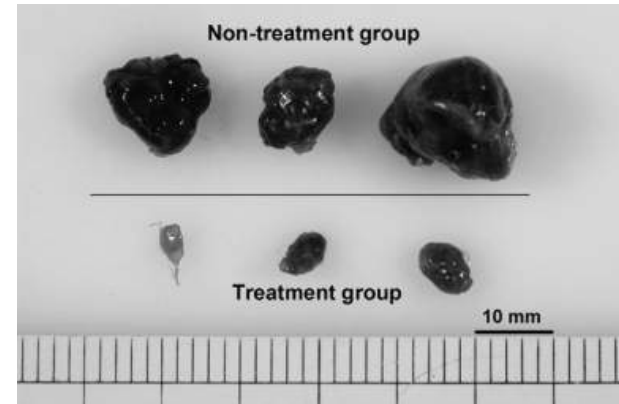
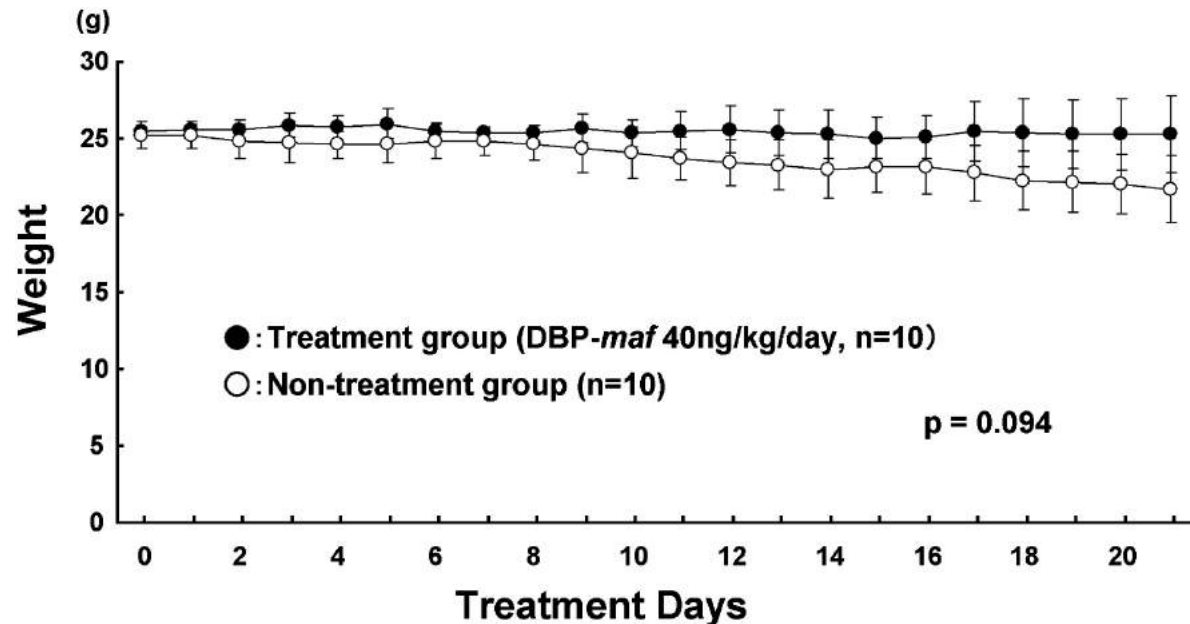
Saisei Mirai

Flow cytometric analysis of dendritic cells (DCs) grown from normal peripheral blood with and without 2nd Generation GcMAF on specific marker expression after 6 and 8 days cultivation

In vivo antitumor activity of 1st Generation GcMAF

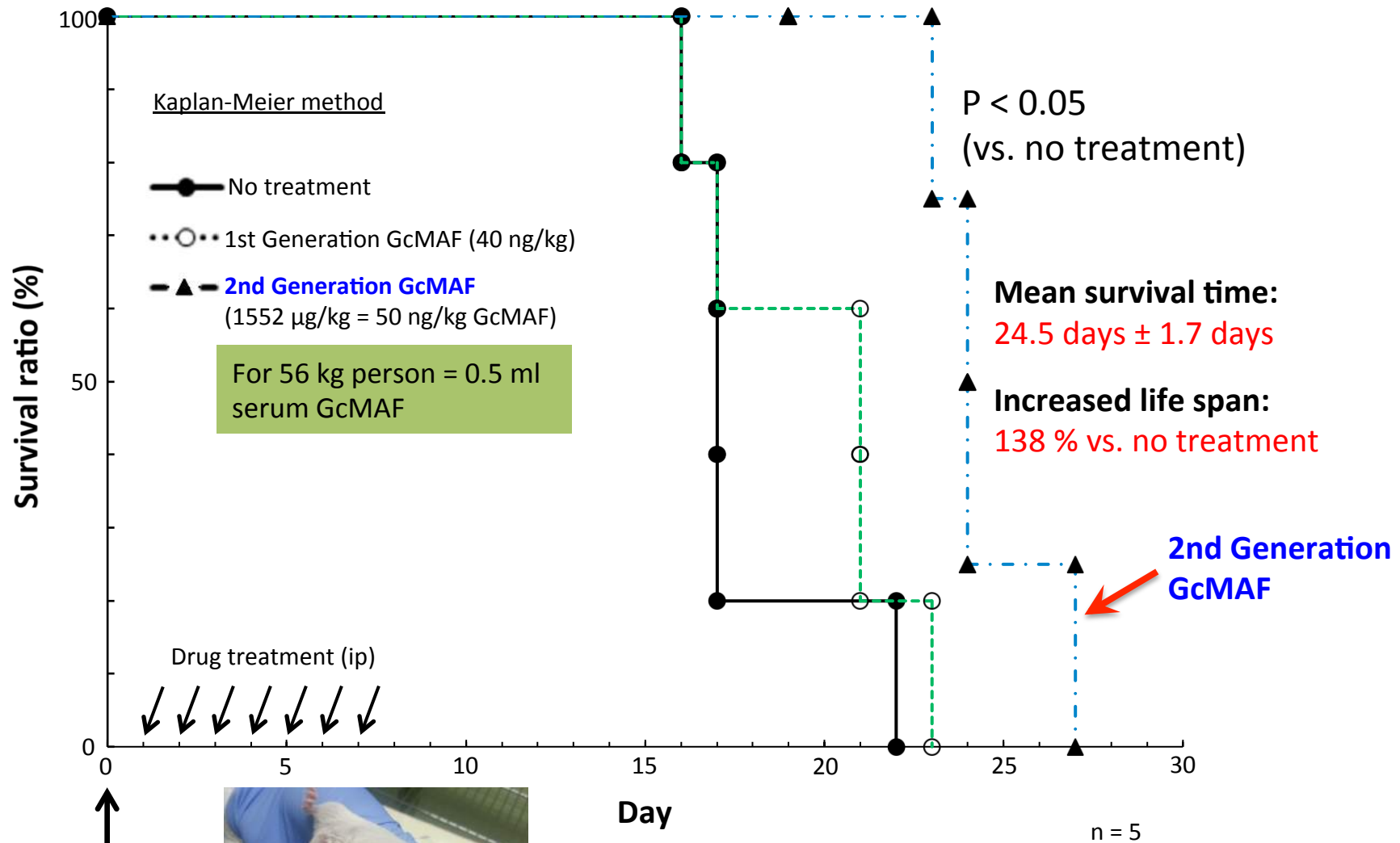


Treatment group
(1st Generation GcMAF)



Nonaka K., Onizuka S., Uto Y., *et al.*,
J. Surgical Res., 172, 116-22, 2012.

In vivo antitumor activity of 2nd Generation GcMAF in Ehrlich Ascites tumor-bearing ICR mice



Kuchiike D., Uto Y., *et al.*, *Anticancer Res.*, 33, 2881-5, 2013.
Uto Y., *et al.*, PCT/JP2012/072884

GcMAF research at Tokushima University

- Tokushima University conducts experiments with 1st Generation and 2nd Generation GcMAF
- 1st generation GcMAF is prepared from Gc protein purified from human serum using vitamin D3 affinity column (Dr Yamamoto)
- GcMAF is sterilized using 0.22 μ m filtration system



Vitamin D3
affinity column

2nd Generation GcMAF developed by Saisei Mirai

- Enzymatically-treated human serum GcMAF
- New patented production process
- GcMAF is sterilized using 0.22μm sterile filtration system



Saisei Mirai



Types of GcMAF

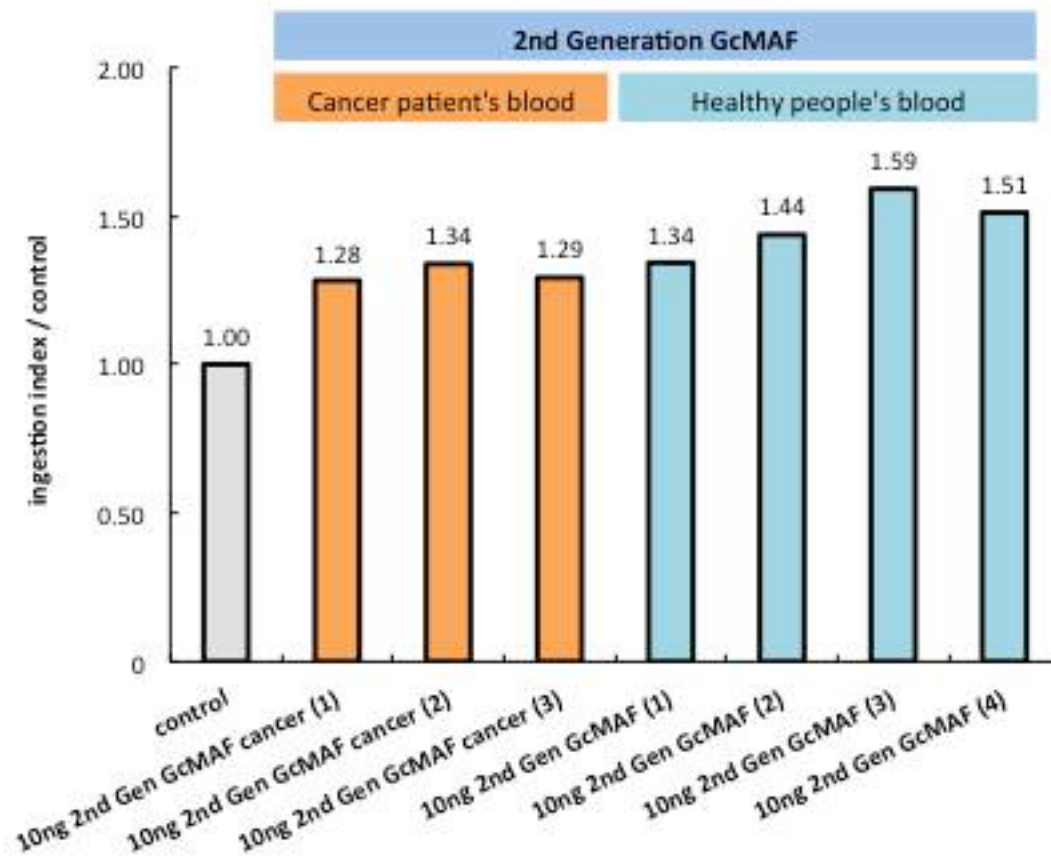
- Serum sources for manufacturing GcMAF
 - Autologous GcMAF produced from the cancer patient's own blood
 - produced from patient's family member's blood
 - produced from the blood of healthy volunteers, such as from Japanese Red Cross



Saisei Mirai

Cancer patients GcMAF activity

- GcMAF made from cancer patient's own blood sample (autologous GcMAF treatment)
- Autologous GcMAF therapy is only done for cancer patients



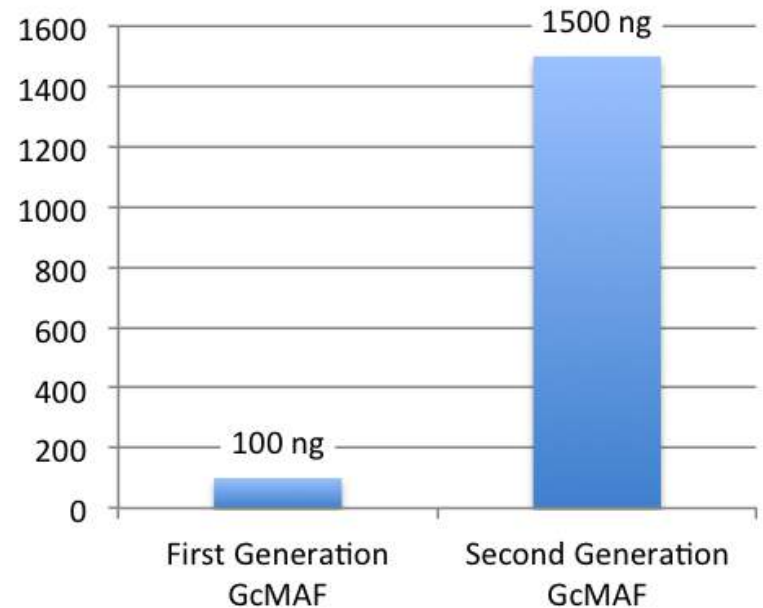
2nd Generation GcMAF made from cancer patients' blood has high activity similar to GcMAF made from healthy people's blood

Second Generation GcMAF:
10 ng total protein, containing approx. 0.01 ng GcMAF

Comparison between 1st Generation and 2nd Generation GcMAF

First Generation GcMAF

- Developed by Dr Yamamoto in 1991
- Low concentration (100 ng/0.25 ml, 1 dose)
- Low stability at room temperature
- 25-(OH) Vitamin D3 Affinity Column
- Unstable in the absence of antioxidants such as albumin and uric acid



Second Generation GcMAF

- Developed by the University of Tokushima and Saisei Mirai in 2010
- High concentration (1500 ng/0.5 ml, 1 dose)
- Significantly higher stability and macrophage activating activity
- New patented production process

Stability of 2nd Generation GcMAF

Temperature	Stability
4 °C (refrigerated)	> 1 year
20 °C (room temperature)	4 weeks
40 °C	1 week



2.5ml GcMAF vials



0.5ml GcMAF vials

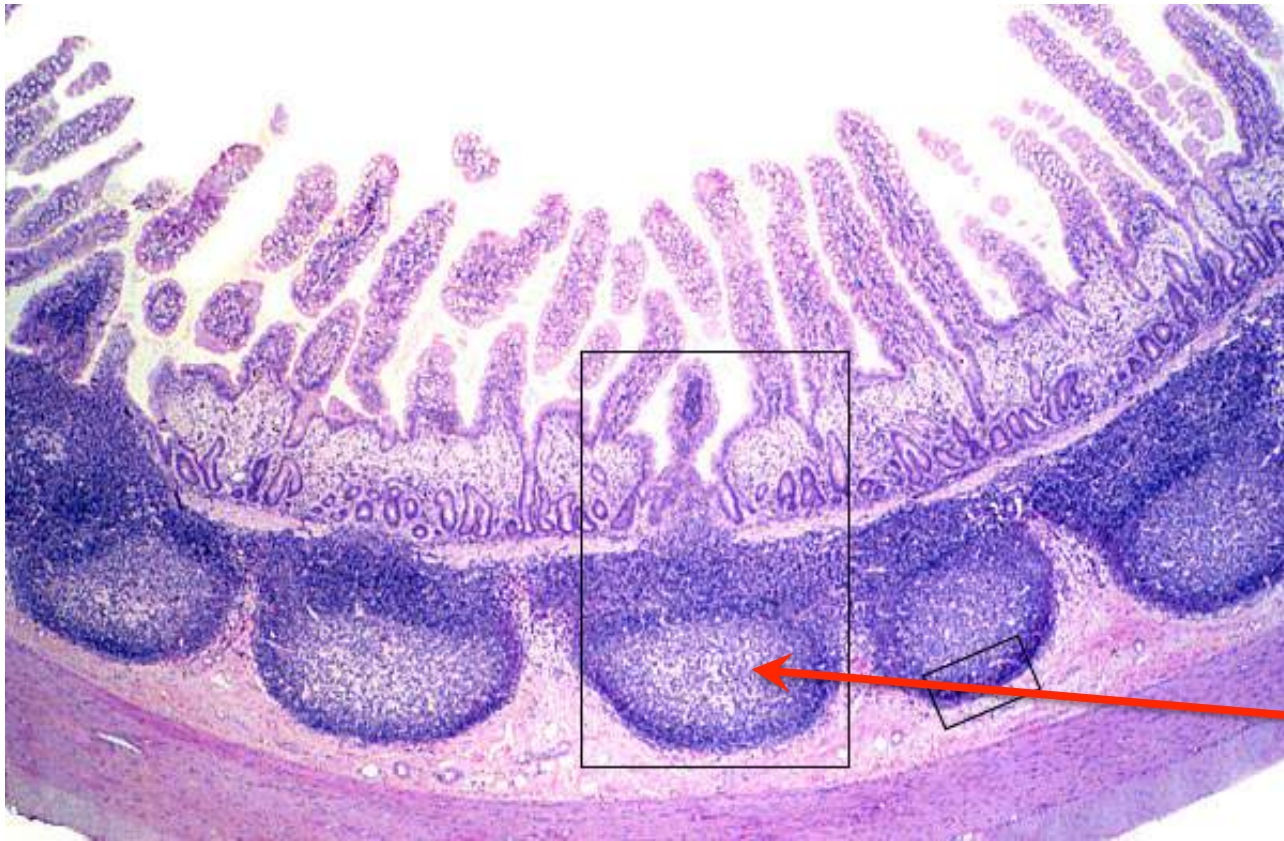
A new take on GcMAF

- What is “3rd generation GcMAF”
 - GcMAF produced from colostrum
 - Colostrum is very similar to serum, very rich in protein and IgA
 - We don't need blood to make 3rd generation GcMAF
 - Enteric capsule for oral administration
 - Target Payer's Patches/gut



Oral GcMAF

Gut Associated Lymphoid Tissue (GALT)



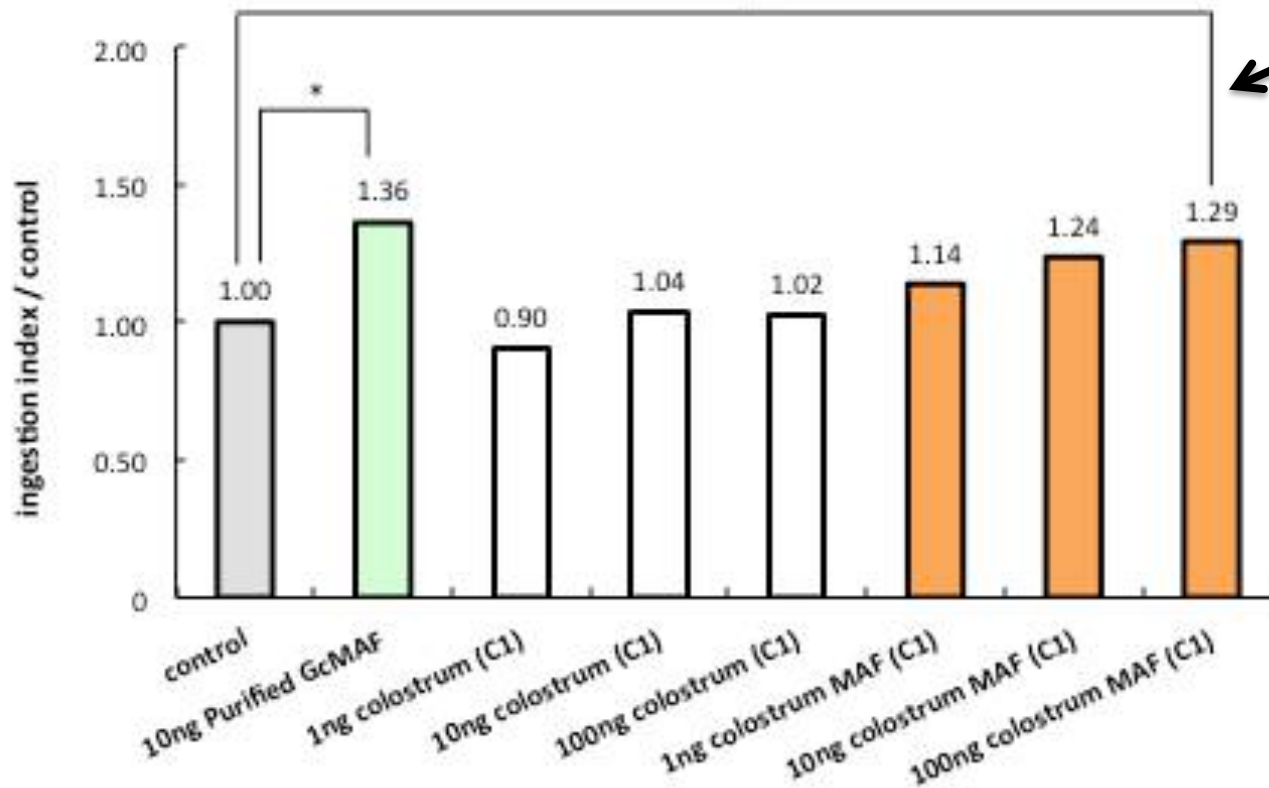
Peyer's Patches
which contain
macrophages

GcMAF possible indications

- Cancer
- Autism
- Chronic fatigue syndrome (CFS)
- Lyme disease
- Various infectious diseases
 - e.g., Influenza, Norovirus, Malaria, Dengue fever, HIV, AIDS, Hepatitis B and C, Tuberculosis
- Autoimmune diseases

Oral GcMAF macrophage activation assay

phagocytosis (0.5% opsonized SRBC)



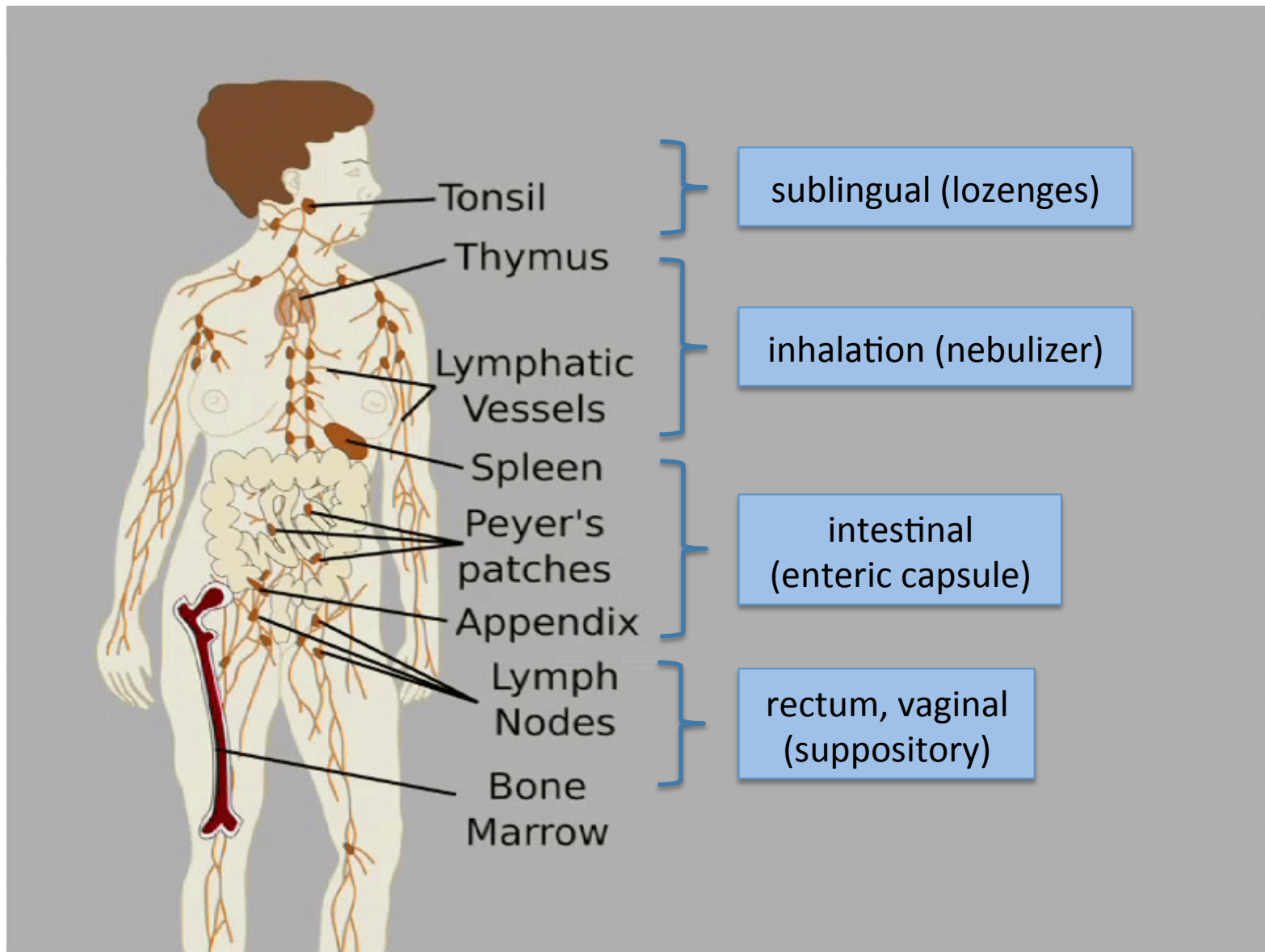
Significant difference between control and 100 ng colostrum MAF

100 ng colostrum MAF has equivalent activity to 10 ng 1st Gen GcMAF

How is GcMAF administered?

- 1st and 2nd generation GcMAF can be used by SC, IM, IV and IT injection
- New form of GcMAF for oral administration
 - Gut associated lymphoid tissue (GALT)
- Other possible administration:
 - lozenges, inhalation, suppository, eye drops, nose drops, patches and ointment

The lymphatic system and GcMAF



Classic Nagalase theories and new facts

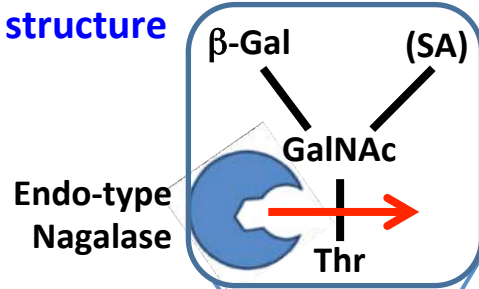
- Alpha-nagalase is an enzyme secreted by cancer cells, virus-infected cells, and normal cells
- 2 types: Exo-type and endo-type Nagalase
- Nagalase has been reported to accumulate in the blood of cancer patients
- Nagalase deglycosylates vitamin D3-binding protein (Gc protein)
- Deglycosylated Gc protein cannot be converted into GcMAF, leading to immunosuppression
- However: GcMAF from cancer patients has demonstrated high macrophage phagocytic activity

2 types of Nagalase

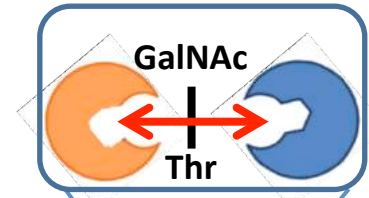
- Exo-type and endo-type
- **Exo-type nagalase** is secreted by: cancer cells, virus-infected cells and normal cells
- **Endo-type Nagalase** is secreted by: cancer cells and virus-infected cells
- Specific to each disease
- We should use both endo-type and exo-type substrate to measure Nagalase

The role of endo/exo-type Nagalase for the digestion of sugar chain of Gc protein and GcMAF

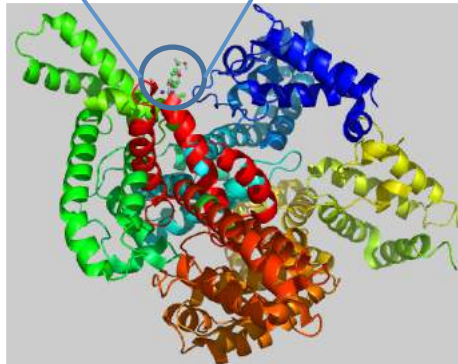
Disaccharide structure



Monosaccharide structure

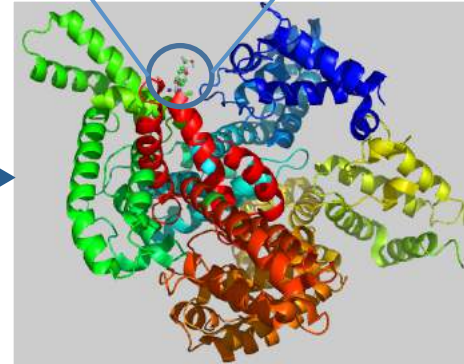


Gc protein



β -galactosidase
Sialidase

GcMAF

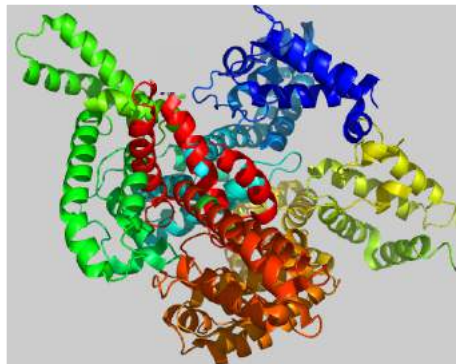


Endo-type Nagalase

Exo-type Nagalase

→ Digestion by enzyme

- Exo-type Nagalase can only digest monosaccharide structure (GcMAF)
- Endo-type Nagalase can digest disaccharide (Gc protein) and monosaccharide structure (GcMAF)



No sugar chain

Injection of GcMAF and Coley Vaccine

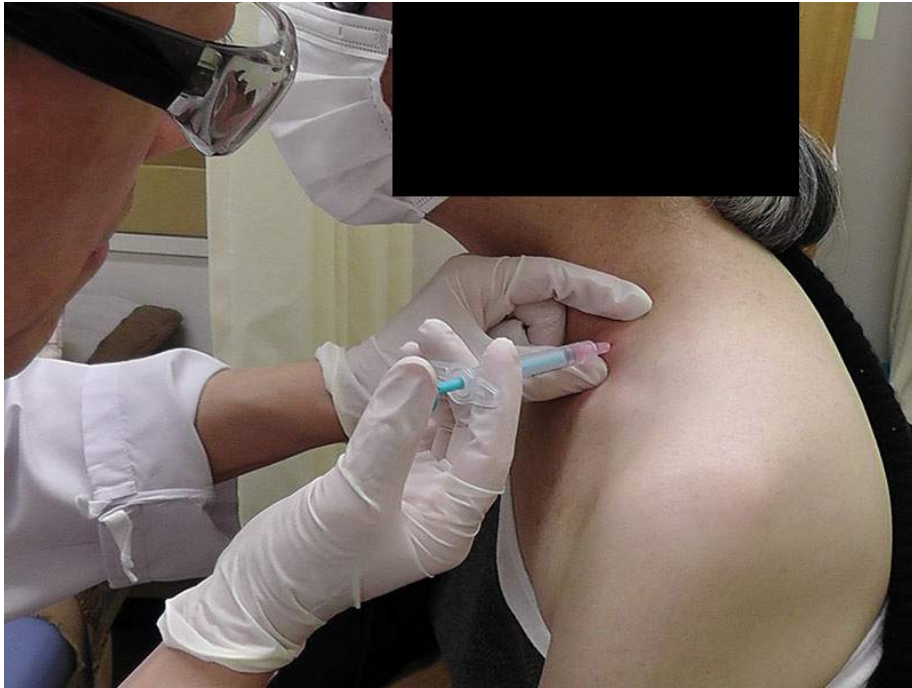


Coley Vaccine IT injection

Coley Vaccine administration leads to
softening of the tumor



Injection of GcMAF and Coley Vaccine



Coley Vaccine IT injection



GcMAF IT injection

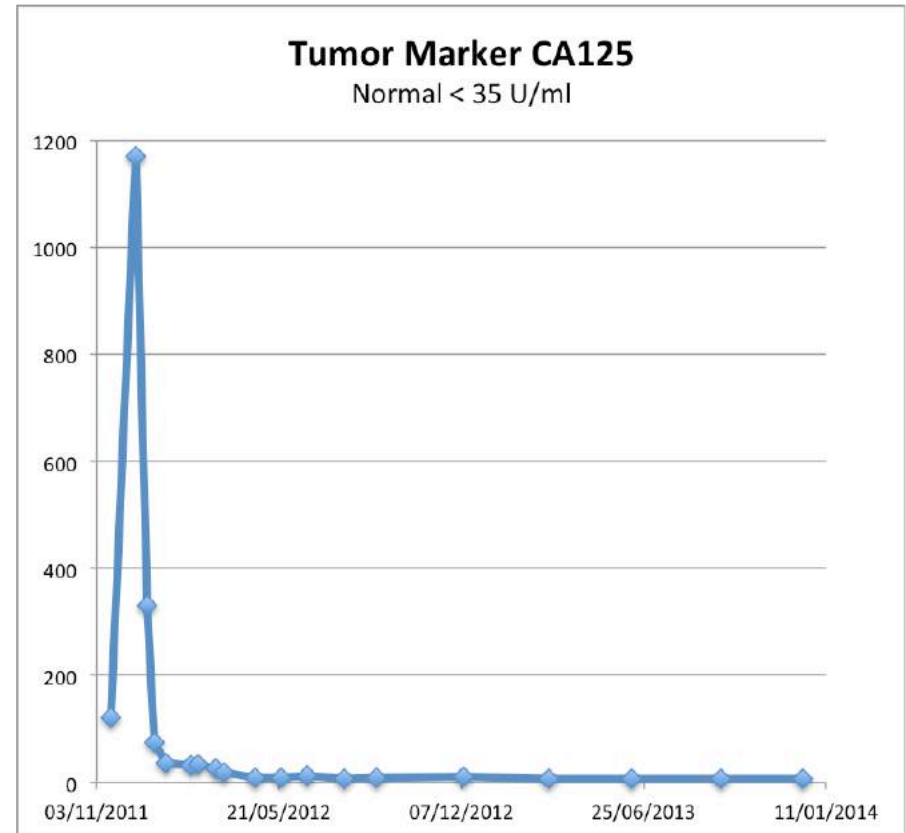
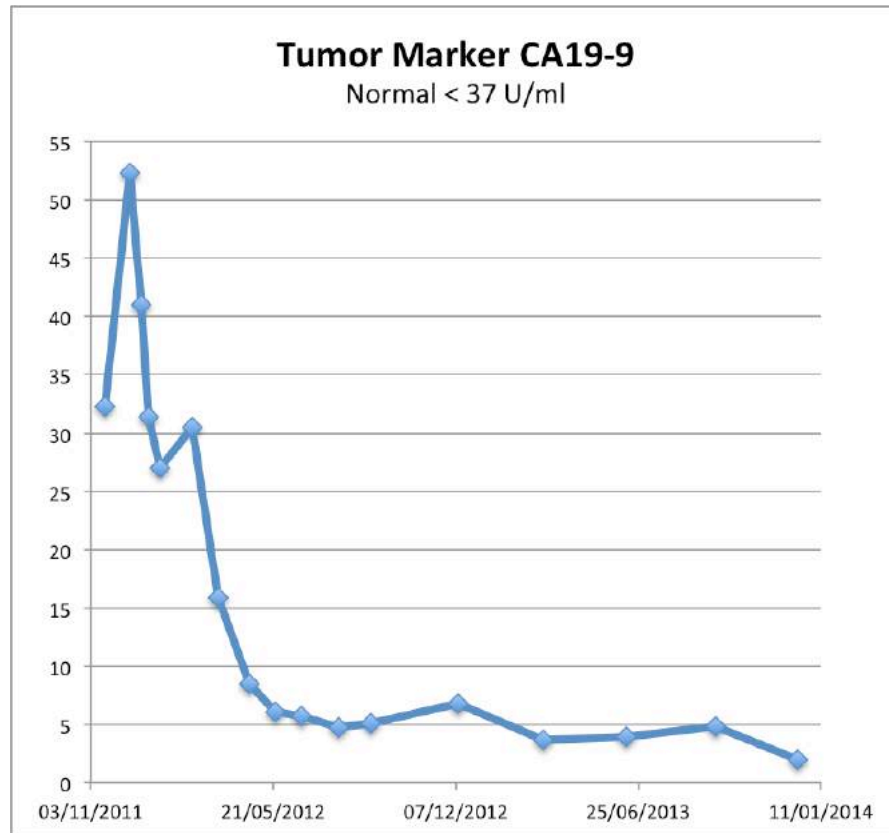
Case report: Uterine cancer

- 63 yo female, uterine cancer recurrence
 - serous adenocarcinoma stage 4b
- 3 times surgery
- 2 times radiation therapy
- CDDP chemotherapy
- Patient discontinued chemotherapy due to side effects
- Dec 2011 presented at Inui Clinic
 - Took 8 times hyperthermia (Thermotron RF-8)
 - 110 times Coley Vaccine
 - 48 times 0.5 ml GcMAF (1500 ng/0.5 ml)

Case report: Uterine cancer (cont.)

Tumor markers

63 yo female, uterine cancer recurrence



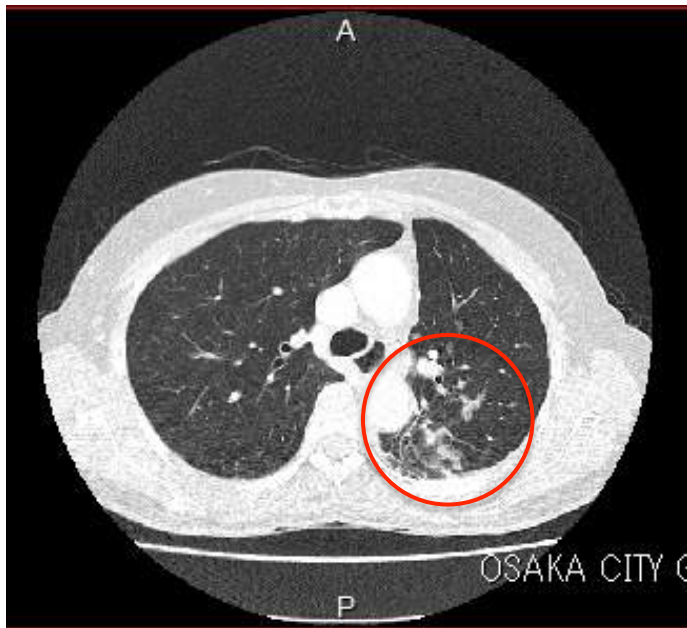
- Patient is in complete remission after treatments.

Case report: Lung cancer

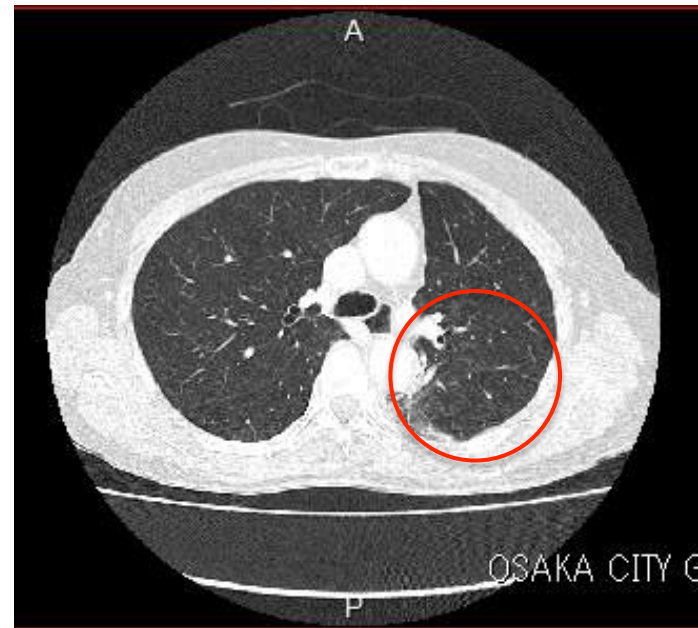
- 64 year old female with lung cancer, adenocarcinoma, stage 4
- Sep 2012 Patient presented at Saisei Mirai
- Integrative Therapies:
 - Iressa targeted therapy
 - 1500 ng High Dose GcMAF 2 times weekly IM injection for 6 months (48 times total)
 - Regional Hyperthermia, 8 times (Thermotron RF8)
 - 4.5 mg Low Dose Naltrexone (LDN) daily

Case report: Lung cancer (cont.)

- Patient achieved complete response by August 2013.
- In remission: 1500 ng High Dose GcMAF 1 time/week (48 doses) for about 1 year to reduce the risk of recurrence.



CT 5-Sep-2012



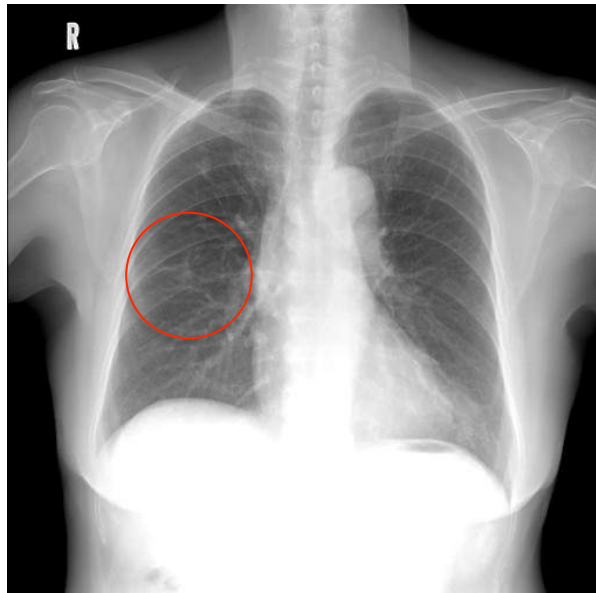
CT 14-Jun-2013

Case report: Pulmonary atypical mycobacteriosis

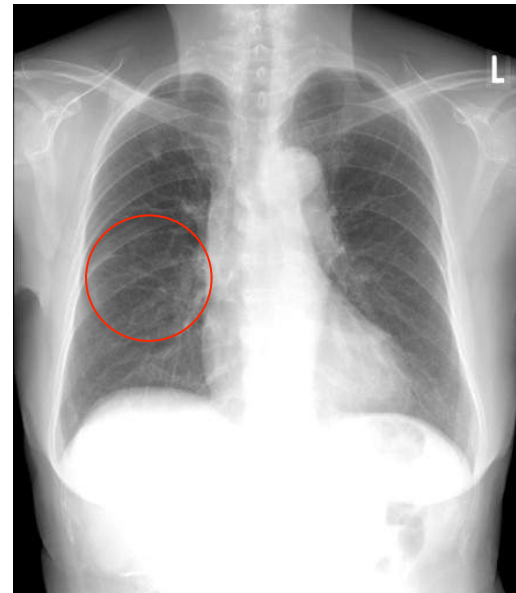
- 76 year old female, pulmonary infections due to nontuberculous mycobacteria of the lung
- Conventional therapy - all available tuberculosis antibiotics for 10 years with good effect initially
- After 10 years of treatment, tolerance to the drugs increased and therapy lost effectiveness
- Jun 2009 - Started high-dose IV vitamin C (25 g) once a week (total 239 times) over a 4 year period

Case report: Pulmonary atypical mycobacteriosis (cont.)

- Feb 2011 - Chest X-ray showed active infiltrations in the lungs
- Oct 2011 - Started high-dose GcMAF (1500 ng, 0.5 ml) 1 time/week (total 41 times) for about 1 year
- Nov 2012 - Chest X-ray showed only scar tissue without active infiltrations in the lungs



3-Feb-2011 Chest X-ray - before GcMAF



22-Nov-2012 Chest X-ray - after GcMAF

Case report: Prostate cancer

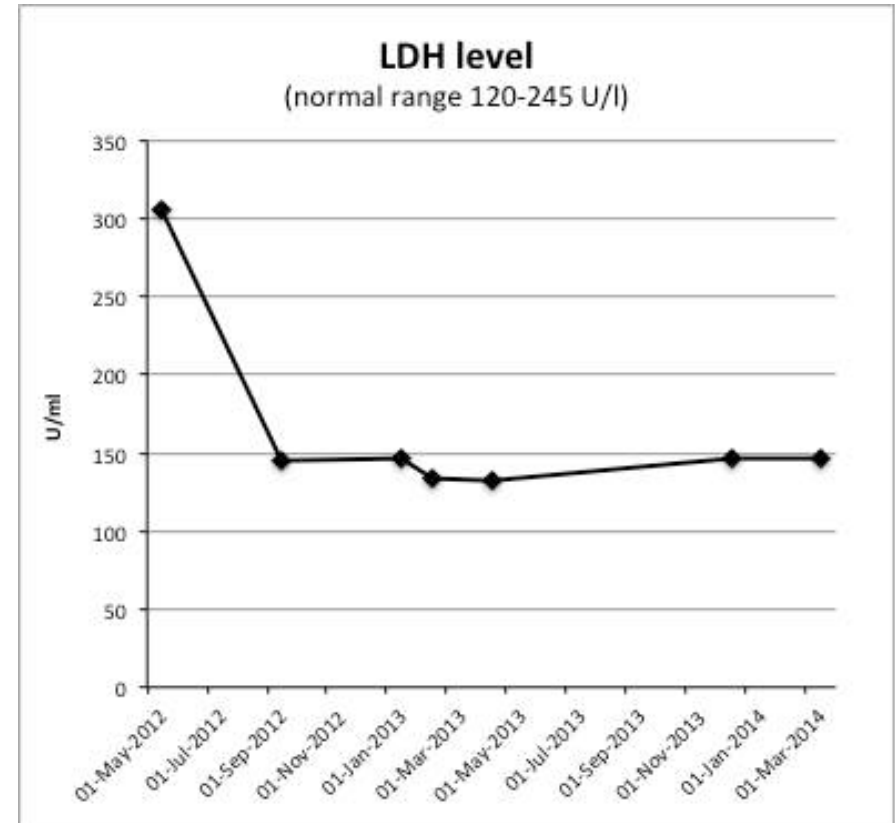
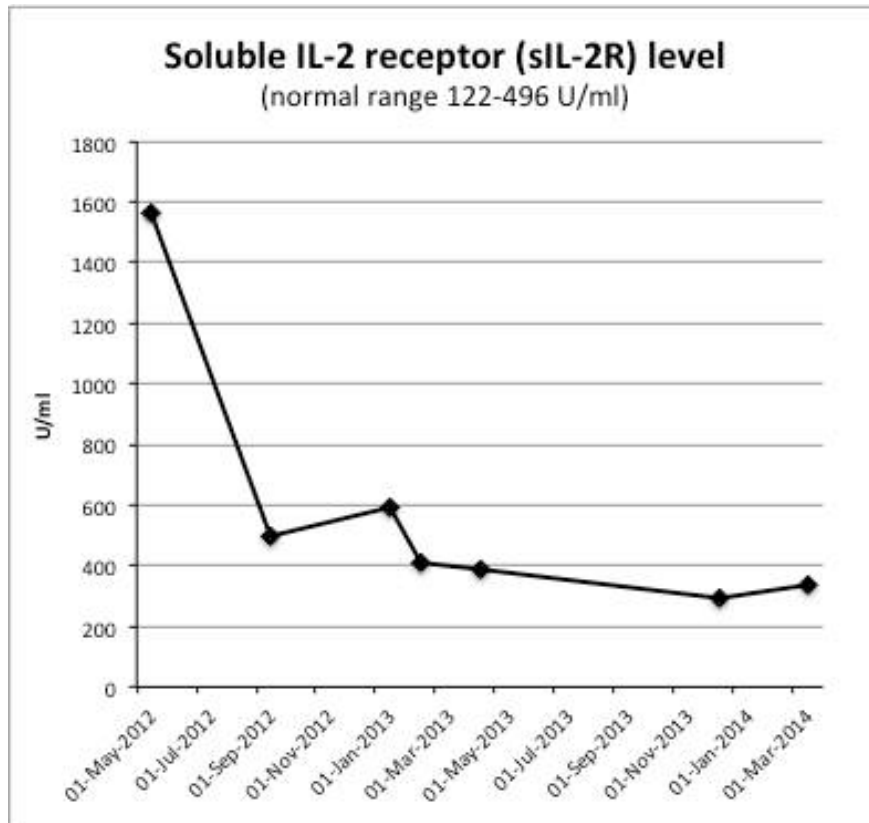
- 60 year old male, prostate cancer
- Gleason score 8 (range 2 to 10)
- Initial diagnosis Feb 2011
- Feb 2011 Radical prostatectomy, lymph node (–)
- No hormonal therapy
- PSA after surgery was increasing
- Oct 2011-Dec 2011 Radiation, 60 Gy dose
- May 2012-Apr 2013 autologous serum GcMAF 72 times, IM (1500 ng/0.5 ml), high-dose IV vitamin C 60 g, 72 times
- Regional Hyperthermia, 21 times (Thermotron RF8)
- Feb 2013 MRI shows no metastatic tumors
- 2014 Normal PSA 0.058 ng/ml, no recurrence

Case report: Hodgkin's lymphoma

- 38 year old female, Hodgkin's lymphoma, stage 2B
- Diagnosed Apr 2012, May sIL-2R 1562 U/ml, LDH 305 U/l
- Chemotherapy, 2 times. Discontinued due to severe side effects (liver dysfunction)
- Jun 2012 presented at Saisei Mirai
- Jul 2012-Jul 2013 high-dose GcMAF 48 times, IM/SC (1500 ng/0.5 ml), high-dose IV vitamin C 60 g, 1 time/wk
- Dec 2012-Jan 2013 Sonodynamic therapy (SDT), 12 times
- Feb 2013 sIL-2R and LDH within normal range
- Jun 2013 PET CT shows no recurrence
- Jan 2014 MRI shows no change from 1 year prior
- Mar 2014 sIL-2R 334 and LDH still within normal range

Case report: Hodgkin's lymphoma

Tumor markers LDH and sIL2 receptor



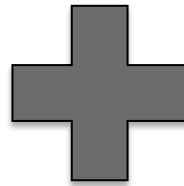
Case report: Esophageal leukoplakia

- 33 year old male, esophageal leukoplakia
- 0.5ml high-dose GcMAF 1 time/week, total 17 times
- IV high-dose vitamin C 25g (13 times) and 50g (5 times), 1 time/week, total 18 times
- Patient was very busy going on business trips overseas
- Endoscopy showed complete disappearance of 3 spots of leukoplakia

Concept of cancer therapy at Saisei Mirai

- Destruction of local cancer tissue + immunotherapy

- Sonophotodynamic therapy
- High Intensity Focused Ultrasound (HIFU)
- Hyperthermia

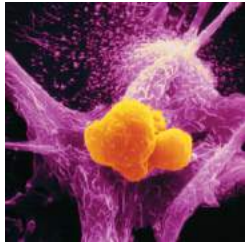


- GcMAF
- Hyper T/NK cell therapy
- Hyperthermia
- Dendritic cell therapy

Cancer treatment solutions at Saisei Mirai

Destruction of local cancer tissue

- Photo Sonodynamic therapy
- Ultrasound guided HIFU



Immunotherapy



- GcMAF
- Hyper T/NK cell therapy
- Dendritic cell therapy



Supportive therapy



- High Dose Vitamin C
- Ozone therapy
- Hyperthermia



Thank you

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