The Road to Total Disease Control

- Regenerative medicine as an ultimate of precision medicine

The principles, present status and scope

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Foundation for Biomedical Research and Innovation at Kobe

*Founded in 2002 by the Ministry of Education, Culture, Sports, Science and Technology (**MEXT**) and **Kobe City**, as the first academic data center for clinical researches in Japan.*

What is learning, for what and how?

學不可以已。學至於行之而止矣。

（荀子）

大上有立徳、其次有立功、其次有立言。

（左伝）

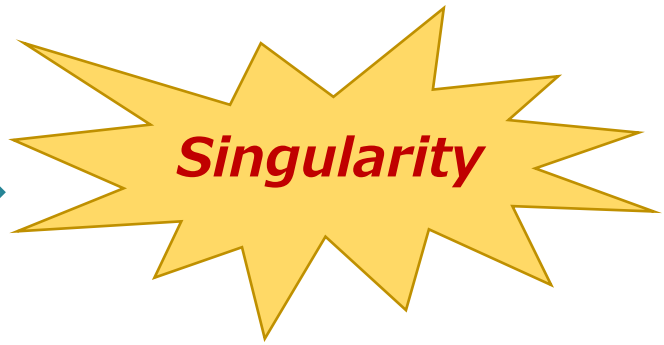
Today's Topics

1. **Introduction – *we are living in unprecedented science revolutionary age***
2. What kinds of services and supports TRI provide, and our accomplishments
3. Explore the life/living organisms as it is
4. Principles of regenerative medicine
5. Examples of regenerative medicine
   – Results from clinical trials
6. What should be done next

Ongoing Science & Technology Revolution in Medical / Healthcare field

1. Genome ・ Immunology
2. Stem cell ・ Exosome
3. Robotics ・ BMI/BCI
4. Nanotechnology ・ Sensing
5. IoT ・ AI

Human exceed human
Machine exceed human



What is SCIENCE ・・・Definition

To observe* the interrelation of things and cause/effect in the universe as it is, describe and explain exactly what you have recognized

*includes to formulate, execute, and observe the experiment

2016.4.12 M. Fukushima

Scientists explore the world as it is, rather than as they would like it to be.

『Nature』449. 25 Oct, 2007
Editorials “Watson’s folly”

唯仏与仏 乃能究尽 諸法実相 （Lotus sutra）

What mankind are doing

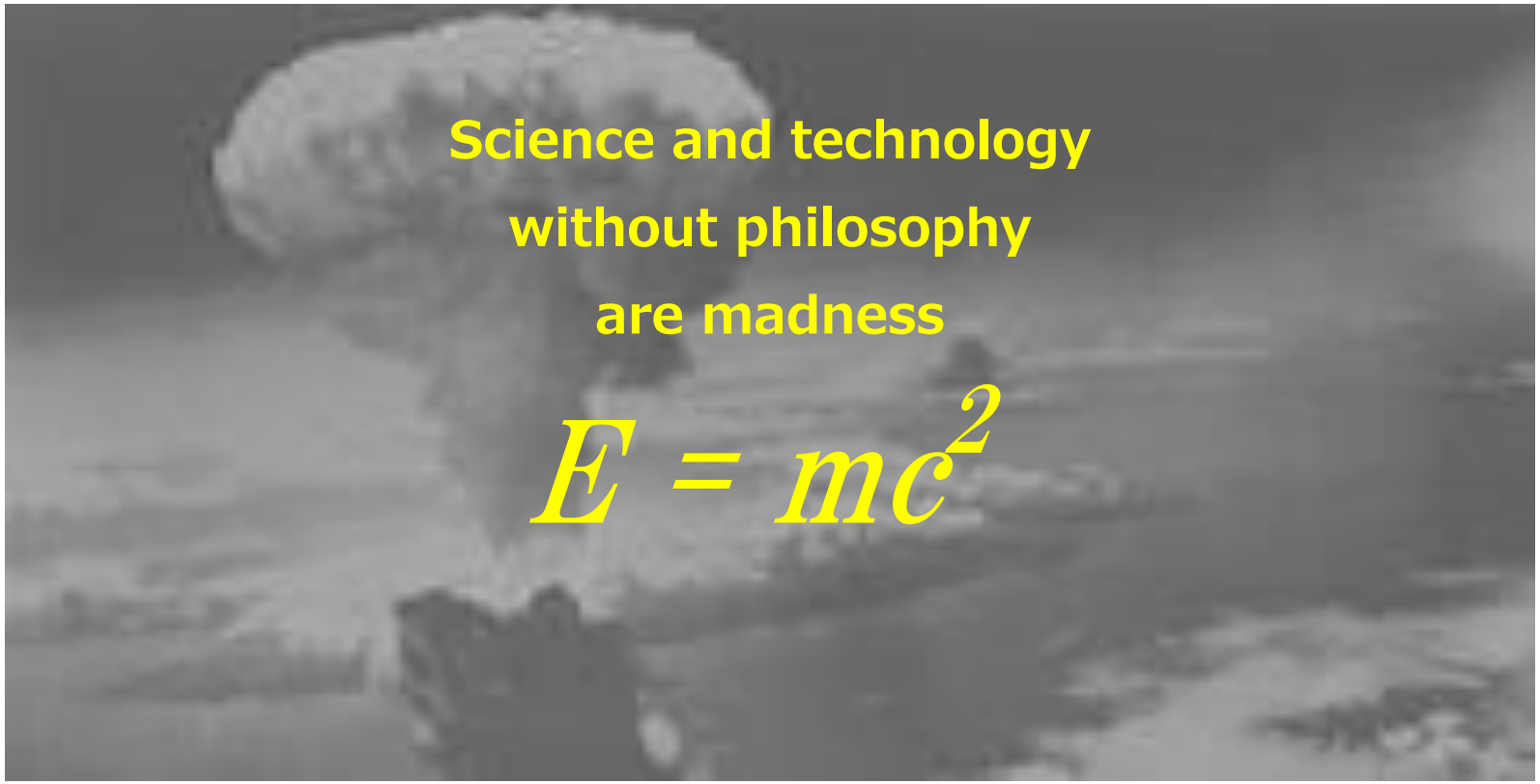
Explain the nature as you have recognized

Predict and intervene nature to

make it as one would like it to be

⇒ Technology

Science and Technology



Right and wrong differs among individual person.

Future of humanity is in your hands.

What do you see beyond AI evolution?

What is ETHICS・・・Definition

Consensus and/or request of the society in which the person belongs to.

The appropriate behavior -words and actions, in which the person lives at that time.

2016.4.7 M. Fukushima

Ethics: a set of moral principles in human conduct
(Ref: Oxford Dictionary)

The School of Athens by Raphaelo illustration cited from Wikipedia:
https://en.wikipedia.org/wiki/The_School_of_Athens

What is CLINICAL SCIENCE・・・Definition

Essential knowledge to solve various problems affecting human health, and the methodology to generate them

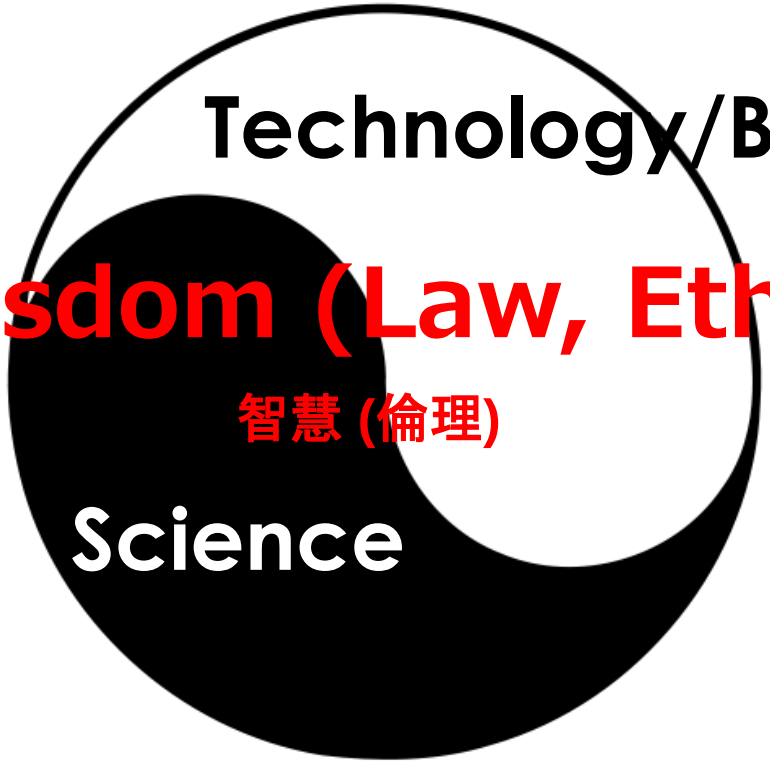
→ To clarify necessary and adequate measures and roadmaps to achieve desirable goal, and to implement them strategically

"Medicine is a science of uncertainty and art of probability"
- Sir William Osler

Science
by the human, for the human, of the human

Science vs Business

Scientists explore the world as it is, technology/business act to the world as they would like it to be ・・・but not necessarily true



→ **Drive R&D**

Required Mindsets

Research & Development (R&D) of drug/medical devices/medical procedure should not be driven **by researcher's own interest**, but is a **product development operation** and **legal process** assembling science & technology based on law, aiming for the goal of attaining approval from the authority.

➡ ***Registration Clinical Trials is the solution***

Under the Pharmaceuticals and Medical Devices Act

What is REGULATORY SCIENCE

Regulatory science requires the state-of-the-art scientific rigor at the time based on humanity, which should be managed and guaranteed by the law.

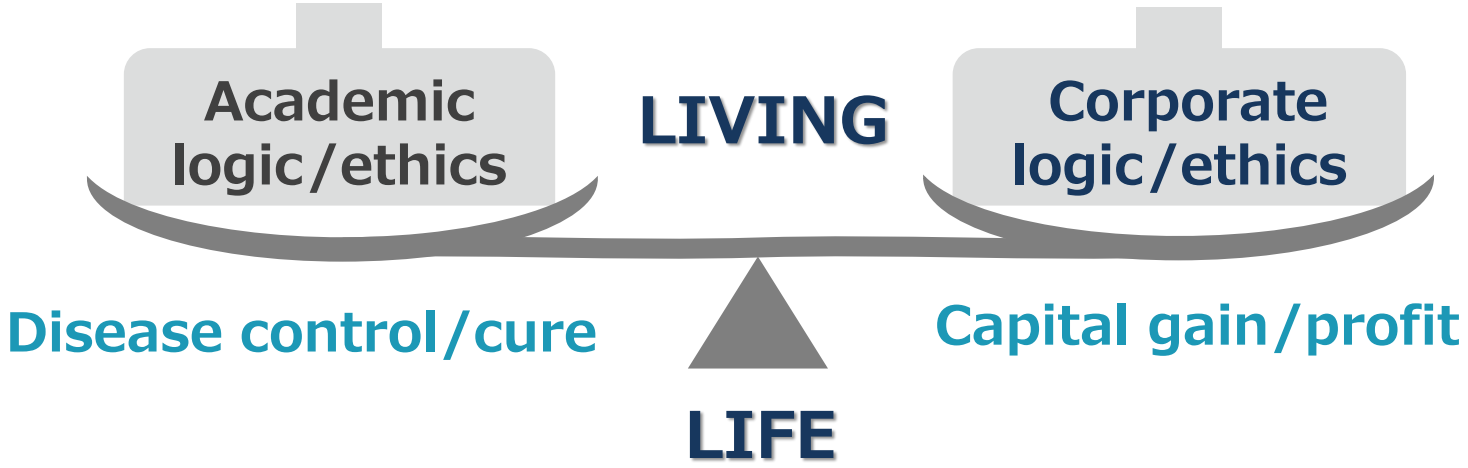
2011.1.8 M. Fukushima

The collision of logic/ethics between Academia and Commercial companies

Invested money should be collected.
Moreover,
should make a profit.

However, medical R&Ds are NOT for making money !

➡ **Return on Investment (ROI)**



Within medical R&D competition---

Is science a slavery of business?

＜我見諸衆生
没在於苦海＞

Science

Business

Liberal ocean on economic market

⇒ ***vicious cycle***

Health care vs. Economics

For total disease control, we need new theory or principles of economics

Issue of re-distribution of wealth

Poverty is the major cause of disease

The question is can we achieve our goal through existing social economic system, per se capitalism for market?

Ongoing Science & Technology Revolution in Medical / Healthcare field

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3. Robotics ・ BMI/BCI
4. Nanotechnology ・ Sensing
5. IoT ・ AI

Janus image cited from Wikipedia
https://en.wikipedia.org/wiki/Janus

Human exceed human
Machine exceed human

Types of innovation

【J. Schumpeter】

Tailored-type Service

Material → Manufacture → Production → Product → Distribution

Philosophy / Science / Technology

- **Individual base**
- **Population base**

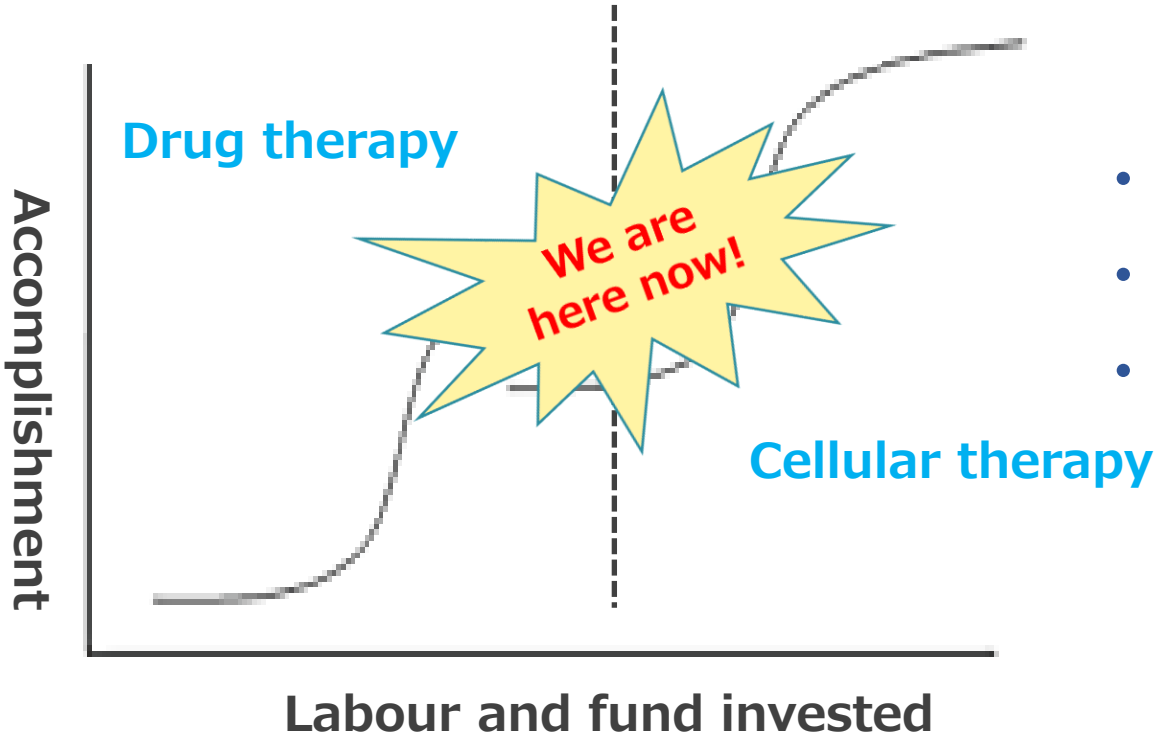
Genome, exosome innovation
Stem cell, immuno-cellular Innovation
Digital Health Innovation

AI assisted science & health care system
(Learning health care system)

Feature of Disruptive Innovation

Disruptive innovation refers to economic activities such as products and services which have the impact of destroying existing market value and converting the market into a whole new value system (Clayton M. Christensen, “The Innovator's Dilemma”)

Double S-curve 【R. Foster】



- **Non-contiguous (change of personnel)**
- **Types**
- **Investments**
- **Entrepreneurs**

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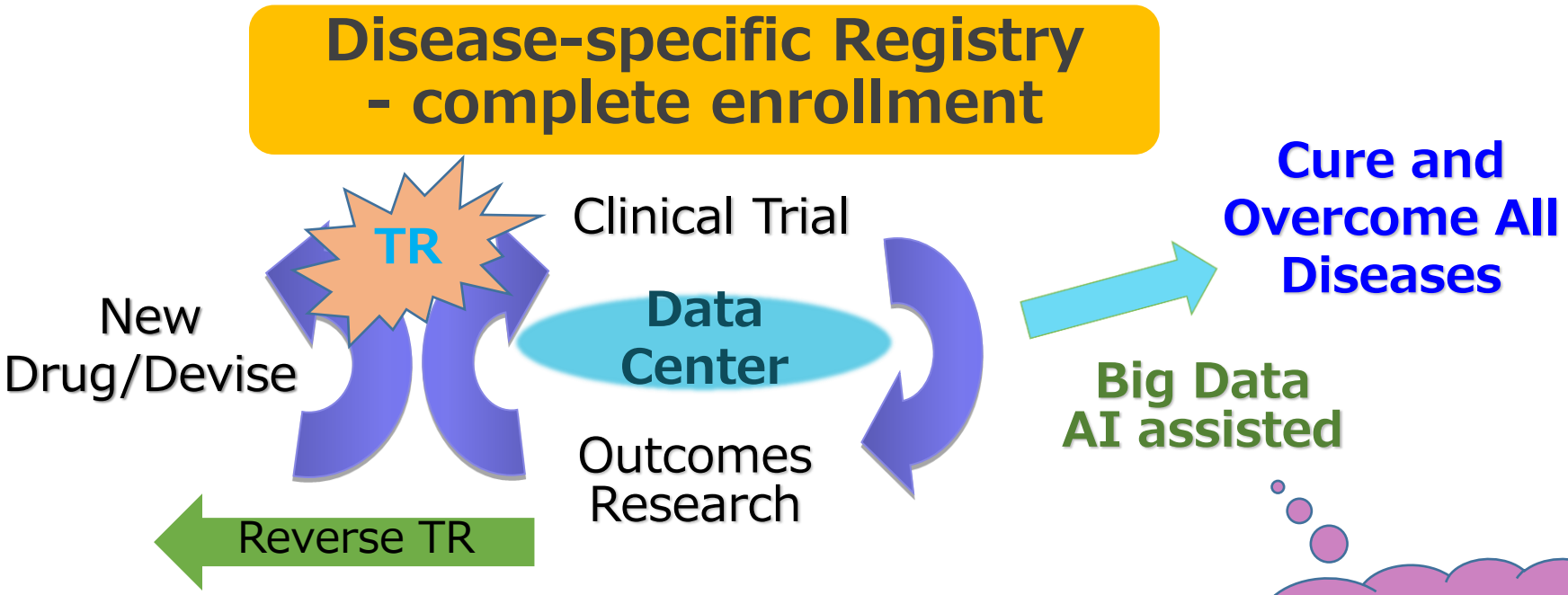
Grand design -Toward Total Disease Control
Central dogma - Practice of Science

We can accelerate this cycle dynamically through global data sharing



TRI's organization structure to achieve its goal

GOAL

- **Healthy Centenarian**
- **0 Bed-ridden**

Division of Health Data Science

projects

Registry → **Guidelines**

Sales & Marketing

Business Development Group

Science Statistics

Orders

R&D Pipeline → **Approvals**

Division of Medical Innovation

projects

Digital Health Innovation

Regenerative therapeutics:

① Vocal cord (titanium bridge)
② Cerebral nerve/ bone marrow (CD105 cell)
③ Eardrum (bFGF/gelatin sponge)
④ Blood vessel (CD34 cell)
⑤ Bone (CD34/atelocollagen)
⑥ Cornea (Mucous membrane cell sheet)
⑦ Cartilage (Cartilage cell/collagen)
…

TRI Operation
- Support Clinical Trials & Medical R&D

➢ The one and only Data Center for academia

Provide comprehensive clinical trial management services for studies of all phases from research planning to data analysis.

R&D Strategy

1. R&D policy
   - Market analysis・Competitive Research
   - R&D scheme・R&D truck
2. Patent strategy
   - Patent consultation
   - Patent research support
3. Non-clinical
   - Efficacy ・Safety ・Test material production
4. Collaborations with joint development (Liaison)
5. ARO framework construction support

Clinical trial

1. First-in-man study protocol development and regulatory relations
2. Clinical trial management – launch and operation of trials
3. Data Management
4. Statistical analysis
5. System development (ex. EDC)
6. Global clinical trial support – planning, launch, and operation
7. Monitoring and Audit

【リンゴ位置変える】TRI Achievement - Pipeline, Approval and Market

TRI aims for disease control and healthy life extension.

From our past activities, some major diseases that cause long-term care, are expected to be overcome.
In the future, we will strongly promote digital health innovation and contribute to the extension of the healthy life span.

* Vocal cords / Ear-drum / Cornea

Ability to communicate is essential to live centenarian society.

Regeneration of eardrum, vocal cords, and cornea make it possible to “hear”, “talk”, and “see”.

＊Vocal cords: TITANBRIDGE, December 15, 2017, approval (The first approval in SAKIGAKE Designation System!!)

** Nerve, Spinal cord / Blood vessel / Carti-lage / Bone

We devote ourselves to reduce bed-ridden, care-needed patients.

Regeneration of nerves, blood vessels, and cartilage will lead to independent living from lower limb amputation caused by stroke, spinal cord injury, Buerger’s disease, and ASO, and other various bed-ridden, care-needed causes.

＊＊Nerve, Spinal cord: Stemirac, December 28, 2018, conditional and time-limited approval

Dementia / Prostate cancer / Colon cancer / Arterial sclerosis

New therapies are under development for the treatment of dementia, prostate cancer, colon cancer, arterial sclerosis.

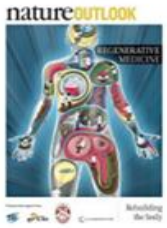
Examples of TRI-supported serves

TRI Advances

https://advances.tri-kobe.org/

https://advances.tri-kobe.org/en/research/8/-a-stem-cell-fix-for-spinal-injury

Nature Outlook 2016 ／ Outline 2017



Vol. 540 No.S49, December 7, 2016

Nature Outlook: Regenerative medicine

www.nature.com/articles/540S49a

Theory

Vol. 544 No.7650_supp_out,April 20, 2017

Corneal repair

https://www.nature.com/collections/pdryjrsvnz/videos

Preparing NDA

Vol. 546 No.7659_supp, June 22, 2017

Eardrum regeneration: membrane repair

https://www.nature.com/collections/rzfrydkflp/videos

Approved on Aug 1. 2019

Vol. 548 No.7668_supp, August 24, 2017

Critical limb ischaemia

https://www.nature.com/collections/vmxkcnxvwg/videos

Registration trial ongoing

Nature Outline 2017 - 2018

Vol. 550 No. S193, October 26, 2017

Non-union bone fracture: a quicker fix

https://www.nature.com/collections/qmpthxknbn/videos

Registration trial ongoing

Vol. 552 No. 7684_supp, December 14, 2017

Spinal-cord injury: spurring regrowth

https://www.nature.com/collections/ctdkppqqnx/videos

Approved / Launched

Vol. 563 No. S33, November 7, 2018

Ulcerative colitis

https://www.nature.com/collections/qwtdpjcrpg/video

Registration trial ongoing

Vol. 564 No. S73, December 20/27, 2018

Liver Cirrhosis

https://www.nature.com/collections/ycpfrvtnhj/video

Registration trial ongoing

First round of regenerative medicine has been completed

	Type of regeneration	Target Disease	PI	SAKIGAKE Designation
Approved **Dec. 20, 2018**	**Nerve** (auto serum-expanded autologous CD105 mesenchymal stem cells)	Spinal cord injury  Vol. 552 No. 7684_supp | December 14, 2017 Spinal-cord injury: spurring regrowth https://www.nature.com/collections/ctdkppqqnx/videos	**Osamu Honmou (Sapporo Medical University)**	★ February 2016
Approved **Aug. 1, 2019**	**Eardrum** (bFGF/gelatin sponge)	Tympanic membrane  Vol. 546 No. 7659_supp | June 22, 2017 Eardrum regeneration: membrane repair http://www.nature.com/collections/rzfrydkflp/videos	**Shinichi Kanemaru (Kitano Hospital)**	
Under trial	**Blood vessel** (CD34/cell)	Critical Limb Ischemia  Vol. 548 No. 7668_supp | August 24, 2017 Critical limb ischaemia www.nature.com/collections/vmxkcnxvwg/videos	Atsuhiko Kawamoto (TRI)	★ March 2018
Under trial	**Bone** (CD34/atelocollagen)	Refractory bone fracture  Vol. 550 No. S193 | October 26, 2017 Non-union bone fracture: a quicker fix https://www.nature.com/collections/qmpthxknbn/videos	Ryosuke Kuroda (Kobe University)	★ March 2018
Preparing NDA	**Cornea** (Mucouse membrane cell sheet)	Corneal epithelial stem cell deficiency  Vol. 544 No. 7650_supp_out | April 20, 2017 Corneal repair http://www.nature.com/collections/pdryjrsvnz/videos	Chie Sotozono (Kyoto Prefectural University of Medicine)	
Under trial	**Cartilage** (Cartilage cell/collagen)	Cartilage injury	Hiroyuki Ishibashi (Hirosaki University)	

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Group blindness evaluation　群盲撫象

・・・ *far from as it is!*

Image cited form Wikipedia https://en.wikipedia.org/wiki/Blind_men_and_an_elephant

Scientific procedure: recognition always under restraint by individual perception

Science is preoccupied with inductive inference as experimental science

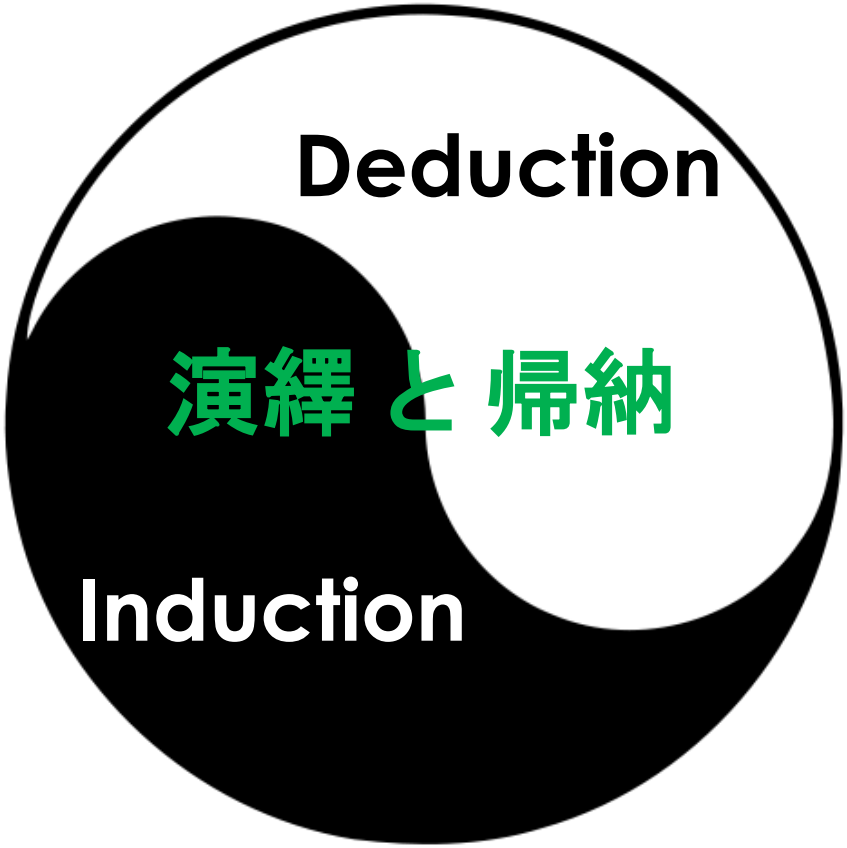
Inductive inference

- ➡ hypothesis/principles/theory
- ➡ deduction
- ➡ explain the nature based on the hypothesis
- ➡ test hypothesis

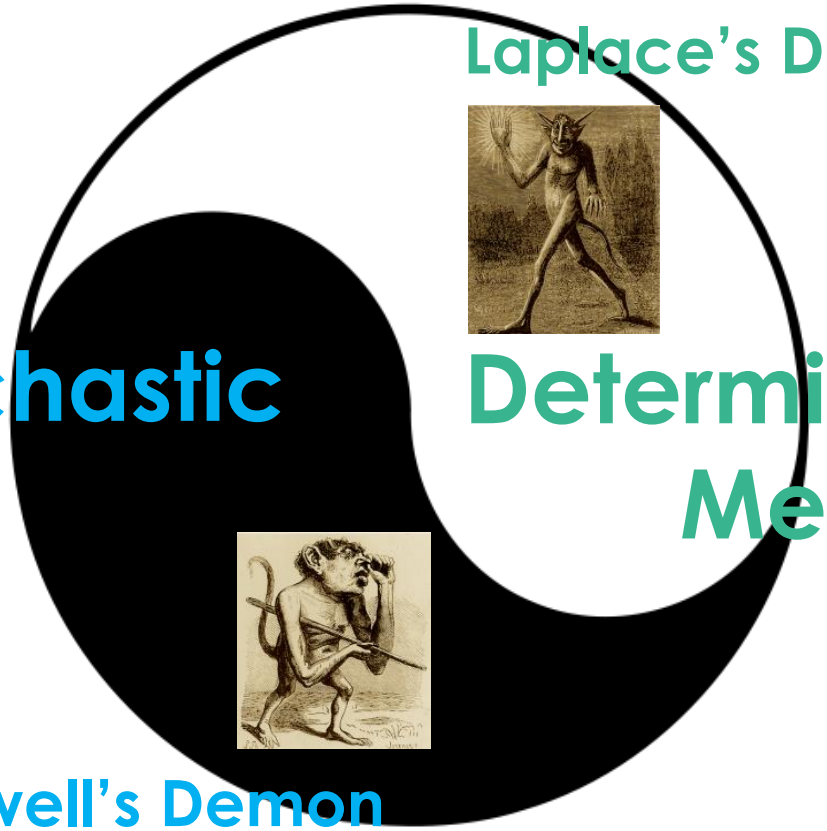
To gain insight deeply, in particular,

the understanding of life, AI, humanity, biological principles derived from the research axiomatic system

Science based on epistemology: theory of knowledge



Understanding the world/life: prediction of phenomena



Demon illustration cited from https://www.atlasobscura.com/articles/demons-illustrations-dictionnaire-infernal

Science: level of recognition

Epistemology
Theory of knowledge

phenomenalism

substantialism

essentialism

Unsolved Questions

1. origin, evolution
2. life duration
3. consciousness

Axiomatic System

- integrity
- homeostasis
- self-organization
- scaffold field
- symbiosis
- rhythm resonance, synchronization
- symmetry

2016.5.9 M. Fukushima

Developmental stage-1
Diagnostics and therapeutics

Level of disease control

science (level of recognition)	diagnosis	treatment	evaluation
phenomenalism	Symptomatic	Symptom control	Surrogate endpoint
substantialism	Patho-physiological	↓	↓
	Genetical		
essentialism	Etiological	Event control	True endpoint

Developmental stage-2
Diagnostics and therapeutics

Level of disease control

science (level of recognition)	Approaches		
phenomenalism	probabilistic	analytic	mechanistic
substantialism	↓	↓	↓
essentialism	deterministic	integrative	holistic

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Stem cell medical revolution

New paradigm of medicine using the essence of natural healing force

Autologous or allogeneic stem cells derived from bone marrow, adipose tissue etc.

→ **Newer conception of disease**

regenerative homeostasis failure

Indicates to majority of intractable diseases

Copernican revolution

→ **Paradigm change of drug discovery & development**

The Biological Principles of tissue regeneration in the body -1

Stem cell which play major role in tissue repairing/regeneration are widely distributed over the body tissues, connective tissues, adipose tissue, bone marrow, and it was preserved through the process of evolution.

The Biological Principles of tissue regeneration in the body -2

Stem Cell is naturally existing in the blood, circulating the whole body and serving turn-over of the tissue cells, repairing and regeneration of the tissues.

The Biological Principles of tissue regeneration in the body -3

When some tissue are damaged, … signaling

Stem cells catch the signals from the damaged tissue … perception

Stem cells are mobilized to blood flow, … mobilization

Stem cells are homing to the damaged area, … homing

Stem cells perform required conditioning of the damaged area,

…conditioning

Triage dying cells and survivable cells, eliminate the former, and protect/ rescue the latter cells, suppressing inflammation

conversion of M1 to M2, control permeability of the micro-vasculature,

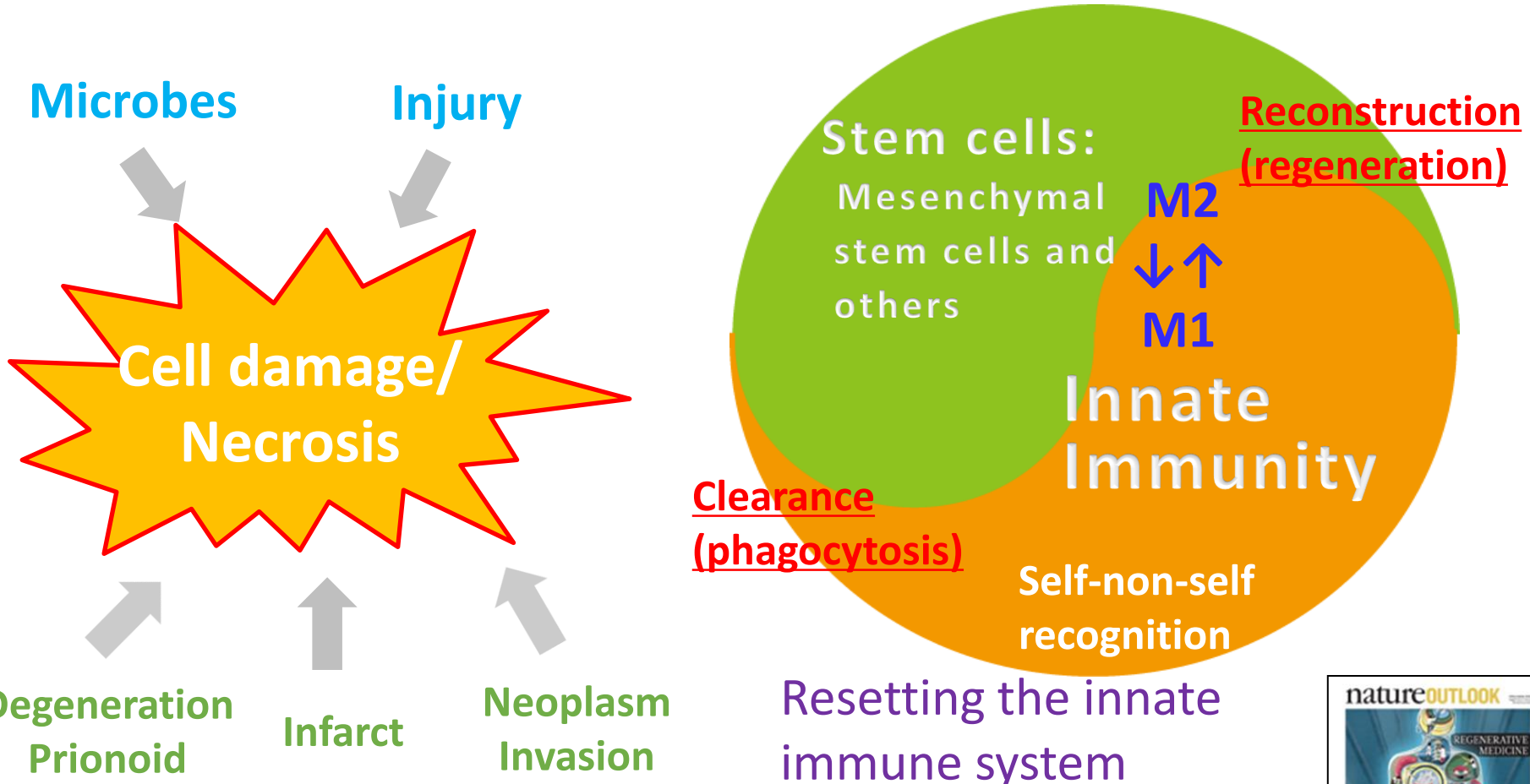
Reconstruct/regenerate micro vascular architecture,

supply oxygen and nutrients for survivors, and then regeneration process begins. … repairing, regeneration

The Biological Principles of tissue regeneration in the body -4

When the tissue damage is massive and beyond natural healing capacity i.e. insufficient number of stem cells which is mobilized as a natural process, re-infusion of expanded stem cells derived from autologous bone marrow or from harvested circulating stem cells help the process of tissue regeneration as natural healing autonomic process.

Multicellular symbiotic system for maintaining homeostasis that inherits self-preserving ability



Ref: Nature Outlook: Regenerative Medicine, December 7, 2016
Figure 2. Mechanisms regulating maintenance of normal functioning of multicellular symbiotic systems.

Physiology and pathology of stem cell and its molecular basis

	Phenomenon	Cell	Cell EV	Cytokine, others
Damage →	Signaling		Exosome mitochondria? miRNA	SDF-1/CXCR4, S1P/S1PR HMGB1/RAGE?, etc.
Necrosis Apoptosis	↓			
	Mobilization	MSC, Muse cell (SSEA3+)		
	↓			
↓	Homing	CD105		
Inflammation	↓	CD34		
	Conditioning Clearance (phagocytosis)			
	・anti inflammatory	M1→M2, HGF, etc.		
	・trophic action			NGF
	・vascularization			VEGF, etc.
	・others			
	↓			
	Differentiation Regeneration			

Current status on regenerative medicine I

First round of regenerative medicine clinical development

- Disruptive Innovation Phase

has been completed, and entered into second round

First round: biological principle concerning tissue regeneration

– ***clarification of cell-generation process and establishment of its utilization***

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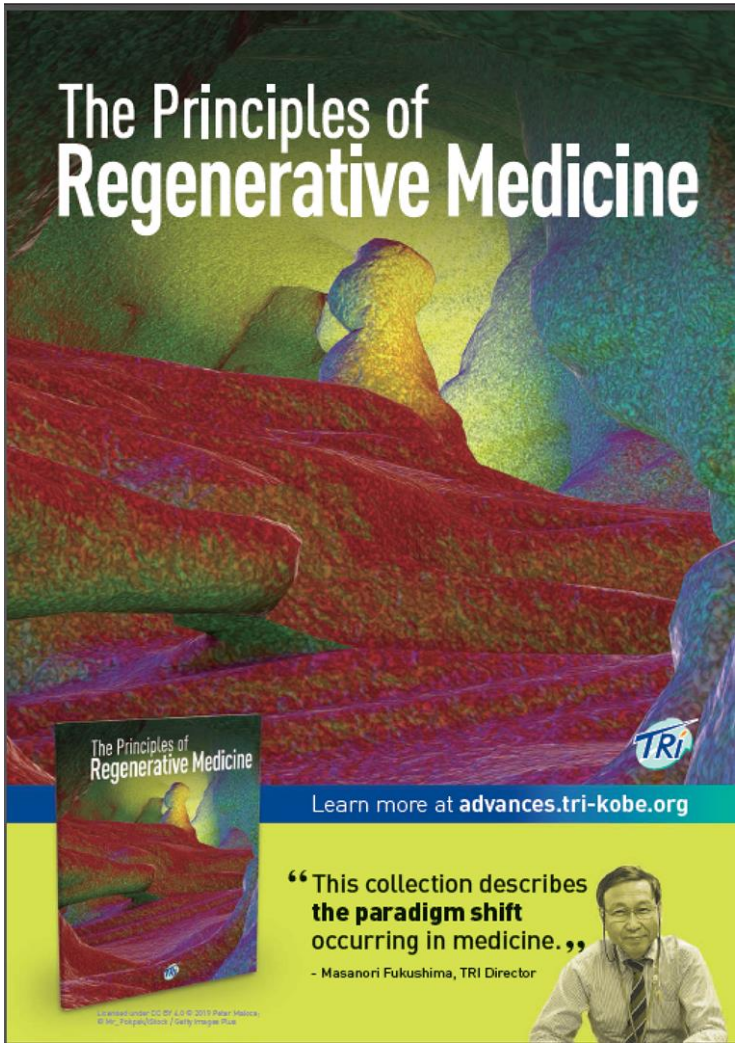
The Principles of Regenerative Medicine



Theory of Disease Control — Masanori Fukushima

Reprinted from *The Principles of Regenerative Medicine*, a collection of articles to be published soon on regenerative medicine research being conducted by researchers associated with the Translational Research Center for Medical Innovation (TRI) in Kobe.

An overview of regenerative medicine: its principles and the scope of the current revolution

Masanori Fukushima
CEO and Chairman, Translational Research Center for Medical Innovation, Kobe, Japan

1. INTRODUCTION

Regenerative medicine medicine represents a coming revolution in the treatment of many illnesses. Emerging therapies that use stem cells harvested from the patients themselves are demonstrating extremely promising results in clinical trials and other studies. Referred to as stem-cell therapies, or just cell therapies, they involve taking stem cells from the body, culturing them, and then putting them back into the body to induce tissue to regrow. Japanese researchers are world leaders in this field and their many years of hard work are bearing fruit as clinical trials progress according to schedule. We can be optimistic that novel therapies will soon make currently untreatable diseases and disorders treatable.

The chapters of this collection describe six regenerative therapies. Before considering specifics, however, in this essay I provide an overview, explaining the principles common to all regenerative therapies: the use of bioactive stem cells in a patient and the provision of framework materials as scaffolds for tissue regeneration using tissue engineering.

2. WHAT IS STEM-CELL THERAPY?

Stem cells are found in various tissues in the body, including the blood, bone marrow, fat, connective tissue, nerves, skin, etc. When stimulated, stem cells have the capacity to produce specific, mature cell types. Stem cells are believed to replenish damaged or dead cells in the body.

The stem cell therapies described here use adult stem cells. Since cell manipulation is not applied, there is no risk of developing tumours. The ethical issues associated with embryonic cells are also avoided. There is no risk of rejection by the immune system, as the patient's own cells or immunotolerant stem cells are used. This means that, unlike organ transplantation, stem-cell therapy does not need adjuvant therapies such as the continuous administration of immunosuppressants. Furthermore, both cell extraction from the body and cell replacement (either as-is or after culturing) can be partially automated. Many procedures can be performed using relatively simple techniques, such as intravenous infusion and other techniques that do not require general anaesthetic.

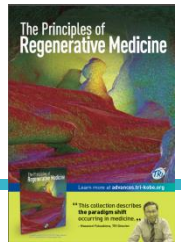
Adult stem cells fall into two broad categories. One is haematopoietic stem cells. As their name indicates, these stem cells can produce blood cells, but they can also generate vascular cells. The other type is currently referred to as mesenchymal stem cells. They are so named because these stem cells were originally thought to produce cells that originate in the mesoderm (mesenchyme), including the bone and fat. However, recent studies have revealed that some mesenchymal stem cells can create nerve cells, which are not of mesodermal origin. Further research is needed to uncover more about these cells.

How do we identify adult stem cells when they exist only in minute quantities in the body? The answer lies in using markers for the glycoproteins that are expressed on their membranes. For example, since haematopoietic stem cells have a glycoprotein called CD34, they can be identified as $CD34^+$ cells. These $CD34^+$ haematopoietic stem cells show potential for treating blood vessels in the legs that have been obstructed in critical limb ischemia (Stem Cell Therapy 3) and for treating intractable fractures (Tissue Engineering 1) in combination with a scaffold. An example of an emerging stem-cell therapy that uses mesenchymal stem cells is one for spinal cord injuries (Stem Cell Therapy 1), in which nerve cells are regenerated using $CD105^+$ cells.

Some adult stem cells defy categorization due to their diverse characteristics. For example, multilineage differentiating stress enduring (Muse) cells, which were discovered by Mari Dezawa of Tohoku University in 2010[1], are thought to create cells of various tissue types and to play a specialized role in the repair of body tissues. Muse cells are the basis for an experimental approach to treating myocardial infarction (Stem Cell Therapy 2) and have been used to regenerate cardiac muscles, which had previously been considered difficult to do. Remarkably, studies have indicated that intravenous infusion of Muse cells is effective for treating myocardial infarction patients and, despite being an allogeneic transplant, immunosuppression is not needed for the initial infusion.

The above-mentioned stem-cell approaches all employ extremely simple medical procedures. They harness the body's innate healing mechanism, by extracting stem cells, boosting them outside the body and then returning them to the patient, as is the case in intravenous infusion of stem cells from the patient. The therapies are based on biological principles known as stem-cell physiology, which have been described in the publications listed in Ref.

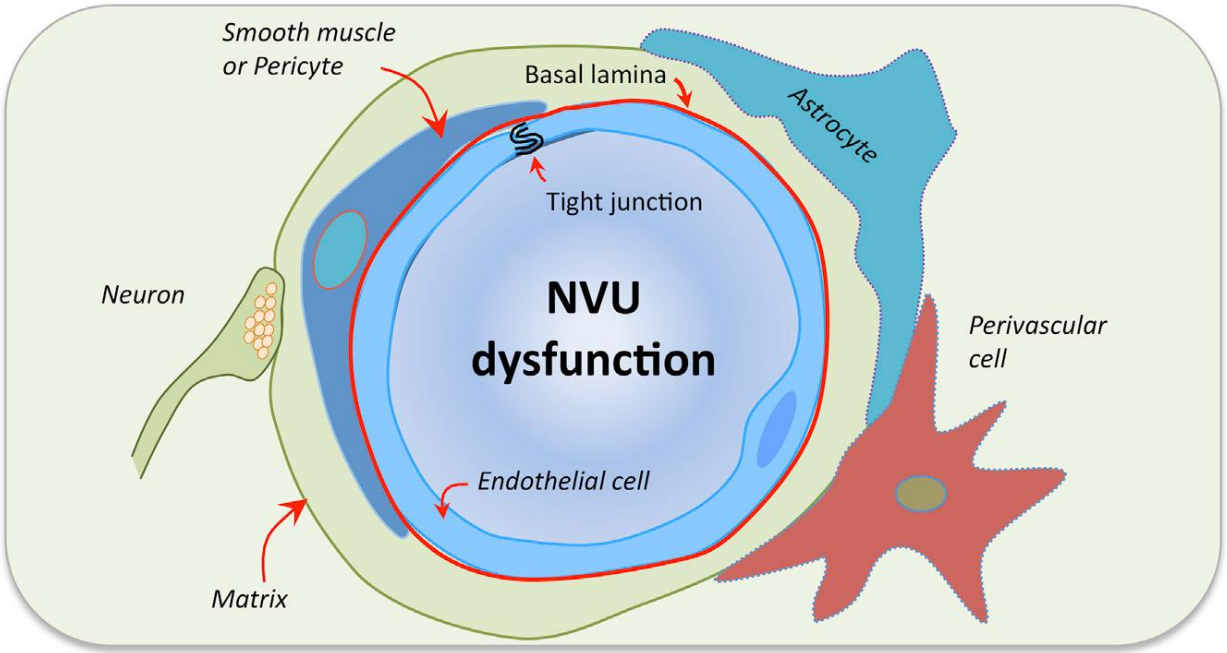
Figure 1. Multicellular symbiotic system for maintaining homeostasis that inherits self-preserving ability



The Principles of Regenerative Medicine
Stem cell therapy and tissue engineering therapy

Preface			
Theory: An overview of regenerative medicine: its principles and the scope of the current revolution			
Stem Cell Therapy	1	**Prof. Honmou,** Spporo medical Univ.	**Nervous system**
	2	**Profs. Minatoguchi & Dezawa,** Gifu Univ. & Tohoku Univ.	**Myocardium**
	3	**Dr. Kawamoto,** TRI	**Vascular**
Tissue Engineering	4	**Prof. Kuroda,** Kobe Univ.	**Bone**
	5	**Prof. Sotozono,** Kyoto pref. Univ.	**Cornea**
	6	**Dr. Kanemaru,** Kitano Hosp.	**Ear drum**

Fine architecture of nervous system: neuro-vascularization complex symbiotic system



Reduced CBF	BBB dysfunction	Trophic failure	Reduced clearance
⇩	⇩	⇩	⇩
Hypoxia	Altered homeostasis	Cell vulnerability	Protein accumulation

Ref : *Neuron.* 2017 Sep 27;96(1):17-42.

Spinal-cord injury: spurring regrowth

Vol. 552 No. 7684_supp, December 14, 2017

Spinal-cord injury: spurring regrowth

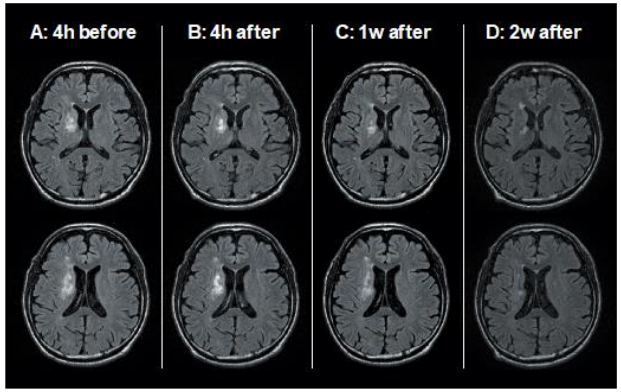
https://www.nature.com/collections/ctdkppqqnx/videos

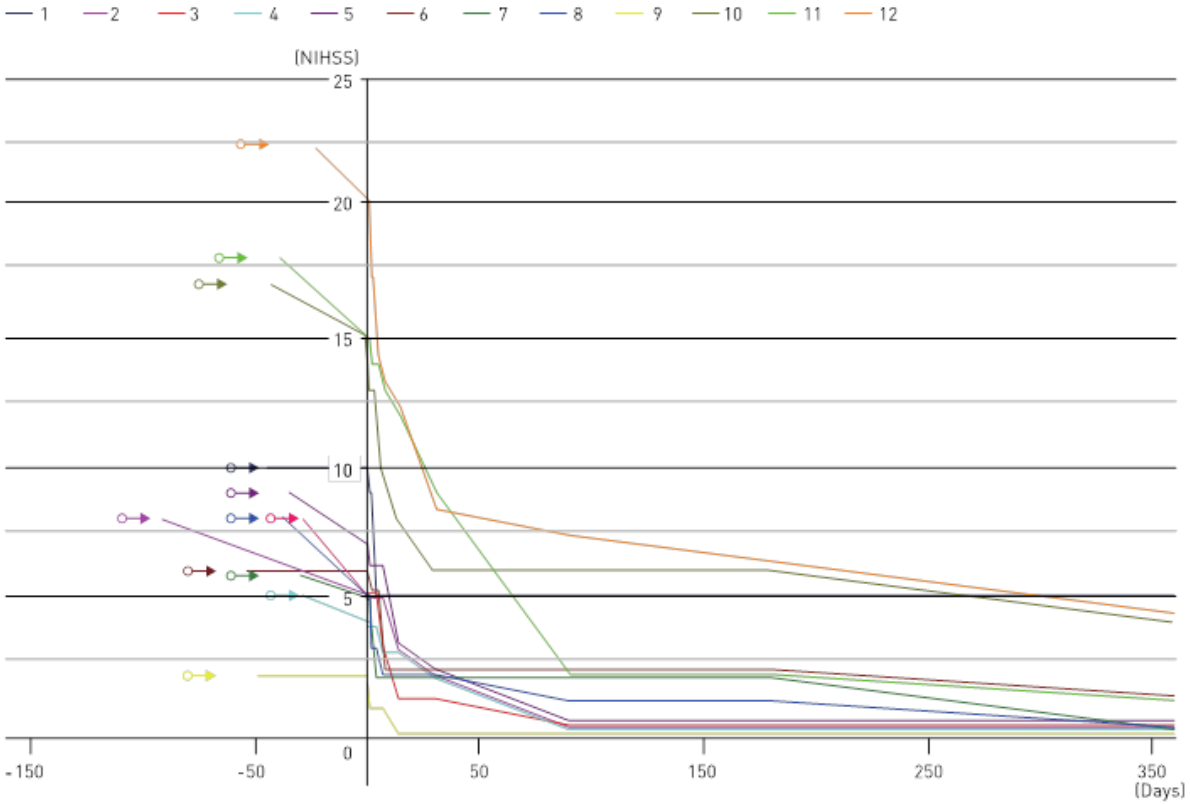
Prof. Osamu Honmou
Department of Neural Regenerative Medicine, Research Institute for Frontier Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan

Publication
Honmou O., et al. **Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke.** Brain 2011: 134; 1790–1807.
Honmou O., et al. **Mesenchymal stem cells: therapeutic outlook for stroke.** Trends in Molecular Medicine 2012 May;18(5); 292-7.

MSC for Stroke

Figure Magnetic resonance imaging images of patients' brains before and after the administration of mesenchymal stem cells.





Ref : Honmou et al., Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke.
Brain 2011: 134; 1790-1807.

Figure Effect of administration of mesenchymal stem cells on the NIH stroke scale (NIHSS) of patients.

ASIA score transition in spinal cord injury - from Guideline of ICCP in US (issued 2007)

cervical	2day	week 4	week 8	week 16	week 26	week 52		From w4 (d28) to w52 (1y)
ASIA A	○	252	221	202	202	188		ASIA A ⇒ B/C/D
ASIA B		29	39	43	47	33		17.2 %
ASIA C		13	21	20	27	25		
ASIA D			4	13	17	19		
ASIA E								
total		294	285	278	293	265		
cervical	2day	week 4	week 8	week 16	week 26	week 52		
ASIA A		9	8	6	7	5		ASIA B ⇒ C/D/E
ASIA B	○	49	34	28	25	23		49.4 %
ASIA C		28	25	19	20	11		
ASIA D		9	25	37	41	44		
ASIA E		1	1	3	6	6		
total		96	93	93	99	89		
cervical		week 4	week 8	week 16	week 26	week 52		
ASIA A			1					
ASIA B								ASIA C ⇒ D
ASIA C	○		22	12	8	6		71.3 %
ASIA D			17	28	32	32		
ASIA E			0					
total			40	40	40	38		

Ref: Guideline of ICCP（International Campaign for Cures of Spinal Cord Injury Paralysis）
Fawcett JW, Curt A, et al. . Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: spontaneous recovery after spinal cord injury and statistical power needed for therapeutic clinical trials. Spinal Cord. (2007) 45:190–205. PMID: 17179973

Stemirac: Approval of autologous MSC for spinal cord injury (issued Nov. 21, 2018)

平成３０年１１月２１日
医薬・生活衛生局
医療機器審査管理課

審議結果報告書

［類　　別］　ヒト細胞加工製品　二　ヒト体性幹細胞加工製品
［一般的名称］　ヒト（自己）骨髄由来間葉系幹細胞
［販 売 名］　ステミラック注
［申 請 者］　ニプロ株式会社
［申 請 日］　平成30年6月29日（製造販売承認申請）

【審議結果】

平成30年11月21日の再生医療等製品・生物由来技術部会の審議結果は次のとおりであり、この内容で薬事分科会に報告することとされた。

本品目を承認して差し支えない。条件及び期限付承認に該当する。条件及び期限は次のとおりとすることが適当である。

承認条件

1. 緊急時に十分対応できる医療施設において、脊髄損傷の診断・治療に対して十分な知識・経験を持つ医師のもとで、本品の使用が適切と判断される患者に対して、バイタルサインの確認、臨床検査によるモニタリングや管理等の適切な対応がなされる体制下で本品を使用すること。
2. 条件及び期限付承認後に改めて行う本品の製造販売承認申請までの期間中は、本品を使用する症例全例を対象として製造販売後承認条件評価を行うこと。

承認の期限

7年

Package insert for Stemirac

2018年12月作成（第１版）

承認番号	23000FZX00001000

ヒト細胞加工製品 02 ヒト体性幹細胞加工製品
ヒト（自己）骨髄由来間葉系幹細胞

再生医療等製品 **ステミラック注**

再使用禁止

〔警　　告〕
1. 製造販売業者が実施する本品に関する講習会を修了し、「用法及び用量又は使用方法」の項の内容を熟知した医師のもとで本品を使用すること。［適正な使用により安全性を確保するため］
2. 緊急時に十分対応できる医療施設において、脊髄損傷の診断・治療に対して十分な知識・経験を持つ医師のもとで、本品の使用が適切と判断される患者に対して、バイタルサインの確認、臨床検査によるモニタリングや管理等の適切な対応がなされる体制下で本品を使用すること。［適正な使用により安全性を確保するため］
3. 本品に関する臨床成績は限られていること及びそれを踏まえた条件及び期限付承認であることを含めた本品の正確な情報について、文書を用いて患者又は家族へ説明し、文書同意を取得した上で使用すること。［患者が本品の有効性及び安全性を理解することが重要であるため］

禁忌・禁止
1. 再使用禁止。
2. 本品は原料として用いる骨髄液及び末梢血を採取した患者本人以外に適用しないこと。［自己細胞由来製品のため］
3. 本品の成分に対し過敏症の既往歴のある患者

成分		分量（１製品中）	
		１バッグ（20mL）/製品	２バッグ（40mL）/製品
構成細胞	自己骨髄間葉系幹細胞	0.5～2.0×10^8個	
副成分	RPMI 1640	8 mL	16mL
	ジメチルスルホキシド	2 mL	4 mL
	デキストラン40	200mg	400mg
	塩化カルシウム水和物	0.4mg	0.8mg
	塩化カリウム	0.6mg	1.2mg
	塩化ナトリウム	12.0mg	24.0mg
	L-乳酸ナトリウム	6.2mg	12.4mg
	自己血清	8 mL	16mL

⑵骨髄採取キット
本品の副構成体であり、１単位あたり、以下の構成体を同梱したものである。

構成体	形状、構造、成分、分量又は本質		分量
骨髄希釈液DMEM	成分名	分量	10本
	骨髄希釈液DMEM	20mL	
	原材料名		

Stemirac : Summary basis of approval (issued on Nov. 21, 2018)

http://www.pmda.go.jp/regenerative_medicines/2019/R20190125001/530100000_23000FZX00001_A100_1.pdf

表 14　被験者毎の有効性データ

症例番号	投与直前 AIS [a]	受傷後 220 日 AIS	AIS の変化			ISCSCI-92					SCIM-III				
						受傷後 220 日目における投与直前からの変化量				受傷後 220 日合計得点	受傷後 220 日目における投与直前からの合計変化量	受傷後 220 日目			
			不変	1 段階改善	2 段階改善	運動機能	表在触覚	ピン痛覚	合計			合計得点	セルフケア（小計）	呼吸と排泄管理（小計）	移動（小計）
STR0103-03	A	C	—	—	●	18	23	26	67	120	4	14	1	10	3
STR0103-04		C	—	—	●	13	44	47	104	149	9	11	0	10	1
STR0103-14		B	—	●	—	0	16	10	26	54	2	4	0	4	0
STR0103-15		B	—	●	—	3	19	11	33	61	2	4	0	4	0
STR0103-16		B	—	●	—	5	20	13	38	66	3	5	0	4	1
STR0103-17		A	●	—	—	0	8	6	14	24	0	0	0	0	0
STR0103-07	B	C	—	●	—	7	1	4	12	183	17	21	2	10	9
STR0103-12		D	—	—	●	57	48	47	152	277	2	12	0	10	2
STR0103-05	C	D	—	●	—	56	21	10	87	252	76	86	15	34	37
STR0103-06		D	—	●	—	51	6	4	61	220	24	34	2	21	11
STR0103-09		D	—	●	—	47	0	0	47	219	65	77	17	33	27
STR0103-10		D	—	●	—	36	38	36	110	286	67	77	14	36	27
STR0103-11		D	—	●	—	39	6	6	51	224	82	92	18	36	38

a：二次症例登録前で治験製品の投与前 7 日以内に評価された。

http://www.pmda.go.jp/regenerative_medicines/2019/R20190125001/530100000_23000FZX00001_A100_1.pdf

MSC Therapy for forthcoming application –Pre-clinical POC

• chronic cerebral infarction	Komatsu, K., et al. Therapeutic time window of mesenchymal stem cells derived from bone marrow after cerebral ischemia. Brain Res. 1334, 84–92 (2010). Namioka, T., Namioka, A., Sasaki, M., Kataoka-Sasaki, Y., Oka, S. et al. Intravenous infusion of mesenchymal stem cells promotes functional recovery in a rat model of chronic cerebral infarction. *J. Neurosurg.* https://doi.org/10.3171/2018.5.JNS18140 (2018).
• cerebral hemorrhage	Nakazaki, M., *et al.* Intravenous infusion of mesenchymal stem cells inhibits intracranial hemorrhage after recombinant tissue plasminogen activator therapy for transient middle cerebral artery occlusion in rats. *J. Neurosurg.* **127,** 917–926 (2017).
• chronic spinal cord injury	Morita, T., et al. Intravenous infusion of mesenchymal stem cells promotes functional recovery in a model of chronic spinal cord injury. Neurosci. 335, 221–231 (2016).
• post-resuscitation encephalopathy	Zheng, W., et al. Therapeutic benefits of human mesenchymal stem cells derived from bone marrow after global cerebral ischemia. Brain Res. 1310, 8–16 (2009).
• Parkinson's disease	Inden, M., et al. Therapeutic effects of human mesenchymal and hematopoietic stem cells on rotenone-treated Parkinsonian mice. J. Neurosci. Res. 91, 62–72 (2013).
• prion disease	Song, C. H., et al. The effect of transplantation of bone marrow-derived mesenchymal stem cells on mice infected with prion. J. Virol. 83, 5918–5927 (2009).
• hypoxic ischemic encephalopathy in the developing brain	Sakai, T., et al. Functional recovery after the systemic administration of mesenchymal stem cells in a rat model of neonatal hypoxia-ischemia. J. Neurosurg. Pediatr. 22, 467–599 (2018).
• epilepsy	Fukumura, S., et al. Intravenous infusion of mesenchymal stem cells reduces epileptogenesis in a rat model of status epilepticus. Epilepsy Res. 141, 56–63 (2018).
• brain tumours	Nakamura, K., et al. Anti-tumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. Gene Ther. 11, 1155–1164 (2004).
• peripheral neuropathy	Matsuda, Y., et al. Intravenous infusion of bone marrow-derived mesenchymal stem cells reduces erectile dysfunction following cavernous nerve injury in rats. Sex. Med. 6, 49–57 (2018). Takayanagi, A., et al. Intravenous preload of mesenchymal stem cells rescues erectile function in a rat model of cavernous nerve injury. J. Sex. Med. 12, 1713–1721 (2015).

Spinal cord injury: Protocol

The first clinical trial for spinal cord injury using MSC

CONFIDENTIAL

研究等実施計画書　　試験コード：UHA_SCI04-01

急性期脊髄損傷に対する培養自家骨髄間質細胞移植による

脊髄再生治療の検討 （第Ⅰ－Ⅱ相臨床試験）

研究等実施計画書

研究責任者　：関西医科大学 救急医学科 教授　中谷 壽男

研究副責任者　：関西医科大学 脳神経外科 講師　岩瀬 正顕

関西医科大学倫理委員会承認日　2005 年 07 月 01 日

MSC intrathecal injection for spinal cord injury

Table 4

Patient information and changes in the AIS and ASIA motor scores

No.	Age	Injury	Day of TX	Size of SCI (mm)		AIS	
				Initial	6 M	Initial	6 M
1	35	C5: DL + FX	13	62 × 8	21 × 11	A	A
2	59	C6: DL	8	23 × 6	5 × 5	B	D
3	45	C4: DL + FX	13	38 × 6	11 × 6	C**	D
4	23	C5: DL + FX	17	ND	70 × 22*	A	A
5	51	C4-6: DL + F	14	65 × 9	45 × 15	A	A

All the five patients were male.

AIS = American Spinal Injury Association Impairment Scale. (A = Sensorimotor complete lesion. No motor c
sacral segments. B = Motor complete lesion. Sensory but not motor function preserved in at least the sacral se

Ref : F. Saito et al. **Administration of cultured autologous bone marrow stromal cells into cerebrospinal fluid in spinal cord injury patients: A pilot study.** Restorative Neurology and Neuroscience 30 (2012) 127-136.

Mononuclear cell for spinal cord injury

Table 6

Changes in the AIS and ASIA scores

Case	AIS grade		Motor score		Light touch score	
	at TX	6 M after TX	at TX	6 M after TX	at TX	6 M after TX
1	A	B	50	50	62	78
2	B	D	33	99	70	106
3	A	A	51	57	77	79
4	B	C	50	57	61	98
5	A	A	50	50	59	67
6	A	A	20	22	26	28
7	B	B	30	32	55	78
8	B	C	19	31	40	76
9	B	B	50	50	76	77
10	A	A	12	16	20	36

Complication refers to adverse events related to this study; Recovery from the anemia in cases 5 and
Abbreviation: TX, transplantation

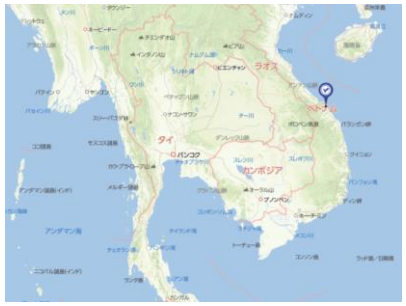
Ref: Suzuki Y, Ishikawa N, Omae K, Hirai T, Ohnishi K, Nakano N, Nishida H, Nakatani T, Fukushima M, Ide C. **Bone marrow-derived mononuclear cell transplantation in spinal cord injury patients by lumbar puncture.** Restor Neurol Neurosci. 2014;32(4):473-82.

Global expansion of regenerative treatment - Vietnam

Confidential

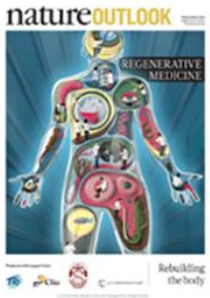
2012年 12月	ダナン病院 Dr. Ngoc Nguyen Ba, Dr. Ho Dac Hanh招聘
2013年 7月	ダナン病院訪問し、実行可能性の検討
2013年 8月	日越友好協会の理事及び副理事とベトナム大使と面談
2014年 2月	ダナン病院、北野病院、先端医療振興財団がMOUの締結
2014年 6月	ベトナム　ダナン市の保健局の承認
2014年 10月	ベトナム　保健省のダナン病院査察
2015年 3月・11月 2016年 1月	骨髄単核球分離のためのトレーニングを北野病院とダナン病院で実施
2015年 7月	共同研究契約の締結
2015年 11月	ダナン病院での調印式
2016年 5月	ベトナム保健省保健大臣承認
2016年 9月	第１症例実施(NCT02923817) 日本経済新聞、日本産業新聞、神戸新聞、ダナン新聞等に掲載
2019年 1月21日	第15症例実施

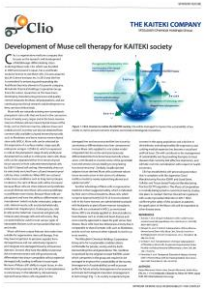
ダナン病院　病院長　Dr. Le Duc Nhan
　　脳神経外科　Dr. Ngoc Ba Nguyen
北野病院　形成外科部長　鈴木義久
神戸医療産業都市推進機構　尾前　薫



No.	年齢・性別	損傷部位	受傷から移植までの日数	ASIA	
				移植前	6カ月後
1	41男	C5-C6	207	A	B
2	20男	T2-T4	310	A	A
3	29男	C4-C5	109	B	D
4	54男	C2-C4	138	B	C
5	中　止				
6	24男	C5-C6	274	A	B
7	23男	T8-T10	212	A	B
8	20男	C6-C7	125	A	A
9	25男	C4-C5	245	A	B
10	25男	T2-T8	170	A	B
11	49男	C3-C4	141	B	C

Muse Cell





Vol. 540 No.S49, December 7, 2016

Nature Outlook: Regenerative medicine

www.nature.com/articles/540S49a

Prof. Shinya Minatoguchi
Department of Circulatory and Respiratory Advanced Medicine,
Gifu University Graduate School of Medicine

Prof. Mari Dezawa
Department of Stem Cell Biology and Histology, Tohoku University
Graduate School of Medicine

Publication
Tanaka T., et al. **Mobilized Muse Cells After Acute Myocardial Infarction Predict Cardiac Function and Remodeling in the Chronic Phase.** Circulation Journal. 2018 ; 82 (2) ; 561-571.
Minatoguchi S., et al. **S1P–S1PR2 Axis Mediates Homing of Muse Cells Into Damaged Heart for Long-Lasting Tissue Repair and Functional Recovery After Acute Myocardial Infarction.** Circulation Research. 2018;122:1069–1083.

Muse Cell
–intravenous injection of Muse cell for acute cardiac infarction–

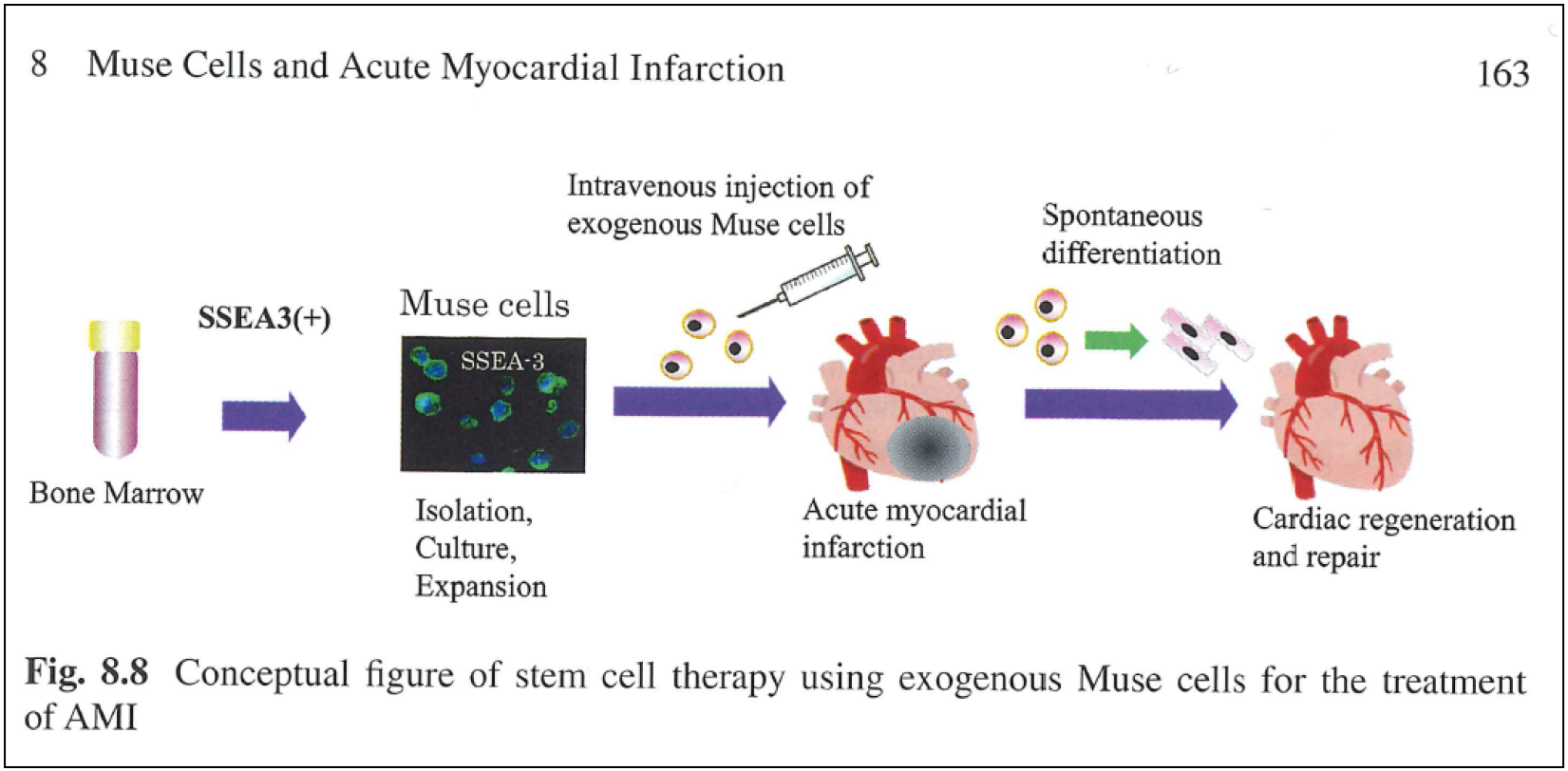


Fig. 8.8 Conceptual figure of stem cell therapy using exogenous Muse cells for the treatment of AMI

Ref：Minatoguchi S., et al.
S1P–S1PR2 Axis Mediates Homing of Muse Cells Into Damaged Heart for Long-Lasting Tissue Repair and Functional Recovery After Acute Myocardial Infarction.
Circulation Research. 2018;122:1069–1083.

Critical limb ischaemia

Vol. 548 No.7668_supp, August 24, 2017

Critical limb ischaemia

https://www.nature.com/collections/vmxkcnxvwg/videos

Prof. Atsuhiko Kawamoto
Institute of Medical Research and Development, Translational Research Center for Medical Innovation, Foundation for Biomedical Research and Innovation at Kobe, Japan

Publication
Kawamoto, A. et al. **Intramuscular transplantation of G-CSF-mobilized CD34(+) cells in patients with critical limb ischemia: a phase I/IIa, multicenter, single-blinded, dose-escalation clinical trial.** Stem Cells 27, 2857-2864 (2009).
Kinoshita, M. et al. **Long-term clinical outcome after intramuscular transplantation of granulocyte colony stimulating factor-mobilized CD34 positive cells in patients with critical limb ischemia.** Atherosclerosis 224, 440-445 (2012).
Fujita, Y. et al. **Phase II clinical trial of CD34+ cell therapy to explore endpoint selection and timing in patients with critical limb ischemia.** Circ J 78, 490-501 (2014).

CLI: G-CSF mobilized autologous peripheral blood mononuclear cell

Advance Publication by-J-STAGE

Circ J
doi:10.1253/circj.CJ-17-1220

ORIGINAL ARTICLE
Peripheral Vascular Disease

Outcome From a Randomized Controlled Clinical Trial

— Improvement of Peripheral Arterial Disease by Granulocyte Colony-Stimulating Factor-Mobilized Autologous Peripheral-Blood-Mononuclear Cell Transplantation (IMPACT) —

Takashi Horie, MD, PhD; Seiji Yamazaki, MD; Sayaka Hanada, MD; Shuzo Kobayashi, MD, PhD; Tatsuo Tsukamoto, MD, PhD; Tetsuya Haruna, MD, PhD; Katsuhiko Sakaguchi, MD, PhD; Ken Sakai, MD, PhD; Hideaki Obara, MD, PhD; Kiyofumi Morishita, MD, PhD; Kenichi Saigo, MD, PhD; Yoshiaki Shintani, MD; Kohmei Kubo, MD, PhD; Junichi Hoshino, MD, PhD; Teiji Oda, MD, PhD; Eiji Kaneko, MD, PhD; Masaharu Nishikido, MD, PhD; Tetsuya Ioji; Hideaki Kaneda, MD, PhD; Masanori Fukushima, MD, PhD
for the Japan Study Group of Peripheral Vascular Regeneration Cell Therapy (JPRCT)

Background: The clinical usefulness of peripheral blood (PB) mononuclear cell (MNC) transplantation in patients with peripheral arterial disease (PAD), especially in those with mild-to-moderate severity, has not been fully clarified.

Methods and Results: A randomized clinical trial was conducted to evaluate the efficacy and safety of granulocyte colony-stimulating factor (G-CSF)-mobilized PBMNC transplantation in patients with PAD (Fontaine stage II–IV and Rutherford category 1–5) caused by arteriosclerosis obliterans or Buerger's disease. The primary endpoint was progression-free survival (PFS). In total, 107 subjects were enrolled. At baseline, Fontaine stage was II/III in 82 patients and IV in 21, and 54 patients were on hemodialysis. A total of 50 patients had intramuscular transplantation of PBMNC combined with standard of care (SOC) (cell therapy group), and 53 received SOC only (control group). PFS tended to be improved in the cell therapy group than in the control group (P=0.07). PFS in Fontaine stage II/III subgroup was significantly better in the cell therapy group than in the control group. Cell therapy-related adverse events were transient and not serious.

Conclusions: In this first randomized, large-scale clinical trial of G-CSF-mobilized PBMNC transplantation, the cell therapy was tolerated by a variety of PAD patients. The PBMNC therapy was significantly effective for inhibiting disease progression in mild-to-moderate PAD.

Key Words: Granulocyte colony stimulating factor; Peripheral arterial disease; Peripheral blood mononuclear cells; Progression-free survival

Horie et al. Outcome from a Randomized Controlled Clinical Trial: Improvement of Peripheral Arterial Disease by Granulocyte Colony-Stimulating Factor-Mobilized Autologous Peripheral-Blood Mononuclear-Cell Transplantation (IMPACT) .
Circ J. 2018;82(8):2165-74.

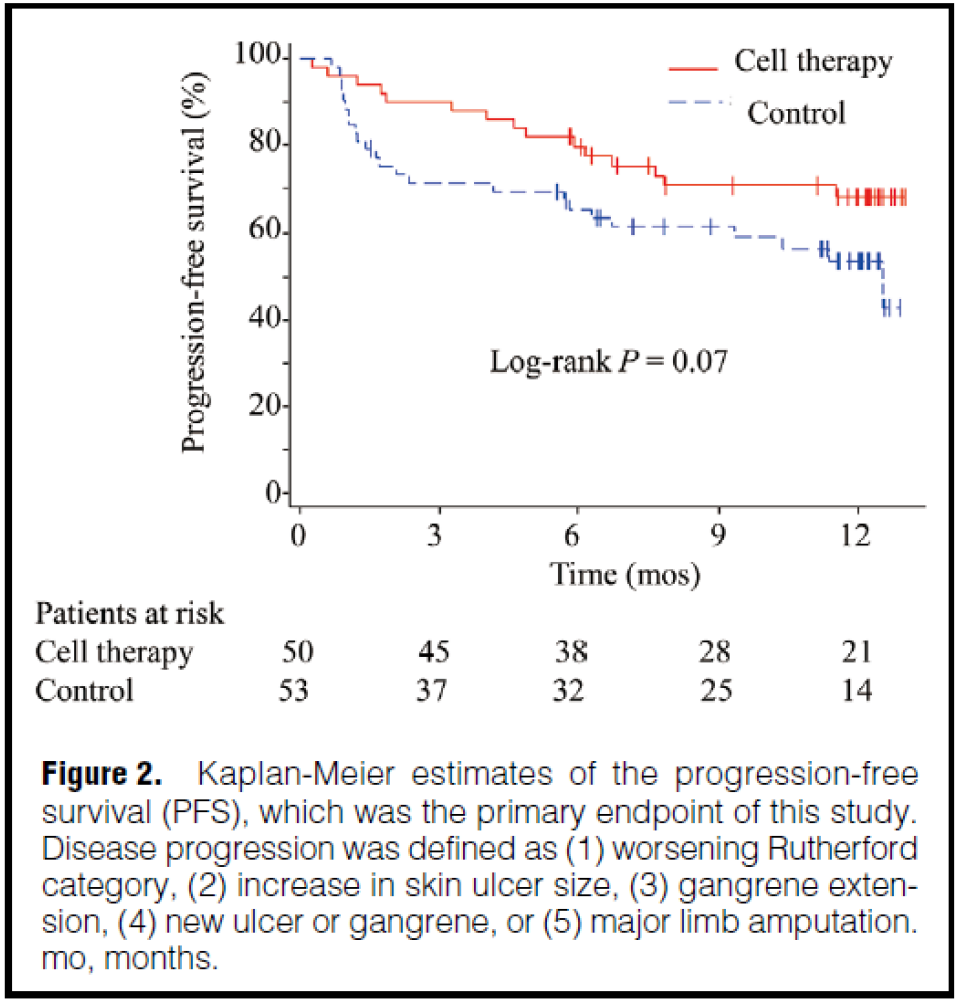


Figure 2. Kaplan-Meier estimates of the progression-free survival (PFS), which was the primary endpoint of this study. Disease progression was defined as (1) worsening Rutherford category, (2) increase in skin ulcer size, (3) gangrene extension, (4) new ulcer or gangrene, or (5) major limb amputation. mo, months.

CLI: G-CSF mobilized peripheral blood mononuclear cells

Bone Marrow Transplantation (2011) 46, 278–284
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www.nature.com/bmt

ORIGINAL ARTICLE

Bone marrow mononuclear cells versus G-CSF-mobilized peripheral blood mononuclear cells for treatment of lower limb ASO: pooled analysis for long-term prognosis

R Onodera[1], S Teramukai[2,3], S Tanaka[2], S Kojima[2,3], T Horie[4], S Matoba[5], T Murohara[6], H Matsubara[5] and M Fukushima[3], BMMNC Follow-Up Study Investigators, M-PBMNC Follow-Up Study Investigators[7]

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Onodera et al. Bone marrow mononuclear cells versus G-CSF-mobilized peripheral blood mononuclear cells for treatment of lower limb ASO: pooled analysis for long-term prognosis.
Bone Marrow Transplant. 2011;46(2):278-84.

Table 3 Prognostic factors affecting overall survival and amputation-free survival, identified using Cox's regression analysis with backward variable selection

Factor		*HR*	*95% CI*	*P-value*
Overall survival				
History of dialysis	–	1	—	—
	+	4.40	2.06–9.41	<0.001
Total no. $CD34^+$ cells collected	Low	1	—	—
	High	0.45	0.21–0.96	0.04
Age	Per year	1.03	1.00–1.06	0.08
Sex	Male	1	—	—
	Female	0.54	0.27–1.08	0.08
Amputation-free survival				
Fontaine classification	III	1	—	—
	IV	3.51	1.83–6.71	<0.001
Total no. $CD34^+$ cells collected	Low	1	—	—
	High	0.48	0.28–0.81	0.006
History of dialysis	–	1	—	—
	+	1.96	1.19–3.25	0.008
Sex	Male	1	—	—
	Female	0.53	0.30–0.94	0.03

Abbreviations: CI, confidence interval; HR, hazard ratio.

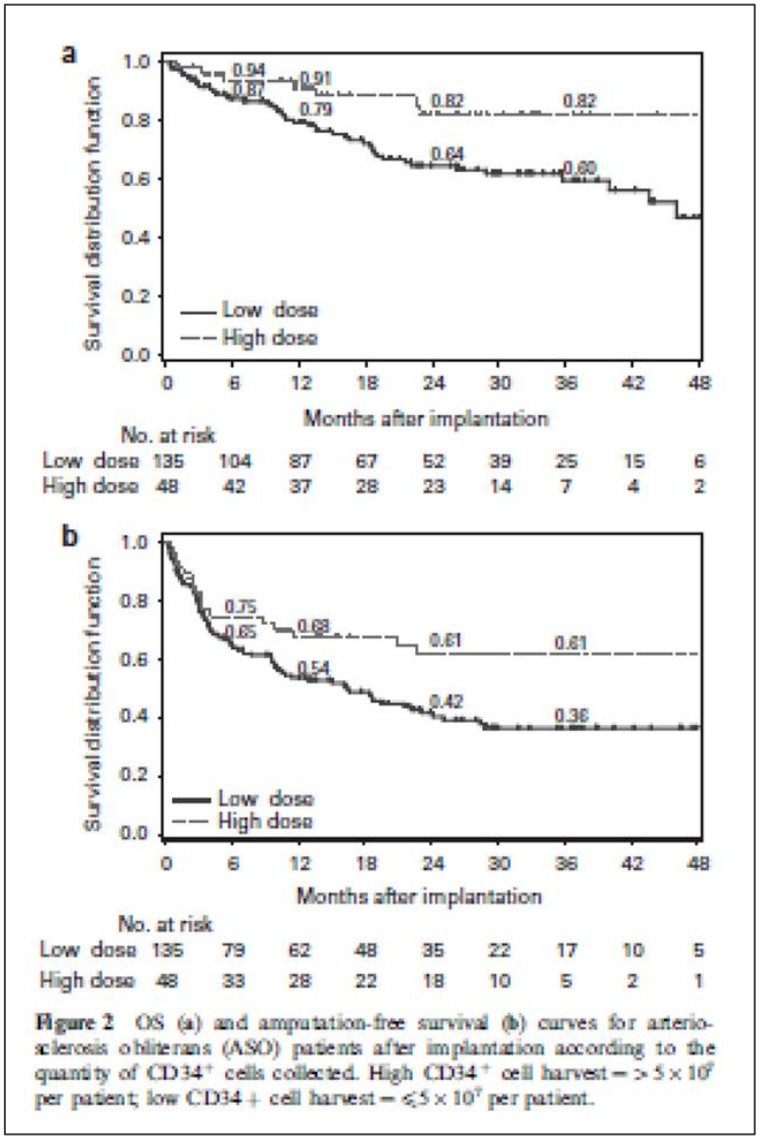
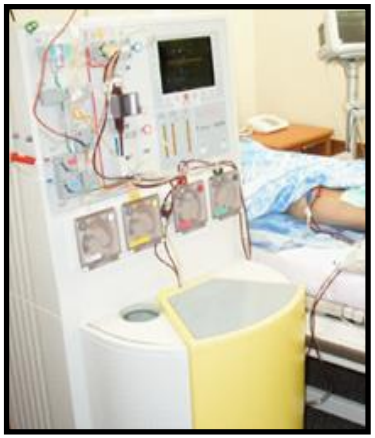


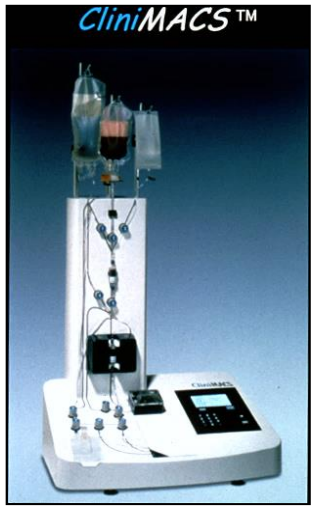
Figure 2 OS (a) and amputation-free survival (b) curves for arteriosclerosis obliterans (ASO) patients after implantation according to the quantity of $CD34^+$ cells collected. High $CD34^+$ cell harvest = $>5\times10^7$ per patient; low $CD34+$ cell harvest = $\leqslant5\times10^7$ per patient.

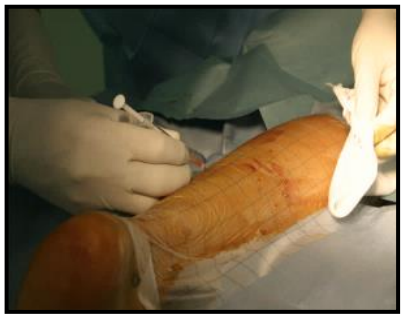
CLI: G-CSF mobilized peripheral blood mononuclear cells –procedure–

Day 1 - 5 | ***Day 5*** | ***Day 6***

G-CSF subcutaneous injection







Leukapheresis

Magnetic sorting of CD34+ cells

Intramuscular injection of CD34+ cells

EPC mobilization | **Total MNCs harvest** | **EPC purification** | **EPC transplantation**

CLI: CD34+ stem cell treatment

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Long-term clinical outcome after intramuscular transplantation of granulocyte colony stimulating factor-mobilized CD34 positive cells in patients with critical limb ischemia

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ABSTRACT

Background: Our phase I/IIa clinical trial revealed that intramuscular transplantation of autologous, GCSF-mobilized CD34+ cells was safe, feasible and potentially effective at week 4 and 12 post cellular therapy in 17 patients with chronic critical limb ischemia (CLI) (5 patients with atherosclerotic peripheral arterial disease (PAD) and 12 with Buerger's disease). However, long-term outcome of the cell therapy has yet to be reported.

Methods and results: Incidence of major clinical events and physiological parameters of limb ischemia were evaluated at week 52, 104, 156 and 208 post CD34+ cell therapy. No patients died by week 104, whereas 3 patients with PAD died by week 156 and 1 patient with Buerger's disease died by week 208 due to cardiac complications. No patients underwent major amputation, whereas 1 patient with Buerger's disease underwent unplanned minor amputation by week 104. CLI-free ratio was 88.2% at week 52 and 104, 92.3% at week 156 and 84.6% at week 208 in all patients. Significant improvement of toe brachial pressure index versus baseline was sustained up to week 208 and that of transcutaneous partial oxygen pressure was kept up to week 156. The Wong-Baker FACES pain rating scale, ulcer size and exercise tolerance significantly improved at week 52, the final evaluation time point, compared with baseline. Subgroup analysis revealed the similar outcome in patients with Buerger's disease.

Conclusions: Favorable clinical outcomes as well as physiological evidences strongly indicate the long-term benefit of GCSF-mobilized CD34+ cell transplantation for retrieval from CLI, especially in patients with Buerger's disease.

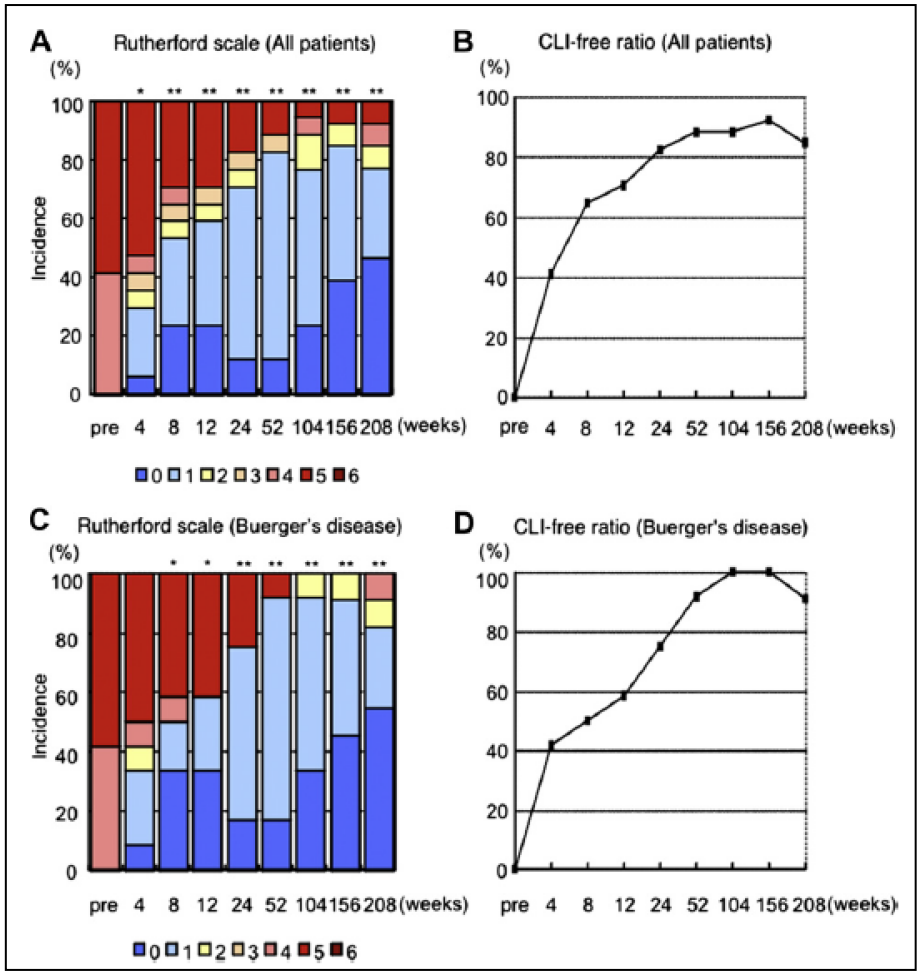

Fig. 1. Serial changes in the proportion of Rutherford scale (0–6) and CLI-free ratio following CD34+ cell transplantation in all patients ($n = 17$ at week 0–104, $n = 13$ at 156–208) (A, B) and patients with Buerger's disease ($n = 12$ at week 0–104, $n = 11$ at week 156–208) (C, D). *, $p < 0.05$ versus baseline; **, $p < 0.01$ versus baseline.

Kinoshita et al. Long-term clinical outcome after intramuscular transplantation of granulocyte colony stimulating factormobilized CD34 positive cells in patients with critical limb ischemia. Atherosclerosis. 2012;224(2):440-5.

CD34+cell treatment for hemodialysis patients

STEM CELLS TRANSLATIONAL MEDICINE

HUMAN CLINICAL ARTICLE

Autologous Granulocyte Colony-Stimulating Factor-Mobilized Peripheral Blood CD34 Positive Cell Transplantation for Hemodialysis Patients with Critical Limb Ischemia: A Prospective Phase II Clinical Trial

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Key Words. CD34 positive cells • Critical limb ischemia • Hemodialysis • Transplantation

ABSTRACT

Critical limb ischemia (CLI) is a devastating disease in patients undergoing hemodialysis (HD). Based on the unsatisfactory results of autologous mononuclear cell transplantation for patients with CLI undergoing HD, we conducted a phase II clinical trial to evaluate the safety and efficacy of granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood-derived autologous purified CD34 positive (CD34+) cell transplantation for CLI in patients undergoing HD. Six patients with CLI (two with Rutherford category 4 and four with Rutherford category 5) were enrolled. As for primary endpoint, there were no major adverse events related to this therapy. As for efficacy, the amputation-free survival rate was 100% at 1 year after cell therapy. Both rest pain scale and ulcer size were significantly improved as early as 4 weeks after therapy compared with baseline ($p < .01$), and three out of five ulcers completely healed within 12 weeks after cell transplantation. Clinical severity, including Fontaine scale and Rutherford category, significantly improved at 24 weeks after cell transplantation ($p < .05$), and further improved at 52 weeks ($p < .01$) compared with baseline. The improvement rate from CLI stage to non-CLI stage was 83.3% at 52 weeks. Toe skin perfusion pressure and absolute claudication distance were also significantly improved. In conclusion, G-CSF-mobilized peripheral blood CD34+ cell transplantation was safe, feasible, and effective for patients with CLI undergoing HD. STEM CELLS TRANSLATIONAL MEDICINE *2018;7:1–9*

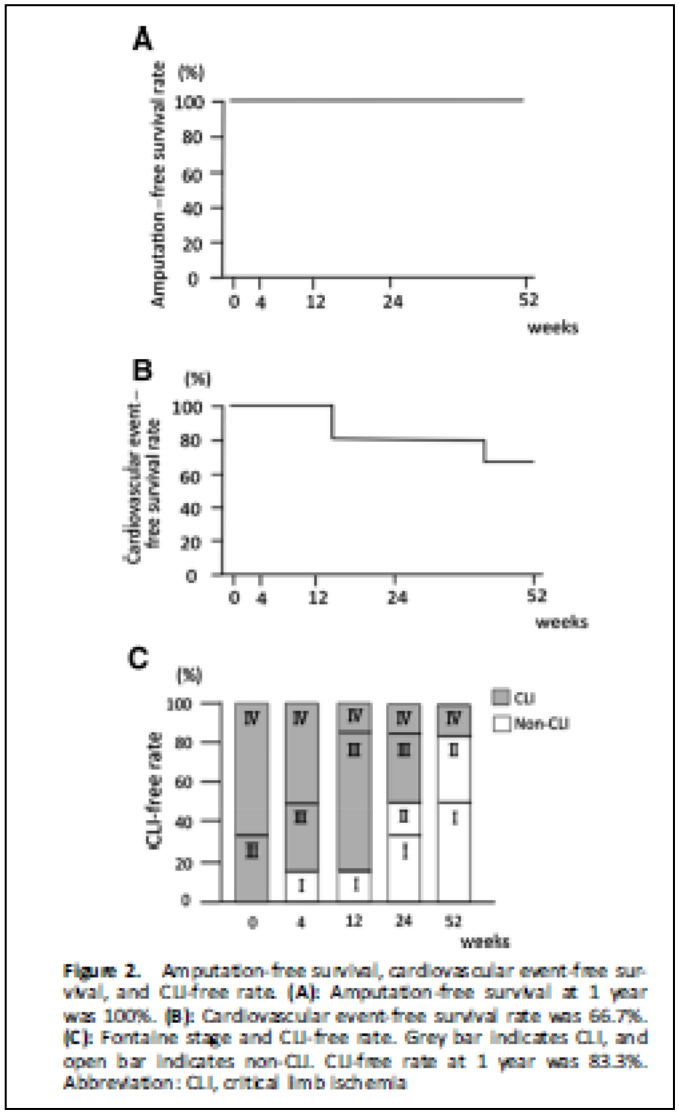


Figure 2. Amputation-free survival, cardiovascular event-free survival, and CLI-free rate. **(A)**: Amputation-free survival at 1 year was 100%. **(B)**: Cardiovascular event-free survival rate was 66.7%. **(C)**: Fontaine stage and CLI-free rate. Grey bar indicates CLI, and open bar indicates non-CLI. CLI-free rate at 1 year was 83.3%. Abbreviation: CLI, critical limb ischemia

Ohtake et al. Autologous Granulocyte Colony-Stimulating Factor-Mobilized Peripheral Blood CD34 Positive Cell Transplantation for Hemodialysis Patients with Critical Limb Ischemia: A Prospective Phase II Clinical Trial.
Stem Cells Transl Med. 2018 Jul 30. [Epub ahead of print]

CLI: bFGF + atellocolagen

Advance Publication

Circulation Journal
doi:10.1253/circj.CJ-18-0815

ORIGINAL ARTICLE
Peripheral Vascular Disease

First-in-Man Clinical Pilot Study Showing the Safety and Efficacy of Intramuscular Injection of Basic Fibroblast Growth Factor With Atelocollagen Solution for Critical Limb Ischemia

Kazunori Ono, MD; Kenji Yanishi, MD, PhD; Makoto Ariyoshi, MD, PhD;
Satoshi Kaimoto, MD, PhD; Motoki Uchihashi, MD, PhD;
Keisuke Shoji, MD; Satoaki Matoba, MD, PhD

Background: Therapeutic angiogenesis with basic fibroblast growth factor (bFGF) with atelocollagen was confirmed in a study using a limb ischemia mouse model. Because the number of elderly patients with critical limb ischemia (CLI) is increasing, particularly that caused by arteriosclerosis obliterans (ASO), the development of less invasive angiogenesis therapies desired.

Methods and Results: This first-in-man clinical study was designed to assess the safety and efficacy of i.m. injection of bFGF with atelocollagen. Human recombinant bFGF (200 μg), combined with 4.8 mL 3% atelocollagen solution, was prepared and injected into the gastrocnemius muscle of the ischemic leg. The primary endpoint was safety, evaluated on all adverse events over 48 weeks after this treatment. The secondary endpoint was efficacy, evaluated by improvement of ischemic symptoms. No serious procedure-related adverse events were observed during the follow-up period. Visual analogue scale (VAS) score was significantly improved at 4, 24 and 48 weeks compared with baseline ($P<0.05$), and 7 patients became pain free during the follow-up period. Fontaine classification was improved in 4 of 10 patients at 48 weeks. Cyanotic lesions disappeared in 2 patients at 4 weeks.

Conclusions: I.m. injection of bFGF with atelocollagen is safe and feasible in patients with CLI. Randomized controlled trials are therefore needed to confirm these results.

Key Words: Basic fibroblast growth factor; Critical limb ischemia; Peripheral artery disease; Therapeutic angiogenesis

Due to the recent rise in the number of diabetic patients, as a result of the aging of the general population worldwide, the prevalence of critical limb ischemia (CLI; Fontaine classification III and IV, or Rutherford classification categories 4, 5, and 6) has also been increasing. Although medical and surgical treatment, including percutaneous transluminal angioplasty (PTA) and bypass procedures, have markedly contributed to the treatment of CLI, many patients are forced to undergo amputation of the lower limbs because of arteriosclerosis obliterans (ASO) or Buerger's disease (thromboangiitis obliterans; TAO). In spite of the remarkable progress in the treatment of CLI, therapeutic outcomes remain far from satisfactory and the prognosis of CLI remains challenging.[1-3] Therefore, new therapeutic approaches are needed for the effective management of CLI.

No effective alternatives to percutaneous or surgical revascularization presently exist for the treatment of CLI. In recent years, cell therapies have been developed and are performed, in clinical practice, as advanced medical treatment. Procedures that facilitate angiogenesis and collateral circulation have recently been assessed for the reduction of tissue damage. These procedures include the use of growth factors (vascular endothelial growth factor, VEGF; hepatocyte growth factor, HGF; and basic fibroblast growth factor, bFGF and FGF-2), and bone marrow cells (CD34+ or mononuclear cells [MNC]).[4-9] The Therapeutic Angiogenesis by Cell Transplantation (TACT) trial reported on the safety and efficacy of i.m. implantation of bone marrow MNC (BM-MNC implantation) as a treatment for CLI, and, in particular, those patients with ASO or TAO with no option for other treatment.[8,10,11] Concerns regarding the effectiveness, immune or inflammatory responses to genetic materials and invasiveness of gene therapy or cell transplantation, however, still exist. Many patients with CLI, especially those with ASO, have ischemic heart dis-

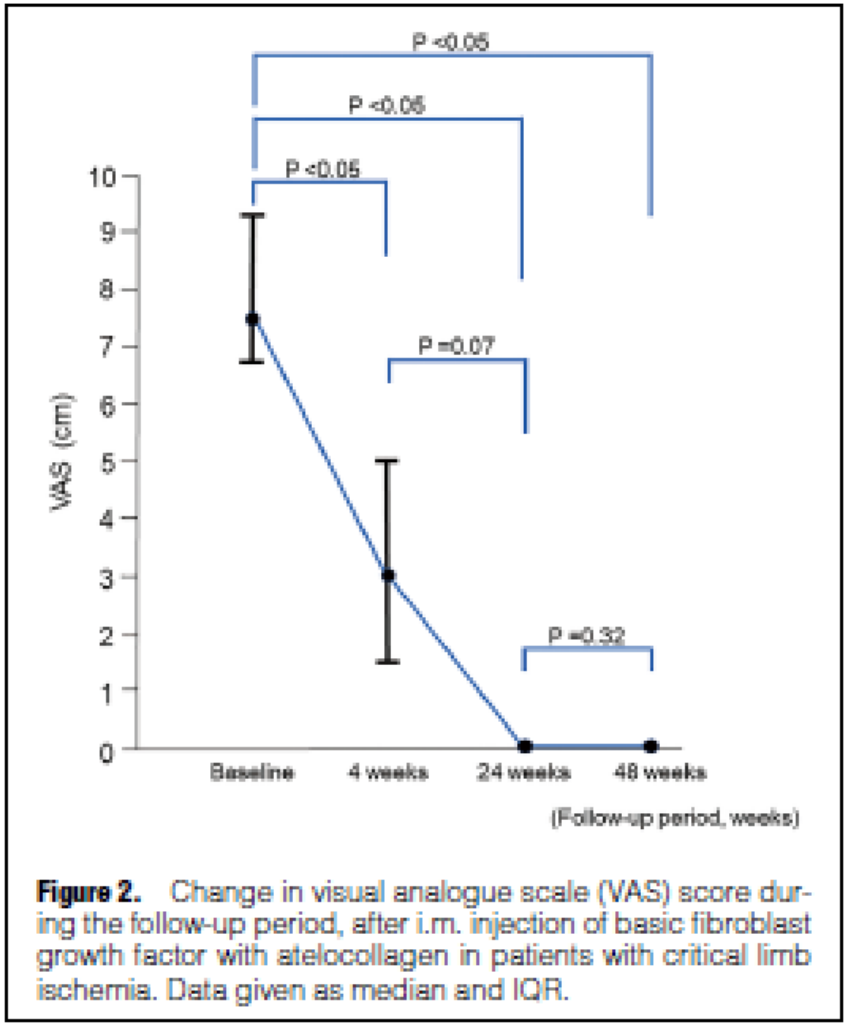
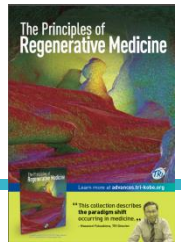


Figure 2. Change in visual analogue scale (VAS) score during the follow-up period, after i.m. injection of basic fibroblast growth factor with atelocollagen in patients with critical limb ischemia. Data given as median and IQR.

Ono K et al., First-in-Man Clinical Pilot Study Showing the Safety and Efficacy of Intramuscular Injection of Basic Fibroblast Growth Factor With Atelocollagen Solution for Critical Limb Ischemia, Circulation Journal, 2018, doi:10.1253/circj.CJ-18-0815



The Principles of Regenerative Medicine
Stem cell therapy and tissue engineering therapy

Preface			
Theory: An overview of regenerative medicine: its principles and the scope of the current revolution			
Stem Cell Therapy	1	**Prof. Honmou,** Spporo medical Univ.	**Nervous system**
	2	**Profs. Minatoguchi & Dezawa,** Gifu Univ. & Tohoku Univ.	**Myocardium**
	3	**Dr. Kawamoto,** TRI	**Vascular**
Tissue Engineering	4	**Prof. Kuroda,** Kobe Univ.	**Bone**
	5	**Prof. Sotozono,** Kyoto pref. Univ.	**Cornea**
	6	**Dr. Kanemaru,** Kitano Hosp.	**Ear drum**

Non-union bone fracture: a quicker fix

Vol. 550 No. S193, October 26, 2017

Non-union bone fracture: a quicker fix

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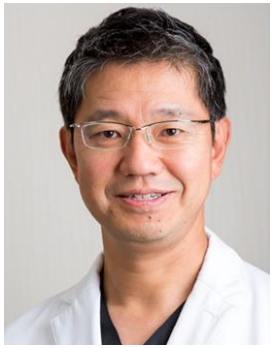
Prof. Ryosuke Kuroda
Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine



Publication
Kuroda R, Matsumoto T, Niikura T, Kawakami Y, Fukui T, Lee SY, Mifune Y, Kawamata S, Fukushima M, Asahara T, Kawamoto A, Kurosaka M. **Local transplantation of granulocyte colony stimulating factor-mobilized CD34+ cells for patients with femoral and tibial nonunion: pilot clinical trial.** Stem Cells Transl Med. 2014;3(1):128-34.

Non-union bone fracture
–procedure–

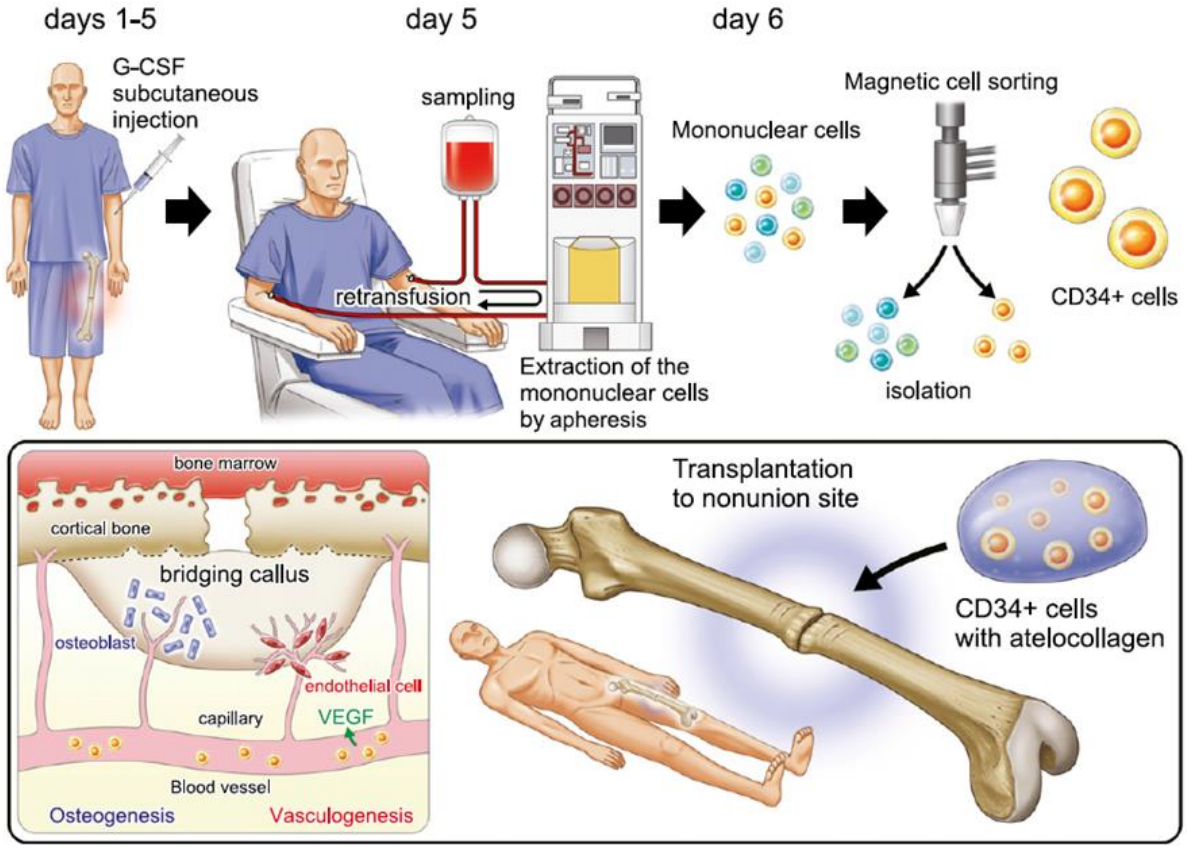
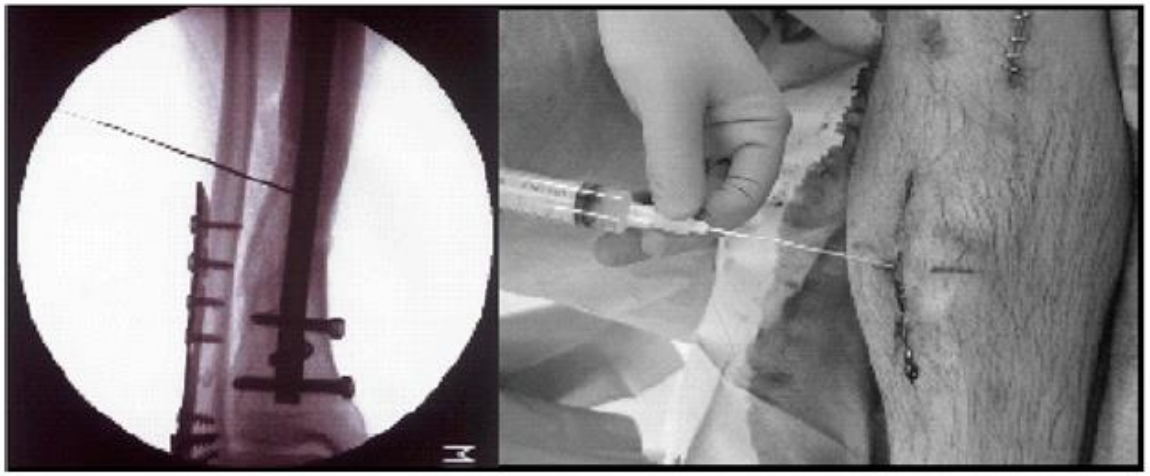
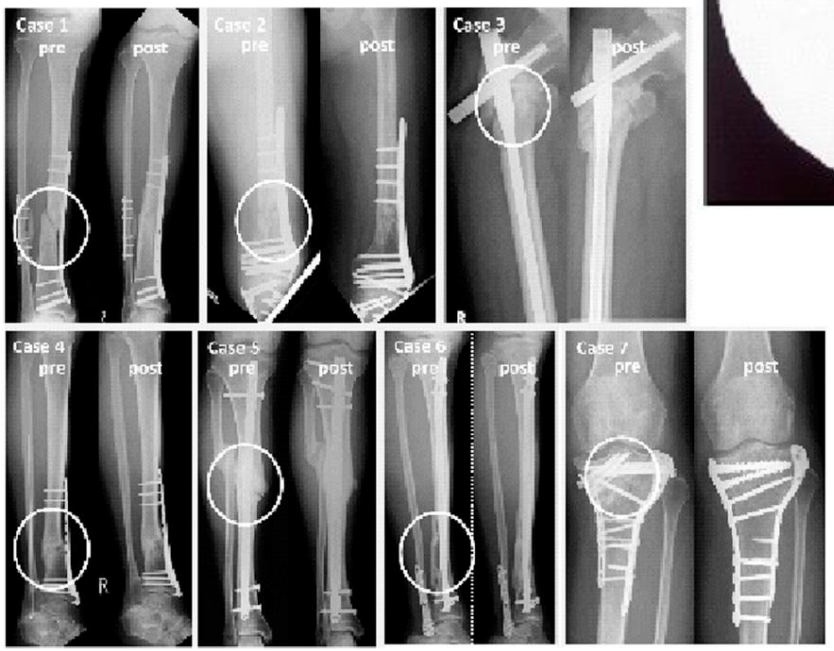


Figure 1. Schema of the treatment procedure. Abbreviations: G-CSF, granulocyte colony stimulating factor; VEGF, vascular endothelial growth factor.

Ref：Kuroda R, Matsumoto T, Niikura T, Kawakami Y, Fukui T, Lee SY, Mifune Y, Kawamata S, Fukushima M, Asahara T, Kawamoto A, Kurosaka M.
Local transplantation of granulocyte colony stimulating factor-mobilized CD34+ cells for patients with femoral and tibial nonunion: pilot clinical trial.
Stem Cells Transl Med. 2014;3(1):128-34.

Non-union bone fracture –procedure and result–





Ref : Kuroda R, Matsumoto T, Niikura T, Kawakami Y, Fukui T, Lee SY, Mifune Y, Kawamata S, Fukushima M, Asahara T, Kawamoto A, Kurosaka M.
Local transplantation of granulocyte colony stimulating factor-mobilized CD34+ cells for patients with femoral and tibial nonunion: pilot clinical trial.
Stem Cells Transl Med. 2014;3(1):128-34.

Corneal repair

Vol. 544 No.7650_supp_out,April 20, 2017

Corneal repair

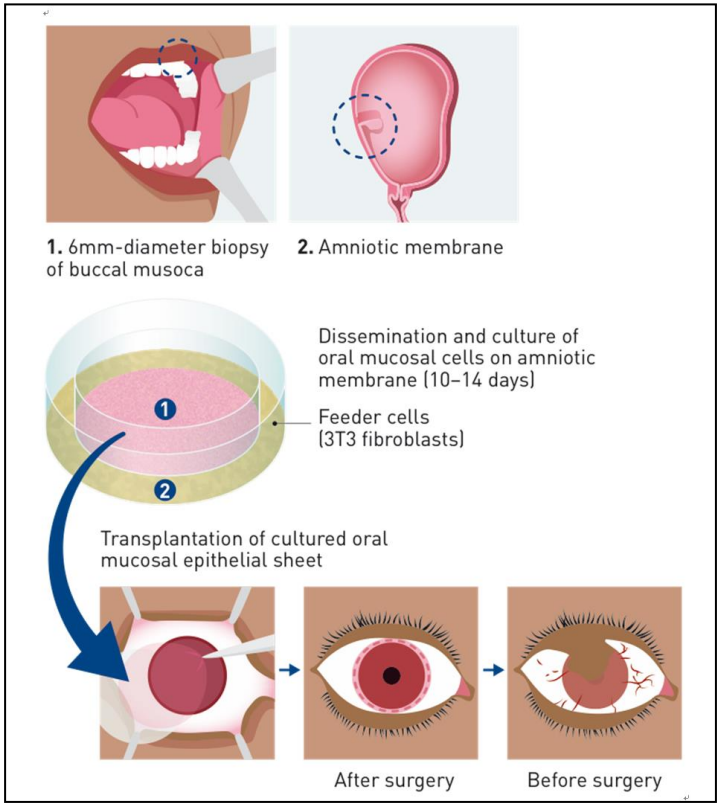
https://www.nature.com/collections/pdryjrsvnz/videos

Prof. Chie Sotozono
Department of Ophthalmology, Kyoto Prefectural University of Medicine

Publication
Sotozono C, Inatomi T, Nakamura T, Koizumi N, Yokoi N, Ueta M, Matsuyama K, Miyakoda K, Kaneda H, Fukushima M, Kinoshita S. **Visual improvement after cultivated oral mucosal epithelial transplantation.** Ophthalmology. 2013;120(1):193-200.
Sotozono C, Inatomi T, Nakamura T, Koizumi N, Yokoi N, Ueta M, Matsuyama K, Kaneda H, Fukushima M, Kinoshita S. **Cultivated oral mucosal epithelial transplantation for persistent epithelial defect in severe ocular surface diseases with acute inflammatory activity.** Acta Ophthalmol. 2014;92(6):e447-53.

Corneal repair
–procedure and result–

Figure 2. Procedure for transplanting cultivated autologous oral mucosal epithelial sheets. A mucosal specimen containing the oral mucosal epithelium was collected to create an oral mucosal epithelial sheet at the Cell Procession Center. After about 2 weeks, this stratified epithelial sheet was used for cultivated oral mucosal epithelial sheet transplantation.



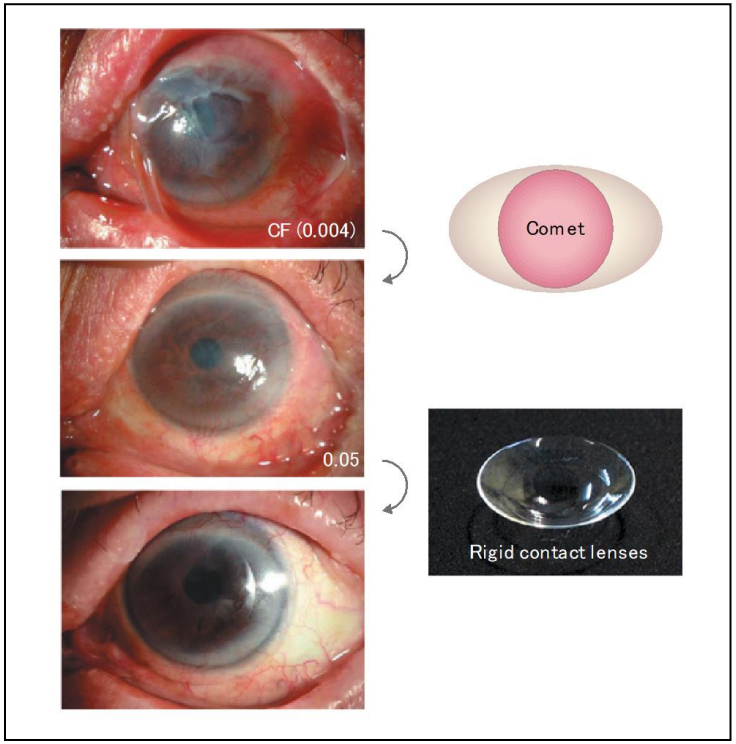


Figure 6. Improvement in visual function using limbal rigid contact lenses. A patient with Stevens–Johnson syndrome with severe adhesion on the ocular surface and a preoperative vision of counting fingers (0.004). The patient's own oral mucosal epithelium transplanted onto the cornea was nearly stabilized 6 months after surgery, improving visual acuity to 0.05; the use of limbal rigid contact lenses further improved visual acuity to 0.9–1.0. This improvement has been maintained for over 7 years since surgery. (Modified from Ref. 43.)
COMET, cultivated oral mucosal epithelial sheet transplantation

Eardrum regeneration: membrane repair

Vol. 546 No.7659_supp, June 22, 2017

Eardrum regeneration: membrane repair

https://www.nature.com/collections/rzfrydkflp/videos

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Department of Otorhinolaryngology/Department of Head and Neck Surgery,
Tazuke Kofukai Medical Research Institute, Kitano Hospital, Osaka, Japan.

Publication
Omae K, Kanemaru SI, Nakatani E, Kaneda H, Nishimura T, Tona R, Naito Y, Kawamoto A, Fukushima M. **Regenerative treatment for tympanic membrane perforation using gelatin sponge with basic fibroblast growth factor.** Auris Nasus Larynx. 2017;44(6):664-71.
Kanemaru SI, Kanai R, Yoshida M, Kitada Y, Omae K, Hirano S. **Application of Regenerative Treatment for Tympanic Membrane Perforation With Cholesteatoma, Tumor, or Severe Calcification.** Otol Neurotol. 2018;39(4):438-44.

Eardrum regeneration
–procedure and result–

Methods and Procedures

The tympanic region was fully anesthetized by applying a cotton ball soaked in 4% lidocaine to the perforation in contact with the residual TM for 15 minutes. Mechanical disruption of the perforation edge was then created under the microscope by a myringotomy knife. A gelatin sponge that was larger than the perforation was immersed in b-FGF (5–30 μg of Trafermin [recombinant human b-FGF] of 100 μg/ml) then inserted into the perforation in contact with the perforation edge of the TM. Fibrin glue was then dripped over the sponge. Figure 3 shows these procedures in detail. In cases in which complete closure of

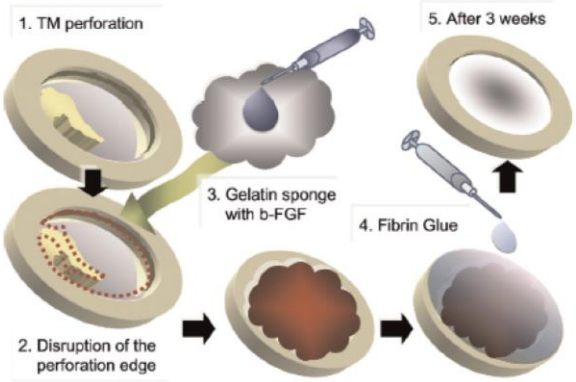

FIG. 3. A schematic diagram showing the method and procedures used in this treatment. *1*, TM perforation. *2*, After local anesthesia with 4% lidocaine, a mechanical disruption of the TM perforation edge is created under the microscope. *3*, A gelatin sponge immersed in b-FGF is placed over the perforation in contact with the residual TM. *4*, Fibrin glue is dripped over the sponge. *5*, Three weeks after the treatment, residual crust is removed. In cases of incomplete closure of the TM perforation, the treatment is performed repeatedly.

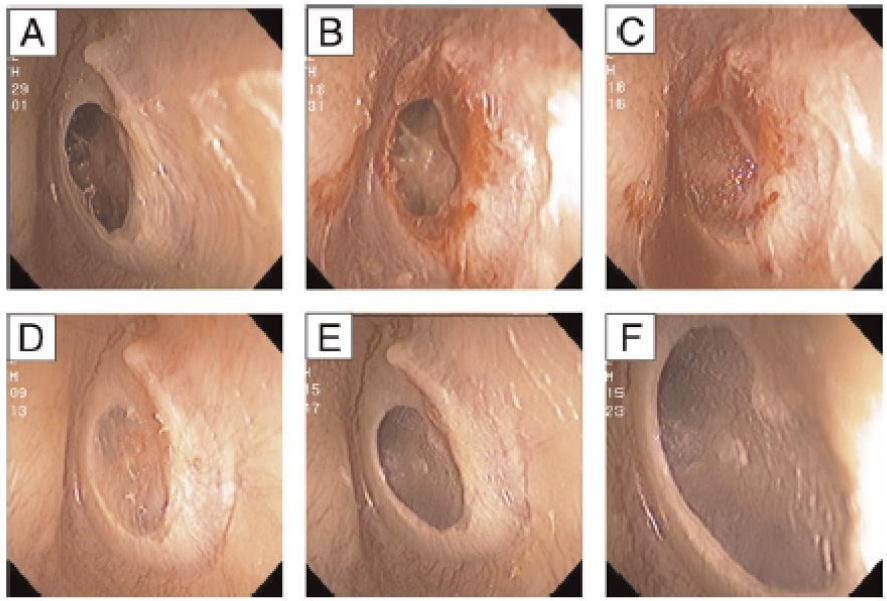

FIG. 4. Case 1: A 65-year-old woman with chronic otitis media persisting for 30 years. *A*, Large, dry, Grade II perforation. *B*, Disruption of the perforation edge. *C*, Gelatin sponge with b-FGF was placed over the TMP and sealed by fibrin glue. *D*, Three weeks after the treatment the TM was perfectly regenerated. *E* and *F*, Four months after the treatment, slightly hypertrophic tissue became thinner, and an almost normal TM with hypervascularity was regenerated.

Ref：Omae K, Kanemaru SI, Nakatani E, Kaneda H, Nishimura T, Tona R, Naito Y, Kawamoto A, Fukushima M.
Regenerative treatment for tympanic membrane perforation using gelatin sponge with basic fibroblast growth factor.
Auris Nasus Larynx. 2017;44(6):664-71.

TRI-supported clinical trial :
Eardrum regeneration treatment for tympanic membrane perforation (US)

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Fibroblast Growth Factor Regeneration of Tympanic Membrane Perforations

⚠ The safety and scientific validity of this study is the responsibility of the study sponsor and investigators. Listing a study does not mean it has been evaluated by the U.S. Federal Government. Read our disclaimer for details.

ClinicalTrials.gov Identifier: NCT02307916

Recruitment Status : Active, not recruiting
First Posted : December 4, 2014
Last Update Posted : March 5, 2019

Sponsor:
Dr. Bradley Welling

Collaborator:
United States Department of Defense

Information provided by (Responsible Party):
Dr. Bradley Welling, Massachusetts Eye and Ear Infirmary

Study Details | Tabular View | No Results Posted | Disclaimer | How to Read a Study Record

Study Description Go to

Brief Summary:
A Phase II randomized trial will be initiated to evaluate closure of the perforated tympanic membrane as the primary measureable outcome. The goal is to determine the safety and efficacy of Fibroblast Growth Factor-2 (FGF-2) in the closure of chronic tympanic membrane perforations (TMP). If FGF-2 is topically applied for the treatment of chronic TMP in humans, it is hypothesized it will be safe, tolerable and effective for use as treatment for tympanic membrane perforation. A total of 60 subjects will be recruited.

Condition or disease	Intervention/treatment	Phase
Tympanic Membrane Perforation	Drug: FGF-2	Phase 2

First round of regenerative medicine has been completed

	Type of regeneration	Target Disease	PI	SAKIGAKE Designation
Approved **Dec. 20, 2018**	**Nerve** (auto serum-expanded autologous CD105 mesenchymal stem cells)	Spinal cord injury  Vol. 552 No. 7684_supp | December 14, 2017 Spinal-cord injury: spurring regrowth https://www.nature.com/collections/ctdkppqqnx/videos	**Osamu Honmou (Sapporo Medical University)**	★ February 2016
Approved **Aug. 1, 2019**	**Eardrum** (bFGF/gelatin sponge)	Tympanic membrane  Vol. 546 No. 7659_supp | June 22, 2017 Eardrum regeneration: membrane repair http://www.nature.com/collections/rzfrydkflp/videos	**Shinichi Kanemaru (Kitano Hospital)**	
Under trial	**Blood vessel** (CD34/cell)	Critical Limb Ischemia  Vol. 548 No. 7668_supp | August 24, 2017 Critical limb ischaemia www.nature.com/collections/vmxkcnxvwg/videos	Atsuhiko Kawamoto (TRI)	★ March 2018
Under trial	**Bone** (CD34/atelocollagen)	Refractory bone fracture  Vol. 550 No. S193 | October 26, 2017 Non-union bone fracture: a quicker fix https://www.nature.com/collections/qmpthxknbn/videos	Ryosuke Kuroda (Kobe University)	★ March 2018
Preparing NDA	**Cornea** (Mucouse membrane cell sheet)	Corneal epithelial stem cell deficiency  Vol. 544 No. 7650_supp_out | April 20, 2017 Corneal repair http://www.nature.com/collections/pdryjrsvnz/videos	Chie Sotozono (Kyoto Prefectural University of Medicine)	
Under trial	**Cartilage** (Cartilage cell/collagen)	Cartilage injury	Hiroyuki Ishibashi (Hirosaki University)	

2nd round of Regenerative Medicine Development

From Disruptive Innovation
to Continuous Innovation

- **Cell Free**
- **CPC Free**
- **One step preparation technique**

2nd round : Clarification of molecular basis of biological principle concerning tissue generation and establishing its utilization

Today's Topics

1. Introduction – *we are living in unprecedented science revolutionary age*
2. What kinds of services and supports TRI provide, and our accomplishments
3. Explore the life/living organisms as it is
4. Principles of regenerative medicine
5. Examples of regenerative medicine –Results from clinical trials
6. **What should be done next**

Next mission is what should be done next

- **Next mission is dissemination of regenerative treatment over the country/world for the patients waiting, including the process of innovation to marketing.**
- **Strategic investment and initiative by government to create an overall infrastructure to provide regenerative medicine is critical.**

→ **Dramatic reduction of need of care and bedridden**

→ **Global expansion**

Regenerative treatment - What can be done within next 5 years

日本再興戦略 “Japan revitalization strategy”

Theme 1 ：**Extension of healthy life expectancy of citizen**・・・・・・・・・・59

①Society which one can age healthy with effective disease prevention and health control services

②**Society which can provide cutting-edge medical treatment by activation of medical industry**

③**Society which one can promptly recover to society from disease/injury with the access to high-quality medical care**

- ✓ **Reduce to just about nil, the bedridden and wheel chaired life caused by spinal cord injury**
- ✓ **Reduce to just about nil, the bedridden and care-needed life caused by stroke**
- ✓ **Reduce to just about nil, the lower limb amputation caused by critical limb ischemia**
- ✓ **And much more…**

Reduction of social burden

BY adequate medical strategic policy and investment !

Grand design of global network

Network management

ECRIN(EU)

Asia ARO Network

Taiwan-Japan ARO Workshop since 2015

NCATS(US)

AAHRPP Accreditation

Global ARO Network

ECRIN Data Centre Certification

Global development of academia medical product candidates

CDISC Standards

- Cancer
- MCI/AD
- ALS
- Stroke
- Others

Global **"Disease-specific"** Consortium

Goal

- Improve Outcomes
- Overcome Diseases

Clinical Trial Network

Mission of Academia

1. Academia should live as per science
   Science is academia's soul & spirit
2. To live is to intersect with the world, to face the world, to work in the world, and to **engage in the world**
3. People should live to a high point of the era, most of all, to live to **the height of philosophy of the era**

Misin de la universidad
Jose Ortega y Gasset, 1930

Our GOAL is total disease control and absolute improvement of prognosis

We the humans are walking on the road toward total disease control at last in 2018.

In order to accelerate disease control through medical innovation, we shall be withdrawing from ineffective competitive effort driven by researcher's individual interest.

Desired new concept featuring "Management Science for Science" aim for stable achievements by :

- gathering the knowledge and wisdom to gain synergy effect
- through strong project management
- bundling medical R&Ds into integrated/synthetic human science enterprise

Ref: Preface from
"Road to total disease control" by M. Fukushima
Book issued in 2019

謝謝

Thank you for your attention !