第27回日本遺伝子細胞治療学会学術集会

The 27th Annual Meeting of Japan Society of Gene and Cell Therapy

Date

Live Conference
September 9 -10, 2021

Web Conference
September 27 - October 15, 2021

Venue

Live Conference
Toranomon Hills Fourm

President

Torayuki Okuyama
Director, Department of Clinical Laboratory Medicine,
Center for Lysosomal Storage Diseases,
National Center for Child Health and Development

Gene and Cell Therapy, Toward Next Generation

Program & Abstracts

JSGCT Chairman's Lecture

Tomoki Todo (The Institute of Medical Science, The University of Tokyo)

ASGCT and ESGCT Special Lecture

Stephen J. Russell (Immediate Past President of ASGCT)
Beverly L. Davidson (President of ASGCT)
Hildegard Büning (President of ESGCT)

Scientific Symposia

Symposium 1: Genetic Disorders
Symposium 2: Basics & Genome Editing
Symposium 3: Vector Development
Symposium 4: Neurological Disorders
Symposium 5: Clinical application
Symposium 6: Cancer

JSGCT

https://www.jsgct2021.jp/index.html

【大会事務局】 国立成育医療研究センター　臨床検査部　〒157-8535　東京都世田谷区大蔵2-10-1
【運営事務局】 株式会社SKアペックスプラン
〒104-0041　東京都中央区新富1-8-6　SSビル5F　TEL：03-3523-3722　FAX：03-3523-3723　e-mail：info@skap.jp

The 27th Annual Meeting of JSGCT 2021

The 27th Annual Meeting of Japan Society of Gene and Cell Therapy

JSGCT2021

Program & Abstracts

Date

Live Conference: September 9 – 10, 2021
Web Conference: September 27 – October 15, 2021

Venue

Live Conference
Toranomon Hills Forum
Mori Tower 5th Floor, 1-1-23 Toranomon, Minato-ku, Tokyo 105-6390, Japan

The 27th Annual Meeting of Japan Society of Gene and Cell Therapy 2021

Meeting President
Torayuki Okuyama

Member of the JSGCT Committee

Chairman of the Board of Director (BOD)

Tomoki Todo

Vice Chairman of BOD

Ryuichi Morishita & Takashi Okada

President-Elect

Yoshikazu Yonemitsu

Members of BOD

Kazunori Aoki, Toshiyoshi Fujiwara, Hiroshi Fukuhara, Yasufumi Kaneda, Yumi Kanegae, Noriyuki Kasahara, Akihiro Kume, Kohnosuke Mitani, Hiroyuki Mizuguchi, Hideki Mochizuki, Ryuichi Morishita, Shin-ichi Muramatsu, Takafumi Nakamura, Yasumoto Nasu, Toya Ohashi, Takashi Okada, Makoto Otsu, Masatoshi Tagawa, Kenzaburo Tani, Tomoki Todo, Eriko Uchida, Masato Yamamoto, Yoshikazu Yonemitsu

Board Composition

＊Chief (underline)

Honorary Chairman of BOD
Yasufumi Kaneda

Auditors Torayuki Okuyama & Katsuto Tamai

Treasurers Ryuichi Morishita, Hiroshi Fukuhara (Vice Chair)
Toshiyoshi Fujiwara, Hideki Mochizuki, Shin-ichi Muramatsu, Mahito Nakanishi, Yasutomo Nasu

Public Relations and International Exchanges
Takafumi Nakamura
Noriyuki Kasahara, Ko Mitani, Toya Ohashi, Masatoshi Tagawa, Masato Yamamoto

Ethical and Safety Issues, COI
Takashi Okada
Kazunori Aoki, Yumi Kanegae, Makoto Otsu, Kenzaburo Tani, Eriko Uchida

Educational and Public Affairs
Yoshikazu Yonemitsu, Hironori Nakagami (Vice Chair)
Akihiro Kume, Hiroyuki Mizuguchi, Hiroyuki Nakai

Working Group Committee for the Next Generation
Makoto Otsu
Hiroshi Fukuhara, Kenya Kamimura, Toshinao Kawai, Hiroshi Kobayashi, Ken-ichiro Kosai, Hironori Nakagami, Takafumi Nakamura, Yozo Nakazawa, Fuminori Sakurai, Shigeki Yagyu

Future Planning Committee
Chairman of the BOD
Vice Chairmen of the BOD, Chair of Committee and JSGCT Administrative Office

JSGCT2021 Scientific Committee　Chair: Torayuki Okuyama

Basic Science Yumi Kanegae, Kohnosuke Mitani, Hiroaki Mizukami, Takashi Nakamura, Eriko Uchida, Masato Yamamoto

Vector Kazunori Aoki, Hiroyuki Mizuguchi, Shin-ichi Muramatsu, Mahito Nakanishi, Masashi Urabe

Cancer Ken-ichiro Kosai, Tomoyasu Nasu, Masatoshi Tagawa, Kenzaburo Tani, Tomoki Todo

Genetic Diseases Hiroshi Kobayashi, Koichi Miyake, Makoto Otsu, Naoya Uchida, Toru Uchiyama

Cardiovascular & Other Diseases
Akihiro Kume, Ryuichi Morishita, Hironori Nakagami, Yoshikazu Yonemitsu

Neurological Disorders
Yasuhiro Ikeda, Takashi Okada, Norio Sakai, Takanori Yamagata

JSGCT2021 Secretariat Office　Ryuichi Mashima, Toru Uchiyama

Department of Clinical Laboratory Medicine, National Center for Child Health and Development (NCCHD)
2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan
E-mail：info@skap.jp　https://www.jsgct2021.jp/

Japan Society of Gene and Cell Therapy Administrative Office

Secretary General
Hiroshi Fukuhara

Vice Secretary General
Makoto Otsu

Message from the President

Dear colleagues;

It is my great pleasure to announce the 27th JSGCT Annual Meeting being held on September 9 to 10, 2021 at the Toranomon Hills Forum, Tokyo, Japan.

To consider the serious pandemic situation, we will hold the meeting as so called" hybrid method", which is a combination of "face to face" meeting and on-line meeting.

The theme of the meeting is "Gene and Cell Therapy, Towards Next Generation". The recent progress in gene and cell therapy is outstanding, and the focus of the gene therapy research is sifting from basic research to clinical study. At the meeting, we are planning six Scientific Symposiums (genetic diseases, neurodegenerative diseases, genome editing, cancer gene therapy, vector development, and clinical gene therapy). We are also planning the special lecture of the presidents of American Society and European Society for Gene and Cell Therapy (ASGCT, ESGCT).

Unfortunately, all of us cannot meet in the Toranomon Hills Forum, but many foreign guests will participate in the meeting with on-line system, I truly hope the 27th Annual Meeting of Japan Gene and Cell Therapy will be stimulating and enjoyable for all participants.

Sincerely yours,

Torayuki Okuyama, M.D., Ph.D.
President of the 27th JSGCT-2021
Director, Clinical Laboratory Medicine
Center for Lysosomal Storage Diseases
National Center for Child Health and Development

Meeting Schedule

Live Conference: September 9 - 10, 2021
Web Conference: September 27 - October 15, 2021

Registration

You could choose pre-registration by on-line or on-site registration as follows schedule.

	Pre-registration Fee & Date (Aug 6th to 31st until 15:00)	On-site registration Fee & Date (Sep 1st to Oct 7th until 15:00)	LIVE	WEB
JSGCT Member	13,000JPY	16,000 JPY	○	○
Non Member	20,000 JPY		○	○
Student	Graduate Student*: 1,000 JPY Student*: Free		○	○

*Graduate student & Student need to adduce verification card.
*Program & Abstracts Book Fee: 3,000 JPY (Free for those who have paid their 2021-Annual Membership Fees)
***September 9th to 10th could be payable on-site registration desk by cash (JPY) only.**
*All Payments must be made in Japanese Yen (JPY).
*Please be sure that all authors need to registration except non-member of the JCGCT,

Method of Payment

Pre-registration: August 6th to 31st, until 15:00
On-site registration: September 1st to October 7th, until 15:00
*September 9th to 10th could be payable on-site registration desk by cash (JPY) only.

Registration fee entitles the participants to all Scientific Sessions and Exhibitions.
Nametags will be send by mail first week of September for the Pre-registrations, and distributed at the Registration Desk for On-site registrations.

JSGCT Board Meetings

Councilors Meeting: September 9 (Thursday) 9:30 to 10:50 at Toranomon Hills Forum "Hall A2"

General Assembly

General assembly will be held from 13:00 to 14:00 on September 9 at Room 1 (Toranomon Hills Forum)
All members will be requested to take part in this assembly.

Exhibition

Exhibition will be held from September 9 to 10.

Corporate Seminars

Due to a limited amount of foods offered at some of these seminars, admission tickets will be provided on the day at the Registration Desk.
For information ask to staffs at the Registration Desk and please note that tickets will be limited in quantity.

Instructions for speakers in oral presentation

1. The language for oral presentation is either Japanese or English.
   *Symposium and Plenary Session's speakers presentation is in English.
2. All slides should be prepared in English. No simultaneous interpretation will be provided.
3. Application software for preparing presentation data should be PowerPoint 2007/2010/2013/2016.
4. If you have prepared your presentation data on a Macintosh PowerPoint, please check that your presentation functions correctly in a windows-based environment, or bring your own PC in order to avoid display problems.
5. Use standard font (e.g. Arial, Helvetica, Times, Times New Roman) in preparing your presentation to avoid conversion errors.
6. All authors required to disclose any conflict of interest with sponsoring companies.
   For oral presentations, please include the slide disclosing the state of COI in your PowerPoint presentation after your title slide.
   (You can download sample template from Call for Abstract page on your website **https://www.jsgct2021.jp/endai.html**)
7. To avoid the possible spread of computer viruses, please scan your presentation files beforehand with update anti-virus software.

For Live Conference Presenters

PC Preview Section

1. Please bring your presentation data in a USB flash memory on your own laptop PC, at least 30 minutes prior to your presentation to the PC Preview Section to complete review of presentation data.
2. On-site operating system will be Windows 10, PowerPoint 2007/2010/2013/2016.
3. Please place all video clips linked with the PowerPoint presentation into a single folder.
   Video file should be WMV or MPEG1, MPEG4.
4. If using a Macintosh or you're PowerPoint presentation includes moving images, please bring your own PC and back-up data to make your presentation.
5. Presentation data loaded on the conference PC will be completely deleted after your presentation by our staffs.

Laptop users

1. Macintosh users are requested to bring your own PC.
2. Turn off any sleep functions and screen savers beforehand.
3. Cable connector used at the venue for image output is D-sub 15 pin connector or HDMI.
   Please bring your own connector conversion adapter if necessary.
4. After checking your data at the PC Preview Section, please bring your PC to the "Operating Desk" near the speakers' podium in the session rooms.
5. Please pick up your PC at the Operation Desk after your presentation.

For Web Conference Presenters

1. All general presentation will be oral presentation.
2. We requested to send your MP4 presentation data with sound by to the secretariat office by e-mail until August 25th.

*You can download "How to prepare your presentation data" from Call for Abstract page on your website.
https://www.jsgct2021.jp/en/abstract

▌Time allocations for presentations

The speakers of Plenary Session will be given 10 minutes in total.
(8 min. talk followed by 2 min. discussion)
The speakers of Oral Sessions will be given 7 minutes.
(Please prepare your presentation data by MP4 within 7 min.)

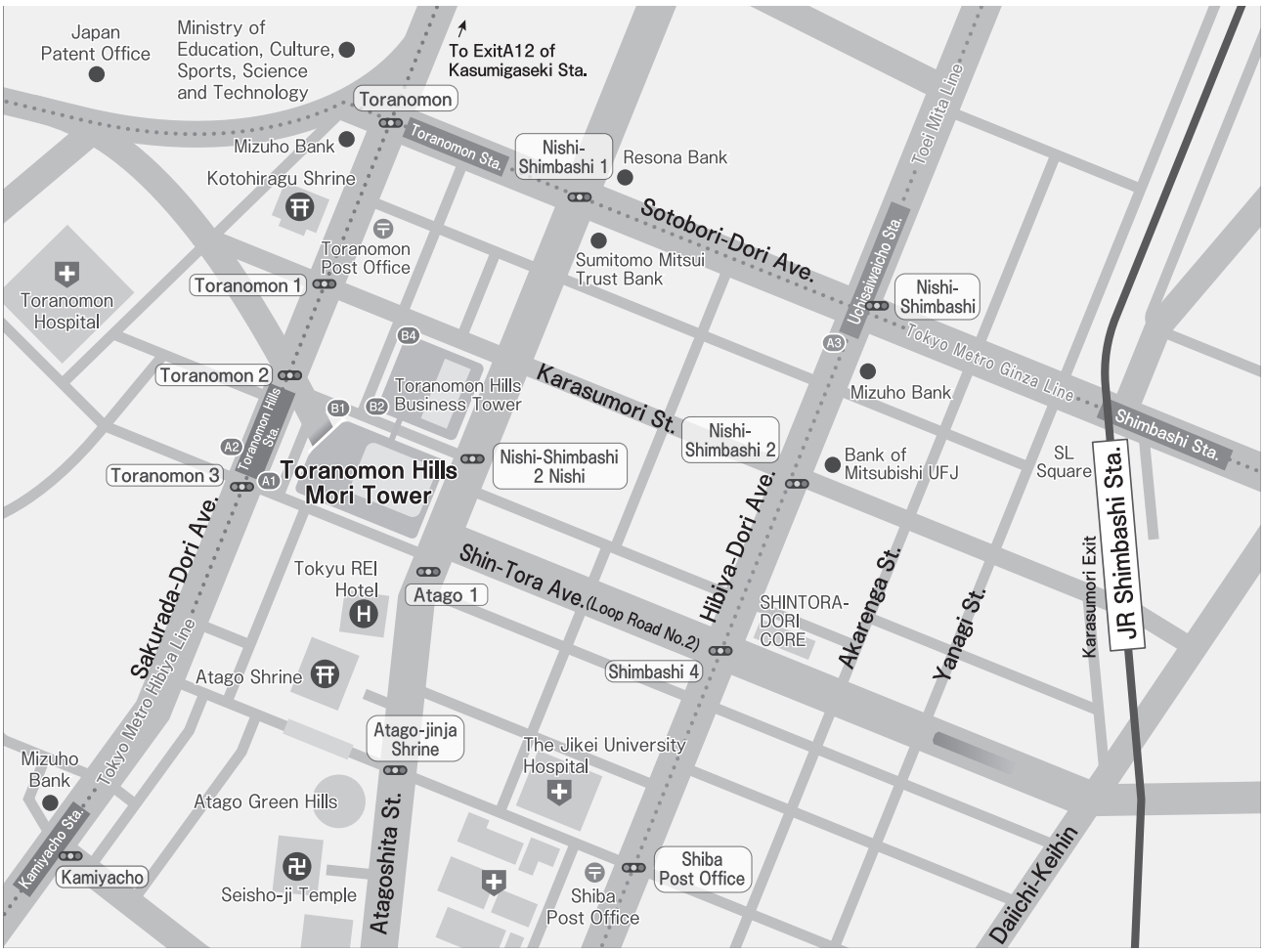
▌Time allocations for presentations

Session Title (Total allocated time)	Talk
Chairman's Lecture (30 min.)	25 min.
ESGCT Special Lecture (30 min)	25 min.
ASGCT Special Lectures (30 min. per person)	25 min.
Symposium 1 (20 min. per person)	17 min.
Symposium 2 (20 min. per person)	17 min.
Symposium 3 (20 min. per person)	17 min.
Symposium 4 (20 min. per person)	17 min.
Symposium 5 (20 min. per person)	17 min.
Symposium 6 (20 min. per person)	17 min.
11th Takara Bio Award Lecture (20 min.)	15 min.
Plenary Session 1&2 (10 min. Per Person)	8 min.

▶Please note that alterations may be informed by the Session's Chairs.

For the latest information about the meeting, please visit the website.
https://www.jsgct2021.jp/

Access to Toranomon Hills Forum



Toranomon Hills Mori Tower 5th Floor, 1-23-3 Toranomon, Minato-ku, Tokyo 105-6305

● Public Transportation

Tokyo Metro Ginza Line 2-minute walk from Exit B4 of Toranomon Sta.

Tokyo Metro Hibiya Line Toranomon Station (Nakameguro gates) 2-minute walk from Exit A1

Exit B1. Exit A2 is not accessible from the Nakamegurogates and Exits A1 and B1 are not accessible from the Kita-Senju gates.

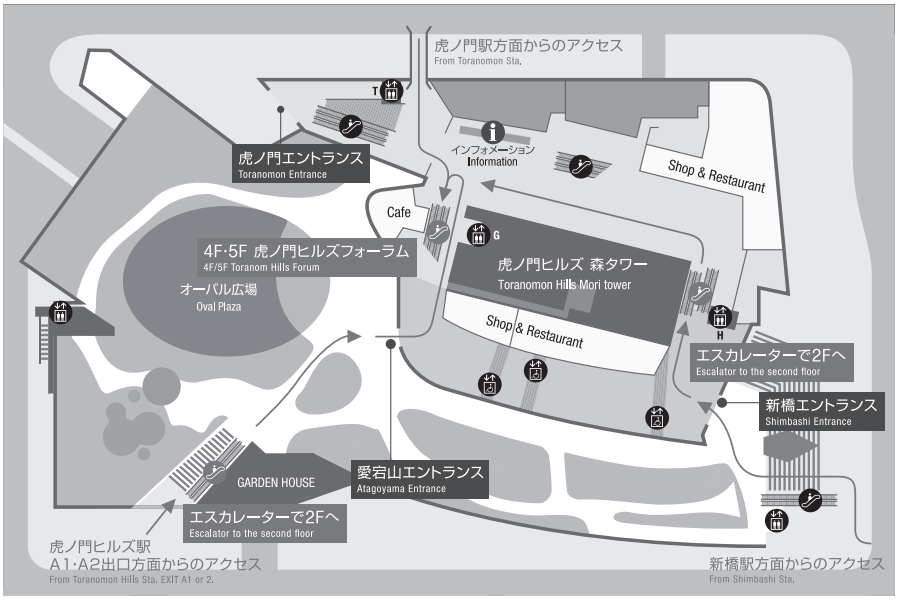
Toranomon Station (Kita-Senju gates) 2-minute walk from Exit A2.

Tokyo Metro Chiyoda Line/Marunouchi Line 8-minute walk from Exit A12 of Kasumigaseki Station.

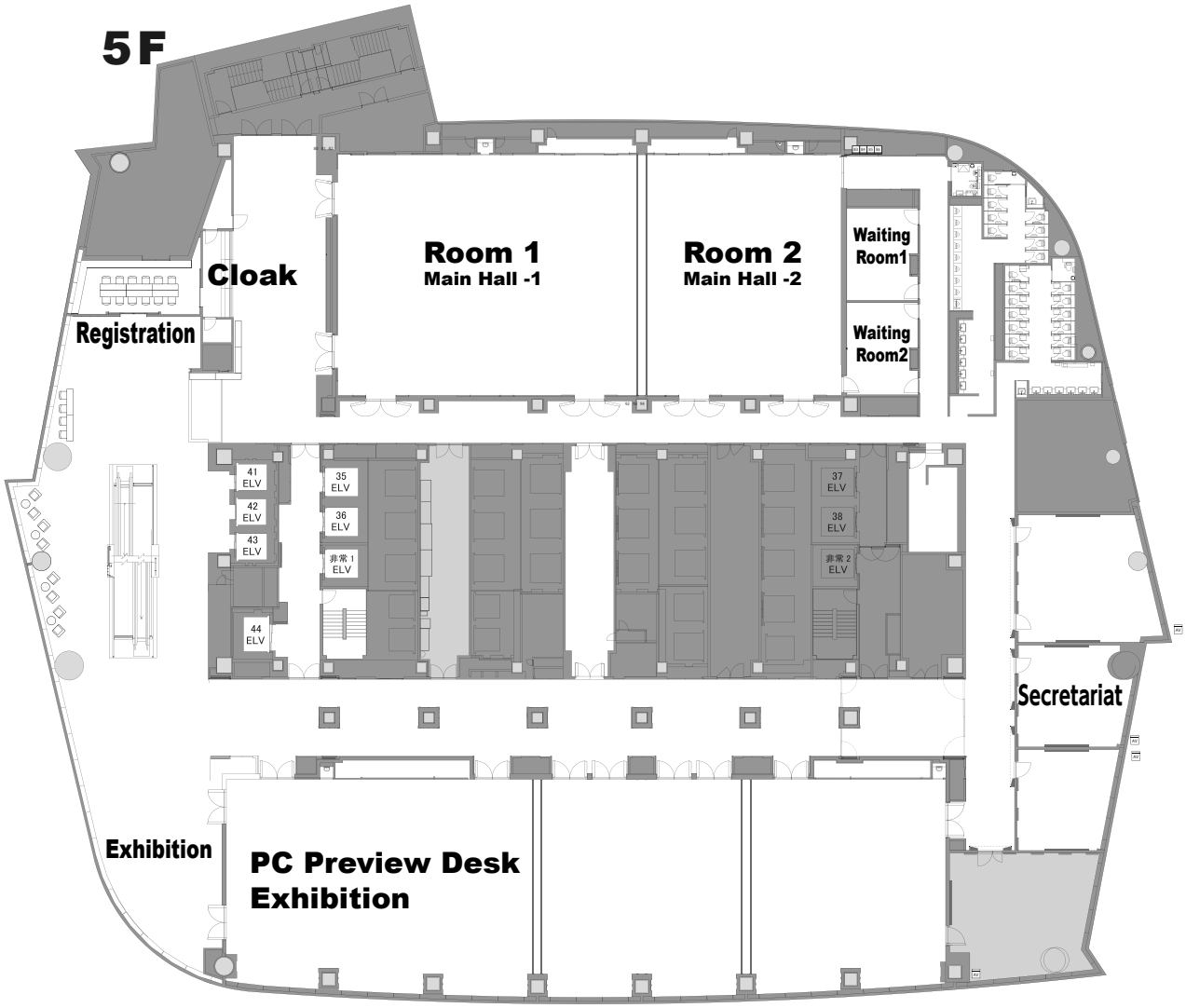
Toei Subway Mita Line 8-minute walk from Exit A3 of Uchisaiwaicho Station.

Ginza Line/Asakusa Line/Yurikamome/Yamanote Line/Keihin-Tohoku Line/Tokaido Line/Yokosuka Line

11-minute walk from Karasumoriguchi Exit ofShimbashi Station



Floor Map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Cloak
Registration
Room 1
Main Hall -1
Room 2
Main Hall -2
Waiting Room1
Waiting Room2
41 ELV
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非常 1 ELV
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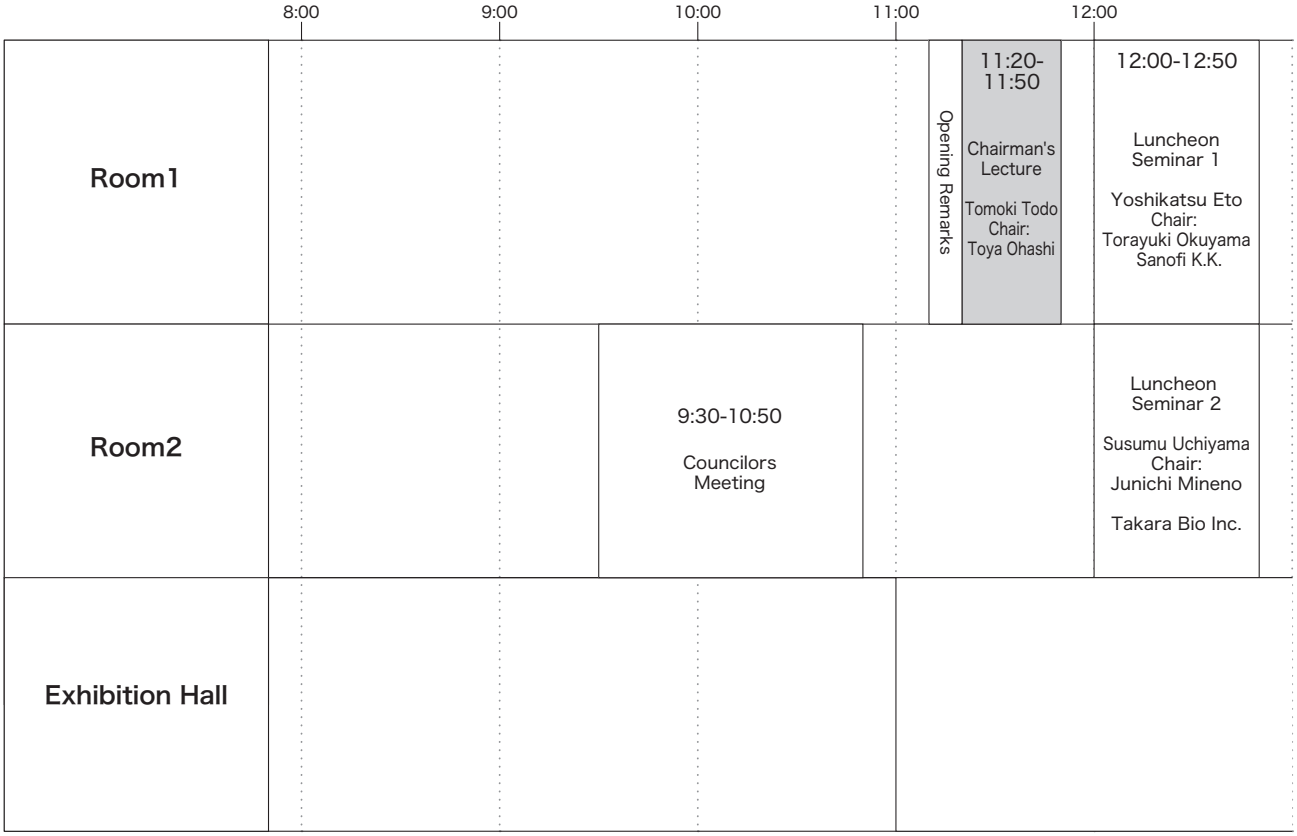
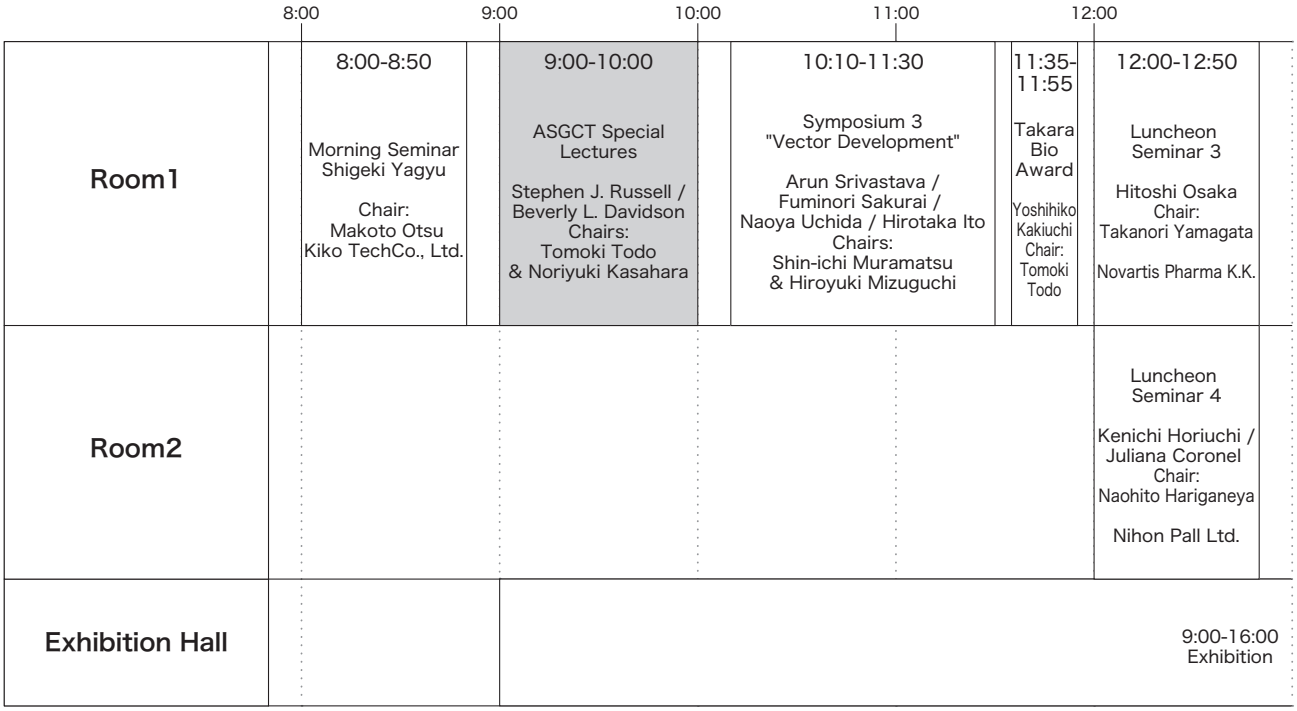
38 ELV
非常 2 ELV
Secretariat
Exhibition
PC Preview Desk
Exhibition

The 27th Annual Meeting of Japan Society of Gene and Cell Therapy

Day 1 (Thursday, September 9)

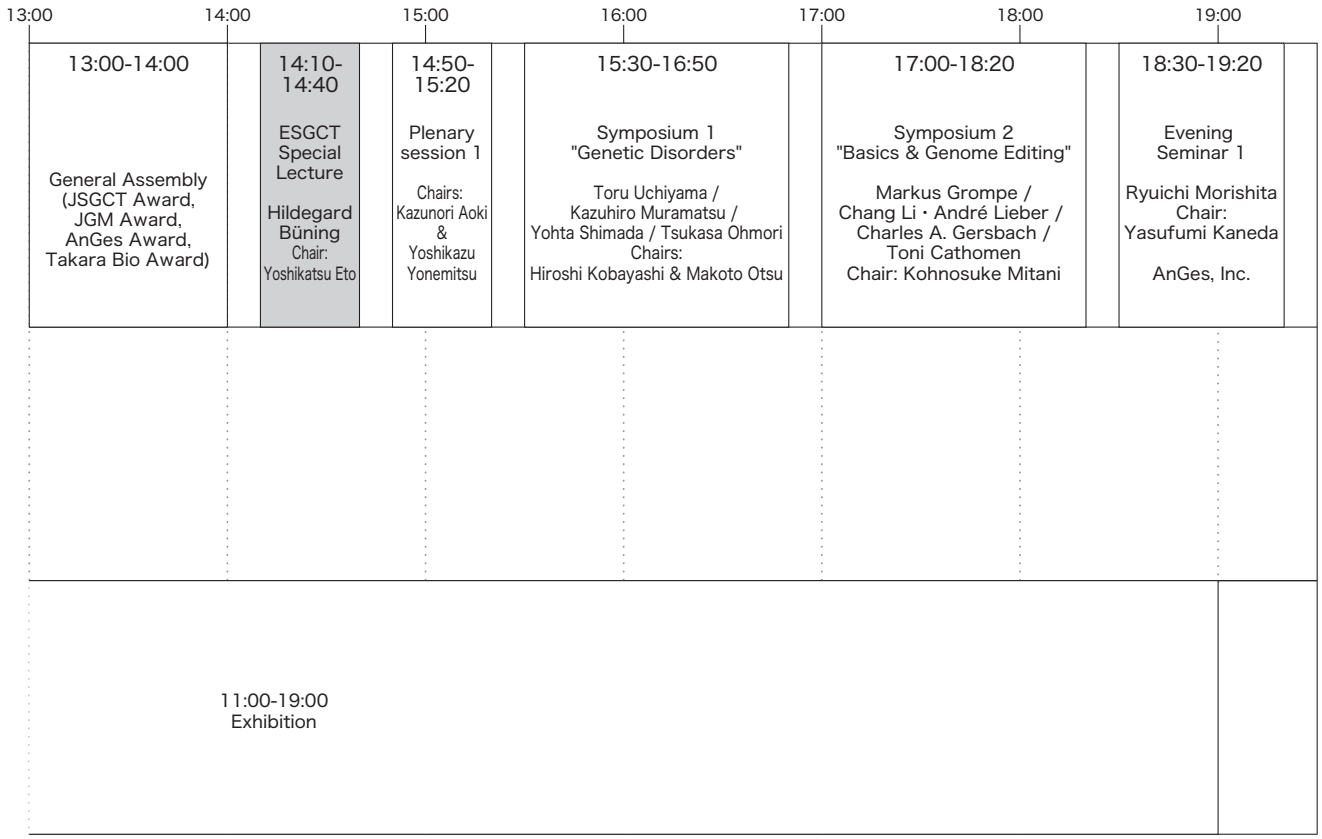


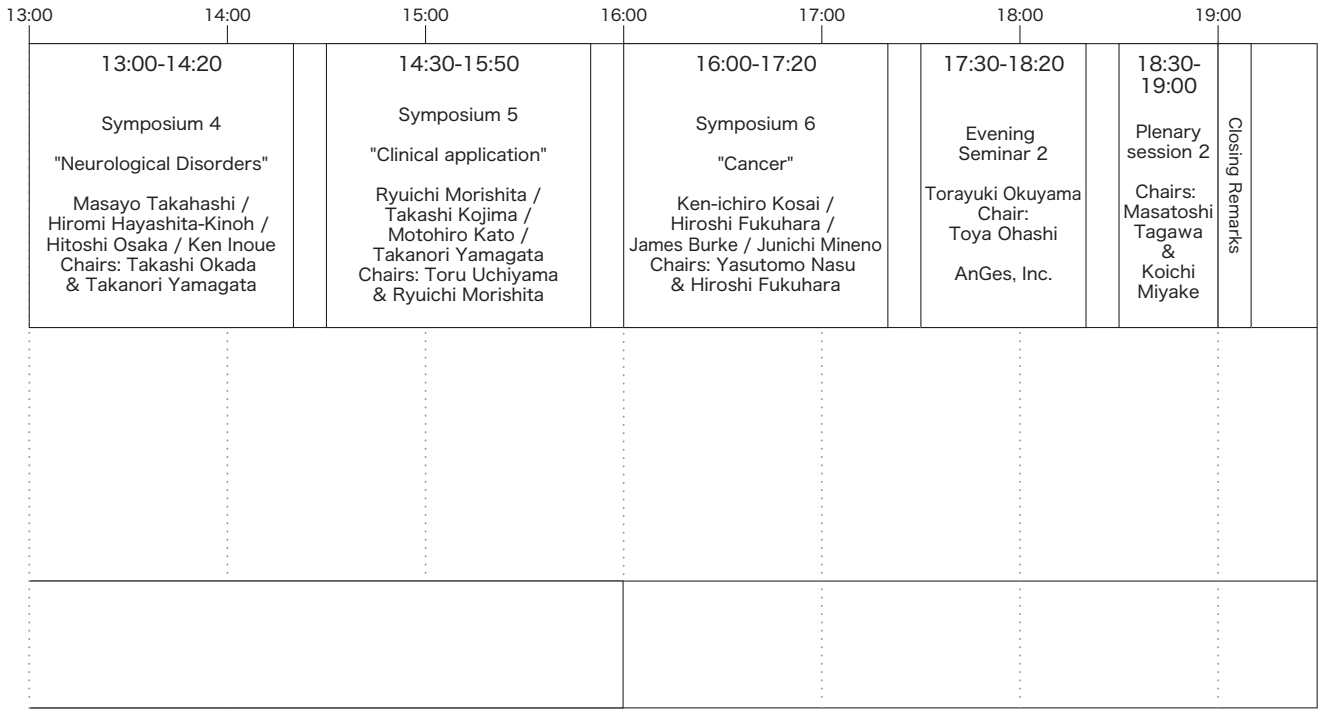
Day 2 (Friday, September 10)



DATE Live Conference: September 9-10, 2021　Web Conference: September 27-October 15, 2021

Venue Live Conference: Toranomon Hills Forum





PROGRAM

Live Conference

September 9 (Thu) – September 10 (Fri), 2021
Venue: Toranomon Hills Forum

Day 1: Thursday, September 9
Room 1

11:10-11:20 **Opening Remarks: President Torayuki Okuyama**
(Department of Clinical Laboratory Medicine, Center for Lysosomal Storage Diseases, National Center for Child Health and Development)

11:20-11:50 **Chairman's Lecture**
Chairperson: Toya Ohashi (The Jikei University School of Nursing)

CL **"Recent progress of oncolytic virus therapy using HSV-1"** 3
Tomoki Todo *(Division of Innovative Cancer Therapy, The Institute of Medical Science, The University of Tokyo)*

12:00-12:50 **Luncheon Seminar 1** Co-sponsored by Sanofi K.K.
Chairperson: Torayuki Okuyama (Department of Clinical Laboratory Medicine, Center for Lysosomal Storage Diseases, National Center for Child Health and Development)

LS1 **"Latest therapy trends for Lysosomal Storage disorders ~ from ERT to Gene Therapy~"**
Yoshikatsu Eto *(Advanced Clinical Research Center, Southern Tohoku Institute for Neuroscience / The Jikei University School of Medicine)*

13:00-14:00 **General Assembly**

14:10-14:40 **ESGCT Special Lecture**
Chairperson: Yoshikatsu Eto (Advanced Clinical Research Center, Southern Tohoku Institute for Neuroscience / The Jikei University School of Medicine)

SL1 **"Capsid-engineered Adeno-Associated Virus (AAV) vectors for in vivo Gene Therapy"** 7
Hildegard Büning *(President of ESGCT / Hannover Medical School, Germany)*

14:50-15:20 **Plenary Session 1**
Chairpersons: Kazunori Aoki (National Cancer Center Research Institute)
Yoshikazu Yonemitsu (Graduate School of Medicine, Kyushu University)

PS1-1 **"Improving the in vivo gene targeting efficiency of liver-directed rAAV vector using the nucleotide analog class of ribonucleotide reductase inhibitors"** 78
Shinnosuke Tsuji *(Daiichi Sankyo Co., Ltd.)*

PS1-2 **"LassoGraft Technology® allows rapid and simple generation of engineered AAV vectors with defined receptor dependency"** 79
Junichi Takagi *(Institute for Protein Research, Osaka University)*

PS1-3 **"Elucidation of the mechanism of reovirus-mediated antifibrotic effects on liver fibrosis"** 80
Ikuho Ishigami *(Graduate School of Pharmaceutical Science, Osaka University)*

15:30-16:50 **Symposium 1: Genetic Disorders**

Chairpersons: Hiroshi Kobayashi (The Jikei University School of Medicine)
Makoto Otsu (Kitasato University School of Medicine)

S1-1 **"Progress of gene therapy for primary immunodeficiency diseases"** 15
Toru Uchiyama *(Division of Molecular Pathogenesis, Department of Human Genetics, National Center for Child Health and Development)*

S1-2 **"Development of gene therapy targeting liver for congenital metabolic disorder based on novel AAV vector"** 17
Kazuhiro Muramatsu *(Department of Pediatrics, Jichi Medical University / Division of Neurological Gene Therapy, Jichi Medical University)*

S1-3 **"Hematopoietic stem cell gene therapy for mucopolysaccharidosis type II"** 19
Yohta Shimada *(Division of Gene Therapy, Research Center for Medical Sciences, The Jikei University School of Medicine)*

S1-4 **"Gene therapy for hemophilia"** 21
Tsukasa Ohmori *(Department of Biochemistry, Jichi Medical University)*

17:00-18:20 **Symposium 2: Basics & Genome Editing**

Chairperson: Kohnosuke Mitani (Saitama Medical University)

S2-1 **"In vivo selection for the enhancement of cell and gene therapy of liver diseases"** 25
Markus Grompe *(Oregon Health and Science University, Portland, USA)*

S2-2 **"*In vivo* HSC genome editing"** 27
Chang Li *(University of Washington, Department of Medicine, Division of Medical Genetics, USA)*
André Lieber *(University of Washington, Department of Medicine, Division of Medical Genetics / Department of Pathology, Seattle, WA, USA)*

S2-3 **"Genome Editing for Duchene Muscular Dystrophy"** 29
Charles A. Gersbach *(Duke University, USA)*

S2-4 **"On- and off-target effects of genome editing tools in clinically relevant human hematopoietic cells"** 31
Toni Cathomen *(Institute for Transfusion Medicine and Gene Therapy, Medical Center, University of Freiburg, Germany)*

18:30-19:20 **Evening Seminar 1** Co-sponsored by AnGes, Inc.

Chairperson: Yasufumi Kaneda (National University Corporation Osaka University)

ES1 **"Plasmid DNA-based Gene Therapy: From Vascular Regeneration to DNA Vaccines"**
Ryuichi Morishita *(Graduate School of Medicine, Osaka University)*

Day 1: Thursday, September 9
Room 2

12:00-12:50 **Luncheon Seminar 2** Co-sponsored by Takara Bio Inc.
Chairperson: Junichi Mineno (Takara Bio Inc.)

LS2 **"Biophysical characterization and quality control of AAV vectors"**
Susumu Uchiyama *(Department of Biotechnology, Graduate School of Engineering, Osaka University Manufacturing Technology Association of Biologics)*

Day 2: Friday, September 10
Room 1

8:00-8:50 **Morning Seminar** Co-sponsored by Kiko Tech Co., Ltd.
Chairperson: Makoto Otsu (Department of Transfusion and Transplantation, Kitasato University School of Medicine)

MS **"Non-viral gene transfer based chimeric antigen receptor T cells for solid tumors"**
Shigeki Yagyu (Department of Pediatrics, Kyoto Prefectural University of Medicine)

9:00-10:00 **ASGCT Special Lectures**
Chairpersons: Tomoki Todo (The Institute of Medical Science, The University of Tokyo)
Noriyuki Kasahara (University of California, San Francisco, USA)

SL2-1 **"Intravenous oncolytic virotherapy using Voyager-V1 (VSV-IFNβ-NIS)"** 9
Stephen J. Russell *(Immediate Past President of ASGCT / Mayo Clinic and Vyriad, Rochester, Minnesota, USA)*

SL2-2 **"Regulated control of gene therapies by drug-induced splicing"** 11
Beverly L. Davidson *(President of ASGCT / Perelman School of Medicine of the University of Pennsylvania / Children's Hospital of Philadelphia, USA)*

10:10-11:30 **Symposium 3: Vector Development**
Chairpersons: Shin-ichi Muramatsu (Open Innovation Center, Jichi Medical University)
Hiroyuki Mizuguchi (Graduate School of Pharmaceutical Sciences, Osaka University)

S3-1 **"Development of the Next Generation of AAV Vectors for Human Gene Therapy"** 35
Arun Srivastava *(Division of Cellular and Molecular Therapy, Department of Pediatrics, Powell Gene Therapy Center, University of Florida College of Medicine, USA)*

S3-2 **"Development of a novel oncolytic adenovirus based on adenovirus serotype 35"** 37
Fuminori Sakurai *(Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University)*

S3-3 **"Lentiviral vector system for hematopoietic stem cell gene therapy"** 39
Naoya Uchida *(National Institutes of Health (NIH), USA / The Institute of Medical Science, The University of Tokyo)*

S3-4 **"Development of an oncolytic herpes simplex virus expressing anti-VEGF antibody bevacizumab"** 41
Hirotaka Ito *(Division of Innovative Cancer Therapy, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo)*

11:35-11:55 **Takara Bio Award**
Chairperson: Tomoki Todo (The Institute of Medical Science, The University of Tokyo)

"Local oncolytic adenovirotherapy produces an abscopal effect via tumor-derived extracellular vesicles" 75
Yoshihiko Kakiuchi *(Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences)*

12:00-12:50 **Luncheon Seminar 3** Co-sponsored by Novartis Pharma
Chairperson: Takanori Yamagata (Department of Pediatrics, Jichi Medical University)

LS3 **"Current status and future perspective of AAV gene therapy for genetic neurological disorders"**
Hitoshi Osaka *(Department of Pediatrics, Jichi Medical University)*

13:00-14:20 **Symposium 4: Neurological Disorders**
Chairpersons: Takashi Okada (The Institute of Medical Science, The University of Tokyo)
Takanori Yamagata (Department of Pediatrics, Jichi Medical University)

S4-1 **"Treatments for outer retinal diseases"** 45
Masayo Takahashi *(Vision Care Inc. & Kobe City Eye Hospital)*

S4-2 **"Gene Therapy Approaches using rAAV for the Treatment of Duchenne Muscular Dystrophy"** 47
Hiromi Hayashita-Kinoh *(Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo)*

S4-3 **"Gene therapy of genetic neurological disorders"** 49
Hitoshi Osaka *(Department of Pediatrics, Jichi Medical University)*

S4-4 **"Gene suppression therapy for Pelizaeus-Merzbacher disease using AAV harboring artificial miRNA: principals and problems"** 51
Ken Inoue *(Department of Mental Retardation & Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry)*

14:30-15:50 **Symposium 5: Clinical application**
Chairpersons: Toru Uchiyama (National Center for Child Health and Development)
Ryuichi Morishita (Department of Clinical Gene Therapy, Osaka University)

S5-1 **"Plasmid DNA-based Gene Therapy: From Collategene to DNA Vaccine"** 55
Ryuichi Morishita *(Department of Clinical Gene Therapy, Osaka University)*

S5-2 **"Oncolytic Virus Therapy as Immunotherapy"** 57
Takashi Kojima *(Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East)*

S5-3 **"Clinical application of chimeric antigen receptor T-cell therapy for leukemia"** 59
Motohiro Kato *(Department of Pediatrics, The University of Tokyo)*

S5-4 **"Clinical experiences of Onasemnogene abeparvovec treatment and worldwide clinical trials of gene therapy for child neurological diseases"** 61
Takanori Yamagata *(Department of Pediatrics, Jichi Medical University)*

16:00-17:20 **Symposium 6: Cancer**
Chairpersons: Yasutomo Nasu (Okayama University)
Hiroshi Fukuhara (Department of Urology, Kyorin University School of Medicine)

S6-1 **"Research and Development, Nonclinical Studies and First-In-Human and Phase I/II Clinical Trials of Conditionally Replicating Adenovirus Targeting and Treating with Multiple Factors (m-CRA) for Next-Generation Oncolytic Virus Immunotherapy"** 65
Ken-ichiro Kosai *(Department of Gene Therapy and Regenerative Medicine / South Kyushu Center for Innovative Medical Research and Application / Center for Innovative Therapy Research and Application, Kagoshima University Graduate School of Medicine and Dental Sciences / Center for Clinical and Translational Research, Kagoshima University Hospital)*

S6-2 **"Phase 1 clinical trial of a third-generation oncolytic HSV-1, G47Δ in patients with castration-resistant prostate cancer"** 67
Hiroshi Fukuhara *(Department of Urology, Kyorin University School of Medicine)*

S6-3 **"CORE-001: Phase 2, Single Arm Study of CG0070 Combined with Pembrolizumab in Patients with Non Muscle Invasive Bladder Cancer (NMIBC) Unresponsive to Bacillus Calmette-Guerin (BCG)"** 69
James Burke *(CG Oncology, USA)*

S6-4 **"Efforts to address the challenges in TCR/CAR gene therapy for cancer"** 71
Junichi Mineno *(Takara Bio Inc.)*

17:30-18:20 **Evening Seminar2** Co-sponsored by AnGes, Inc.
Chairperson: Toya Ohashi (The Jikei University School of Nursing)

ES2 **"Present Status and Future Prospect of Novel Therapeutic Approach for Rare Genetic Diseases"**
Torayuki Okuyama *(Department of Clinical Laboratory Medicine, Center for Lysosomal Storage Diseases, National Center for Child Health and Development)*

18:30-19:00 **Plenary session 2**
Chairpersons: Masatoshi Tagawa (Graduate School of Medicine, Chiba University)
Koichi Miyake (Nippon Medical School)

PS2-1 **"Fusogenic oncolytic vaccinia virus enhances systemic antitumor immune response and sensitivity to immune checkpoint blockade by remodeling the tumor microenvironment"** 81
Motomu Nakatake *(Department of Molecular Medicine, Graduate School of Medical Sciences, Tottori University)*

PS2-2 **"Hematopoietic Stem Cell Gene Therapy Corrects Lysosomal Storage in CNS in Murine Model of GM1-gangliosidosis"** 82
Toshiki Tsunogai *(Department of Pediatrics / Division of Gene Therapy, Research Center for Medical Science, Jikei University School of Medicine)*

PS2-3 **"Sustained fetal hemoglobin induction allowed by BCL11A interference with a truncated erythropoietin receptor"** 83

Naoya Uchida *(Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo / Cellular and Molecular Therapeutics Branch, National Heart Lung and Blood Institutes (NHLBI)/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), USA)*

19:00-19:10 **Closing Remarks: President Torayuki Okuyama**

(Department of Clinical Laboratory Medicine, Center for Lysosomal Storage Diseases,National Center for Child Health and Development)

Day 2: Friday, September 10
Room 2

12:00-12:50 **Luncheon Seminar 4** Co-sponsored by Nihon Pall Ltd.

Chairperson: Hariganeya Naohito (Nihon Pall Ltd.)

LS2-1 **"Practical use of single-use bioreactor technology in gene therapy – from Process development to Commercial manufacturing"**

Kenichi Horiuchi *(Nihon Pall Ltd.)*

LS2-2 "**Scale-up of AAV production using ELEVECTA, the fully stable AAV production platform"**

Juliana Coronel *(CEVEC Pharmaceuticals GmbH)*

Web Conference

September 27 (Mon) – October 15 (Fri) until 15:00

Session A: Basic science and technologies

Session B: Vector development and gene transfer technologies

Session C: Preclinical studies for gene and cell therapy (include studies for molecular pathogenesis)

Session D: Clinical studies for gene and cell therapy

JSGCT Chairman's Lecture

Abstract & Curriculum Vitae

Chairperson: Toya Ohashi

CURRICULUM VITAE

Name Tomoki Todo, M.D., Ph.D.

Address (Affiliation) Division of Innovative Cancer Therapy and Department of Surgical Neuro-Oncology
The Institute of Medical Science, The University of Tokyo
4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639 Japan

Field of Research Neurosurgery, Neuro-oncology, Oncolytic virus therapy, Translational research

Education

1985 M.D. The University of Tokyo, Faculty of Medicine
1994 Ph.D. Medical Science The University of Tokyo, Graduate School of Medicine

Professional Experience

1985 Resident and clinical associate, Department of Neurosurgery, University of Tokyo
1989 Staff Neurosurgeon, Department of Neurosurgery, International Medical Center of Japan, Tokyo
1990 Research fellow, Dept. of Neurosurgery, University of Erlangen-Nürnberg, Erlangen, Germany
1992 Staff Neurosurgeon (resumed), Department of Neurosurgery, International Medical Center of Japan
1995 Instructor, Department of Neurosurgery, University of Tokyo
1995 Research fellow, Department of Neurosurgery, Georgetown University Medical Center, Washington, DC, USA
1998 Research Assistant Professor, Department of Neurosurgery, Georgetown University Medical Center
2000 Assistant Professor of Neurosurgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
2003 Senior Assistant Professor of Neurosurgery, Graduate School of Medicine, The University of Tokyo
2008 Project Professor, Translational Research Center, The University of Tokyo
2011 Professor and Chairman, Division of Innovative Cancer Therapy, and Department of Surgical Neuro-Oncology, The Institute of Medical Science, The University of Tokyo, Japan

Recent Related Publications (5 Papers)

1. Uchihashi T, Nakahara H, Fukuhara H, Iwai M, Ito H, Sugauchi A, Tanaka M, Kogo M, Todo T: Oncolytic herpes virus G47Δ injected into tongue cancer swiftly traffics in lymphatics and suppresses metastasis. ***Mol Ther Oncolytics*** (in press).
2. Fukuhara H, Takeshima Y, Todo T: Triple-mutated oncolytic herpes virus for treating both fast- and slow-growing tumors. ***Cancer Sci*** [Epub 2021 May 25. doi: 10.1111/cas.14981]
3. Sugawara K, Iwai M, Ito H, Tanaka M, Seto Y, Todo T: Oncolytic herpes virus G47Δ works synergistically with CTLA-4 inhibition through dynamic intratumoral immune modulation. ***Mol Ther Oncolytics*** (in press).
4. Sugawara K, Iwai M, Yajima S, Tanaka M, Yanagihara K, Seto Y, Todo T: Efficacy of a third-generation oncolytic herpes virus G47Δ in advanced stage models of human gastric cancer. ***Mol Ther Oncolytics*** 17: 205-215, 2020. [DOI:10.1016/j.omto.2020.03.022]
5. Yamada T, Tateishi R, Iwai M, Koike K, Todo T: Neoadjuvant use of oncolytic herpes virus G47Δ enhances the antitumor efficacy of radiofrequency ablation. ***Mol Ther Oncolytics*** 18: 535-545, 2020. [DOI:10.1016/j.omto.2020.08.010]

Recent progress of oncolytic virus therapy using HSV-1

Tomoki Todo

Division of Innovative Cancer Therapy, The Institute of Medical Science, The University of Tokyo

Oncolytic virus therapy is the next major breakthrough in cancer treatment following the success in immunotherapy using immune checkpoint inhibitors. Oncolytic viruses are defined as genetically engineered or naturally occurring viruses that selectively replicate in and kill cancer cells without harming the normal tissues. In reality, in order to completely abolish the replication capability of the virus in normal cells while retaining its high capability to replicate in cancer cells, one needs to 'design' the viral genome based on elucidated viral gene functions and construct a man-made virus carefully and cleverly. T-Vec (talimogene laherparepvec), a second-generation oncolytic herpes simplex virus type 1 (HSV-1) armed with GM-CSF, was approved for progressive melanoma in 2015-2016 as the first oncolytic virus drug in the United States and Europe. In Japan, we have been developing a triple-mutated, third-generation oncolytic HSV-1, G47Δ (teserpaturev). The clinical development of G47Δ was initiated solely by academia and recently succeeded in crossing the so-called death valley of new drug development. The interim analysis of the investigator-initiated phase II trial for recurrent glioblastoma showed that the 1-year survival rate was tremendously higher than the set control value based on meta-analysis of historical data. The trial was terminated early due to high efficacy and 19 patients in total were registered. It was considered the pivotal study, and G47Δ received a conditional approval as a new drug for malignant glioma in Japan in June 2021. An investigator-initiated, phase I/II trial of G47Δ-based oncolytic HSV-1 armed with interleukin-12 (T-hIL12) in patients with malignant melanoma is in progress. Whereas numerous oncolytic viruses have been subjected to clinical trials in the world, the common feature playing a major role in prolonging the survival of cancer patients is an induction of specific antitumor immunity in the course of tumor specific viral replication. Oncolytic virus therapy is expected to become a standard therapeutic option for all cancer patients in the near future.

ESGCT & ASGCT Special Lecture

Abstract & Curriculum Vitae

ESGCT Special Lecture

Chairperson: Yoshikatsu Eto

ASGCT Special Lecture

Chairpersons: Tomoki Todo & Noriyuki Kasahara

CURRICULUM VITAE

Name Hildegard Büning, Ph.D.

Address (Affiliation) Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany

Field of Research Adeno-associated virus (AAV) vector development, in vivo Gene Therapy

Education

2015-Present	Professor for Infection Biology & Gene Transfer at Hannover Medical School
2015-Present	Head of the Laboratory for Infection Biology & Gene Transfer at the Institute of Experimental Hematology (Hannover Medical School)
2012-Present	Member of the German Center for Infection Research (DZIF)
2008	***venia legendi*** for "Molecular Medicine" (Faculty of Medicine, University of Cologne)
2004-2015	Head of the Laboratory for AAV Vector Development at the Clinic I of Internal Medicine and Center for Molecular Medicine Cologne (ZMMK, University of Cologne)
1997-2003	Post-doctoral Fellow at the Gene Center (Ludwig-Maximilians-University (LMU) Munich)
1997	Ph.D. (Dr. rer. nat.) (Faculty of Chemistry and Pharmacy, LMU Munich)
1993-1997	Dissertation at the Institute of Biochemistry (LMU Munich)
-1993	Diploma in Biology (Faculty of Biology, LMU Munich)
1988-1993	Studies in Biology (University of Münster and LMU Munich)

Professional Experience

2021-Present	Member of Board, American Society of Gene and Cell Therapy (ASGCT)
2019-Present	President, Commission "Gene and/or Cell Therapy of Rare Disorders" of the Association Francaise contre les Myopathies (AFM-Telethon)
2019-Present	Member, Permanent Committee of the Scientific Council of AFM
2019-Present	European Editor, Human Gene Therapy
2019-2021	Chair, Infectious Diseases and Vaccines Committee of the American Society for Gene and Cell Therapy (ASGCT)
2018-Present	President, European Society of Gene and Cell Therapy (ESGCT)
2016-2018	Vice-President, ESGCT
2014-2019	Editor, Human Gene Therapy Methods
2014-Present	Scientific Secretary, German Society for Gene Therapy (DG-GT e.V.)
2013-Present	Scientific Advisory Board, Paul-Ehrlich-Institute, Langen, Germany
2013-2015	Deputy Coordinator, DZIF Academy, partner site Bonn-Cologne
2013-2015	Co-coordinator, Interdisciplinary Program Molecular Medicine (IPMM) of the University of Cologne
2010-2014	President, DG-GT e.V.
2009-2013	Co-coordinator, SPP 1230 – Mechanisms of Gene Vector Entry and Persistence
2008-2010	Scientific Secretary, DG-GT e.V.

Recent Related Publications (5 Papers)

1. Pavlou M.* and Schön C.*, L. M. Occelli, A. Rossi, N. Meumann, R. F. Boyd, J. T. Bartoe, J. Siedlecki, M. J. Gerhardt, S. Babutzka, J. Bogedein, J. E. Wagner, S. G Priglinger, M. Biel, S. M. Petersen-Jones, H. Büning# and S. Michalakis#. Novel AAV capsids for intravitreal gene therapy of photoreceptor disorders. ***EMBO Mol. Med.*** (2021); online ahead of print. (*equal contribution; #co-senior authors)
2. Krooss S.A.* and Dai Z.*, F. Schmidt, A. Rovai, J. Fakhiri, A. Dhingra, Q. Yuan, T. Yang, A. Balakrishnan, L. Steinbrück, S. Srivaratharajan, M.P. Manns, A. Schambach, D. Grimm, J. Bohne, A.D. Sharma, H. Büning# and M. Ott#. Ex vivo/in vivo gene editing in hepatocytes using "All-in-One" CRISPR-Adeno-associated virus vectors with a self-linearizing repair template. ***iScience*** (2020): 23: 100764. (*equal contribution, # equal contribution)
3. Hagedorn C.* and Schnödt-Fuchs M.*, P. Boehme, H. Abdelrazik, H.J. Lipps, H. Büning. S/MAR Element facilitates episomal long-term persistence of adeno-associated virus vector genomes in proliferating cells. ***Hum. Gene Ther.*** (2017): 28: 1169-1179. (*equal contribution)
4. Hösel M., A. Huber, S. Bohlen, J. Lucifora, G. Ronzitti, F. Puzzo, F. Boisgerault, U.T. Hacker, W.J. Kwanten, N. Klöting, M. Blüher, A. Gluschko, M. Schramm, O. Utermöhlen, W. Bloch, F. Mingozzi* and O. Krut* and H. Büning*. Autophagy determines efficiency of liver-directed gene therapy with adeno-associated viral vectors. ***Hepatology*** (2017): 66: 252-265. (*equal contribution)
5. Münch R.C.* and Muth A.*, T. Friedel, J. Schmatz, B. Dreier, A. Trkola, A. Plückthun, H. Büning# and C. Buchholz#. Off-target-free gene delivery by affinity-purified receptor-targeted viral vectors. ***Nat. Commun.*** (2015): 6: 6246 (*equal contribution, # equal contribution)

Capsid-engineered Adeno-Associated Virus (AAV) vectors for in vivo Gene Therapy

Hildegard Büning [1,2]

[1] Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany
[2] REBIRTH Center for Translational Regenerative Medicine, Hannover Medical School, Hannover, Germany

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Vectors based on the Adeno-Associated Virus (AAV) are the most widely used delivery systems for in vivo gene therapy. Already three AAV vector-based gene therapies have received market authorization and more are expected soon. Despite this success, prevalence of pre-existing neutralizing antibodies in the human population and the need for high vector doses to obtain therapeutic relevant transduction in the actual target cell population are challenges that need to be addressed to improve efficacy and safety of AAV vector-based gene therapies. As the viral capsid is not only the target of antibodies but also mediates pre- and post entry steps in cell transduction, capsid engineering appears as suitable strategy in this regard. Rational design as well as library-based capsid engineering strategies are followed with great success. Here, data will be presented on novel AAV capsid variants with improved efficacy of cell transduction and with re-directed tropism. Finally, the impact of such virions in deciphering host-AAV interactions as well as the possible impact of capsid-engineering on host immune responses will be discussed.

CURRICULUM VITAE

Name Stephen J. Russell, M.B.Ch.B., Ph.D., FRCP (UK), FRCPath (UK), LLD (Hon).
Stephen J. Russell, M.D., Ph.D., Founder, Director, CEO & President

Dr. Russell is a Professor of Molecular Medicine, Consultant Hematologist at Mayo Clinic Rochester and serves on the Board of Trustees of Buena Vista University, Iowa. He obtained his MD from the University of Edinburgh, Scotland and his PhD from the University of London, England. He trained at University College Hospital and the Royal Marsden Hospital, London, then moved to Cambridge, England where he led a research team in the MRC Laboratory for Molecular Biology and was a practicing consultant hematologist at Addenbrooke's Hospital. In 1998 he moved to Mayo Clinic where he built a comprehensive translational gene and virus therapy program, established the Department of Molecular Medicine, developed innovative technologies for targeting viruses to cancer cells, and orchestrated the first-in-human testing of oncolytic Measles and Vesicular stomatitis viruses. In addition to his academic faculty role, he is currently serving as CEO of Vyriad, an oncolytic virotherapy company.

His research is focused on developing engineered viruses for the treatment of disseminated malignancies and demonstrating their efficacy in clinical trials. Research themes in his laboratory include the engineering of viral membrane glycoproteins, targeting cell to cell fusion, targeting virus entry, using cellular microRNAs to reprogram virus tropism, modulating and exploiting virus-tumor-immune system interactions, pharmacokinetic and pharmacodynamic studies of virus-based therapies, noninvasive monitoring and imaging of viral gene expression in vivo, and radiovirotherapy.

Intravenous oncolytic virotherapy using Voyager-V1 (VSV-IFNβ-NIS)

Stephen J. Russell

Immediate Past President of ASGCT / Mayo Clinic and Vyriad Rochester, Minnesota, USA

Oncolytic viruses (OV) are promising tumor targeted agents that can mediate in situ tumor cell killing, thereby inflaming the tumor microenvironment and amplifying the host antitumor response. Intratumoral (IT) herpes simplex virus (HSV) based OVs have been approved for metastatic melanoma, and recently in Japan for glioma therapy but not yet for other common metastatic cancers where IT therapy cannot efficiently stimulate abscopal antitumor immune responses to impact uninjected tumors.

Systemic OV therapy can potentially overcome the limitations of IT therapy but is a more challenging approach since the viruses need to survive in the bloodstream, extravasate from tumor neovessels and propagate efficiently in the tumor. Unsurprisingly, both in rodent tumor models and in patients with metastatic cancer, intravenous delivery and subsequent intratumoral OV spread is highly heterogeneous. Systemic delivery is impacted by (i) serum titers of neutralizing antibodies and/or complement, (ii) virus sequestration by professional phagocytic cells in the liver and spleen, and (iii) variable extravasation of viruses from tumor neovessels with different sized fenestrations. Intratumoral spread is primarily impacted by innate antiviral defence mechanisms in the tumor cells themselves which vary greatly both between different cancer histologies and between different patients with a given cancer histology.

Voyager-V1 is a recombinant vesicular stomatitis virus encoding both interferon-beta and the thyroidal sodium iodide symporter (NIS). The virus encoded IFNβ enhances its proinflammatory activity and serves as a quantifiable soluble reporter that reflects the number of virus infected cells in the body. NIS serves as an imagable reporter used for in-life localization of virus infected cells. Preclinical imaging studies with Voyager-V1 helped us to elucidate its mechanism of action, wherein intravenously administered viruses seed into tumor deposits, nucleating multiple radially expanding infectious centers which (if they expand sufficiently) eventually coalesce to mediate inflammatory tumor destruction. A mathematical algorithm wasvdeveloped based on these studies has subsequently been used to stress-test and better understand the relationship between virus dose, tumor response (or nonresponse) and potential toxicities of Voyager-V1 (eg. an unusual form of tumor lysis syndrome) arising in different mouse models.
Additional insights into the mechanisms and heterogeneity of tumor responses in human subjects receiving intravenous OV therapy have recently been gained through the analysis of serum interferon beta profiles and peak interferon levels from over 100 cancer patients who received a single intravenous infusion of Voyager-V1. Multiple tumor histologies and stages of tumor progression are represented and the impact of interpatient and intertumor heterogeneity is very well π, providing valuable insights for the continued development of effective systemic OV therapy. These data and additional relevant preclinical studies will be presented.

CURRICULUM VITAE

Name Beverly L. Davidson, Ph.D.

Address (Affiliation) The Raymond G. Perelman Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia,
3501 Civic Center Boulevard, 5060 Colket Translational Research Building, USA

Areas of Research Interest

Research in my laboratory is focused on inherited genetic diseases that cause central nervous system dysfunction, with a focus on (1) recessive, childhood onset neurodegenerative disease, in particular the lysosomal storage diseases such as the mucopolysaccharidoses and Battens disease; and (2) dominant genetic diseases for example the CAG repeat disorders, Huntington's disease and spinal cerebellar ataxia, and (3), understanding how noncoding RNAs participate in neural development and neurodegenerative diseases processes.

Our research on childhood onset neurodegenerative diseases is focused on experiments to better understand the biochemistry and cell biology of proteins deficient in these disorders, and to develop gene and small molecule based medicines for therapy. In recent work we demonstrated that the application of recombinant viral vectors to animal models of storage disease reversed CNS deficits.

Therapies for dominant disorders are an exciting challenge and require that the dominant disease allele be silenced. To approach this, we developed reagents for expressing inhibitory RNA in vivo. This approach improved disease phenotypes in relevant models of dominantly inherited human neurodegenerative diseases.

Finally, we investigate how naturally occurring noncoding RNAs, miRNAs, participate in cell fate decisions in normal development, and how their expression is altered in disease states. We find that miRNAs with altered expression in Huntington's disease or spinocerebellar ataxia brains target proteins that themselves contribute to disease phenotypes. This work may reveal new targets for drug therapy. We are also developing the tools to understand how miRNAs may participate in brain development using the mouse as a model organism.

Appointments

2001-2014; adjunct 2014 Professor, Internal Medicine, Phys & Biophysics, Neurology, U of I, --
2001-2004 Co-Director, Iowa Biosciences Advantage Program, U of I
2004-2014 Vice Chair for Research, Internal Medicine, UI
2014- Professor, Pathology and Laboratory Medicine, Perelman School of Medicine of the University of Pennsylvania
2014- Director, Raymond G. Perelman Center for Cellular and Molecular Therapeutics, Children's Hospital of Philadelphia
2015- Professor of Genetics, Perelman School of Medicine of the University of Pennsylvania-
2016- Chief Scientific Strategy Officer, Children's Hospital of Philadelphia Research Institute-

Honors and Awards:

2013 Chair, Medical Sciences, AAAS
2013 Member, External Scientific Panel, NIH Common Fund
2013 University of Iowa, Inventor Award
2014- Arthur V Meigs Chair in Pediatrics, CHOP
2015 The Leslie Gehry Brenner Prize for Innovation in Science
2017 Member, American Academy of Arts and Sciences
2018 F.E. Bennett Memorial Lectureship Award, American Neurological Association
2018 Member, Electorate Nominating Committee for Section V (Neuroscience), AAAS
2019 Chair, Gordon Research Conference on Lysosomal Storage Disease
2019 Frontiers in Neuroscience Plenary Session presenter, AAN
2019 Member, National Academy of Medicine
2020-2021 President-Elect, American Society for Cell and Gene Therapy (President; 2021-2022)

Regulated control of gene therapies by drug-induced splicing

Beverly L. Davidson

President of ASGCT
Perelman School of Medicine of the University of Pennsylvania / Children's Hospital of Philadelphia, USA

So far, gene therapies have relied on complex constructs that cannot be finely controlled[1,2]. Here we report a universal switch element that enables precise control of gene replacement or gene editing after exposure to a small molecule. The small-molecule inducers are currently in human use, are orally bioavailable when given to animals or humans and can reach both peripheral tissues and the brain. Moreover, the switch system, which we denote X^{on}, does not require the co-expression of any regulatory proteins. Using X^{on}, the translation of the desired elements for controlled gene replacement or gene editing machinery occurs after a single oral dose of the inducer, and the robustness of expression can be controlled by the drug dose, protein stability and redosing. The ability of X^{on} to provide temporal control of protein expression can be adapted for cell-biology applications and animal studies. Additionally, owing to the oral bioavailability and safety of the drugs used, the X^{on} switch system provides an unprecedented opportunity to refine and tailor the application of gene therapies in humans.

References
1. Yen, L. et al. Exogenous control of mammalian gene expression through modulation of RNA self-cleavage. Nature 431, 471–476 (2004).
2. Dow, L. E. et al. Inducible in vivo genome editing with CRISPR–Cas9. Nat. Biotechnol. 33, 390–394 (2015).

Symposium 1

Abstract & Curriculum Vitae

Genetic Disorders

Chairpersons: Hiroshi Kobayashi & Makoto Otsu

CURRICULUM VITAE

Name Toru Uchiyama, M.D., Ph.D.

Address (Affiliation) Division of Molecular Pathogenesis, Department of Human Genetics, National Center for Child Health and Development
2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

Field of Research Gene Therapy, Pediatric Hematology/Oncology, Immunology

Education

1993-1999	M.D.	Medicine	Niigata University, School of Medicine
2002-2005	Ph.D.	Pediatrics	Tohoku University, Graduate School of Medicine

Professional Experience

1999-2000	Resident in Pediatrics, Niigata University School of Medicine, Japan
2001-2002	Medical staff in Pediatrics, Niigata Prefectural Central Hospital, Japan
2007-2010	Research Fellow in National Institutes of Health (NIH), USA
2011-2012	Assistant Professor in Pediatrics, Tohoku University School of Medicine, Japan
2013-Present	Chief, Department of Human Genetics, National Center for Child Health and Developemt (NCCHD), Japan
2020-Present	Director, Research and Development, Gene and Cell Therapy Promotion Center, NCCHD

Recent Related Publications (5 Papers)

1. **Uchiyama T**, Kawakami S, Masuda H, Yoshida K, Niizeki H, Mochizuki E, Edasawa K, Ishiguro A, Onodera M. A Distinct Feature of T Cell Subpopulations in a Patient with CHARGE Syndrome and Omenn Syndrome. ***J Clin Immunol*** 41: 233-237, 2021. doi: 10.1007/s10875-020-00875-7.
2. Ishikawa T, Okai M, Mochizuki E, Uchiyama T, Onodera M, Kawai T. BCG infections at high frequency in both AR-CGD and X-CGD patients following BCG vaccination. Clin Infect Dis 2020. doi: 10.1093/cid/ciaa1049.
3. Kanamaru Y, Uchiyama T, Kaname T, Yanagi K, Ohara O, Kunishima S, Ishiguro A. ETV-6-related thrombocytopenia associated with a transient decrease in von Willebrand factor. Int J Hematol. 2021. doi: 10.1007/s12185-021-03136-4.
4. Igarashi Y, Uchiyama T, Minegishi T, Takahashi S, Watanabe N, Kawai T, Yamada M, Ariga T, Onodera M. Single cell-based vector tracing in patients with ADA-SCID treated with stem cell gene therapy. ***Mol Ther Methods Clin Dev*** 6: 8-16, 2017.
5. Ikawa Y, Uchiyama T, Jagadeesh GJ, *Candotti F. The long terminal repeat negative control region is a critical element for insertion oncogenesis after gene transfer into hematopoietic progenys with Moloney murine leukemia viral vectors. ***Gene therapy***. 2016; 23(11):815-818

Progress of gene therapy for primary immunodeficiency diseases

Toru Uchiyama

Division of Molecular Pathogenesis, Department of Human Genetics, National Center for Child Health and Development

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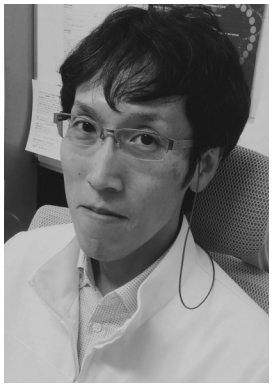
Transfer of therapeutic gene into hematopoietic stem cells reconstitutes the functional immune system in the same manner as allogeneic HSCs in patients with primary immunodeficiency diseases (PIDs). A series of retroviral vector-mediated gene therapy trials confirmed this expectation, and most patients recovered from refractory infections. Whereas the use of retrovirus revealed the fundamental issue of vector integration, including the insertional mutagenesis, a lentiviral vector with a deletion of enhancer element has shown the improvements in the clinical manifestations without overexpression of proto-oncogene. The safety and efficacy feature now allows lentiviral gene therapy to be close to approval for PIDs. Gene editing using engineered nucleases shows the potentials of targeted endogenous gene modifications, which could provide significant benefits to some PIDs. Direct modification of the mutant allele could address PIDs with dominant-negative mutations. Moreover, it should also be noted that the transcription by the endogenous enhancer/promoter element on the chromosome enables a complete physiological expression of the target gene. These features allow genome editing to be potentially applicable to diseases which the gene addition strategy using viral vectors could not treat. Recently, clinical trials of CRISPR-based cell therapy have been launched for blood disorders, such as sickle cell diseases and beta thalassemia, and their clinical efficacy also implies the promising results of this new strategy for primary immunodeficiencies.

CURRICULUM VITAE



Name Kazuhiro Muramatsu, M.D., Ph.D.

Address (Affiliation) Department of Pediatrics, Jichi Medical University,
3311-1 Yakushiji, Shimotsuke-city, Tochigi, 329-0498 Japan

Field of Research Pediatric Neurology, Autophagy, Neuroscience,

Education

1998	M.D.	Gunma University School of Medicine, Gunma
2008	Ph.D.	Gunma University Graduate School of Medicine, Gunma

Professional Experience

1998-2003	Dept. of Pediatrics and Neonatology, Japanese Red Cross Medical Center
2003-2004	Dept. of Pediatrics, Gunma University Hospital
2005-2008	Japan Society for Promotion of Science Research Fellowship for Young Scientists
2008-2009	Dept. of Pediatrics, Gunma University Hospital
2009-2016	Assistant Professor, Gunma University Graduate School of Medicine, Pediatrics
2011-2013	Dept. of Neurology, Philipps University Marburg, Germany
2016-2017	Lecture, Gunma University Hospital, Pediatrics
2017-Present	Associate Professor, Jichi Medical University, Pediatrics

Recent Related Publications (5 Papers)

1. Kurokawa Y, Osaka H, Kouga T, Jimbo E, Muramatsu K, Nakamura S, Takayanagi Y, Onaka T, Muramatsu SI, Yamagata T. Gene Therapy in a Mouse Model of Niemann-Pick Disease Type C1. ***Hum Gene Ther.*** 2021 Feb 22. doi: 10.1089/hum.2020.175. PMID: 33256498.

2. Kidokoro H, Yamamoto H, Kubota T, Motobayashi M, Miyamoto Y, Nakata T, Takano K, Shiba N, Okai Y, Tanaka M, Sakaguchi Y, Maki Y, Kawaguchi M, Suzuki T, Muramatsu K, Natsume J. High-amplitude fast activity in EEG: An early diagnostic marker in children with beta-propeller protein-associated neurodegeneration (BPAN). ***Clinical Neurophysiology.*** 2020; 131(9):2100-2104. DOI: https://doi.org/10.1016/j.clinph.2020.06.006

Development of gene therapy targeting liver for congenital metabolic disorder based on novel AAV vector

Kazuhiro Muramatsu[1,2], Shin-ichi Muramatsu[2,3], Eriko Jinbo[1], Hitoshi Osaka[1],Takanori Yamagata[1]

[1] Department of Pediatrics, Jichi Medical University
[2] Division of Neurological Gene Therapy, Jichi Medical University
[3] Center for Gene & Cell Therapy, The Institute of Medical Science, The University of Tokyo

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The development of gene therapy for intractable diseases has progressed worldwide, and adeno-associated virus (AAV) vector has been used due to their safety and efficiency. We have developed gene therapy for AADC deficiency and demonstrated its safety and efficacy (Kojima K, et al. Brain 2019;142:322-333). Gene therapy is also under development for GLUT1 deficiency (Nakamura S, et al. Gene Ther. 2021; 28:329-338.) and Niemann-Pick disease type C (Kurokawa Y, et al. Hum Gene Ther. 2021; 32:589-598).
Otherwise, inborn errors of metabolism are often caused by the enzyme expressing in the liver. Only few of them are curable such as enzyme replacement therapy. There are many incurable diseases for which development of new curative treatment methods is desired. Conventional vector AAV8 are not efficient for gene transfer to the liver, making therapeutic application difficult.

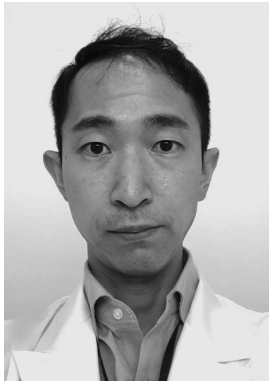
In order to establish a therapeutic vector for the liver, a newly developed AAV vector (AAV.GT5) (Ito M, et al. Sci Rep 2021;11:9322) were verified for culture cells, primary cultured human hepatic stellate cell, and animal models. Then, we focused on ornithine transcarbamylase (OTC) deficiency, which causes hyperammonemia in infancy. At present, living-donor liver transplantation is the only curative treatment for OTC deficiency. Medication and reduced protein diet for a lifetime cannot avoid central nervous system sequelae. Therefore, the development of a curative therapy is urgent. If gene therapy by intravascular administration is established, it can be a non-invasive curative treatment.

We confirmed the introduction efficiency of AAV.GT5 was 75 times higher than that of AAV8 in the cultured cell system and 40-fold higher than that of AAV8 in primary cultured human hepatic stellate cell and proved a clear superiority of AAVGT5 over AAV8. In the United States, Ultragenyx Pharmaceutical Inc. has started clinical trials of gene therapy for OTC deficiency using AAV8 vector, but the AAV.GT5 has overwhelmingly superior gene transfer efficiency to human hepatocytes than AAV8. Next, we verified the enzymatic activity of OTC, which also showed improvement.

The AAV.GT5-OTC and AAV.GT5-GFP were administered to PXB-OTC mice transplanted with hepatocytes from human OTC patients as an animal model of OTC deficiency. Data such as decrease in ammonia, improvement in liver function, and increase in blood glucose were obtained after vector administration.

AAV.GT5 appears highly potential vector for treatment of congenital metabolic disorders. Achieve a non-invasive radical treatment for OTC deficiency and improve central nervous system prognosis as a guideline first choice.

CURRICULUM VITAE



Name Yohta Shimada, Ph.D.

Address (Affiliation) Research Associate, Division of Gene Therapy, Reserch Center for Medical Sciences, The Jikei University School of Medicine
3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan.

Field of Research Gene therapy, Lysosomal storage disease, Biochemisrty

Education

2004 B.S. Faculty of Science, Toho University
2006 M.S. Graduate School of Science, Toho University
2009 Ph.D. The Jikei University Graduate School of Medicine

Professional Experience

2009-Present Research associate, Division of Gene Therapy, Research Center for Medical Sciences, The Jikei University School of Medicine

Recent Related Publications (5 Papers)

1. Wada M, Shimada Y, Iizuka S, et al. Ex Vivo Gene Therapy Treats Bone Complications of Mucopolysaccharidosis Type II Mouse Models through Bone Remodeling Reactivation. ***Mol Ther Methods Clin Dev.*** 2020; 19:261-274.
2. Miwa S, Watabe AM, Shimada Y, et al. Efficient engraftment of genetically modified cells is necessary to ameliorate central nervous system involvement of murine model of mucopolysaccharidosis type II by hematopoietic stem cell targeted gene therapy. ***Mol Genet Metab.*** 2020;130(4):262-273.
3. Shimada Y, Wakabayashi T, Akiyama K,et al. A method for measuring disease-specific iduronic acid from the non-reducing end of glycosaminoglycan in mucopolysaccharidosis type II mice. ***Mol Genet Metab***. 2016;117(2):140-3.
4. Wakabayashi T, Shimada Y, Akiyama K, et al. Hematopoietic Stem Cell Gene Therapy Corrects Neuropathic Phenotype in Murine Model of Mucopolysaccharidosis Type II. Hum Gene Ther. 2015;26(6):357-66.
5. Akiyama K, Shimada Y, Higuchi T, et al. Enzyme augmentation therapy enhances the therapeutic efficacy of bone marrow transplantation in mucopolysaccharidosis type II mice. ***Mol Genet Metab***. 2014;111(2):139-46.

Hematopoietic stem cell gene therapy for mucopolysaccharidosis type II

Yohta Shimada[1], Takashi Higuchi[1], Saki Matsushima[1], Yumi Kanegae[2], Toya Ohashi[1,3], Hiroshi Kobayashi[1]

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[3] The Jikei University School of Nursing

Mucopolysaccharidosis type II (MPS II) is an X-linked recessive lysosomal storage disease (LSD) caused by a deficiency of iduronate-2-sulfatase (IDS), leading to progressive accumulation of glycosaminoglycans (GAG) subsequently various symptoms such as hepatosplenomegaly and central nervous system (CNS) involvement. Enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT) are available for patients with MPS II and effective for peripheral tissues in them. In particular, HSCT is also expected to have therapeutic effect to CNS involvement, because HSCT bring a clinical benefit to patients with several neuropathic LSDs. However, efficacy of HSCT for CNS in patients with MPS II is controversial and therapy for CNS involvement is still ongoing challenge in MPS II.

We previously demonstrated that neither ERT nor HSCT improve the GAG accumulation in brain from murine model of MPS II. To enhance the efficacy of HSCT for MPS II mice, we performed ex vivo gene therapy to HSC of disease mice using lentiviral vector. Ex vivo HSC gene therapy decreased the level of accumulated GAG in brain as well as peripheral tissues in MPS II mice. Amelioration of neuronal functions was also observed in MPS II mice treated with ex vivo gene therapy. To further analysis of potential clinical application, we developed an immunodeficient mouse model with MPS II. We found that lentiviral gene transduced human CD34+ cells improve GAG accumulation in several tissues of xenograft model of MPS II mice. These results indicate that ex vivo HSC gene therapy is a promising approach for treatment of MPS II. We are currently trying to establish a production process of gene-modified human CD34+ cells using lentiviral vector toward clinical application.

CURRICULUM VITAE

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Field of Research Thrombosis and Haemostasis, Gene therapy, Molecular Biology

Education

1994	M.D.	Jichi Medical University
2003	Ph.D.,	Yamanashi Medical University

Professional Experience

1994-1995	Resident in the Department of Internal Medicine, Yamanashi Prefectural Hospital
1996-1998	Department of Internal Medicine, Nanbu Clinic, Yamanashi, Japan
1999	Research Fellow, Department of Laboratory Medicine, Yamanashi Medical University
2000-2003	Department of Internal Medicine, Minobusan Hospital, Yamanashi, Japan
2004-2007	Research Associate, Research Division of Cell and Molecular Medicine, Center for Molecular Medicine, Jichi Medical University
2007-2015	Assistant Professor, Research Division of Cell and Molecular Medicine, Center for Molecular Medicine, Jichi Medical University
2015-2016	Associate Professor, Department of Biochemistry, Jichi Medical University
2017-Present	Professor, Department of Biochemistry, Jichi Medical University

Recent Related Publications (5 Papers)

1. Baatartsogt N, Kashiwakura Y, Hayakawa M, Kamoshita N, Hiramoto T, Mizukami H, and Ohmori T. Development of a sensitive and reproducible cell-based assay using secNanoLuc to detect neutralizing antibody against adeno-associated virus vector capsid. ***Mol Ther Methods Clin Dev***. 2021 (in press).
2. Byambaa S, Uosaki H, Ohmori T, Hara H, Endo H, Nureki O, Hanazono Y. Non-viral ex vivo genome-editing in mouse bona fide hematopoietic stem cells with CRISPR/Cas9. ***Mol Ther Methods Clin Dev***. 2021;20:451-462.
3. Reiss UM, Zhang L, Ohmori T. Hemophilia gene therapy – New country initiatives. ***Haemophilia*** 2021 Suppl 3:132-141.
4. Ohto-Ozaki H, Hayakawa M, Kamoshita N, Maruyama T, Tominaga S-I, Ohmori T. Induction of IκBζ augments cytokine and chemokine production by IL-33 in mast cells. ***J Immunol***. 2020 Apr 15;204(8):2033-2042.
5. Ohmori T. Advances in gene therapy for hemophilia: basis, current status, and future perspectives. ***Int J Hematol***. 2020;111: 31-41.

Gene therapy for hemophilia

Tsukasa Ohmori

Department of Biochemistry, Jichi Medical University

Hemophilia is considered suitable for gene therapy because it is caused by a single gene abnormality, and therapeutic coagulation factor levels may vary across a broad range. Recently, successful clinical studies have been conducted on hemophiliacs with systemic administration of adeno-associated virus (AAV) vectors to express coagulation factors from the liver. These demonstrated that hemophilia could be cured by employing gene therapy, but several challenges have to overcome in the future. In this symposium, we will introduce our recent research works listed below.

1) Neutralizing antibodies: The presence of neutralizing antibodies against AAV reduces the efficacy of AAV-mediated gene therapy. We analyzed the threshold of neutralizing antibody titer to inhibit the therapeutic effect. Treatment with a high-dose AAV8 vector enabled evasion of the inhibitory effect of neutralizing antibodies in mice. Conversely, gene transduction was dramatically influenced in the mice treated with low-dose vector, suggesting the therapeutic effect of systemic gene therapy is determined according to vector dose and neutralizing antibody titer. Further, the second intravenous administration of an identical AAV vector seems not to be possible, because of the emergence of neutralizing antibodies after the first administration. We confirmed maintenance of a high-titer neutralizing antibody in the course of a long-term follow-up of AAV8-treated monkeys, while we successfully re-administered the other serotype in combination with intra-portal injection of the vector.

2) Identification of coagulation factor VIII (FVIII)-producing cells: Most coagulation factors are produced from hepatocytes, whereas FVIII expresses from endothelial cells. Current gene therapies to express FVIII target hepatocyte, a non-physiological producing cell. The increased expression of FVIII could activate ER stress response through the formation of amyloid-like fibrils, leading to cell death. Gene therapy targeting FVIII-producing cells may resolve these problems. We tried to characterize primary FVIII-producing organ and cell species using genetically engineered mice and found FVIII-producing cells were existed only in the thin sinusoidal layer of the liver and characterized as $CD31^{high}$, $CD146^{high}$, lymphatic vascular endothelial hyaluronan receptor 1^{+} and C-type lectin-like receptor-2^{+} .

3) Genome editing: Another issue in AAV-mediated systemic gene therapy targeting the liver is the application to affected children where liver hepatocytes are proliferating. AAV vectors are predominantly maintained episomally; therefore, cell division will dilute the AAV genome, resulting in the loss of therapeutic expression. We previously described treatment strategies for hemophilia B (factor IX deficiency) mice using CRISPR-Cas9. Recently, we have directly restored a point mutation in induced pluripotent stem cells (iPSCs) from a hemophilia B patient using a base-editing approach. Gene-corrected iPSCs differentiated into hepatocyte-like cells *ex vivo* and expressed robust F9 mRNA after subrenal capsule transplantation into immunodeficient mice.

Symposium 2

Abstract & Curriculum Vitae

Basics & Genome Editing

Chairperson: Kohnosuke Mitani

CURRICULUM VITAE

Name Markus Grompe, M.D.

Address (Affiliation) Papé Family Pediatric Research Institute
Department of Pediatrics, Oregon Health & Science University
L321, 3181 Sam Jackson Park Road, Portland, Oregon 97239, USA

Field of Research Cell and gene therapy for metabolic liver diseases

Education

1976-1983	M.D. Medicine	University of Ulm, School of Medicine, Germany
1983-1984	Clinical Physiology	University of Ulm, Ulm, Germany
1984-1987	Pediatric Residency	Oregon Health Sciences University, Portland, OR
1987-1991	Medical Genetics Fellowship	Baylor College of Medicine, Houston, TX

Medical Medical & Biochemical Genetics. In practice since 1987.

Professional Experience

1983-1984	Research Assistant, Department of Clinical Physiology, Ulm
1984-1987	Pediatric Residency, Oregon Health Sciences University
1987-1991	Postdoctoral fellowship in the Institute for Molecular Genetics at Baylor College of Medicine, Houston. Mentor: Dr. C. Thomas Caskey
1991-1999	Assistant/Associate Professor in the Department of Molecular and Medical Genetics and Pediatrics at the Oregon Health Sciences University (OHSU) in Portland, Oregon
2008-2018	Director, Papé Family Pediatric Research Institute and Ray Hickey Chair, OHSU
2011-2018	Vice Chair for Research, Dept. of Pediatrics, OHSU
1999-Present	Professor, Dept. of Molecular and Medical Genetics and Pediatrics, OHSU
2004-Present	Director, Oregon Stem Cell Center, OHSU

Recent Related Publications (5 Papers)

1. Grompe, M. (2017). Fah Knockout Animals as Models for Therapeutic Liver Repopulation. ***Adv Exp Med*** Biol 959, 215-230.
2. Nygaard, S., Barzel, A., Haft, A., Major, A., Finegold, M., Kay, M.A., and Grompe, M. (2016). A universal system to select gene-modified hepatocytes in vivo. ***Sci Transl Med*** 8, 342ra379.
3. Vonada, A., Tiyaboonchai, A., Nygaard, S., Posey, J., Peters, A.M., Winn, S.R., Cantore, A., Naldini, L., Harding, C.O., and Grompe, M. (2021). Therapeutic liver repopulation by transient acetaminophen selection of gene-modified hepatocytes. ***Sci Transl Med*** 13.
4. Yin, H., Xue, W., Chen, S., Bogorad, R.L., Benedetti, E., Grompe, M., Koteliansky, V., Sharp, P.A., Jacks, T., and Anderson, D.G. (2014). Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. ***Nat Biotechnol*** 32, 551-553.
5. Zhang, Q.S., Tiyaboonchai, A., Nygaard, S., Baradar, K., Major, A., Balaji, N., and Grompe, M. (2021). Induced Liver Regeneration Enhances CRISPR/Cas9-Mediated Gene Repair in Tyrosinemia Type 1. ***Hum Gene Ther*** 32, 294-301.

In vivo selection for the enhancement of cell and gene therapy of liver diseases

Markus Grompe, Anne Vonada, Amita Tiyaboonchai

Oregon Health & Science University, Portland, Oregon, USA

Gene therapy by integrating vectors is promising for monogenic liver diseases, especially in children where episomal vectors remain transient. However, reaching the therapeutic threshold with integrating vectors is challenging. Therefore, a method to expand hepatocytes bearing therapeutic transgenes was developed. The common fever medicine acetaminophen becomes hepatotoxic via cytochrome p450 metabolism. Hepatocytes lacking the essential co-factor of Cyp enzymes, NADPH-cytochrome p450 reductase (Cypor), were selected in vivo by acetaminophen administration replacing up to 50% of the hepatic mass. Lentiviral vectors with transgenes linked in cis to a Cypor shRNA were administered to neonatal mice. Acetaminophen treatment resulted in over 30-fold expansion of transgene-bearing hepatocytes and achieved therapeutic thresholds in hemophilia B and phenylketonuria. We conclude that therapeutically modified hepatocytes can be selected safely and efficiently with a transient regimen of moderately hepatotoxic acetaminophen.

CURRICULUM VITAE

Name Chang Li, Ph.D.

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Field of Research Gene therapy for genetic and infectios diseases

Education

2009-2015	Ph.D.	Biochemistry and Molecular Biology Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China
2003-2007	B.Eng.	Biotechnology Hunan Agricultural University, Changsha, China

Professional Experience

2020-Present	Acting Instructor (Junior Faculty), University of Washington, Div. Medical Genetics, Seattle, WA, USA
2016-2020	Senior Fellow, University of Washington, Div. Medical Genetics, Seattle, WA, USA
2015-2016	Research Associate, University of Maryland, College Park, MD, USA

Recent Related Publications (5 Papers)

1. Li C*, Georgakopoulou A, Mishra A, Gil S, Hawkins RD, Yannaki E, Lieber A. (2021) ***In vivo*** HSPC gene therapy with base editors allows for efficient reactivation of fetal γ globin in β YAC mice. (*corresponding author). ***Blood Adv***. 5(4):1122 1135.
2. Li C, Wang H, Georgakopoulou A, Gil S, Yannaki E, Lieber A. (2021) In vivo HSC gene therapy using a bi modular HDAd5/35++ vector cures Sickle Cell Disease in a mouse model. Mol Ther. 29(2):822 837. Commented by Townes TA. (2021) A 'Shot in the Arm' for Sickle Cell Disease. ***Mol Ther***. 29(2):416 417.
3. Li C, Course MM, McNeish IA, Drescher CW, Valdmanis PN, Lieber A. (2019) Prophylactic in vivo hematopoietic stem cell gene therapy with an immune checkpoint inhibitor reverses tumor growth in a syngeneic mouse tumor model. ***Cancer Re***s. 80(3):549 560.
4. Li C, Mishra AS, Gil S, Wang M, Georgakopoulou A, Papayannopoulou T, Hawkins RD, Lieber A. (2019) Targeted integration and high level transgene expression in AAVS1 transgenic mice after in vivo HSC transduction with HDAd5/35++ vectors. ***Mol Ther***. 27(12):2195 2212.
5. Li C, Psatha N, Sova P, Gil S, Wang H, Kim J, Kulkarni C, Valensisi C, Hawkins RD, Stamatoyannopoulos G, Lieber A. (2018) Reactivation of γ-globin in adult β-YAC mice after ***ex vivo*** and ***in vivo*** hematopoietic stem cell genome editing. ***Blood***. 131(26):2915-2928

In vivo HSC genome editing

Chang Li[1], André Lieber[1,2]

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[2] University of Washington, Department of Pathology, Seattle, WA, USA

We have developed an *in vivo* hemopoietic stem cell (HSC) gene therapy approach without the need for myelosuppressive conditioning and autologous HSC transplantation. It involves HSC mobilization and an intravenous injection of a helper-dependent adenovirus HDAd5/35++ vector system. These vectors target CD46, a receptor that is expressed on primitive HSCs. We used HDAd5/35++ vectors for *in vivo* gene addition with random integration, mediated by a *Sleeping Beauty* transposase, or targeted integration, mediated by homology-directed DNA repair. Furthermore, HDAd5/35++ vectors were employed for *in vivo* genome editing by transient expression of designer nucleases, base editors, and prime editors. We have demonstrated the safety and efficacy of *in vivo* HSC transduction in mouse disease models for thalassemia, Sickle Cell Disease, and hemophilia A, where we achieved a phenotypic correction of the diseases. We have performed studies in five mobilized rhesus macaques that were *in vivo* transduced with integrating HDAd5/35++ vectors expressing gamma-globin or secreted therapeutic proteins. In these studies, using a new prophylaxis regimen (dexamethasone, IL-6R, IL-1betaR antagonists, saline bolus IV), intravenous delivery of HDAd5/35++ was well tolerated without significant cytokine activation. Flow cytometry analyses showed efficient, preferential HSC transduction and re-homing of transduced $CD34^+/CD45RA^-/CD90^+$ cells to the bone marrow. At week 4, about 5% of progenitor colony-forming cells demonstrated stable transduction with integrated vector. Transgene marking and expression levels increased after *in vivo* selection with low-dose O^6BG/BCNU. Thus far, the longest follow up is 6 months (in two animals). Our NHP studies indicate that in vivo HSC gene therapy could be feasible in humans.

CURRICULUM VITAE

Name Charles Alan Gersbach, Ph.D.

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Departments of Biomedical Engineering and Surgery
Director, Center for Advanced Genomic Technologies
Director, Center for Biomolecular and Tissue Engineering,
Duke University
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Field of Research Genome Engineering

Education

2007-2009	Post-Doctoral Research Fellow		The Scripps Research Institute
May 2006	Ph.D.	Biomedical Engineering	Georgia Institute of Technology and Emory University
Aug 2001	B.S.	Chemical Engineering	Georgia Institute of Technology

Professional Experience

2021-Present	Professor (with tenure)
2015-2020	Associate Professor (with tenure)
2009-2015	Assistant Professor, Department of Biomedical Engineering, Duke University, Durham, NC
2019-Present	Department of Surgery, Duke University Medical Center, Durham, NC
2009-2015	Center for Genomic and Computational Biology, Duke University, Durham, NC
2013-Present	Department of Orthopaedic Surgery, Duke University Medical Center (secondary)
2020-Present	Department of Cell Biology, Duke University Medical Center (secondary)
2019-Present	Director, Center for Advanced Genomic Technologies, Duke University
2015-Present	Director, Center for Biomolecular and Tissue Engineering, Duke University

Recent Related Publications (5 Papers)

1. Kwon JB, Ettyreddy AR, Vankara A, Bohning JD, Delvin G, Hauschka SD, Asokan A, and Gersbach CA. In vivo gene editing of muscle stem cells with adeno-associated viral vectors in a mouse model of Duchenne muscular dystrophy. ***Molecular Therapy Methods and Clinical Development*** 19:320-329 (2020).
2. Kocak DD, Josephs EA, Bhandarkar V, Adkar SS, Kwon JB, Gersbach CA. Engineered Guide RNA Secondary Structure Increases the Specificity of Diverse CRISPR Systems. ***Nature Biotechnology*** 37(6):657-666 (2019).
3. Nelson CE, Wu Y, Gemberling MP, Oliver ML, Waller MA, Bohning JD, Robinson-Hamm JN, Bulaklak K, Castellanos Rivera RM, Collier JH, Asokan A, and Gersbach CA. Long-term Evaluation of AAV-CRISPR Genome Editing for Duchenne Muscular Dystrophy. ***Nature Medicine*** 25(3):427-432 (2019).
4. Thakore PI, Kwon JB, Nelson CE, Rouse DC, Gemberling MP, Oliver MO, Gersbach CA. RNA-Guided Transcriptional Silencing In Vivo with S. aureus CRISPR-Cas9 Repressors. ***Nature Communications*** 9(1):1674 (2018).
5. CE Nelson, CH Hakim, DG Ousterout, PI Thakore, EA Moreb, RM Castellanos Rivera, S Madhavan, X Pan, FA Ran, WX Yan, A Asokan, F Zhang, D Duan, CA Gersbach. In Vivo Genome Editing Improves Muscle Function in a Mouse Model of Duchenne Muscular Dystrophy. ***Science*** 351(6271):403-7 (2016).

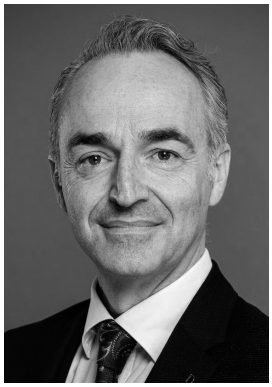
Genome Editing for Duchene Muscular Dystrophy

Charles A. Gersbach

Duke University, USA

The advent of genome editing technologies, including the RNA-guided CRISPR/Cas9 system, has enabled the precise editing of endogenous human genes. For example, we engineered CRISPR/Cas9-based nucleases to correct the mutated human dystrophin gene in cells isolated from Duchenne muscular dystrophy (DMD) patients. When delivered directly to a mouse model of this disease, gene editing by the CRISPR/Cas9 system led to gene restoration and improvement of biochemical and mechanical muscle function. Genome editing and dystrophin protein restoration is sustained in the mdx mouse model of DMD for one year after a single intravenous administration of AAV-CRISPR. We also confirmed immunogenic host response to Cas9 when administered via AAV vectors to adult mice and observed unintended genome and transcript alterations induced by AAV-CRISPR. We have observed gene editing in muscle satellite cells following AAV-CRISPR delivery in vivo, supporting the possibility of long-term gene correction despite muscle cell turnover. More recently, we have developed novel humanized models of this disease for the preclinical development of therapies that will correct human disease-causing mutations. New constructs have been developed and validated for significant levels of gene correction and dystrophin restoration in this model. Moreover, we have demonstrated in vivo gene editing that restores a full-length dystrophin gene, in contrast to previous approaches that restore a truncated, partially functional protein. These studies demonstrate the potential for genome editing to be used to treat DMD and other neuromuscular disorders, and also highlight issues for further study and development.

CURRICULUM VITAE



Name Toni Cathomen, Ph.D.

Address (Affiliation) Medical Center – University of Freiburg
Institute for Transfusion Medicine & Gene Therapy,
Freiburg, Germany

Field of Research Genome Editing

Education

1997	Ph.D.	Molecular Virology
1992	M.Sc.	Molecular Biology

Professional Experience

Since 2019	Scientific Director, Center for Chronic Immunodeficiency, Medical Center – University of Freiburg, Freiburg, Germany
Since 2012	Director, Institute for Transfusion Medicine and Gene Therapy, Medical Center – University of Freiburg, Freiburg, Germany
Since 2012	Professor of Cell and Gene Therapy, University of Freiburg, Freiburg, Germany
2009-2012	Associate Professor of Experimental Hematology, Hannover Medical School, Germany
2003-2009	Assistant Professor of Molecular Virology, Charité Medical School, Berlin, Germany

Recent Related Publications (5 Papers)

1. Turchiano G, Andrieux G, Klermund J, Blattner G, Pennucci V, El Gaz M, Monaco G, Poddar S, Mussolino C, Cornu TI, Boerries M, and Cathomen T (2021). Quantitative evaluation of chromosomal rearrangements in gene-edited human stem cells by CAST-Seq. ***Cell Stem Cell*** 28, online ahead of print (DOI: 10.1016/j.stem.2021.02.002)
2. Alzubi J, Lock D, Rhiel M, Schmitz S, Wild S, Mussolino C, Hildenbeutel M, Brandes C, Rositzka J, Lennartz S, Haas SA, Chmielewski KO, Schaser T, Kaiser A, Cathomen T, and Cornu TI. (2021) Automated generation of gene-edited CAR T cells at clinical scale. ***Mol Ther Methods Clin Dev*** 20, 379-88.
3. Craig-Muller N, Hammad R, Elling R, Alzubi J, Timm B, Kolter J, Knelangen N, Bednarski C, Gläser B, Ammann S, Ivics Z, Fischer J, Speckmann C, Schwarz K, Lachmann N, Ehl S, Moritz T, Henneke P, and Cathomen T. (2020) Modeling MyD88 deficiency in vitro provides new insights in its function. ***Front Immunol 11***, 608802.
4. Patsali P, Turchiano G, Papasavva P, Romito M, Loucari CC, Stephanou C, Christou S, Sitarou M, Mussolino C, Cornu TI, Antoniou MN, Lederer CW*, Cathomen T*, and Kleanthous M* (2019). Correction of IVS I-110(G>A) beta-thalassemia by CRISPR/Cas- and TALEN-mediated disruption of aberrant regulatory elements in human hematopoietic stem and progenitor cell. ***Haematologica*** 104, e497-501 (*corresponding authors)
5. Dettmer V, Bloom K, Gross M, Weissert K, Aichele P, Ehl S, and Cathomen T (2019). Retroviral UNC13D gene transfer restores cytotoxic activity of T cells derived from familial hemophagocytic lymphohistiocytosis type 3 patients in vitro. ***Hum Gene Ther*** 30, 975-84.

On- and off-target effects of genome editing tools in clinically relevant human hematopoietic cells

Toni Cathomen

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Therapeutic genome editing with designer nucleases has shown great promise for clinical translation but also revealed the risk of genotoxicity caused by creating mutations at off-target sites or by triggering chromosomal rearrangements. Recently developed methods to identify off-target activity in a genome-wide manner confirmed those perils but also revealed the restrictions of some of these assays, including insufficient sensitivity or specificity, or the failure to detect gross chromosomal aberrations. To overcome these limitations, we developed a novel assay, CAST-Seq, which is capable of detecting chromosomal rearrangements derived from on- and off-target activity of genome editing tools in clinically relevant cell types. I will present data that show that designer nucleases, such as CRISPR-Cas and TALEN, not only induce deletions, inversions and translocations, but also novel on-target activity mediated aberrations, such as homology-mediated translocations, acentric and dicentric translocations between homologous chromosomes, and very large deletions. Validation by deep sequencing and digital PCR confirmed that CAST-Seq is quantitative, that chromosomal translocations can occur in up to 0.5% of edited stem cells, and that up to 20% of target loci harbor large chromosomal aberrations. Furthermore, our assay reveals that also other genome editing tools, such as nickases, trigger chromosomal translocations. In conclusion, CAST-Seq provides new insights into chromosomal rearrangements associated with genome editing in clinically relevant human cells, so enabling a thorough risk assessment before clinical application of gene editing products

Symposium 3

Abstract & Curriculum Vitae

Vector Development

Chairpersons: Shin-ichi Muramatsu & Hiroyuki Mizuguchi

Symposium 3
S3-1

CURRICULUM VITAE

Name Arun Srivastava, Ph.D.

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Departments of Pediatrics, Molecular Genetics & Microbiology
Powell Gene Therapy Center, Cancer and Genetics Research Complex
2033 Mowry Road, Room 492-A
University of Florida College of Medicine
Gainesville, FL 32611-3633, USA

Field of Research Gene Therapy

Education

1971	BSc	Ewing Christian College, University of Allahabad, Allahabad, India
1973	MSc	Department of Biochemistry, University of Allahabad, Allahabad, India
1979	PhD	Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore, India

Professional Experience

1994-2004	Professor, Departments of Microbiology & Immunology and Medicine, Indiana University School of Medicine, Indianapolis, IN, USA
2004-Present	George H. Kitzman Professor of Genetics and Chief, Division of Cellular & Molecular Therapy, Departments of Pediatrics, Molecular Genetics & Microbiology, Powell Gene Therapy Center, University of Florida College of Medicine, Gainesville, FL, USA
2007-2022	Member, Scientific Review Board, Gene Therapy Resource Program, NHLBI, NIH
2010-Present	Member, Editorial Board, Journal of Integrative Medicine
2020-Present	Member, Editorial Board, Molecular Therapy

Recent Related Publications (5 Papers)

1. S.R.P. Kumar, J. Xie, S. Hu, J. Ko, Q. Huang, H.C. Brown, A. Srivastava, D.M. Markusic, C.B. Doering, H.T.Spencer, A. Srivastava, G. Gao, and R.W. Herzog. Coagulation factor IX gene transfer to non-human primates using engineered AAV3 capsid and hepatic optimized expression cassette. ***Molecular Therapy Methods & Clinical Development***, in review, 2021.
2. H.C. Brown, C.B. Doering, R.W. Herzog, C. Ling, D.M. Markusic, H.T. Spencer, A. Srivastava, and A. Srivastava. Development of a clinical candidate AAV3 vector for gene therapy of hemophilia B. ***Human Gene Therapy***, 31: 1114-1123, 2020.
3. H. Rambhai, F.J. Ashby, III, K. Qing, and A. Srivastava. Role of essential metal ions in AAV vector mediated transduction. ***Molecular Therapy Methods & Clinical Development***, 18: 159-166, 2020.
4. H. Yang, K. Qing, G.D. Keeler, L. Yin, M. Mietzsch, C. Ling, B.E. Hoffman, M. Agbandje-McKenna, M. Tan, W. Wang, and A. Srivastvava. Enhanced transduction of human hematopoietic stem cells by AAV6 vectors: Implications in gene therapy and genome editing. ***Molecular Therapy-Nucleic Acids***, 20: 451-458, 2020.
5. H.-J. Kwon, K. Qing, S. Ponnazhagan, X.-S. Wang, D.M. Markusic, S. Gupte, S.E. Boye, and A. Srivastava. Adeni-associated virus D-sequence-mediated suppression of expression of a human major histocompatibility class II gene: Implications in the development of adeno-associated virus vectors for modulating humoral immune response. ***Human Gene Therapy***, 31: 565-574, 2020.

Development of the Next Generation of AAV Vectors for Human Gene Therapy

Arun Srivastava

Division of Cellular and Molecular Therapy, Department of Pediatrics, Powell Gene Therapy Center, USA

Recombinant vectors based on a non-pathogenic parvovirus, the adeno-associated virus (AAV), have gained center stage in gene therapy of several human diseases. To date, 255 Phase I/II/III clinical trials have been or are currently being performed using the first generation of AAV vectors. Several AAV serotype vectors are now available, which have shown clinical efficacy in a number of human diseases, such as Leber's congenital amaurosis (LCA), lipoprotein lipase deficiency, hemophilia B, aromatic L-amino acid decarboxylase deficiency, choroideremia, Leber hereditary optic neuropathy, hemophilia A, and spinal muscular atrophy (SMA). Remarkable clinical efficacy has also been achieved in gene therapy of LCA and SMA, and two AAV "drugs"– Luxturna, and Zolgensma – have been approved by the United States Food and Drug Administration (USFDA) for gene therapy of LCA and SMA, respectively. However, in some cases, relatively large vector doses are needed to achieve clinical efficacy. The use of high doses of the first generation of AAV vectors has been shown to provoke host immune responses culminating in serious adverse events, and more recently, in the deaths of four patients. Thus, although remarkable progress has been made, it has also become increasingly clear that none of the first generation of AAV vectors currently in use is optimal for the following reasons: (i) AAV evolved as virus, and not as a vector for the purposes of delivery of therapeutic genes; (ii) The use of the first generation of AAV vectors composed of naturally occurring capsids induces immune responses, especially at high doses because the host immune system cannot distinguish between AAV as a virus versus AAV as a vector; and (iii) Most of the first generation of AAV serotype vectors, although effective in animal models, lack selective tropism for primary human cells and tissues. We have developed the next generation ("NextGen") of AAV serotype vectors that overcome most, if not all, of the limitations of the first generation of AAV serotype vectors. For example, we have previously reported that although liver was the predominant target of AAV2 vectors injected intravenously in mice, transgene expression occurred in fewer than 5% of hepatocytes. In subsequent studies, persistent expression of human clotting factor IX (FIX) was reported following intravenous injection in hemophilia B mice and in hemophilia B dogs. Based on these encouraging preclinical studies, a Phase I clinical trial for hemophilia B was performed with AAV2 vectors expressing FIX. Unfortunately, however, the low and medium vector doses failed to express human FIX in two patients. At a high dose of AAV2 vectors, production of the therapeutic levels of FIX was observed in one patient, but it was short-lived due to induction of AAV2 capsid-specific CD8+ memory T cell response, leading to the destruction of transduced hepatocytes. Thus, AAV2 vectors, although effective in mice and dogs, failed to provide durable clinical efficacy for hemophilia B in humans. In subsequent years following these initial studies, additional AAV serotypes became available, and among the 10 most commonly used serotype vectors, AAV8 vectors were reported to be highly efficient in transducing the mouse liver. A subsequent clinical trial with AAV8 vectors yielded encouraging results in that therapeutic levels of FIX were sustained over a 3-year follow-up period. However, a similar immune response, as seen previously with high vector doses, was again observed. In recently published studies, clinical trials with AAV5 and AAV8 vectors have been reported to lead to clinical efficacy in patients with hemophilia B, but these AAV serotype vectors are sub-optimal transducing human hepatocytes. In a head-to-head comparison, both in primary human hepatocytes in vitro and in a ''humanized'' mouse model in vivo, we have reported that AAV3 vectors are significantly more efficient than AAV5 and AAV8 vectors in human hepatocytes. We have also developed an optimized AAV3-FIX vector and evaluated its efficacy in hemophilia B mice, in ''humanized'' mice, and in non-human primate models, and achieved therapeutic levels of FIX in both models. The results of these studies will be presented and discussed. Based on these encouraging data, plans are also currently underway to initiate a Phase I/II clinical trial at relatively low vector doses without the need for immune-suppression. The clinical candidate NextGen AAV3-FIX vector is predicted to be safer and more effective in clinical gene therapy of hemophilia B in humans.

Symposium 3
S3-2

CURRICULUM VITAE

Name Fuminori Sakurai, Ph.D.

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Field of Research Gene Therapy and Oncolytic virotherapy

Education

1993-1996		Kyoto University Faculty of Pharmaceutical Sciences
1996-2001	Ph.D.	Kyoto University Graduate School of Pharmaceutical Sciences

Professional Experience

2001-2003	Postdoctoral fellow, Division of Biological Chemistry and Biologicals, National Institute of Health Sciences, Tokyo, Japan.
2003-2004	Researcher, Division of Biological Chemistry and Biologicals, National Institute of Health Sciences, Tokyo, Japan.
2005-2010	Researcher, Laboratory of Gene Transfer and Regulation, National Institute of Biomedical Innovation, Osaka, Japan.
2010	Visiting researcher, Department of Pharmacology, University of Texas, Southwestern medical center.
2011-Present	Associate Professor, Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University.
2014-2016	Program Officer, Ministry of Education, Culture, Sports, Science and Technology, Japan

Recent Related Publications (5 Papers)

1. Ono R, Takayama K, Sakurai F*, Mizuguchi H*. Efficient antitumor effects of a novel oncolytic adenovirus fully composed of species B adenovirus serotype 35. ***Mol Ther Oncolytics***. 20, 399-409. (2021) (*Corresponding author)
2. Sakurai F#*, Nishimae F#, Takayama K, Mizuguchi H*. Optimization of an E1A Gene Expression Cassette in an Oncolytic Adenovirus for Efficient Tumor Cell Killing Activity. ***Anticancer Res***. 41, 773-782. (2021) (*Corresponding author, #equally contributed)
3. Shimizu K#, Sakurai F*#, Iizuka S, Ono R, Tsukamoto T, Nishimae F, Nakamura SI, Nishinaka T, Terada T, Fujio Y, Mizuguchi H*. Adenovirus Vector-Induced IL-6 Promotes Leaky Adenoviral Gene Expression, Leading to Acute Hepatotoxicity. ***J Immunol***. 206, 410-421. (2021) (*Corresponding author, #equally contributed)
4. Machitani M, Sakurai F*, Wakabayashi K, Nakatani K, Tachibana M, Kato N, Fujiwara T, Mizuguchi H*. Suppression of Oncolytic Adenovirus-Mediated Hepatotoxicity by Liver-Specific Inhibition of NF-κB. ***Mol Ther Oncolytics***. 7, 76-85. (2017) (*Corresponding author)
5. Machitani M#, Sakurai F#*, Wakabayashi K, Tachibana M, Fujiwara T, Mizuguchi H*. Enhanced Oncolytic Activities of the Telomerase-Specific Replication-Competent Adenovirus Expressing Short-Hairpin RNA against Dicer. ***Mol Cancer Ther***. 16, 251-259. (2017) (*Corresponding author, #equally contributed)

Development of a novel oncolytic adenovirus based on adenovirus serotype 35

Fuminori Sakurai, Ryosuke Ono, Fumitaka Nishimae, Hiroyuki Mizuguchi

Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University,

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Oncolytic adenoviruses (OAds) are among the most promising oncolytic viruses. Almost all oncolytic adenoviruses are composed of human adenovirus serotype 5 (Ad5) (OAd5). However, expression of the primary infection receptor for Ad5, coxsackievirus-adenovirus receptor (CAR), often declines on malignant tumor cells, resulting in inefficient infection in CAR-negative tumor cells. In addition, at least 80% of adults have neutralizing antibodies against Ad5 due to natural infection. In this study, in order to overcome the drawbacks of OAd5, we developed a novel OAd fully composed of human adenovirus serotype 35 (OAd35), which belongs to species B2. OAd35 recognizes CD46, which is a complement regulatory protein ubiquitously expressed on almost all human cells and is often upregulated on malignant tumor cells, as an infection receptor. Moreover, 20% or fewer adults have neutralizing antibodies against Ad35, indicating that it is unlikey that OAd35 infection is inhibited by pre-existing antibodies. In order to develop an OAd35, a human telomerase reverse transcriptase (hTERT) promoter-driven E1A gene expression cassette was inserted into the OAd35 genome. OAd35 mediated efficient cell lysis activities at levels similar to OAd5 in CAR-positive tumor cells, while OAd35 showed higher levels of cell lysis activities than OAd5 in CAR-negative tumor cells. Anti-Ad5 serum significantly inhibited in vitro tumor cell lysis activities of OAd5, whereas OAd35 exhibited comparable levels of in vitro tumor cell lysis activities in the presence of anti-Ad5 and naïve serum. OAd35 significantly suppressed growth of not only subcutaneous CAR-positive H1299 tumors but also the CAR-negative T24 tumors following intratumoral administration. Furthermore, intravenous administration of OAd35 in human pancreatic BxPC-3 tumors resulted in efficient suppression of tumor growth. These results indicated that OAd35 is a promising alternative oncolytic virus for OAd5.

Next, we tried to find small chemical compounds which enhanced OAd infection in tumor cells by high-throughput screening of FDA-approved drug library (1134 compounds). We found that a compound X significantly enhanced the tumor cell lysis activities of not only OAd5 but also OAd35 in human pancreatic tumor cells by more than 2-fold. A compound X alone did not apparently exhibit the cytotoxic effects on the human tumor cells. Further analysis is now underway.

Symposium 3
S3-3

CURRICULUM VITAE

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Field of Research Hematopoietic stem cell transplantation, Gene therapy, Genome editing, Lentiviral vector

Education

Academic Degree

2009	Ph.D.	Medicine	Nippon Medical School, Tokyo, Japan
1999	Medical Doctor		Nippon Medical School, Tokyo, Japan

Professional Experience

Research Experience

2020-Present	Associate Professor in Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo, Japan
2020-Present	Part-time Staff Scientist in Cellular and Molecular Therapeutics Branch, NHLBI, NIH, Bethesda, MD, USA
2018-2020	Staff Scientist in Cellular and Molecular Therapeutics Branch, NHLBI, NIH, Bethesda, MD, USA
2017-2018	Staff Scientist in Sickle Cell Branch, NHLBI, NIH, Bethesda, MD, USA
2009-2017	Staff Scientist in Molecular and Clinical Hematology Branch, NHLBI, NIH, Bethesda, MD, USA
2012-2014	Adjunct Instructor in Molecular Biology, Nippon Medical School, Tokyo, Japan
2008-2009	Research Fellow in Molecular and Clinical Hematology Branch, NHLBI, NIH, Bethesda, MD, USA

Recent Related Publications (5 Papers)

1. Uchida N*, Ferrara F, Drysdale CM, Yapundich M, Gamer J, Nassehi T, DiNicola J, Shibata Y, Wielgosz M, Kim YS, Bauler M, Throm RE, Haro-Mora JJ, Demirci S, Bonifacino AC, Krouse AE, Linde NS, Donahue RE, Ryu B, Tisdale JF. Sustained fetal hemoglobin induction in vivo is achieved by BCL11A interference and coexpressed truncated erythropoietin receptor. ***Sci Transl Med***. 2021 Apr 28;13(591):eabb0411.
2. Uchida N*, Li L, Nassehi T, Drysdale CM, Yapundich M, Gamer J, Haro-Mora JJ, Demirci S, Leonard A, Bonifacino AC, Krouse AE, Linde NS, Allen C, Peshwa MV, De Ravin SS, Donahue RE, Malech HL, Tisdale JF. Preclinical evaluation for engraftment of CD34+ cells gene-edited at the sickle cell disease locus in xenograft mouse and non-human primate models. ***Cell Reports Medicine***. 2021;2(4).
3. Uchida N*, Drysdale CM, Nassehi T, Gamer J, Yapundich M, DiNicola J, Shibata Y, Hinds M, Gudmundsdottir B, Haro-Mora JJ, Demirci S, Tisdale JF. Cas9 protein delivery non-integrating lentiviral vectors for gene correction in sickle cell disease. ***Mol Ther Methods Clin Dev***. 2021 Mar 3;21:121-132.
4. Drysdale CM, Nassehi T, Gamer J, Yapundich M, Tisdale JF*, Uchida N. Hematopoietic-Stem-Cell-Targeted Gene-Addition and Gene-Editing Strategies for β-hemoglobinopathies. ***Cell Stem Cell***. 2021 Feb 4;28(2):191-208. (DCM, NT, GJ, and YM contributed equally to this work.)
5. Uchida N*, Hsieh MM, Raines L, Haro-Mora JJ, Demirci S, Bonifacino AC, Krouse AE, Metzger ME, Donahue RE, Tisdale JF. Development of a forward-oriented therapeutic lentiviral vector for hemoglobin disorders. ***Nat Commun***. 2019 Oct 2;10(1):4479.

Lentiviral vector system for hematopoietic stem cell gene therapy

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[1] Cellular and Molecular Therapeutics Branch, National Heart Lung and Blood Institutes (NHLBI)/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), Bethesda, MD, USA.
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Hematopoietic stem cell (HSC)-targeted gene therapy is curative for various hereditary hematopoietic diseases, including immunodeficiencies, hemoglobinopathies, metabolic diseases, and congenital cytopenia. HSCs reconstitute peripheral blood for life due to their self-renewal and multipotency specific for hematopoietic lineages; therefore, a replacement or repair of pathogenic mutations/deletions in HSCs allows for one-time cure of genetic diseases. A human immunodeficiency virus type-1 (HIV-1)-based lentiviral vector system has been developed to deliver a normal or therapeutic gene to the target cell genome, and lentiviral integration can allow for long-term therapeutic gene expression in HSCs. In preliminary HSC gene therapy trials in immunodeficiencies, a γ-retroviral vector system was used for a therapeutic gene delivery; however, hematological malignancies were developed in several patients due to insertional mutagenesis (Howe SJ. J Clin Invest. 2008). The γ-retroviral vector system is generated from murine 'leukemia' virus (likely enhancing target cell expansion), which favor integration into transcription start sites (near to a promoter) in activated genes, and if the γ-retroviral vector is integrated into an oncogene, the oncogene expression can increase by the viral enhancer in the long terminal repeat (LTR), thereby inducing leukemia (Wu X. Science. 2003). In contrast, a new generation of lentiviral vector system has a minimal risk of leukemia development, since this vector was generated from human 'immunodeficiency' virus (likely reducing target cell expansion), which are more equally integrated into activated genes, and the viral enhancer is removed from the LTR to produce self-inactivation. The HSC gene therapy trials with lentiviral transduction result in phenotypic correction in patients with various diseases without insertional mutagenesis in more than 200 patients (Eichler F. N Engl J Med. 2017, Cavazzana M. Nat Rev Drug Discov. 2019, Kohn DB. Nat Med. 2020, Kohn DB. N Engl J Med. 2021). Recently, leukemia development was reported in an HSC gene therapy for a patient with sickle cell disease (SCD) (Leonard A. Mol Ther. 2021). The lentiviral integration site in the leukemia cells is not related to oncogenes, and patients with SCD have a risk of myeloid malignancies in their natural history as well as post-transplantation. Therefore, this leukemia development is thought to be caused by disease status and/or conditioning instead of lentiviral integration. In conclusion, HSC gene therapies should improve the outlook for patients with genetic diseases.

Conflict of interest
The author declares no conflicts of interest.

Symposium 3
S3-4

CURRICULUM VITAE

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Field of Research Oncolytic virotherapy for brain tumors, Surgical Neuro-oncology

Education

2006	M.D.		School of Medicine, Yokohama City University
2017	Ph.D.	Medicine	Graduate School of Medicine, The University of Tokyo

Professional Experience

2019-Present	Assistant Professor at Division of Innovative Cancer Therapy and Dept. of Surgical Neuro Oncology, The Institute of Medical Science, The University of Tokyo
2017-2019	Research Fellow at Harvey Cushing Neuro-oncology Laboratories, Department of Neurosurgery, Brigham and Women's Hospital
2013-2017	Neurosurgeon at Division of Innovative Cancer Therapy and Dept. of Surgical Neuro-Oncology, The Institute of Medical Science, The University of Tokyo
2012-2013	Neurosurgeon at Department of Neurosurgery, NTT Medical Center Tokyo
2011-2012	Neurosurgeon at Department of Neurosurgery, Tokyo Metropolitan Neurological Hospital

Recent Related Publications (5 Papers)

1. Perlman O, Ito H, Gilad AA, McMahon MT, Chiocca EA, Nakashima H, Farrar CT: Redesigned reporter gene for improved proton exchange-based molecular MRI contrast. ***Sci Rep***. Nov 26;10(1):20664. (2020)
2. Mineo M, Lyons SM, Zdioruk M, von Spreckelsen N, Ferrer-Luna R, Ito H, Alayo QA, Kharel P, Giantini Larsen A, Fan WY, Auduong S, Grauwet K, Passaro C, Khalsa JK, Shah K, Reardon DA, Ligon KL, Beroukhim R, Nakashima H, Ivanov P, Anderson PJ, Lawler SE, Chiocca EA: Tumor interferon signaling is regulated by a lnc INCR1 transcribed from the PD-L1 locus. ***Mol Cell***. Jun 18;78(6):1207-1223. (2020)
3. Alayo QA, Ito H, Passaro C, Zdioruk M, Mahmoud AB, Grauwet K, Zhang X, Lawler SE, Reardon DA, Goins WF, Fernandez S, Chiocca EA, Nakashima H: Glioblastoma infiltration of both tumor- and virus-antigen specific cytotoxic T cells correlates with experimental virotherapy responses. ***Sci Rep***. Mar 20;10(1):5095. (2020)
4. Ito H, Nakashima H, Chiocca EA: Molecular responses to immune checkpoint blockade in glioblastoma. ***Nat Med***. 25, 359-361. (2019)
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Development of an oncolytic herpes simplex virus expressing anti-VEGF antibody bevacizumab

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Oncolytic virus (OV) therapy is a promising class of cancer therapy utilizing virus that can selectively replicate in and destroy malignant cells while leaving normal tissues essentially unharmed. A variety of genetically modified viruses have been investigated and started to put on the clinical pipeline. As most of OVs showed a favorable toxicity profile but have a limited efficacy as a single agent in the clinical trials, modification of OV therapy such as arming OVs with therapeutic transgenes or combining with other therapies, is a strategy being investigated clinically and preclinically.

Glioblastoma is one of the most malignant tumors resistant to the current multimodal treatment, and there is an urgent need for innovative therapeutics. We have developed a triple-mutated, third-generation oncolytic Herpes Simplex Virus type I (HSV-1), G47Δ, that exhibits enhanced replication capability in a variety of cancer, efficient induction of specific antitumor immunity, and high safety features. The interim analysis of the phase II clinical trial of G47Δ for glioblastoma that started in 2015 showed an unprecedented high therapeutic efficacy, awaiting approval as the first-in-the-world OV product for the treatment of malignant glioma. In the series of clinical trials of G47Δ, the "swelling" of tumors immediately after intratumoral administration of the virus was observed without exception, which can impede application of this treatment for such brain tumors that are large, multiple or infratentorial. Although corticosteroid efficiently alleviates brain edema, it causes immunosuppression that can hamper therapeutic efficacy of OV therapy. Bevacizumab, a humanized monoclonal anti-human vascular endothelial growth factor (VEGF) antibody that has been used as a standard therapy for many cancers including glioblastoma, is known as a robust suppressant of brain edema, however, systemic administration accompanied by delayed wound healing makes it difficult to be combined with OV therapy that requires surgical procedure. These limitations provide rationale for developing an oncolytic HSV-1 expressing bevacizumab using the G47Δ backbone (T-BV) as a next generation OV with improved efficacy and utility. MRI of an intracranial mouse glioma model after intratumoral virus inoculation revealed that T-BV showed an anti-swelling effect comparable to control virus combined with systemic administration of bevacizumab. The expression of bevacizumab is also restricted in the tumor microenvironment, which leads to a lower risk of side effects seen by systemic administrations of bevacizumab.

These results suggest that T-BV will be able to improve the feasibility of OV therapy for brain tumors via its unique efficacy and that selecting appropriate strategy for modifying OV therapy based on the characteristics of disease and transgene product is of great importance.

【COI】
Tomoki Todo owns the patent right for G47Δ in multiple countries including Japan.

Symposium 4

Abstract & Curriculum Vitae

Neurological Disorders

Chairpersons: Takashi Okada & Takanori Yamagata

CURRICULUM VITAE

Name Masayo Takahashi, M.D., Ph.D.

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650-0047 Japan

Field of Research Regenerative medicine, Retinal degeneration, Macular degeneration

Education

1992	Ph.D.	Graduate School of Medicine, Kyoto University, Japan
1986	M.D.	Faculty of Medicine, Kyoto University, Japan

Professional Experience

2019-Present	President, Vision Care Inc.
2006-2019	Team Leader (2012-Project leader), RIKEN
2001-2006	Associate professor, Team Leader of retinal regeneration project, Translational Research Center, Kyoto University Hospital
1995-1996	Post-doc in Laboratory of Genetics, the Salk Institute

Recent Related Publications (5 Papers)

1. Mandai M, Watanabe A, Kurimoto Y, Hirami Y, Morinaga C, Daimon T, Fujihara M, Akimaru H, Sakai N, Shibata Y, Terada M, Nomiya Y, Tanishima S, Nakamura M, Kamao H, Sugita S, Onishi A, Ito T, Fujita K, Kawamata S, Go MJ, Shinohara C, Hata KI, Sawada M, Yamamoto M, Ohta S, Ohara Y, Yoshida K, Kuwahara J, Kitano Y, Amano N, Umekage M, Kitaoka F, Tanaka A, Okada C, Takasu N, Ogawa S, Yamanaka S, Takahashi M. Autologous Induced Stem-Cell-Derived Retinal Cells for Macular Degeneration. ***N. Engl J. Med***. 376(11):1038-1046
2. Mandai M, Fujii M, Hashiguchi T, Sunagawa GA, Ito SI, Sun J, Kaneko J, Sho J, Yamada C, Takahashi M. iPSC-derived retina transplants improve vision in rd1 end-stage retinal degeneration mice. ***Stem Cell Reports***. 8(1):69-83
3. Akiba R, Matsuyama T, Tu HY, Hashiguchi T, Sho J, Yamamoto S, Takahashi M, Mandai M. Quantitative and Qualitative Evaluation of Photoreceptor Synapses in Developing, Degenerating and Regenerating Retinas. ***Front Cell Neurosci***. 13(16):1-20
4. Ochiai K, Motozawa N, Terada M, Horinouchi T, Masuda T, Kudo T, Kamei M, Tsujikawa A, Matsukuma K, Natsume T, Kanda GN, Takahashi M, Takahashi K. A Variable Scheduling Maintenance Culture Platform for Mammalian Cells. ***SLAS Technol***. 26(2):209-217.
5. Sugita S, Mandai M, Kamao H, Takahashi M. Immunological aspects of RPE cell transplantation Prog Retin ***Eye Res***. in press.

Treatments for outer retinal diseases

Masayo Takahashi

Vision Care Inc. & Kobe City Eye Hospital

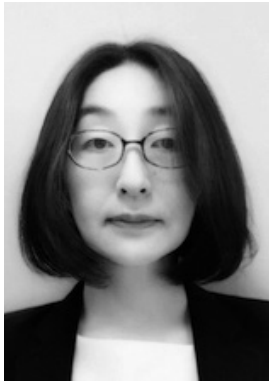
Our aim is to develop therapies for outer retinal diseases. The first in man application of iPS cells started in 2013 for age-related macular degeneration. Since then we conducted three clinical studies using iPS cells: 1) autologous retinal pigment epithelial (RPE) sheets, 2) HLA matched allogeneic RPE suspensions, and 3) retinal organoid including photoreceptor cells transplantation.

To start the clinical studies, not only cell risks but also risks from treatments such as immunosuppression or surgery should be considered in a risk matrix (frequency x severity). Also, the benefit matrix is needed to make decision.

As for retinal organoid, we proved (1) maturation of the grafted immature retinal sheet after transplantation, (2) synapse formation with host secondary neurons, (3) electrophysiological light responses in the host ganglion cells, and (4) positive results of behavior test after transplantation. With those preclinical findings from animal experiments as POC, we can imagine the effect and learned the way how to select the patients.

Now we are developing gene therapy for the early stage of autosomal dominant retinitis pigmentosa. Channel rhodopsin gene therapy will restore vision in some extent in the late phase, and photoreceptor cell replacement therapy in the middle. Thus, we will have tools to treat future patients with incurable retinal degenerative diseases at any stages.

CURRICULUM VITAE



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Field of Research Molecular Biology, AAV

Education

2002 Ph.D. Medical Science The University of Tokyo, Graduate School of Medicine

Professional Experience

2002 Postdoctoral fellow, University Research Institute for Diseases of Old Age, Juntendo University School of Medicine
2007 Postdoctoral fellow, Cell-Matrix Frontier Laboratory, Hiroshima University
2008 Postdoctoral fellow, Department of Molecular Therapy, National Institute of Neuroscience, NCNP
2014 Postdoctoral fellow, Department of Biochemistry and Molecular Biology, Nippon Medical School
2017 Assistant Professor, Division of Cell and Gene Therapy, Nippon Medical School
2020 Project Assistant Professor, Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo

Recent Related Publications (5 Papers)

1. Improved transduction of canine X-linked muscular dystrophy with rAAV9-microdystrophin via multipotent MSC pretreatment. Hayashita-Kinoh H, Posadas-Herrera Guillermo, Nitahara-Kasahara Y, Kuraoka M, Okada H, Chiyo T, Takeda S, Okada T. Molecular therapy. ***Methods & clinical development*** 20 133-141(2021)
2. rAAV8 and rAAV9-Mediated Long-Term Muscle Transduction with Tacrolimus (FK506) in Non-Human Primates. Ishii A, Okada H, Hayashita-Kinoh H, Shin JH, Tamaoka A, Okada T, Takeda S Molecular therapy. ***Methods & clinical development*** 18 44-49 (2020)
3. Intra-amniotic rAAV-mediated microdystrophin gene transfer improves canine X-linked muscular dystrophy and may induce immune tolerance. Hayashita-Kinoh H, Yugeta N, Okada H, Nitahara-Kasahara Y, Chiyo T, Okada T, Takeda S. ***Molecular therapy*** (2015)
4. Robust Long-term Transduction of Common Marmoset Neuromuscular Tissue With rAAV1 and rAAV9. Okada H, Ishibashi H, Hayashita-Kinoh H, Chiyo T, Nitahara-Kasahara Y, Baba Y, Watanabe S, Takeda S, Okada T. ***Molecular therapy Nucleic acids*** (2013)
5. Improvement of cardiac fibrosis in dystrophic mice by rAAV9-mediated microdystrophin transduction. Shin JH, Nitahara-Kasahara Y, Hayashita-Kinoh H, Ohshima-Hosoyama S, Kinoshita K, Chiyo T, Okada H, Okada T, Takeda S. ***Gene therapy*** (2011)

Gene Therapy Approaches using rAAV for the Treatment of Duchenne Muscular Dystrophy

Hiromi Hayashita-Kinoh[1], Atsushi Takagi[2], Guillermo Posadas-Herrera[1], Yuko Nitahara-Kasahara[3], Akiko Ishii[4], Shin'ichi Takeda[4], Takashi Okada[1]

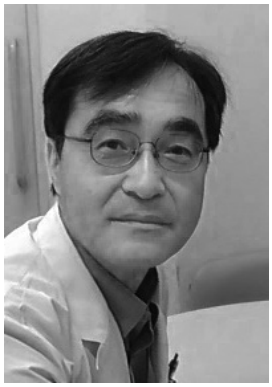
[1] Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo
[2] Department of Pediatrics, Nippon Medical School
[3] Department Biochemistry and Molecular Biology, Nippon Medical School
[4] Department of Molecular Therapy, National Institute of Neurosciences, NCNP

Duchenne muscular dystrophy (DMD) is a congenital disease causing progressive deterioration of skeletal and cardiac muscles because of mutations in the dystrophin gene. DMD is a rare and intractable pediatric muscular dystrophy that presents with progressive and severe symptoms, and has a high incidence of 1 in 3,500 live births in boys, one third of which are de novo mutations with no family history. At present, there is no curative therapy, and the main treatment is respiratory management. Exon-skipping therapies using artificial nucleic acid have been approved in the United States and Japan. DMD is caused by mutations or deletions in the DMD gene that encodes a 427-kDa protein called dystrophin. The DMD gene is the largest human gene consisting of 79 exons and there are many different types of mutations associated with it. Given that different patients have distinct mutations in the gene, exon-skipping drugs need to be designed for each mutation type, AAV-based dystrophin replacement therapy can be used for all mutations.

Supplementation of dystrophin using rAAV-microdystrophin is sufficient to improve pathogenesis of mouse models of DMD. However, we previously reported that local injection of rAAV8 or rAAV9 into canine or monkey skeletal muscles without immunosuppression resulted in insufficient transgene expression with potent immune responses. The rAAV vector has been used in many clinical trials but an important obstacle in clinical translation is the activation of both innate and adaptive immune responses to the rAAV capsid itself, the vector genome and rAAV-derived transgene product. While the extent of immune response can be influenced by the dosage, administration route and target organs, this can cause reduction of efficacy, and other unwanted side effects such as local tissue damage. Luxturna and Zolgensma, the two rAAV products with licensed regulatory approval in Europe and the United States, use prednisolone pre- and post-administration of the rAAV. In clinical trials of DMD, AAV administration protocol includes prophylactic use of both anti-complement inhibitor eculizumab and C1 esterase inhibitor with prednisone.

We used somatic stem cells (mesenchymal stromal cells, MSCs) to investigate strategies for inducing immune modulation to the rAAV9 vector and transgene expression in the DMD dog, CXMDJ. MSCs have been employed in various inflammatory diseases including graft-versus-host disease (GvHD) for their immunosuppressive effects. The CXMDJ pre-treated with MSCs and rAAV9-microdystrophin showed higher transduction with functional improvement than other DMD dogs of the same age. This strategy would be a practical approach to increase the expression and function of the transgene in vivo. These findings also support the future feasibilities of rAAV-mediated protein supplementation strategies to treat DMD.

CURRICULUM VITAE



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Education

1987	M.D.	Tohoku University School of Medicine,
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Professional Experience

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1989-1996	Department of Pediatrics, Yokohama City University School of Medicine
1996-1999	Postdoctoral fellow, Department of Pharmacology, School of Medicine, University of California, San Diego, CA
1999-2002	Research fellow, Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry
2002-	Investigator of Information and Cellular function, PRESTO, JST
2009-	Chief of Neurology, Kanagawa Children's Medical Center
2014-	Professor, Jichi Medical Univ., Dept of Pediatrics

Recent Related Publications (5 Papers)

1. Y. Kurokawa, H. Osaka, T. Kouga, E. Jimbo, K. Muramatsu, S. Nakamura, Y. Takayanagi, T. Onaka, S.I. Muramatsu, T. Yamagata, Gene Therapy in a Mouse Model of Niemann-Pick Disease Type C1. ***Human gene therapy*** 32 (2021) 589-598.
2. S. Nakamura, H. Osaka, S.I. Muramatsu, N. Takino, M. Ito, E.F. Jimbo, C. Watanabe, S. Hishikawa, T. Nakajima, T. Yamagata, Intra-cisterna magna delivery of an AAV vector with the GLUT1 promoter in a pig recapitulates the physiological expression of SLC2A1. ***Gene therapy*** (2020).
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4. S. Nakamura, S.I. Muramatsu, N. Takino, M. Ito, E.F. Jimbo, K. Shimazaki, T. Onaka, S. Ohtsuki, T. Terasaki, T. Yamagata, H. Osaka, Gene therapy for Glut1-deficient mouse using an adeno-associated virus vector with the human intrinsic GLUT1 promoter. ***The journal of gene medicine*** 20 (2018) e3013.
5. S. Nakamura, H. Osaka, S. Muramatsu, S. Aoki, E.F. Jimbo, T. Yamagata, Mutational and functional analysis of Glucose transporter I deficiency syndrome. ***Mol Genet Metab*** 116 (2015) 157-162

Gene therapy of genetic neurological disorders

Hitoshi Osaka[1], Sachie Nakamura[1], Yoshie Kurokawa[1], Karin Kojima[1], Akihiko Miyauchi[1], Kazuhiro Muramatsu[1], Eriko Jinbo[1], Takeshi Nakajima[2], Hiroaki Mizukami[3], Shin-ichi Muramatsu[4,5], Takanori Yamagata[1]

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Majorities of genetic neurological disorders are caused by loss of function and are good targets for gene therapy. Recently, we examined an adeno-associated virus (AAV) vector to treat 8 patients with Aromatic L-amino acid decarboxylase deficiency. This enzyme is expressed in a limited area of the brain, and local injection improved dramatically the clinical phenotypes in all of the patients (Kojima et al., Brain 2019). Most other neurological diseases require diffuse brain expression of causative protein.

As such, glucose transporter 1 deficiency syndrome (GLUT1DS) is an autosomal dominant disorder caused by a haplo-insufficiency of *SLC2A1*, a gene encoding GLUT1 that is expressed in all brain areas. We have established a functional assay for glucose transporter activity (Nakamura et al., Mol Genet Metab. 2015). We generated an AAV vector, AAV-glut1-SLC2A1, involving SLC2A1 under its intrinsic promoter, and injected this vector into the cerebral ventricles of the GLUT1$^{+/-}$ model mouse. One injection improved cerebrospinal fluid glucose levels and motor functions to comparable to wild type levels, as assessed by rota-rod tests (Nakamura et al., J Gene Med. 2018). In preparation for clinical application, we injected AAV-GLUT1 (1.63×10^{12} vector genomes/kg) into the cisterna magna of pigs. After injection, exogenous GLUT1 was expressed in the wide areas of central nervous system (CNS). At the cellular level, exogenous GLUT1 was mainly expressed in the endothelium, followed by glia and neurons. Intra-cisterna magna injection of AAV-GLUT1 appears to be a feasible approach for gene therapy of GLUT1DS (Nakamura et al., Gene Ther. 2020).

Niemann-Pick disease type C1 (NPC1) is a neurodegenerative disorder caused by mutations in the *NPC1* gene, which is involved in cholesterol transport in lysosomes. Clinical manifestations include liver failure, pulmonary disorder, neurological deficits, and psychiatric symptoms. The main cause of death in NPC1 patients involves CNS dysfunction. We generated a AAV 9/3 vector that expresses human *NPC1* under a CMV promotor and injected it into the left lateral ventricle and cisterna magna of *Npc1*$^{-/-}$ mice. Each mouse received total 1.35×10^{11} vector genome on day 4 or 5 of life. AAV-treated *Npc1*$^{-/-}$ mice had an average survival of >28 weeks, while all saline-treated *Npc1*$^{-/-}$ mice died within 16 weeks. AAV-treated *Npc1*$^{-/-}$ mice also showed a significant improvement in Rotarod test performance. A pathological analysis showed that cerebellar Purkinje cells were preserved in AAV-treated *Npc1*$^{-/-}$ mice. In contrast, untreated *Npc1*$^{-/-}$ mice showed an almost total loss of cerebellar Purkinje cells (Kurokawa et al., Hum Gene Ther 2021).

For GLUT1-DS and NPC1, we are preparing for clinical trials under the AMED grants. Patients with rare genetic neurological diseases comprise a large proportion of unmet medical needs. AAV gene therapy can provide great promises for these patients and families.

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Field of Research Neurogenetics

Education

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Professional Experience

1994-1997	Staff Physician, Hinatadai Hospital, Yokohama, Japan
1997-2002	Postdoctoral Fellowship, Baylor College of Medicine, Houston, Texas
2002-2004	Assistant Professor, Baylor College of Medicine, Houston, Texas
2004-Present	Division Chief, National Institute of Neuroscience, NCNP, Tokyo, Japan

Recent Related Publications (5 Papers)

1. Kashiki H, Li H, Miyamoto S, Ueno H, Tsurusaki Y, Ikeda C, Kurata H, Okada T, Shimazu T, Imamura H, Enomoto Y, Takanashi JI, Kurosawa K, Saitsu H, Inoue K. POLR1C variants dysregulate splicing and cause hypomyelinating leukodystrophy. ***Neurol Genet***. 2020 Oct 13;6(6):e524. doi:10.1212/NXG.0000000000000524.
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Gene suppression therapy for Pelizaeus-Merzbacher disease using AAV harboring artificial miRNA: principals and problems

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Gene overexpression is one form of the molecular etiology in genetic diseases, and suppressing the expression of causative genes would serve as the primary molecular therapy for such diseases. Pelizaeus-Merzbacher disease (PMD) is one such example. Genomic duplication of the *PLP1* gene, encoding a major myelin membrane protein in the CNS, leads to overexpression of PLP1, which causes congenital failure of the CNS myelin production. Although the exact mechanism as to how one extra-copy of the structural protein-coding gene results in such severe pathology in oligodendrocytes, the myelin-producing cells in the CNS, targeted gene suppression therapy of *PLP1* in oligodendrocytes may serve as a fundamental therapeutic approach to PMD. We have been developing an AAV-based gene therapy for PMD caused by *PLP1* duplication using an artificial miRNA driven by oligodendrocyte-specific promoter to down-regulate the expression of *PLP1* to mitigate the disease phenotype. Using a Tg mouse mode of PMD overexpressing the *PLP1* gene, we observed oligodendrocyte-specific expression of AAV-delivered gene, efficient suppression of PLP1 expression, reduction of pathological intracellular accumulation of PLP1, recovery of the oligodendrocyte number, increase in number of myelinated axons, rescue of motor function and longer life span. Although these therapeutic effects are encouraging, we still have several issues to be resolved for the clinical application of this technology, including optimization of serotype and delivery for better distribution.

Symposium 5

Abstract & Curriculum Vitae

Clinical Application

Chairpersons: Toru Uchiyama & Ryuichi Morishita

CURRICULUM VITAE

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Education

1981-1987	M.D.	Medicine	Osaka University Medical School, Osaka, Japan
1987-1991	Ph.D.	Medicine	Osaka University Medical School, Osaka, Japan

Professional Experience

1996-1998	Assistant Professor, Department of Geriatric Medicine (T. Ogihara), Osaka University Medical School
1998-2004	Associate Professor, Division of Gene Therapy Science (Y. Kaneda), Osaka University Medical School
2003-Present	Professor, Department of Clinical Gene Therapy, Osaka University Medical School

Recent Related Publications (5 Papers)

1. Koriyama H, Nakagami H, Nakagami F, Osako MK , Kyutoku M, Shimamura M, Miyake T, Katsuya T, Rakugi H, Morishita R. Long term blood pressure reduction by Angiotensin II DNA vaccine in Spontanouly Hypertnsive Rats. ***Hypertension*** 2015;66:167-174
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5. Yoshida S, Nakagami H, Hayashi H, Ikeda Y, Sun J, Tenma A, Tomioka H, Kawano T, Shimamura M, Morishita R, Rakugi H. The CD153 vaccine is a senotherapeutic option for preventing the accumulation of senescent T cells in mice. ***Nature Communication*** 2020 May 18;11(1):2482

Plasmid DNA-based Gene Therapy: From Collategene to DNA Vaccine

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Gene therapy has emerged as a novel therapy to promote angiogenesis in patients with critical limb ischemia (CLI) caused by peripheral artery disease. We focused on hepatocyte growth factor (HGF) as pro-angiogenic factors. In phase III clinical trial, naked plasmid DNA encoding HGF showed the safety and their potential for symptomatic improvement in CLI patients. Based on phase III data, HGF gene therapy drug, Collategene, has been approved by PMDA in Japan. Collategene was launched in Japan market as the first gene therapy drug at 2019. In this session, we would like to discuss about future application of HGF gene therapy.

In addition, we recently focused on the therapeutic vaccination which has extended its scope from infectious diseases to chronic diseases. We reported that angiotensin (Ang) II vaccine for hypertension successfully attenuated the high blood pressure in animal models (PLoS One 2013, Sci Rep 2017, Stroke 2017). Increasing the effectiveness of drug adherence interventions may have a great impact on the health of the population, because approximately 50% may not take medications. This poor adherence to medication leads to increased morbidity and death. As a result, the vaccine-induced anti-Ang II antibodies can efficiently ameliorate Ang II-induced high blood pressure and perivascular fibrosis in mice. Phase I/II clinical trial demonstrated good safety profile and the production of antibody against Ang II. In next step, we will start phase IIb study to test the anti-hypertensive efficacy.

Based on plasmid DNA platform technology, we have applied to develop DNA vaccine against COVID-19. Successfully, we have developed DNA vaccine against SARS-Cov2. Now, phase II/III clinical trial using our DNA vaccine was already started, from 4Q on 2020. As the safety profile of DNA vaccine was very well, in this lecture, I would like to discuss about DNA vaccine against COVID-19.

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Field of Research GI Oncology

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Professional Experience

2003-2007	National Cancer Center Hospital East, Gastrointestinal Oncology, Resident
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2009-2010	National Cancer Center Hospital East, Department of Endoscopy, Physician
2010-2011	National Cancer Center Hospital East, Digestive Oncology, Gastrointestinal Oncology Physician and Head and Neck Oncology/ Plastic Surgery Physician
2011-2015	National Cancer Center Hospital East, Gastrointestinal Oncology, Physician
2015-Present	National Cancer Center Hospital East, Gastrointestinal Oncology / Head

Recent Related Publications (5 Papers)

1. Kojima T, Yamazaki K, Kato K, Muro K, Hara H, Chin K, et al. Phase I dose-escalation trial of Sym004, an anti-EGFR antibody mixture, in Japanese patients with advanced solid tumors. ***Cancer Sci***. 2018;109(10):3253-62.
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5. Kato K, Doki Y, Ura T, Hamamoto Y, Kojima T, Tsushima T, et al. Long-term efficacy and predictive correlates of response to nivolumab in Japanese patients with esophageal cancer. ***Cancer Sci***. 2020;111(5):1676-84.

Oncolytic Virus Therapy as Immunotherapy

Takashi Kojima

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Oncolytic viruses are a new class of therapeutic agents that are thought to preferentially infect and replicate in cancer cells, resulting in direct killing of infected cells and induction of systemic antitumor immunity. The first oncolytic virus to receive FDA approval was a treatment for melanoma known as talimogene laherparepvec (Imlygic®), or T-VEC. The treatment, which is injected into tumors, was engineered to produce a protein that stimulates the production of immune cells in the body and to reduce the risk of causing herpes. In Japan, teserpaturev/G47Δ (Delytact®) had received conditional and time-limited approval from PMDA for the treatment of patients with malignant glioma in 2021.

Oncolytic Viral Therapy (OVT) is one of the novel approaches for treating cancer and has a preferable safety profile, but there are two points to be worried about. One is the biosafety problem, and the other is that most of OVT used as local treatment by intralesional injection, so therapeutic effect might be limited to local lesions.

Concerning biosafety, it is important to mention the Cartagena Protocol on Biosafety. The Cartagena Protocol on Biosafety is the international agreement to regulate the trans-boundary movements of genetically engineered organisms including the viruses used for OVT. Japan has ratified this Protocol and there are regulatory requirements to prevent viral shedding during clinical trials using OVT as well as gene therapy. This means that special attention must be paid to these matters in the clinical treatment of OVT.

On the other hand, to overcome of limitation of local treatment, there is attempting to expand the benefit of OVT by combining with check point inhibitors. ICIs also have limited effectiveness, so looking for the partner to combine with.

Based on the mechanisms underlying oncolytic virotherapy, Oncolytic viruses have the potential to induce T cell priming and infiltration, activate local immune responses, and change the immunosuppressive status of the tumor microenvironment. Thus, Oncolytic viruses are ideal for combining with ICI s. Based on the phase Ib trail of T-VEC + Pembrolizumab, a phase 3 clinical trial involving 600 patients with melanoma who will receive T-VEC with or without pembrolizumab is under way to investigate the combination therapy in a large, randomized study. Including our institution, many kinds of study of oncolytic virus with anti-PD1 antibody are now on going.

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Field of Research Pediatric leukemia, Stem cell transplantation, Genomic medicine

Education

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Professional Experience

2021-Present	Professor, Department of Pediatrics, the University of Tokyo
2015-2020	Chief, Department of Transplantation and Cell Therapy, National Center for Child Health and Development
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2003-2006	Saitama Children's Medical Center
2002-2003	Ome General Hospital
2001-2003	Chiba Nishi General Hospital
2000-2001	University of Tokyo Hospital

Recent Related Publications (5 Papers)

1. Osumi T, Yoshimura S, Sako M, Uchiyama T, Ishikawa T, Kawai T, Inoue E, Takimoto T, Takeuchi I, Yamada M, Sakamoto K, Yoshida K, Kimura Y, Matsukawa Y, Matsumoto K, Imadome K, Arai K, Deguchi T, Imai K, Yuza Y, Matsumoto K, Onodera M, Kanegane H, Tomizawa D, Kato M. A prospective study of allogeneic hematopoietic stem cell transplantation with post-transplantation cyclophosphamide and anti-thymoglobulin from HLA-mismatched related donors for non-malignant diseases. ***Biol Blood Marrow Transplant*** 26(11):e286-e291, 2020
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4. Osumi T, Tsujimoto SI, Tamura M, Uchiyama M, Nakabayashi K, Okamura K, Yoshida M, Tomizawa D, Watanabe A, Takahashi H, Hori T, Yamamoto S, Hamamoto K, Migita M, Ogata-Kawata H, Uchiyama T, Kizawa H, Ueno-Yokohata H, Shirai R, Seki M, Ohki K, Takita J, Inukai T, Ogawa S, Kitamura T, Matsumoto K, Hata K, Kiyokawa N, Goyama S, Kato M: Recurrent RARB Translocations in Acute Promyelocytic Leukemia Lacking RARA Translocation. ***Cancer research*** 78:4452-4458,2018
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Clinical application of chimeric antigen receptor T-cell therapy for leukemia

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Chimeric antigen receptor (CAR) is genetically engineered to recognize and eradicate cells expressing target antigens. Numerous CAR-transduced T-cell therapies have been investigated for use in relapsed/refractory malignant diseases. Among them, CD19-CAR-T manufactured from autologous T cells transduced to express costimulatory domain(s) (4-1BB/CD28) and a CD3ζ T-cell activation signaling domain, demonstrated dramatic response for chemo-refractory B-cell malignancies. Clinical trials showed that more than 90% of patients with refractory leukemia achieved remission. Given the excellent response rate, CD19-CAR-T has been approved for the use in children and young adults with B-cell acute lymphoblastic leukemia (B-ALL) and adults with diffuse large B-cell lymphoma (DLBCL). Although CAR-T therapy is often accompanied by severe adverse events, such as cytokine-releasing syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), efficacy of CAR-T has high impact on therapeutic strategy of B-cell malignancies. In this symposium, we should discuss real-world experience of CD19-CAR-T, including efficacy and adverse events.

COI disclosure: No conflict of interest related to this presentation.

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Field of Research Pediatric neurology

Education

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Professional Experience

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1997 Research Fellow at Department of Molecular and Human Genetics, Baylor College of Medicine
2010 Professor, Department of Pediatrics, Jichi Medical University
2014 Director, Jichi Children's Medical Center Tochigi
2018 Director, Center for Gene Therapy Research, Jichi Medical University

Recent Related Publications (5 Papers)

1. Kurokawa Y, Osaka H, Kouga T, Jimbo E, Muramatsu K, Nakamura S, Takayanagi Y, Onaka T, Muramatsu SI, Yamagata T. Gene Therapy in a Mouse Model of Niemann-Pick Disease Type C1. ***Hum Gene Ther***. 2021 Online ahead of print.
2. Nakamura S, Osaka H, Muramatsu SI, Takino N, Ito M, Jimbo EF, Watanabe C, Hishikawa S, Nakajima T, Yamagata T. Intra-cisterna magna delivery of an AAV vector with the GLUT1 promoter in a pig recapitulates the physiological expression of SLC2A1. ***Gene Ther***. 2020 Online ahead of print.
3. Kojima K, Nakajima T, Taga N, Miyauchi A, Kato M, Matsumoto A, Ikeda T, Nakamura K, Kubota T, Mizukami H, Ono S, Onuki Y, Sato T, Osaka H, Muramatsu S, Yamagata T. Gene therapy improves the motor and mental function in various types of aromatic L-amino acid decarboxylase deficiency. ***Brain*** 2019;42:322-333.
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Clinical experiences of Onasemnogene abeparvovec treatment and worldwide clinical trials of gene therapy for child neurological diseases

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Gene therapy for many child neurological diseases and congenital metabolic disorders using adeno-associated viral (AAV) vectors have been developed and started clinical trials worldwide. Among them, Onasemnogene abeparvovec, AAV9 vector with SMN1 gene, to treat spinal muscular atrophy (SMA) has approved for clinical use. SMA is caused by SMN1 gene deletion that induces progressive loss of motor neuron that induces muscular atrophy. Patients with SMA type 1, severest type, shows progressive muscle weakness and hypotonia from early infancy and die within two years without respiratory support. Onasemnogene abeparvovec was very effective for SMA that after $1x10^{14}$ vg/kg intravenous injection, patients have improved their muscle strength and motor development. Patients treated before the onset or very early phase were reported to be able to walk. On the other hand, patients treated later showed limited effects that bed ridden patients remained in bed ridden. Newborn mass-screening is necessary to diagnose and treat patients earlier. As adverse events, most patients had fever, liver dysfunction and thrombocytopenia around four to seven days, and one month after the injection, although they were well. All patients recovered without sequel, but four patients were reported to be suffered from thrombotic microangiopathy in the world. Further, three patients died from severe liver dysfunction in the clinical trial of gene therapy for X-linked myotubular myopathy. In this trial, AAV8 vector was used and injected intravenously, and this treatment was very effective for muscular weakness. Patients were divided into two groups of $1x10^{14}$ and $3x10^{14}$ vg/kg and 3/14 patients in high dose group were died. All three dead patients were relatively older and heavy weight that required higher total injection dose. High dose intravenous injection was considered to be high risk.

Many clinical trials of gene therapy for child neurological and metabolic diseases were listed in ClinicalTrials gov (https://clinicaltrials.gov/). Most targets were lysosomal diseases that qqlysosomal enzymes could move from cells to another cells. Therefore, lysosomal diseases are good target for gene therapy. The rout of injection to induce genes into more neuronal cells and get maximum effect is important issue. Intravenous, intraparenchymal or intraventricular injection had been considered. Recently, intra cisterna magna injection was considered to be a most effective rout, and several trials such as for mucopolysaccharidosis type I and II have been started.

In this presentation, reported results and our experiences of Onasemnogene abeparvovec treatment, and clinical trials for child neurological diseases in the world will be introduced. And problem of Japanese situation will be discussed.

COI; Lecture fee paid by Novartis Pharma K.K.

Symposium 6

Abstract & Curriculum Vitae

Cancer

Chairpersons: Yasutomo Nasu & Hiroshi Fukuhara

CURRICULUM VITAE

Name Ken-ichiro Kosai, M.D., Ph.D.

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Field of Research Oncolytic virus immunotherapy, Vector development, Regenerative medicine, Stem cell biology, Growth factor (biology and application), Translational research

Education

1988 M.D. Kurume University School of Medicine
1992 Ph.D. Kurume University Graduate School of Medicine

Professional Experience

1992 Assistant Professor, Department of Pathology, Kurume University School of Medicine
1993 Post Doc.> Visiting Assistant Professor (1994), Baylor College of Medicine, USA
1996 Visiting Assistant Professor, Division of Biochemistry, Osaka University Medical School
1997 Assistant Professor, Kurume University Research Center for Innovative Cancer Therapy, (and Departments of Pediatrics, Human Genetics and Surgery), Kurume University
2000 Associate Professor, Department of Gene Therapy and Regenerative Medicine, Gifu University School of Medicine
2003 Professor, Cognitive and Molecular Research Institute for Brain Diseases, (and Department of Pediatrics), Kurume University
2006 Professor and Chairman, Department of Gene Therapy and Regenerative Medicine, Kagoshima University Graduate School of Medicine and Dental Sciences

(Secondary appointment)
Director, South Kyushu Center for Innovative Medical Research and Application,
Director, Center for Innovative Therapy Research and Application,
Kagoshima University Graduate School of Medicine and Dental Sciences
Director, Center for Clinical and Translational Research, Kagoshima University Hospital
Visiting Faculty, Kurume University School of Medicine
Special Affiliated Research Associate, Gifu University School of Medicine
Advisory Board Member, Biologics Center for Research and Training

Recent Related Publications (5 Papers)

1. Mitsui K, Takahashi T, Ide K, Matsuda E, Kosai K.: Optimization of Adenoviral Gene Transfer in Human Pluripotent Stem Cells. ***Biochem Biophys Res Commun***. 541:78-83, 2021
2. Matsuda E, Obama Y, Kosai K.: Safe and low-dose but therapeutically effective adenovirus-mediated hepatocyte growth factor gene therapy for type 1 diabetes in mice. ***Life Sci***. 268:119014. 2021
3. Suzuki S, Kofune H, Uozumi K, Yoshimitsu M, Arima N, Ishitsuka K, Ueno S and Kosai K.: A survivin-responsive, conditionally replicating adenovirus induces potent cytocidal effects in adult T-cell leukemia/lymphoma. ***BMC Cancer***. 19(1):516. doi:10.1186/s12885-019-5730-1, 2019
4. Ide K, Mitsui K, Irie R, Matsushita Y, Ijichi N, Toyodome S, Kosai K.: A Novel Construction of Lentiviral Vectors for Eliminating Tumorigenic Cells from Pluripotent Stem Cells. ***Stem Cells***. 36:230-239, 2018 (Featured Article in this issue; Best of Japan, Virtual Issues, Stem Cells)
5. Sakamoto K, Khai NC, Wang Y, Irie R, Takamatsu H, Matsufuji H, Kosai K.: Heparin-binding epidermal growth factor–like growth factor and hepatocyte growth factor inhibit cholestatic liver injury in mice via different actions. ***Int J Mol Med***. 38(6):1673-1682, 2016

Research and Development, Nonclinical Studies and First-In-Human and Phase I/II Clinical Trials of Conditionally Replicating Adenovirus Targeting and Treating with Multiple Factors (m-CRA) for Next-Generation Oncolytic Virus Immunotherapy

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A couple of oncolytic viruses (OVs), inducing selective tumor killing and potentially systemic antitumor immunity, were regularly and conditionally approved by FDA, EMA and PMDA. Currently, there is a worldwide race to develop the best performing OVs and OV immunotherapy, but breakthroughs such as the discovery of immune checkpoint inhibitors have yet to be identified in clinical trials.

We first, in 1990s in USA, developed combination immune gene therapy strategies, which efficiently induced systemic antitumor immunity after a local injection of replication-defective adenovirus vector expressing a suicide gene and cytokine genes in animals (*PNAS* 1995, 1996, *Cancer Res* 1996 et al.). This expertise and concept led us to the development of the following OV immunotherapies. In early 2000s in Japan, we developed an original platform technology of "m-CRA", that is, "Conditionally replicating adenovirus (CRA) that targets and/or treats tumor cells with multiple factors", for efficiently developing candidates in the best OVs and the next-generation OV immunotherapies (*Gene Ther* 2005).

Using this platform technology, we have generated and tested numbers of m-CRAs as anticancer agents, and found that one of the best was survivin-responsive m-CRAs (Surv.m-CRAs). On basic research, Surv.m-CRAs induced more potent and cancer-specific (safer) effects against most of malignant tumors than other competing CRAs, including telomerase reverse transcriptase (Tert)-responsive m-CRAs (*Cancer Res* 2005). Moreover, Surv. m-CRAs induced increased effectiveness against cancer stem cells, which are resistant to conventional therapies (*J. Trans. Med.* 2014). We also demonstrated that OV, including Surv.m-CRA, can specifically eliminate tumorigenic pluripotent stem cells; the eradication of tumorigenesis is the most important issue in regenerative medicine (*Mol Ther Methods Clin Dev* 2017).

As a next step of translational research, we generated GMP products and preformed GLP nonclinical studies based on ICH guidelines. We performed and completed ICH-GCP First-In-Human clinical trials of Surv. m-CRA-1 (Surv.m-CRA without transgene) for refractory malignant bone and soft tissue tumors (Phase I: single-arm, open label study included 9 patients) in Kagoshima University. Patients underwent a single intratumoral injection of either 1×10e10 viral particle (vp), 1×10e11 vp or 1×10e12 vp. As a result, Surv.m-CRA-1 was well tolerated and showed more remarkable antitumor effects for prolonged periods than previously reported CRAs. Based on this result, in January 2021, we started Phase II multicenter clinical trial of Surv.m-CRA-1 for malignant bone tumors toward approval. We have been simultaneously doing Phase I/II clinical trial of Surv. m-CRA-1 for pancreatic cancer patients in Kagoshima University since 2020. All these investigator-initiated clinical trials have been funded by AMED.

We are also doing nonclinical development of Surv.m-CRA-2 (armed with a cytokine gene), which more strongly induced systemic antitumor immunity for metastatic cancers in animal experiments. On basic research, we have been developing a series of next-generation OV immunotherapies.

CURRICULUM VITAE

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Field of Research Cancer, Urology, oncolytic virus therapy

Education

1995 M.D. The University of Tokyo, Faculty of Medicine
2002 Ph.D. Medical Science The University of Tokyo, Graduate School of Medicine

Professional Experience

1995 Resident and clinical associate, Department of Urology, The University of Tokyo
1998 Research resident, National Cancer Center Research Institute
2001 Research fellow, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
2003 Instructor, Department of Urology, The University of Tokyo
2006 Assistant Professor, Department of Urology, Graduate School of Medicine, The University of Tokyo
2014 Associate Professor, Department of Urology, Graduate School of Medicine, The University of Tokyo
2018 Professor and Chairman, Department of Urology, Kyorin University School of Medicine

Recent Related Publications (5 Papers)

1. Fukuhara H, Takeshima Y, Todo T. Triple-mutated oncolytic herpes virus for treating both fast- and slow-growing tumor. ***Cancer Sci***, 2021, in press.
2. Matsumura S, Nakamori M, Tsuji T, Kato T, Nakamura M, Ojima T, Fukuhara H, Ino Y, Todo T, Yamaue H. Oncolytic virotherapy with SOCS3 enhances viral replicative potency and oncolysis for gastric cancer. ***Oncotarget***, 12, 344-354, 2021.
3. Taguchi S, Uemura Y, Fujimura T, Morikawa T, Naito A, Kawai T, Suzuki M, Kume H, Fukuhara H. Quantification of the individual risk of each Gleason pattern, including tertiary Gleason pattern 5, after radical prostatectomy: development of the modified Gleason grade grouping (mGGG) model. ***BMC Cancer***, 20, 371, 2020.
4. Watanabe S, Hayashi K, Toh K, Kim HJ, Liu X, Chaya H, Fukushima S, Katsushima K, Kondo Y, Uchida S, Ogura S, Nomoto T, Takemoto H, Cabral H, Kinoh H, Tanaka HY, Kano MR, Matsumoto Y, Fukuhara H, Uchida S, Nangaku M, Osada K, Nishiyama N, Miyata K, Kataoka K. In vivo rendezvous of small nucleic acid drugs with charge-matched block catiomers to target cancers. ***Nat Commun***. 10, 1894, 2019.
5. Taguchi S, Fukuhara H, Todo T. Oncolytic virus therapy in Japan: progress in clinical trials and future perspectives. ***Jpn J Clin Oncol***. 49, 201-209, 2019.

Phase 1 clinical trial of a third-generation oncolytic HSV-1, G47Δ in patients with castration-resistant prostate cancer

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We report the findings of a phase I dose-escalating study of a third-generation, triple-mutated oncolytic herpes virus type 1 (HSV-1), G47Δ, in patients with castration-resistant prostate cancer (CRPC). This is the first clinical trial in which oncolytic HSV-1 was used for the treatment of prostate cancer. G47Δ is engineered to selectively replicate in and kill cancer cells, and preclinical studies have shown G47Δ to be effective in prostate cancer models. G47Δ was recently approved in Japan as a new drug for malignant glioma.

In this single-armed phase I study, patients with prostate cancer that had not received prostatectomy and recurred after hormonal therapy, with or without prior record of chemotherapy and with or without remote metastases, are included. The clinical-grade G47Δ was manufactured at the GMP Vector Production Facility at the Institute of Medical Science, the University of Tokyo. The quality tests were performed extensively under GLP at four steps of manufacture.

A total of 6×10^8 to 1.2×10^9 plaque-forming units (pfu) of G47Δ were administered directly into the prostate tumor across two to four cycles in nine patients divided into three cohorts. The primary end point was the evaluation of treatment-related toxicity. G47Δ was well-tolerated in all patients, and only mild fever and transient changes in laboratory data were seen. The only reported toxicity over grade 3 was lymphocytopenia, which appeared in seven cases, all of which normalized by day 2. In four of nine cases a decrease in prostate-specific antigen (PSA) levels of over 50% were seen, for a response rate of 44%. In one patient, PSA levels remained below 50% of baseline for over 300 days post-treatment with no alternative therapy.

We conclude that intratumoral delivery of G47Δ in patients with CRPC was well-tolerated and feasible, and warrants further investigation.

Disclosure of COI:
Grants for commissioned/joint research; Ono Pharmaceutical Co. Ltd., Bayer Yakuhin, Ltd., Takeda Pharmaceutical Co. Ltd., Astellas Pharma Inc. for HF

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Field of Research Gene therapy, oncolytic viruses, oncology

Education

2000-2003 Hematology and Oncology Fellowship, University of California at San Francisco
1997-2000 Internal Medicine Residency, University of California at San Diego
1997 Doctor of Medicine Georgetown University School of Medicine,
1992 Bachelor of Science Biochemistry and Cell Biology, University of California at San Diego

Professional Experience

2019-Present CG Oncology: Chief Medical Officer
2018-Present Kalivir: Consulting Chief Medical Officer
2018-Present Oncomyx: Medical Advisor
2018-2019 Turnstone Bio: Senior Advisor
2015-2018 Turnstone Bio: Chief Medical Officer
2014-2018 Jennerex (later Sillajen): Chief Medical Officer
2010-2014 Jennerex (later Sillajen): VP of Clinical Research
2010-Present Consulting Physician, Billings Clinic, Department of Hematology and Oncology
2006-2010 Director Clinical Research, Billings Clinic, Department of Hematology and Oncology
2002-2006 Cell Genesys, Inc: Associate Director Clinical Research
2003-2005 Veterans Administration Medical Center, San Francisco: Attending Physician

Recent Related Publications (5 Papers)

1. Moehler M, Heo J, Lee HC, Tak WY, Chao Y, Paik SW, Yim HJ, Byun KS, Baron A, Ungerechts G, Jonker D, Ruo L, Cho M, Kaubisch A, Wege H, Merle P, Ebert O, Habersetzer F, Blanc JF, Rosmorduc O, Lencioni R, Patt R, Leen AM, Foerster F, Homerin M, Stojkowitz N, Lusky M, Limacher JM, Hennequi M, Gaspar N, McFadden B, De Silva N, Shen D, Pelusio A, Kirn DH, Breitbach CJ, Burke JM. Vaccinia-based oncolytic immunotherapy Pexastimogene Devacirepvec in patients with advanced hepatocellular carcinoma after sorafenib failure: a randomized multicenter Phase IIb trial (TRAVERSE). ***Oncoimmunology***. 2019 Jun 3;8(8):1615817
2. Burke J, Nieva J, Borad M, Breitbach C. Oncolytic Viruses: Perspectives on Clinical Development. ***Curr Opin Virol***. 2015 May 16;13:55-60.
3. Breitbach C, Bell, J, Wang T, Kirn D, Burke J. The Emerging Therapeutic Potential of the Oncolytic Immunotherapeutic Pexa-Vec (JX-594). ***Oncolytic Virotherapy***. 2015:4 25-31
4. Burke JM, Lamm DL, Meng MV, Nemunaitis JJ, Stephenson JJ, Arseneau JC, Aimi J, Lerner S, Yeung AW, Kazarian T, Maslyar DJ, McKiernan JM. A First in Human Phase 1 Study of CG0070, a GM-CSF Expressing Oncolytic Adenovirus, for the Treatment of Nonmuscle Invasive Bladder Cancer. ***Journal of Urology***. 2012 Dec;188(6):2391-7.
5. Breitbach CJ, Burke J, Jonker D, Stephenson J, Haas AR, Chow LQ, Nieva J, Hwang TH, Moon A, Patt R, Pelusio A, Le Boeuf F, Burns J, Evgin L, De Silva N, Cvancic S, Robertson T, Je JE, Lee YS, Parato K, Diallo JS, Fenster A, Daneshmand M, Bell JC, Kirn DH. Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. ***Nature***. 2011 Aug 31;477(7362):99-102.

CORE-001: Phase 2, Single Arm Study of CG0070 Combined with Pembrolizumab in Patients with Non Muscle Invasive Bladder Cancer (NMIBC) Unresponsive to Bacillus Calmette-Guerin (BCG)

James Burke[1], Roger Li[2], Donald Lamm[3], Ed M. Uchio[4], Gary Steinberg[5], Vignesh T. Packiam[6], Ashish M. Kamat[7], Michael J. Chisamore[8], John McAdory[1], Paola Grandi[1], Jee-Hyun Kim[1]

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INTRODUCTION AND OBJECTIVE: CG0070, an oncolytic vaccine available as an intravesical therapy, is a serotype 5 adenovirus engineered to express GMCSF and replicate selectively in tumor cells with mutated or deficient RB. The CG0070 mechanism of action includes direct cell lysis in conjunction with immune mediated cell death which is enhanced in the presence of GM-CSF. In an open label phase 2 study, an overall CR rate of ~62% and a CR at 12 months (m) of 29% have been observed in patients with high risk NMIBC previously treated with BCG. Intravenous pembrolizumab, a PD-1 checkpoint inhibitor, was recently approved by the FDA for patients with BCG-unresponsive CIS (with or without papillary tumors) with an overall complete RR of 41% and a 12 m CR rate of ~20%. This phase 2 study will assess the potential synergy of the two agents in the treatment of BCG-unresponsive NMIBC.

METHODS: 35 patients with BCG-unresponsive CIS with or without concurrent Ta or T1 disease will be treated with intravesical (IVE) CG0070 at a dose of 1x1012 vp in combination with pembrolizumab at a dose of 400 mg IV q6 weeks. CG0070 will be administered weekly x 6 as induction followed by weekly x 3 maintenance instillations at months 3, 6, 9, 12, and 18. Patients with persistent CIS or HG Ta at 3 m may receive re-induction with weekly x 6 of CG0070. Pembrolizumab will be administered up to 24 m. Assessment of response will include q 3 m cystoscopy with biopsy of areas suspicious for disease, urine cytology, CTU/MRU, and mandatory bladder mapping biopsies at 12 m. Recurrence of HG disease will be enumerated as disease recurrence. The primary endpoint of the study is CR at 12 m. Secondary endpoints will include CR at any time, progression free survival, duration of response, cystectomy free survival and the safety of the combination. Correlate assessments will include changes in the tumor immune microenvironment, systemic immune induction reflected in the peripheral blood and urine, as well as viral replication and transgene expression. Baseline expression of PD-L1, coxsackie adenovirus receptor, E2F transcription factor as well as anti-adenovirus antibody titer will be correlated with tumor response.

RESULTS: At this time there have been 6 patients accrued to this study, of whom 5 are evaluable for 3 m CR and 6 for safety. Assessment of these 5 patients demonstrates 100% 3 m CR. Thus far, treatment related AE have been limited to transient grade 1-2 urinary frequency (3 patients) and grade 1 bladder spasm, hematuria, painful urination, thyroiditis, and flu-like symptoms (one patient each). No grade 3, 4, or 5 AE or SAE were observed.

CONCLUSIONS: Based on preliminary data, the combination of CG0070 and pembrolizumab for BCG unresponsive NMIBC has been well tolerated with encouraging early efficacy at 3 m. At the time of presentation, safety and efficacy results will be further updated.

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Field of Research Molecular Biology

Education

1984	B.Sc.	Kyoto University
2007	Ph.D.	Kagoshima University

Professional Experience

1984-1989	Stuff, Kusu Factory, Takara Shuzo Co., Ltd, Japan
1989-1993	Researcher, Bioproducts Development Center, Takara Shuzo Co., Ltd, Japan
1993-1998	Manager, Marketing, Takara Shuzo Co., Ltd, Japan
1998-2004	Senior Scientist, DNA Function Analysis Center, Takara Bio Inc., Japan
2004-2011	Director, Center for Cell and Gene Therapy, Takara Bio Inc., Japan
2011-2014	Vice President of Gene Medicine Unit, Takara Bio Inc., Japan
2014-2019	President of Bioindustry Business Unit, Takara Bio Inc., Japan
2019-Present	COO, Takara Bio Inc., Japan

Recent Related Publications (5 Papers)

1. Sato Y, et al. Integrative immunogenomic analysis of gastric cancer dictates novel immunological classification and the functional status of tumor-infiltrating cells, ***Clin Transl Immunology***. 2020 Oct 17;9(10)
2. Kawamura K, et al. Development of a Unique T Cell Receptor Gene-Transferred Tax-Redirected T Cell Immunotherapy for Adult T Cell Leukemia, ***Biol Blood Marrow Transplant***. 2020 Aug;26(8):1377-1385.
3. Tomono T, et al. Infectivity Assessment of Recombinant Adeno-Associated Virus and Wild-Type Adeno-Associated Virus Exposed to Various Diluents and Environmental Conditions., ***Hum Gene Ther Methods***. 2019 Aug;30(4):137- 143.\
4. Okamoto S, et al. Highly efficient genome editing for single-base substitutions using optimized ssODNs with Cas9-RNPs., ***Sci Rep***. 2019 Mar 18;9(1):4811.
5. Uchibori R, et al. Functional Analysis of an Inducible Promoter Driven by Activation Signals from a Chimeric Antigen Receptor, ***Mol Ther Oncolytics***. 2018 Dec 1;12:16-25.

Efforts to address the challenges in TCR/CAR gene therapy for cancer

Junichi Mineno

Takara Bio Inc.

Adoptive T cell therapy using the genetically engineered lymphocytes to express tumor antigen-specific T-cell receptor (TCR) /chimeric antigen receptor (CAR) is an attractive strategy for treating cancer patients. To date, CAR-T therapies targeting CD19 and BCMA have been approved for several indications such as B-ALL, DLBCL, MCL, and more than 400 CAR-T trials and around 40 TCR -T trials are recruiting patients. In spite of recent clinical efficacy of TCR/CAR-T therapies, further optimization and modification will be needed to improve the efficacy and safety.

In TCR-T therapy, surface expression level of TCRs affect the therapeutic efficacy, and the existence of endogenous TCRs is one of the reasons for inefficient expression of the introduced TCRs. Furthermore, miss-paired TCR dimers with unknown specificities can cause autoimmunity. To overcome those problems, we have developed siTCR® vector which can simultaneously express siRNAs to silence endogenous TCRs and tumor antigen-specific TCRs. T cells transduced with siTCR® vectors could efficiently express the introduced TCR with reduced expression of the endogenous TCR at relatively low proviral copy number. TCR -T therapy using the siTCR® vector expressing HLA-A*0201 restricted NY-ESO-1-specific TCR genes for synovial sarcoma was conducted as an investigator-initiated clinical trial in Japan.

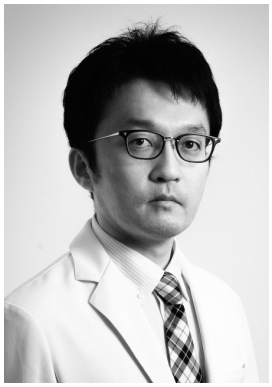
Despite recent successes of CAR-T therapy, considerable clinical challenges remain to be addressed. Excessive T cell activation leads exhaustion and depletion of naïve/memory subsets important for durable clinical responses. Thus, the CAR construct needs to be optimized so that transduced T cells persist and induce potent antigen-specific response with minimal non-specific activation. Recently, Dr. Hirano's group at Princess Margaret Cancer Centre has developed a new generation JAK-STAT CAR composed of a truncated cytoplasmic domain of the IL-2 receptor β chain and STAT3/5 binding motifs, and the JAK-STAT CAR-T cells showed antigen-specific activation of the JAK-STAT signaling pathway, enhanced proliferation, and limited terminal differentiation compared to second generation CAR-T cells, which has the potential to demonstrate improved clinical efficacy. In regard to the target antigen, the tumor associated antigens which are also expressed weakly in normal tissues are used for the major targets of CAR-T cells, and on-target, off-tumor toxicity may arise the safety concerns. Particularly when targeting antigens expressed on the T cells, CAR expressed on T cells react with the cognate antigen on the CAR-T cells, result in fratricide, limited expansion, cell differentiation, and exhaustion of CAR-T cells during cell manufacturing. Recently, we have developed the CD38 CAR-T cells manufacturing method combining JAK-STAT construct, siRNAs to knockdown CD38, and tyrosine kinase inhibitor dasatinib treatment and the resultant CAR-T cells may persist longer and induce potent antigen-specific response with minimal excessive unfavorable activation. This approach can be applied to other CAR-T cells targeting the shared antigen expressed on T cells.

11th Takara Bio Award

Abstract & Curriculum Vitae

Chairperson: Tomoki Todo

CURRICULUM VITAE



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Field of Research Extracellular vesicles, Oncolytic virus, Gastroenterology

Education

2003-2009	M.D.	Saga University School of Medicine. Saga, Japan
2017-Present		Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. Okayama, Japan

Professional Experience

2009-2011	Resident in General Medicine, Okayama Saiseikai Hospital, Japan
2011-2014	Resident in General Surgery, Okyama Saiseikai Hospital, Japan
2014-2016	Chief in General Surgery, Shobara Red Cross Hospital, Japan
2016-2017	Clinical Fellow in Gastroenterological Surgery, Okayama University
2019-Present	Clinical Fellow in Gastroenterological Surgery, Okayama University

Recent Related Publications (5 Papers)

1. Kakiuchi Y, Kuroda S, Kanaya N, Kumon K, Tsumura T, Hashimoto M, Yagi C, Sugimoto R, Hamada Y, Kikuchi S, Nishizaki M, Kagawa S, Tazawa H, Urata Y, Fujiwara T. Local oncolytic adenovirotherapy produces an abscopal effect via tumor-derived extracellular vesicles. ***Mol Ther***. 2021 May 20:S1525-0016(21)00267-7.
2. Kanaya N, Kuroda S, Kakiuchi Y, Kumon K, Tsumura T, Hashimoto M, Moriniro T, Kubota T, Aoyama K, Kikuchi S, Nishizaki M, Kagawa S, Tazawa H, Mizuguchi H, Urata Y, Fujiwara T. Immune modulation by telomerase-specific oncolytic adenovirus synergistically enhances antitumor efficacy with anti-PD1 antibody. ***Mol Ther***. 2020 Mar 4;28(3):794-804.
3. Morihiro T, Kuroda S, Kanaya N, Kakiuchi Y, Kumon K, Tsumura T, Hashimoto M, Yagi C, Sugimoto R, Hamada Y, Kikuchi S, Nishizaki M, Kagawa S, Tazawa H, Urata Y, Fujiwara T. PD-L1 expression combined with microsatellite instability/CD8+ tumor infiltrating lymphocytes as a useful prognostic biomarker in gastric cancer. ***Sci Rep***. 2019 Mar 15;9(1):4633.
4. Kubota T, Kuroda S, Kanaya N, Morihiro T, Aoyama K, Kakiuchi Y, Kikuchi S, Nishizaki M, Kagawa S, Tazawa H, Fujiwara T. HER2-targeted gold nanoparticles potentially overcome resistance to trastuzumab in gastric cancer. ***Nanomedicine***. 2018 Aug;14(6)1919-1929.
5. Aoyama K, Kuroda S, Moriro T, Kanaya N, Kubota T, Kakiuchi Y, Kikuchi S, Nishizaki M, Kagawa S, Tazawa H, Fujiwara T. Liposome-encapsulated plasmid DNA of telomerase-specific oncolytic adenovirus with stealth effect on the immune system. ***Sci Rep***. 2017 Oct 26;7(1):14177.

Local oncolytic adenovirotherapy produces an abscopal effect via tumor-derived extracellular vesicles

Yoshihiko Kakiuchi, Shinji Kuroda, Nobuhiko Kanaya, Kento Kumon, Tomoko, Tsumura, Masashi Hashimoto, Chiaki Yagi, Ryoma Sugimoto, Yuki Hamada, Satoru, Kikuchi, Masahiko Nishizaki, Shunsuke Kagawa, Hiroshi Tazawa, Yasuo Urata, Toshiyoshi Fujiwara

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Extracellular vesicles (EVs) play important roles in various intercellular communication processes. The abscopal effect is an interesting phenomenon in cancer treatment, in which immune activation is generally considered a main factor. We previously developed a telomerase-specific oncolytic adenovirus, Telomelysin (OBP-301), and occasionally observed therapeutic effects on distal tumors after local treatment in immunodeficient mice. Here, we hypothesized that EVs may be involved in the abscopal effect of OBP-301. EVs isolated from the supernatant of HCT116 human colon carcinoma cells treated with OBP-301 were confirmed to contain OBP-301, and showed cytotoxic activity (apoptosis and autophagy) similar to OBP-301. In bilateral subcutaneous HCT116 and CT26 tumor models, intratumoral administration of OBP-301 produced potent antitumor effects on tumors that were not directly treated with OBP-301, involving direct mediation by tumor-derived EVs containing OBP-301. This indicates that immune activation is not the main factor in this abscopal effect. Moreover, tumor-derived EVs exhibited high tumor tropism in orthotopic HCT116 rectal tumors, in which adenovirus E1A and adenovirus type 5 proteins were observed in metastatic liver tumors after localized rectal tumor treatment. In conclusion, local treatment with OBP-301 has the potential to produce abscopal effects via tumor-derived EVs.

Plenary Session

Abstracts

Plenary Session 1

Chairpersons: Kazunori Aoki & Yoshikazu Yonemitsu

Plenary Session 2

Chairpersons: Masatoshi Tagawa & Koichi Miyake

Plenary Session

PS1-1

Improving the in vivo gene targeting efficiency of liver – directed rAAV vector using the nucleotide analog class of ribonucleotide reductase inhibitors

Shinnosuke Tsuji[1,2], Calvin Stephens[1], Giulia Bortolussi[3], Feijie Zhang[1], Katja Pekrun[1], Gabriele Baj[4], Gustavo Alencastro[1], Andrés Muro[3], Mark Kay[1]

[1] Departments of Pediatrics and Genetics, Stanford University, Stanford, California, USA
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While the liver is one of the most attractive target organs for gene therapy using recombinant adeno-associated viral (rAAV) vectors, their use in neonatal or pediatric patients presents several challenges. These include loss of episomal vector genomes resulting from normal or disease related liver growth. Second, the potential for insertional mutagenesis and vector-promoter activation of a proto-oncogene induced hepatocellular carcinoma remains a concern. In order to expand the clinical application of rAAV, our group previously reported a strategy using a rAAV vector for homologous recombination (AAV-HR) without the use of nucleases (Barzel, et al, Nature, 2015) in which we can specifically target a therapeutic coding sequence onto the end of an endogenous coding gene before the translation termination signal. Thus, a chimeric mRNA is produced that makes both the endogenous and therapeutic protein. This technology has been used to treat mouse models of hemophilia B, Crigler-Naajar, ZZ alpha-1- antitrypsin deficiency and methylmalonic acidemia.

In current and prior studies, we and others found that the efficiency of AAV-HR in mouse liver occurs at best in ~1% of hepatocytes. This limits the clinical application of the technology for some disorders. Here, we tested a series of small molecule compounds with various different mechanisms of action in the context of AAV-HR and identified that ribonucleotide reductase (RNR) inhibitors significantly enhanced the AAV-HR efficiency in mouse and human liver cell lines by 2 to 5 fold. Because these drugs may have multiple modes of action, we used RNAi knockdown to confirm reduction of RNR activity contributed to the increased HR efficiency. Furthermore, short term administration of an FDA-approved nucleotide analog type of RNR inhibitor, fludarabine, increased the in vivo efficiency of AAV-HR by about 4-5 fold in murine liver at a dose, which did not cause serious toxicity. In contrast, hydroxyurea failed to show in vivo efficacy possibly related to its mechanism of action as well as its low accumulation in the liver. In addition, we were able to show that fludarabine administration induced transient DNA damage signaling in both proliferating and quiescent hepatocytes. Surprisingly, in vivo BrdU labeling implicated that the majority of AAV-HR events occurred in non-proliferating hepatocytes in both the fludarabine and no drug treatment animals. These data suggest that induction of transient DNA repair signaling in non-dividing hepatocytes is responsible for enhancing the efficiency of AAV-HR in mice treated with fludarabine. We also demonstrated that fludarabine treatment significantly increased the in vivo CRISPR/Cas9-mediated hepatic gene editing efficiencies. In total, we show that the transient use of RNR inhibitors at the time of AAV vector administration can mainly act on quiescent cells and provide a safe strategy to enhance genome editing events for therapeutic purposes.

LassoGraft Technology® allows rapid and simple generation of engineered AAV vectors with defined receptor dependency

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We have recently developed a novel method called LassoGraft Technology®(LGtech for short), which combines a highly efficient macrocylclic peptide discovery system (RaPID system) and a sophisticated structure-based protein engineering to convert any proteins into a specific and high-affinity binder against a target molecule1). The conversion is achieved by “grafting” the selected peptide sequence at surface-exposed loop in the base proteins, leading to a modified protein with a new binding function without losing its original activity. LGtech has already been applied successfully onto many base proteins including IgG, Fc, albumin, and many more extracellular proteins, and these novel engineered molecules have potential to become the next generation biologics, collectively called “Neobiologics”. We also applied this technology to the capsid protein of adeno-associated virus (AAV), to obtain desirable vector for gene therapy with altered cellular/tissue tropism. AAV capsid engineering involving mutagenesis-based random screening and/or insertion of non-viral protein moieties into the capsid has been attempted yielding some successes, but problematically it is labor-demanding if one wishes to implant new specificity on AAV. On the other hand, the modularity of the LGtech method enabled us to readily alter the specificity of AAV. First, we eliminated the native infectivity of AAV2 or AAV1 by inserting an inert 12-residue tag peptide into the ""loop IV"" of the Cap proteins. Then, this ""infection-null"" Cap subunit was co-expressed with mutant Cap harboring targeting peptides at the same loop to obtain chimeric capsid particles. We have used two targeting peptides, aMD4 (YRQFNRRTHEVWNLD) binding to MET receptor and m6A9 (WRPYIERWTGRLIV) binding to Plexin B1. Both chimeric AAVs did not transduce parental cell lines devoid of MET or Plexin B1 expression, but they successfully transduced cells stably expressing these receptors. Thus, we achieved rapid and one-step generation of engineered AAV vectors that can infect cells solely dependent on the targeted receptor.

1) Mihara et al. Lasso-grafting of macrocyclic peptide pharmacophores yields multi-functional proteins. Nature Commun. 12: 1543 (2021)

Plenary Session
PS1-3

Elucidation of the mechanism of reovirus-mediated antifibrotic effects on liver fibrosis

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Liver fibrosis is induced by various types of hepatic damages, including virus infection and alcoholic hepatitis. Hepatic damages lead to the production of transforming growth factor-b (TGF-b). TGF-b activates hepatic stellate cells, which play a crucial rule in liver fibrosis by differentiating into myofibroblasts and producing large amounts of extracellular matrices (ECM). Overexpression of ECM results in liver fibrosis. Advanced liver fibrosis and cirrhosis are major risk factors for hepatocellular carcinoma. Although there are many patients suffering from liver fibrosis, there are few effective anti-fibrotic drugs.

Mammalian Orthoreovirus, (hereafter reovirus), which is a non-enveloped virus containing a 10 segmented dsRNA genome, has attracted much attention as an oncolytic virus due to the promising properties, including efficient antitumor effects and lack of pathogenicity. Clinical trials against various types of cancers have been internationally going. We previously demonstrated that reovirus showed efficient antifibrotic effects on the CCl4-induced liver fibrosis mice following intravenous administration (JSGCT annual meeting 2018), however, it had remained to be clarified how reovirus ameliorated liver fibrosis. In this study, we elucidated the mechanism of reovirus-mediated antifibrotic effects on liver fibrosis.
First, we examined the intrahepatic distribution of reovirus following intravenous administration. Immunohistochemical and western blotting analysis of virus capsid protein demonstrated that reovirus was taken up by not only hepatocytes but also hepatic stellate cells in the liver.

Next, we examined the antifibrotic effects of reovirus on a human hepatic stellate cell line, LX-2 cells. Pre-incubation of LX-2 cells with TGF-b significantly increased the expression levels of fibrotic marker genes, including a-smooth muscle actin (a-SMA) and type I collagen (Col1A1). Treatment with reovirus at a multiplicity of infection (MOI) of 20 significantly reduced the mRNA levels of a-SMA and Col1A1 genes by more than 50%. Viabilities of LX-2 cells were not largely decreased by treatment with reovirus. Apparent apoptosis of hepatic stellate cells were not found in the liver fibrosis mice following reovirus administration. These data indicated that reovirus was taken up by hepatic stellate cells and directly suppressed the expression of fibrotic marker genes in liver satellite cells without apparent cytotoxicity.

Finally, we investigated which cellular signal molecules were involved in reovirus-mediated antifibrotic effects. We found that Smad2/3, which are transcriptional factors on the downstream of TGF-b signal, were downregulated by reovirus infection in activated LX-2 cells. Furthermore, we revealed that molecule X regulated Smad2/3 expression, and molecule X expression was reduced by reovirus infection. Downregulation of molecule X was also found in CCl4-induced liver fibrosis mice following reovirus administration. These results indicated that following intravenous administration, reovirus was taken up by activated hepatic stellate cells, resulting in downregulation of molecule X expression, followed by reduction in Smad2/3 expression and amelioration of liver fibrosis.

Fusogenic oncolytic vaccinia virus enhances systemic antitumor immune response and sensitivity to immune checkpoint blockade by remodeling the tumor microenvironment

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Oncolytic virotherapy is a novel anti-cancer strategy which shows remarkable clinical benefits. Oncolytic virus lyses tumors and subsequently induces anti-tumor immunity. Oncolytic viruses are most often treated intratumorally. This approach provides powerful anti-tumor effect against the injected tumors via anti-tumor immune response following oncolysis. However, in the non-injected tumors, such as metastatic tumor region, their therapeutic potentials are reduced because it depends only on indirect anti-tumor immunity elicited after injected tumor lysis. In this study, we present novel phenotypic oncolytic vaccinia virus, which enhanced oncolytic and immunotherapeutic potential by cell-cell fusion.
Vaccinia virus, basically known as a smallpox vaccine and a non-fusogenic virus, is recently used as an oncolytic agent. We previously developed MAPK-dependent recombinant vaccinia virus (MDRVV), which achieved tumor specific viral replication by deletion of two viral growth factors, VGF and O1L. We recently have isolated a mutant clone which induces cell-cell fusion from non-fusogenic MDRVV. Whole-genome sequencing identified that the fusogenic oncolytic vaccinia virus (FUVAC) has nonsense mutation in viral K2L gene encoding cell-cell fusion inhibitor, although the deletions of VGF and O1L were maintained. FUVAC enhanced the cytopathic effect against multiple human and murine cancer cell lines in vitro, compared with MDRVV. Furthermore, FUVAC efficiently caused apoptosis, necrosis and especially immunogenic cell death than MDRVV.

The in vivo oncolytic activity was examined in syngeneic murine tumor model, which has bi-flank subcutaneous CT26 tumors. In the injected tumors, immunohistochemical analysis showed FUVAC had larger viral replication than MDRVV. FUVAC-treated tumors showed the fusogenic phenotype at the boundary between infected and non-infected regions. Both viruses did not exist in non-injected tumors. Nevertheless, FUVAC significantly inhibited the tumor growth not only in the injected tumors, but also in the non-injected tumors. For immunological analysis, tumor-infiltrating lymphocytes were examined by flow cytometry. FUVAC remarkably increased the $CD8^+$ T cell infiltration especially in the non-injected tumors, compared with MDRVV. Furthermore, FUVAC reduced tumor-associated immune suppressive cells such as regulatory T cells, myeloid-derived suppressor cells and tumor-associated macrophages in the injected tumors. In accordance with these symptoms, oncolytic activity of FUVAC was completely suppressed by depletion of $CD8^+$ T cells, but not $CD4^+$ T cells. On the other hand, combination of FUVAC and PD-1 blockade strongly promoted their anti-cancer function. 3 of 6 mice treated with FUVAC and αPD-1 antibody showed complete response in both tumors, while MDRVV and αPD-1 treated mice could not eliminate the non-injected tumors.

Our study demonstrates that FUVAC has higher potential for oncolytic immunotherapy. FUVAC induces cell-cell fusion and modulates tumor immune microenvironment through decreasing the tumor-associated immune suppressive cells locally and increasing the cytotoxic T lymphocytes systemically. Therefore, FUVAC would be better therapeutic platform and maximize systemic antitumor immune response in combination with immune checkpoint blockades.

Plenary Session

PS2-2

Hematopoietic Stem Cell Gene Therapy Corrects Lysosomal Storage in CNS in Murine Model of GM1-gangliosidosis

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Introduction: GM1 gangliosidosis is progressive neurodegenerative glycoshingolipidosis due to a mutation in GLB1 gene, causing the deficiency of lysosomal enzyme β-galactosidase (β-gal), which leads to the abnormal accumulation of GM1 ganglioside primarily in the central nervous system (CNS). In most severe phenotype, excessive ganglioside accumulation results in a rapid decline in neurological and psychomotor function, and death occurs within two years of age. Currently, there are no effective therapies for the treatment of GM1-gangliosidosis. In this study, we evaluated the therapeutic efficacy of ex vivo lentiviral gene therapy on the neurological involvement of GM1 gangliosidosis mice.

Materials and Methods: We constructed the recombinant lentivirus vector (LV) to transduce the cells with the normal GLB1 cDNA or enhanced green fluorescent protein (eGFP). Bone marrow cells were harvested, and lineage negative cells were separated from GM1 gangliosidosis mice. Separated cells were transduced with LV and cultivated for 24 hours. Transduce lineage-negative cells (2.0x10^6 cells) were administered through the tail vein to 8 weeks-old GM1 gangliosidosis mice after conditioning with 9Gy of total body irradiation (LV-GLB1 or LV-eGFP). Non-treated model mice (KO) and wild type C57B6/J (WT) were used as control. Serum was collected just before injection and every four weeks. Behavioral analysis was conducted at 8 and 30-32 weeks old. Visceral organs and brain were collected at 24 weeks old. The β-gal activity was measured in each organ, and GM1 ganglioside content was measured in the brain. An immunohistological evaluation was performed in the cerebrum, cerebellum, and hippocampus.

Result: LV-GLB1 group displayed a superphysiological level of β-gal activity in visceral organs. In CNS, LV-GLB1 group showed a significant increase in β-gal activity ($p<0.05$) and a decrease in accumulation of GM1 ganglioside in cerebrum and cerebellum ($p<0.05$) compared to KO group. In behavioral analysis, a rota-rod test at 32 weeks old detected an improvement in motor function for LV-GLB1 group compared with LV-eGFP group ($p<0.05$). In histological analysis, reduction in GM1 ganglioside content and improvement in neuroinflammatory response was observed, especially in the cerebrum.

Conclusion: These results suggest that ex vivo gene therapy with LV can ameliorate biochemical abnormalities in impaired organs, inhibit motor function decline and neurodegeneration in GM1 gangliosidosis mice. We have shown that ex vivo gene therapy could be a feasible therapeutic strategy in GM1 gangliosidosis.

Sustained fetal hemoglobin induction allowed by BCL11A interference with a truncated erythropoietin receptor

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Background: Hematopoietic stem cell gene therapy for hemoglobin disorders, such as sickle cell disease, requires high-level gene marking and robust therapeutic globin expression in erythroid cells (>20% of γ- or β-globin production). We previously demonstrated that lentiviral transduction of a truncated human erythropoietin receptor (thEpoR) gene allows for erythropoietin-dependent selective proliferation of gene-modified human erythroid cells during in vitro differentiation and in xenograft mice (ASH 2017, 2019). In this study, we evaluated whether thEpoR can enhance the phenotypic effect of a therapeutic vector in erythroid cells in a non-human primate model.

Methods: To investigate this hypothesis, we designed lentiviral vectors encoding both thEpoR and BCL11A-targeting micro RNA-adapted short hairpin RNA (shmiBCL11A) driven by an erythroid specific ankyrin 1 (ANK1) promoter. Rhesus CD34+ cells were collected and transduced with shmiBCL11A vector (n=2, compared to a GPA promoter-derived shmiBCL11A vector) or thEpoR-shmiBCL11A vector (n=2, compared to a Venus-encoding vector). Transduced CD34+ cells were transplanted into autologous rhesus macaques following 10Gy total body irradiation.

Results: Efficient transduction was observed in CD34+ cells in vitro among all 4 macaques (VCN 3.8-8.7), similar to the previous rhesus transplantation with successful gene marking in vivo. In shmiBCL11A transduction animals, blood recovery was observed around 1 month post-transplant. Engraftment of gene-modified cells (VCN 0.2-2.6) and robust HbF induction (14-16%) were observed at early timepoints (1-2 months) post-transplant. Lentiviral gene marking remained positive at VCN 0.1-0.4; however, HbF levels gradually reduced to less than 1% in both animals 6 months post-transplant. We also observed low HbF-positive percentages (%F-cell) (1.6-1.9%) in both animals ~1 year post-transplant.

In thEpoR-shmiBCL11A transduction, we observed blood recovery around 1 month post-transplant, and importantly, baseline red blood cell counts, hemoglobin concentration, and reticulocyte counts were not elevated. Gene-modified cells were engrafted in both animals, with stable gene marking (VCN 0.6-1.4) 1 year post-transplant. In contrast to shmiBCL11A transduction animals, robust HbF induction (16-18%) was observed 1 month post-transplant, and HbF levels climbed to absolute levels of 23-31% 1 year post-transplant in both animals. We also observed persistently high %F-cell (30-35%) by flow cytometry. These data demonstrate that shmiBCL11A transduction results in transient HbF induction in gene-modified erythroid cells, while thEpoR-co-expression allows for sustained HbF induction with shmiBCL11A.

Conclusion: We developed erythroid-specific thEpoR-shmiBCL11A vectors, enhancing HbF induction in rhesus macaques. The sustained therapeutic-level HbF induction allowed by addition of thEpoR represents a viable gene therapy strategy for hemoglobin disorders.

Oral Session

Abstracts

Sequence analysis of off-target mutation sites induced by SpCas9 using guide RNAs with low specificity

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Genome editing is expected to be a powerful tool for the treatment of intractable human diseases. However, off-target mutation has been recognized as a major safety concern in clinical settings. To know the basis of off-target mutation prediction, we investigated the sequence features of off-target mutation sites induced by the frequently used Streptococcus pyogenes Cas9 (SpCas9). For this purpose, we intentionally designed guide RNAs with low specificity to human genomic sequences to examine as much sequences of off-target mutation sites as possible. The guide RNAs were next used for SpCas9-mediated genome DNA cleavage in vitro, and the cleavage sites were analyzed by next-generation sequencing. To classified the sequences of off-target mutation sites, a complementarity measure called the 'distance' (d), which is defined as the total number of mismatches, insertions, or deletions between the guide RNA and the complementary DNA sequences was used (see reference below). As a result, we found that most of the cleavage sites had sequences with up to d=6 complementarity with the guide RNA. We also noticed that cleavage occurred even when the protospacer adjacent motif (PAM) sequence was not typical (5'-NGG-3').

Reference: Yoshida et al, Evaluation of off-target effects of gapmer antisense oligonucleotides using human cells. Genes to Cells, 24, 827-835 (2019) doi: 10.1111/gtc.12730

Development of an efficient drug delivery system for artificial nucleotide-based anti-sense oligos with recombinant adeno-associated virus empty particles

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Background: Artificial nucleotides can induce gene specific transcriptional modifications and they are more resistant to intracellular degradation and have a higher thermal stability than DNA and RNA, making them an ideal option in the therapeutic regulation of gene expression as anti-sense oligonucleotides (ASOs). Nevertheless, their efficient delivery to target tissues remains challenging. Therefore, we hypothesized that recombinant adeno-associated virus empty particles (rAAV-EPs) can be used as a delivery system for artificial nucleotides. In this work, we investigated if artificial nucleotide ASOs can be packaged into rAAV-EPs by adding a partial Inverted Terminal Repeat (pITR) sequence, allowing for their specific delivery to target cells. The use of a partial sequence instead of the full ITR region of the AAV genome has been reported to reduce the risk of integration into the host cell genome, also decreasing the activation of the innate immunity in the target cells, so it can improve the safety and efficacy of the delivery.

Material and Methods: We conjugated the pITR packaging sequence to artificial nucleotide ASO sequences, to allow for the encapsidation to rAAV-EPs. Two different ASOs were used, including (1) a gapmer incorporating Locked Nucleic Acids (LNAs) to degrade ZsGreenDR mRNA and (2) a Mixmer including ethylene-bridged nucleic acids (ENAs) to induce exon-skipping (exon 23) in the dystrophin gene as a therapeutic option for Duchenne muscular dystrophy (DMD). We produced rAAV8-EPs including LNA ASO/pITR sequences by transfection of HEK293EB cells and the efficacy of packaging as well as their biological activity was evaluated.

Results: We confirmed that conjugation between the LNA ASO and the pITR sequence did not reduce its mRNA interfering activity (29% efficacy for both ASO and ASO/pITR), and after preparation of ASO/ITRs encapsidated into rAAV8-EPs we evaluated the inclusion of the ASO/pITR sequences in the EPs by quantitative RT-PCR (1.47E+13 genome copies/mL). Transduction with the LNA-ASO/pITR-rAAV8-EPs decreased the expression of ZsGreenDR on the reporter cells (36.1% vs. 42.8% positive cells by cytometry, $p<0.05$). For the ENA ASOs we examined the activity of the conjugated ENA-ASO/pITR mixmers when directly transfected to C2C12 myoblasts using digital droplet PCR quantification, observing dystrophin exon23 skip (in 4.78% of the cell's dystrophin mRNA), we are currently in the process of producing rAAV8-EPs packaging the ENA-ASO/pITR.

Conclusions: We show that the addition of a short packaging signal sequence allows for a rAAV-EP-based delivery of artificial nucleotide ASOs to target cells without compromising their function. The rAAV-EP-based method would allow an efficient delivery of therapeutic ASOs in various diseases, offering a method for improving safety and efficacy.

Neuroprotective effects of induced pluripotent stem cell-derived mesenchymal stem cells on brain injury following focal cerebral ischemia in rats

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Background: Mesenchymal stem cells (MSCs) transplantation is expected to improve motor and cognitive functions after stroke. We have demonstrated that bone marrow mesenchymal stem cells (BMMSCs) and dental pulp mesenchymal stem cells showed the neuroprotective effects in focal cerebral ischemia models. However, MSC therapy for brain injury remains problematic due to the scalability limitation and changes in cell properties during in vitro culture and passaging. Therefore, an alternative cell source of MSC therapy is needed. Induced pluripotent stem cells (iPSCs) offer a self-renewable cell source that can be established from autologous somatic cells and differentiated into various types of cells including MSCs. It remains unclear whether iPSC-derived mesenchymal stem cells (iMSCs) have neuroprotective effects in acute cerebral infarction, and if so, by what mechanism. In this study, we investigated the effect of iMSCs via iPSC-derived NCCs (iNCCs) using a rat focal cerebral ischemia model.

Methods: iMSCs were generated from human iPSCs via iNCCs as intermediates. For differentiation of iPSCs into iNCCs, iPSCs were incubated with a chemically defined medium and FACS-sorted by CD271 expression. The CD271-positive iNCCs were further differentiated into iMSCs by incubation with α-MEM supplemented with 10% FBS. iMSCs were characterized with MSC-specific markers by flow cytometry. Male Sprague-Dawley rats underwent transient (90 min) middle cerebral artery occlusion. BMMSCs (1 x 10⁶), iMSCs (1 x 10⁶) or vehicle were administered via the femoral vein immediately after ischemia-reperfusion. Infarct volumes were assessed at 3 days (n=5), and neurological deficits were evaluated at 3, 7, 14, 28, and 56 days (n=8), as well as cognitive dysfunction at 28 and 56 days after reperfusion (n=8). Immunological analyses were also performed at 3 days after reperfusion (n=5).

Results: Flow cytometry analysis showed the downregulation of TRA-1-60 and the upregulation of CD73, CD90 and CD105 in iMSCs. We found that rats administered with BMMSCs and iMSCs showed the significant reduction in infarct volumes at 3 days after reperfusion compared to vehicle treated rats. In addition, the administration of BMMSCs and iMSCs greatly improved the motor function at 3, 7, 14, 28, and 56 days and the cognitive function at 28 and 56 days. Compared with the vehicle group, the microglial activation and pro-inflammatory cytokine expression were suppressed in the MSC-administered groups. Moreover, the MSC-treated groups attenuated oxidative stress markers and neuronal degeneration. There was no significant difference in therapeutic effects between BMMSCs and iMSCs group.

Conclusions: Our results demonstrated that the intravenous administration of iMSCs reduced the ischemia-reperfusion injury and promoted functional improvement through the modulation of inflammation and oxidative stress in a rat focal cerebral ischemia, suggesting that iMSCs can be harnessed as an alternative source of hMSCs to overcome the concerns of existing MSC-based therapy for brain injury.

Effect of suicide gene therapy against brain metastasis of non-small cell lung cancer using stem cells from human exfoliated deciduous teeth (SHED) expressing HSV1-TK

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Introduction: Lung cancer is one of the most common cancers. Despite improved systemic disease control, the frequency of intracranial metastases is increasing and, therefore, the development of new treatments is required. We have reported efficacy of the herpes simplex virus 1 thymidine kinase (HSV1-TK)/ganciclovir (GCV) suicide gene therapy to treat glioma using several types of stem cells as delivery vehicles. In recent years, stem cells from human exfoliated deciduous teeth (SHEDs) have been studied in the field of regenerative medicine because of their easy availability. In this study, we examined the effectiveness of the TK/GCV system using SHEDs as a gene vehicle in a brain metastasis model of non-small cell lung cancer.

Materials and Methods: We modified HSV1-TK in our laboratory and therapeutic SHEDs expressing the modified TK (SHEDs-TK) were produced by lentiviral transduction. The viability of SHED-TK was compared with that of SHEDs expressing wild-type TK. Non-small cell lung cancer (NSCLC) cell lines (H1299, A549, H460) and SHED-TK cells were co-cultured at various ratios in the presence of GCV and we evaluated the inhibition of NSCLC tumor cell growth due to the bystander effect. The migration of SHED-TK cells towards NSCLC tumor cells was evaluated using the Matrigel migration assay. We implanted a mixture of H1299 expressing luciferase (H1299-luc, 5x104 cells) and SHED-TK (5x104 cells) or H1299-luc (5x104 cells) alone in the right brain hemisphere of nude mice. The size of tumors was subsequently measured using luminescence and overall survival was also evaluated.

Results: Transduction of modified TK was less toxic to SHEDs compared with that of wild-type TK. SHED-TK cells were highly sensitive to GCV. In vitro, a significant antitumor effect was observed in co-culture of SHED-TK with H1299, A549, and H460 cells in the presence of GCV due to a strong bystander effect. SHED-TK cells showed significant migration toward the conditioned media of H1299 and A549. In the nude mouse brain tumor model, only the co-implantation group of SHED-TK and H1299-luc cells administered with GCV showed a stable luminescence and the other control groups showed an increased luminescence suggesting tumor growth. All mice in the control groups died in 60 days after tumor implantation because of tumor growth, while all mice in the co-implantation group survived more than 100 days.

Conclusion: The modified HSV1-TK gene was safely transduced into SHEDs. SHED-TK had considerable migratory ability. Treatment with GCV showed a strong bystander effect against NSCLC cell lines both in in vitro and in vivo conditions.

Optimization of Highly Efficient Gene Transfer with Adenoviral Vectors in Human Pluripotent Stem Cells

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Human pluripotent stem cells (hPSCs) such as human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), are indefinitely self-renewing and pluripotent and show great potential not only for basic research in developmental biology by differentiating into a wide variety of cells in vitro, but also as a source of donor cells for cell transplantation therapy. To understand the process of differentiation from pluripotent stem cells into functional cells, it is necessary to efficiently and safely transfer and express exogenous genes. Adenoviral vectors have been widely used not only for experimental studies, but also for clinical trials, both because it is feasible to prepare high titers of adenoviral vectors and because these vectors provide high gene transfer efficiencies in many types of somatic, stem, and cancer cells. The adenoviral vector-mediated genetic manipulation method in hPSCs may provide a powerful new strategy not only for efficiently studying developmental biology but also for advancing regenerative medicine. However, the efficiency of adenoviral vectors, in particular, adenoviral vector-mediated gene transfer of undifferentiated hPSCs, has not been extensively investigated.

In this study, we attempted to optimize the efficient transfer of genes into hESCs and hiPSCs using adenoviral vectors. First, comparative study of the activities of three representative ubiquitously active promoters revealed that only the CA promoter, unlike the RSV and CMV promoters, allowed robust transgene expression in hPSCs. In addition, comparisons among various gene transfer protocols demonstrated that highly efficient gene transfer at lower MOI by infecting floating cells (single-cell suspension) with adenoviral vector; consequently, viability remained high, without losing the undifferentiated state of hPSCs. The high efficiency of gene transfer might be the result of the larger surface area of suspended cells relative to adherent cells, allowing more uniform gene transfer cells. In conclusion, our study showed that adenoviral vectors are an excellent tool for highly efficient and safe gene transfer into hPSCs. Accordingly, adenoviral vectors will be an invaluable tool for both regenerative medicine and basic research using hPSCs.

Elucidation of the mechanism of adenovirus vector – induced hepatotoxicity during the early phase after systemic administration –involvement of inflammatory cytokine-induced leaky adenoviral gene expression –

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An adenovirus (Ad) vector has been widely used as a gene delivery vehicle due to the superior transduction properties, however, preclinical and clinical studies have reported that Ad vectors induce hepatotoxicity as a major adverse event following in vivo application. In mice, two peaks of serum alanine aminotransferase (ALT), an enzymatic biomarker of hepatotoxicity, occur at around 2 and 10 days following Ad vector administration. The hepatotoxicity during the early phase is considered to involve Ad vector-induced inflammatory cytokines, including interleukin (IL)-6; however, the precise mechanism remains to be clarified. In this study, we examined the mechanism of Ad vector-induced hepatotoxicity during the early phase by using a firefly luciferase-expressing conventional Ad vector, Ad-CAL2, and a modified Ad vector, Ad-E4-122aT-CAL2. Ad-E4-122aT-CAL2 harbors 4 copies of the sequences complementary to the liver-specific miR-122a in the 3'-untranslated region (UTR) of the E4 gene, leading to significant suppression of leaky Ad gene expression in the liver via miR-122a-mediated post-transcriptional gene silencing and a significant reduction in late-phase hepatotoxicity (Shimizu, Sakurai et al., Mol.Ther.Methods Clin.Dev., 2014).

First, we examined the acute hepatotoxicity profiles of Ad vectors following intravenous administration in mice. We found that serum ALT levels were significantly reduced for Ad-E4-122aT-CAL2 48 h following intravenous administration in wild-type mice, compared with Ad-CAL2, although Ad-E4-122aT-CAL2 and Ad-CAL2 induced comparable levels of inflammatory cytokine expression in the liver and spleen. Ad-CAL2 exhibited much lower levels of inflammatory cytokine expression in the spleen, acute hepatotoxicity, and leaky Ad gene expression in immune-deficient Rag2/Il2rγc double-knockout mice, which were lacking T, B, and NK cells, compared with wild-type mice. These data indicated that while Ad vector-induced inflammatory cytokines were involved in early-phase Ad vector-mediated hepatotoxicity, they were not the sole determinant of the acute hepatotoxicity.

Next, we examined the effects of IL-6, a major inflammatory cytokine induced by Ad vectors, on leaky Ad gene expression and Ad vector-induced hepatotoxicity in primary mouse hepatocytes. IL-6 significantly enhanced leaky Ad gene expression and cytotoxicity in primary mouse hepatocytes following Ad-CAL2 transduction, but not Ad-E4-122aT-CAL2 transduction. Furthermore, leaky Ad gene expression and cytotoxicity in Ad-CAL2-treated hepatocytes in the presence of IL-6 were significantly suppressed upon inhibition of JAK and STAT3. Ad vector-mediated acute hepatotoxicities and leaky Ad expression were significantly reduced following systemic administration in IL-6 knockout mice, compared with those in wild-type mice. Thus, Ad vector-induced IL-6 promotes leaky Ad gene expression in the liver, leading to acute hepatotoxicity, while leaky Ad gene expression in the liver is suppressed via miR-122a-mediated post-transcriptional gene silencing even in the presence of IL-6, leading to attenuation of the acute hepatotoxicity. Ad-E4-122aT is a promising vector that induces less hepatotoxicity during both the early and the late phase for gene therapy and basic research.

Evaluation of purification and storage conditions for herpes simplex virus-based vectors

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Background: Genetic engineering has enabled the creation of a wide variety of herpes simplex virus (HSV) vectors for gene and oncolytic virus therapy over the past several decades. However, high-yield production of these HSV vectors is challenging, thereby limiting their clinical potential. To address this issue, we have recently improved the upstream process for production of a non-toxic HSV vector, JΔNI5. Here we explored the critical parameters of the downstream process of HSV vector purification and evaluated different storage and recovery conditions for both replication-defective and replication-competent HSV vectors to maximize purity and yields.

Methods: Following growth, replication-defective and replication-competent HSV vector preparations were produced and clarified under different centrifugation conditions and using different types of filters. The clarified HSV supernatants were concentrated by centrifugation at various centrifugal forces. The viral titers were evaluated by quantitative PCR for viral genomes and standard plaque assay. The storage conditions of HSV vector stocks were evaluated and functional recovery was examined.

Results: We found that optimal centrifugal conditions significantly increased the physical and biological titers of HSV vectors. Notably, these optimal conditions also yielded greater oncolytic effect on a pancreatic cancer cell line, PANC-1, and elevated transgene expression in cultured primary dorsal root ganglion neurons. Evaluation of the various vector storage conditions confirmed that repeated freezing and thawing of purified viruses dramatically decreased their biological titers.

Proposal for novel immunological classification of lung cancer based on tumor-infiltrating lymphocytes of tumor microenvironment

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The lung cancers are the leading cause of cancer deaths in Japan and worldwide. Although the identification of immune checkpoint blockade (ICB) is changing the approaches to cancer treatment including non-small cell lung cancer (NSCLC), recent progress in clinical studies has revealed a new issue in clinical oncology, that of drug-resistant cases; just 20% of NSCLC patients respond to anti PD-1/PD-L1 therapy. Since the responsiveness of immune therapy is mainly determined by the composition and activation status of tumor-infiltrating lymphocytes (TIL), it is promising to establish the immunological subtype from the viewpoint of TIL to develop the novel immune therapies.

To examine the immunological characteristics of tumor microenvironment, we constructed the integrated data base of TIL profiling, RNA-seq, whole exsome seq and clinico-pathological findings using 198 fresh resected tissues of lung cancer under AMED project (GAPFREE). The multicolor flow cytometry differentiated 67 cell types of TIL, and to understand the characteristics of TILs in NSCLC, we first compared the number of each immune cell type per tumor volume (%vol) in tumor specimens with those in matched non-tumor tissues. NSCLC specimens showed increased composition of %vol of distinct immune cell types including B cells, CD4+ NKT cells and effector (Fr II) Tregs, and conversely the composition of myeloid cells, macrophage, monocytic myeloid-derived suppressor cells, dendritic cells (DCs), myeloid DCs and NK cells was decreased in NSCLC tissue than non-tumor tissue.

The unsupervised clustering of TIL showed that adenocarcinoma as well as squamous cell carcinoma were divided into 3 types of cluster (cold-, myeloid cell-, CD8 T cell-dominant type), respectively. The patient prognosis was significantly correlated with the immunological types and the CD8+ T cell-type showed the better patient outcome compared to other 2 types. In CD8+ T cell-types in both adenocarcinoma and squamous cell carcinoma, the immune reaction-related pathways such as adaptive immune response were mainly activated, which should link to the good patient prognosis. Gene enrichment analysis showed that specific pathways were activated or suppressed in respective immune subtypes. Integrated data analysis of OMICs and TIL data could be useful to identify the target pathways to develop a personalized immune therapy system for lung cancer.

Generation of retroviral vector-producing human amniotic mesenchymal stem cells (VP-hAMSCs) for cancer cell therapy

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Background: Human mesenchymal stem cells (hMSCs)-based cell therapy is a promising strategy for cancer treatment because of the inherent tumor-homing ability of hMSCs. We previously reported that retroviral vector-producing bone marrow-derived hMSCs (VP-hBMSCs) administered to tumor-bearing mice systemically were able to achieve efficient transduction of therapeutic genes to cancer cells through their accumulation at tumor site and in situ production of infectious progeny viral particles. However, since the clinical preparation requires large-scale transduction of hMSCs, invasive acquisition of hMSCs from bone marrow would be a limitation of this strategy. In the present study, we asked whether human fetal appendage (amnion)-derived MSCs (hAMSCs), which are less invasive to prepare than hBMSCs, are feasible for use in this in situ cancer cell therapy and determined an optimal protocol to generate high-titer retroviral vector-producing hAMSCs (VP-hAMSCs).

Methods: In order to optimize the transfection of hAMSCs for generation of VP-hAMSCs, a GFP expression plasmid and retrovirus packaging plasmids (pGP, pVSV-G and pLTR-luciferase) were co-transfected into hAMSCs by electroporation with 24 different electrical conditions, i.e., different combinations of amplitude, duration and number of stimulus pulse. Four days after electroporation, the resultant VP-hAMSCs and the supernatants were collected. The transfection efficiency and cell viability of VP-hAMSCs per se were determined by measuring GFP-positive and PI-negative cells with flow cytometry, respectively. The genomic and biological titer of the retroviral vectors produced from VP-hAMSCs were elucidated by quantitative real-time RT-PCR and measuring the transduction efficiency of HEK293 cells, respectively. To further validate whether the retroviral vectors produced from VP-hAMSCs effectively transduce to human cancer cells, the VP-hAMSCs electroporated at selected stimulus conditions were co-cultured with a mCherry-expressing pancreatic cancer cell line (PANC1-mCherry). The transduction efficiency of the retroviral vectors to PANC1-mCherry cells were measured by luciferase reporter assay.

Results: The evaluation of hAMSCs electroporation revealed that the stimulation with larger amplitude, longer duration or greater number of electrical pulse resulted in the efficient production of retroviral vector in physical titer from VP-hAMSCs. A positive correlation was observed between the transfection efficiency of hAMSCs, the vector genomic titer and biological titer of the produced retroviral vector. On the other hand, the viability of VP-hAMSCs post electroporation had negative correlation with these three parameters, especially the vector genomic titer. Of note, the VP-hAMSCs generated by electroporation with 2 electrical pulses, rather than 1 or 3, showed the lower genome titer to biological titer ratio. In addition, the VP-hAMSCs generated with the optimal conditions was also capable of efficient transduction of PANC1-mCherry cells in co-culture of VP-hAMSCs and PANC1-mCherry cells.

Conclusion: Our findings support the generation of highly-active VP-hAMSCs and the use for in situ cancer cell therapy.

Systemic therapy for Fabry disease using AAV vector

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Objective: Fabry disease is an X-linked lysosomal storage disease caused by deficiency of α-galactosidase A(α-GLA). Enzyme replacement therapy and molecular chaperone therapy are available, but they need to be administrated permanently because of short half-time. The aim of this study is to assess efficacy of adeno-associated virus (AAV) -mediated gene therapy for Fabry disease.

Methods: We generated AAV2 and AAV9 vector containing human α-GLA gene. Six-week-old male α-GLA knockout mice were intravenously injected with 1×1011 viral genomes of AAV solution or phosphate-buffered saline (PBS). Plasma α-GLA activity was analyzed by fluorescence-enzymatic activity assay 3 and 8 weeks after injection. Animals were killed 8 weeks after injection and α-GLA activity of brain, heart, liver, and kidney was analyzed.

Results: The α-GLA activity in the brain, heart, liver, and kidney tended to be higher in AAV9 group than AAV2 group (brain:[wild type] 62.62 ± 11.83 nmol/h/mg protein, [PBS] undetectable, [AAV2] undetectable, [AAV9] 1.73 ± 1.99 nmol/h/mg protein; heart: [wild type] 2.37 ± 0.47 nmol/h/mg protein, [PBS] undetectable, [AAV2] 0.54 ± 1.54 nmol/h/mg protein, [AAV9] 1.39 ± 2.28 nmol/h/mg protein; liver: [wild type] 26.57 ± 2.22 nmol/h/mg protein, [PBS] 1.80 ± 0.76 nmol/h/mg protein, [AAV2] 3.41 ± 2.13 nmol/h/mg protein, [AAV9] 11.88 ± 16.90 nmol/h/mg protein; kidney: [wild type] 16.71 ± 3.89 nmol/h/mg protein, [PBS] 1.55 ± 3.42 nmol/h/mg protein, [AAV2] 0.43 ± 0.71 nmol/h/mg protein, [AAV9] 3.33 ± 6.04 nmol/h/mg protein).

Conclusions: Intravenous injection of AAV vector will be a promising gene therapy for patients with Fabry disease. Because the α-GLA activity expressed by the delivered gene was not much in this study, we need further experiment to find the appropriate titer of AAV vector.

Development of the lentiviral vector production system with improved safety and high productivity

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Lentiviral vectors are powerful tool for gene modification in both research and clinical applications, as they can infect both dividing and non-dividing cells, integrate transgenes into the genome, and facilitate long-term gene expression. Lentiviral vectors, derived from the human immunodeficiency virus (HIV), have been investigated to increase the safety. Due to the potential risk for the generation of replication competent lentiviruses (RCL), the second-generation lentivirus vectors have been developed by eliminating all accessory proteins from the packaging system, The third-generation system have been developed in which genes required for packaging have been split into separate plasmids, rev expressing plasmid and gag/pol encoding plasmid. And 5'LTR have been modified to a chimeric LTR with enhancer and promoter, which allowed to remove the Tat from the packaging system. Furthermore the U3 region of 3' LTR has truncated to improve the safety. The third-generation, self-inactivating lentiviral vectors have recently been used in multiple clinical trials, it is desired to eliminate maximally the sequence of HIV genome from the lentiviral vector and packaging system to improve the safety, while maintaining the viral titer for large scale vector production.

In this study, to develop the safer and high titer lentiviral vector production system, we have modified the second-generation, self-inactivating lentiviral vector, pLVSIN, and examined the importance of each element by deleting or changing the order. To convert the vector into the Tat-independent third-generation lentiviral vector, the U3 promoter region in the 5'LTR was replaced by CMV promoter, Furthermore to eliminate the sequence of HIV genome and reduce the chance of RCL generation, we have evaluated the essential sequence for viral production by gradually shortening the gag sequence in lentiviral plasmid, and we could develop the viral vector constructs with improved safety without lowering the viral titer, having different internal promoter, MSCV-U3 or EF1α promoter. It is very important to select a suitable promoter according to the size and expression level of the transgene in each target cell type. We have also decided the optimal ratio for lentiviral packaging plasmids to yield the maximum titer of viral vectors. Using our novel third-generation lentiviral production system, we could produce the high titer lentiviral vector encoding a chimeric antigen receptor (CAR) and transduce T cells with high efficiency. The resulting CAR-T cells showed antigen-specific cytotoxicity and cytokine production against tumor cells.

Lymphocytic choriomeningitis virus-based pseudotyped retrovirus is tropic to endogenous neural stem cells to trigger regeneration after spinal cord injury

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Retroviral vectors deliver transgenes only in mitotic neural stem cells (NSCs). Lymphocytic choriomeningitis virus (LCMV) is a neurotropic RNA virus, which preferentially infects neural stem and progenitor cells. We generated a pseudotyped retroviral vector with a glycoprotein-envelope derived from LCMV. The vector was designed to transduce replicating NSCs de-differentiated from ependymal cells after spinal cord injury and therein transfer a transgene.

Treating traumatic spinal cord injury (SCI) is difficult, and individuals often have permanent and severe disabilities. These disabilities partially result from the human body's limited ability to repair and regenerate neural tissue in the spinal cord. However, after SCI, the ependymal cells lining the central canal of the spinal cord can dedifferentiate between the acute and subacute phases. Indeed, the ependymal cells can revert into NSCs, and redifferentiate into glia and neurons. Therefore, we hypothesized that nerve regeneration may be achievable if endogenous injury-induced dedifferentiated NSCs can be reprogrammed into neurons.

The transcriptome analysis of injured Xenopus laevis (X. laevis) tadpole and mice suggested that Neurod4L.S., a basic helix-loop-helix transcription factor, was the most promising transcription factor to exert neuroregeneration after spinal cord injury (SCI) in mammals. We delivered murine Neurod4 to mice undergoing SCI using retrovirus with LCMV envelope. SCI induced ependymal cells to neural stem cells in the central canal. The LCMV-based pseudotyped retroviral vector preferentially introduced Neurod4 into activated neural stem cells, which converted to neurons with axonal regrowth and suppressed the scar-forming glial lineage. Neurod4-induced inhibitory neurons predominantly projected to the subsynaptic domains of motor neurons at the epicenter, and Neurod4-induced excitatory neurons predominantly projected to subsynaptic domains of motor neurons caudal to the injury site suggesting the formation of functional synapses. Thus, Neurod4 is a potential therapeutic factor that can improve anatomical and functional recovery after SCI.

Expression of therapeutic iduronate-2-sulfatase enzyme with a novel single-stranded RNA vector

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Sendai virus vector has received a lot of attention due to its broad tropism for mammalian cells. This vector is capable to express multiple genes with high protein expression efficiency in a single vector. To prove this benefit, we examined the efficacy of this novel RNA vector harboring the human iduronate-2-sulfatase (IDS) gene, a causative gene for mucopolysaccharidosis type II, also known as a disorder of lysosomal storage disorders. First, as expected, we found a markedly high expression of the human IDS gene, together with EGFP, when the vector was infected with mammalian cells. Second, the BHK-21 transformant cells stably expressing the human IDS gene persistently generated an active human IDS enzyme extracellularly. Importantly, the exogenously added enzyme was incorporated into mammalian cells with significant inhibition of cellular uptake by mannose-6-phosphate, suggesting that the produced enzyme may be used for therapeutic use by cross-correction-based mechanism. Taken together, these results suggest that our novel RNA vector may apply to various clinical applications.

Optimization of vector design and biodistribution of non-cytotoxic herpes simplex virus-based vectors in vivo

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Background: Herpes simplex virus (HSV)-based vectors are capable of delivering large or multiple therapeutic genes to a wide range of cell types, supporting their potential utility for the treatment of a variety of diseases. To improve the safety of HSV as a gene delivery vehicle, we recently generated a set of non-cytotoxic HSV vectors that are devoid of all immediate-early gene expression, yet are capable of vigorous transgene expression from the latency-associated transcript (LAT) locus at one end of the linear viral genome or the deleted ICP4 locus (TR) at the other end. In previous presentations, we have described the in vivo transduction efficiencies of JΔNI vectors harboring a luciferase expression cassette in either locus. In this study, we implemented design modifications in the LAT locus to enhance LAT-based transgene expression and examine the vector biodistribution in vivo.

Methods: Different transgene expression cassettes were engineered and inserted into the LAT locus of a representative JΔNI vector via genetic recombination. The recombinant vectors were administered intraperitoneally into neonatal mice. Transgene expression was monitored by in vivo and ex vivo imaging technology. The biodistribution of vector genomes was explored by quantitative PCR analysis.

Results: In vivo imaging studies revealed variable duration and expression levels depending on the expression cassette. At 1 week post injection, transgene expression was mainly observed in the liver and spinal cord, and in skin and muscle at the injection site. At 4 weeks post injection, transgene expression was no longer detectable in the liver, yet remained robust in the spinal cord and at the injection site, suggesting a degree of tissue specificity of transcriptional activity at the LAT locus. Interestingly, we found that transgene expression levels in male mice were lower than in females. We further studied the distribution of transgene expression in the spinal cord and dorsal root ganglions (DRGs) by ex vivo imaging analysis. Transgene expression was mainly observed in thoracic level 11-13 DRGs with modest expression in the spinal cord. Notably, histological examination detected no serious adverse effects in vector-injected mice.

Conclusion: Taken together, our results support the efficacy and safety of our JΔNI vectors in vivo and suggest suitable target tissues for JΔNI-mediated gene therapy.

Cancer cell specific expression of Cas9 gene using Ad5F35 vector

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The CRISPR-Cas9 is thought that a promising clinical potential for cancer th erapy. However, off-target effects of Cas9 is the most concern for the appli cation. At present, various studies are in progress to control the off-targe t effects, but total control of off-target effects is seemed to be very diff icult. Therefore, we thought that if the Cas9 gene could be specifically exp ressed in cancer cells, the adverse effects of off-target gene editing could be minimized. Moreover, by introducing the Cas9 gene and guide RNA using se parate vectors, the probability of gene editing occurring in non-target tiss ues is lower than when Cas9 and guide RNA are loaded into a single vector. I n this study, we constructed the chimeric adenoviral vector of type 5 and 35 adenovirus, named Ad5F35-MKp-hCas9. It infects via the CD46, which is highl y expressed in cancer cells, and human codon-optimized SpCas9 (hCas9) gene w as placed under the promoter of the midkine, which is a highly expressed gro wth factor in cancer cells. This vector is capable of expressing the hCas9 g ene with high specificity into cancer cells, and we believe that the risk of off-target effects on normal tissues can be minimized.

Efficient extraction and purification of AAV vectors both from culture supernatant and host cells & efficient extraction of AAV vectors using freeze-thaw extraction buffer

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Introduction: Recombinant Adeno-Associated Virus vectors (rAAVs) are known to be one of the most useful viral vectors for research and clinical use, because it is non-pathogenic, leads to mild immune response, and infects to dividing cells and non-dividing cells. For extraction and purification of AAV vectors from AAV-producing cells, it is common to perform extraction by freeze-thaw cycles, and then purify by CsCl ultracentrifugation. However, these methods are time consuming, and require specialized equipments and advanced techniques. Therefore, we addressed to develop two efficient methods to extract and purify rAAVs.

Purification of AAV vectors both from culture supernatant and host cells: As the localization of rAAVs in the cells varies depending on the serotypes and harvest time after transfection, it is effective to extract rAAVs both from cells and culture supernatant. Therefore, we developed a simple rAAVs purification protocol from both of them.

First, rAAVs harboring fluorescent gene ZsGreen1 were produced by the transient transfection using AAVpro® helper free system (Takara Bio) into 293T cells. Next, rAAVs were harvested from both cell lysate and cell culture medium by a detergent mediated extraction. The crude lysate was further purified by using concentrating solution and two precipitating solutions followed by ultra-filtration. The obtained rAAVs showed significant higher titers than the rAAVs obtained from only transfected cells by using the existing purification kit in several serotypes. This protocol would provide a simple and an effective rAAV preparation method applicable to a variety kind of serotypes.

Efficient extraction of AAV vectors by one freezing and thawing cycle: We have succeeded to develop new extraction buffer for freeze-thaw which can efficiently extract AAV by single cycle of freezing and thawing. rAAV were produced as above, and after transfection, the rAAVs were harvested from AAV producing-cells by freezing and thawing process in the freeze-thaw extraction buffer, instead of PBS or culture medium. The genome titer of the extracted rAAVs of multiple serotypes using the freeze-thaw extraction buffer was higher than that of the conventional method while maintaining their infectivity. In fact, the rAAV infectivity showed no significant difference between these extraction methods. The extracted rAAVs can be further purified by the commercially available AAVpro® Purification Pack Maxi (All Serotypes) (Takara Bio). The purified rAAVs represented higher biological titer compared to the rAAVs obtained by the conventional extraction method.

In summary, we could establish an efficient rAAVs extraction and a simple purification protocol, which can be performed in only 4 hours by using newly developed freeze-thaw extraction buffer.

Smart Coating of AAV vector with Tannic Acid and Phenylboronic Acid-Modified Copolymer Reduces Hepatotoxicity/Nephrotoxicity and Thwart AAV-Neutralizing Antibodies

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Adeno-associated viral (AAV) vectors are among most effective tools for managing intractable diseases therapy due to their low invasiveness and long-term gene expression. Particularly, AAV-based gene therapy has been approved in Japan for spinal muscular atrophy (Zolgensma). However, 30-50% of the population present neutralizing antibodies against AAV, and the massive dose of AAV9 induces serious side-effects, such as hepatotoxicity and nephrotoxicity. Therefore, a novel AAV delivery system, which suppresses the accumulation in normal tissues, such as liver and kidney, and avoids the inactivation by the neutralizing antibodies is expecting.

We have recently developed a novel biomolecules delivery system comprising tannic acid (TA) and phenylboronic acid-conjugated polymers [Biomacromolecules 2020 Sep 14;21(9):3826-3835, PCT/JP2020/021301]. TA is a polyphenol that forms a complex with biomolecules, such as proteins via hydrophobic interaction and hydrogen bond in aqueous solution. Moreover, TA can form boronate esters with phenylboronic acid-conjugated polymers resulting in the construction of nanoparticles having the biomolecules encapsulated in the core surrounded by the biocompatible polymer. Nanoparticle loading a model GFP protein with a 10-20nm in diameter selectively delivered their cargo to a subcutaneous tumor model when injected intravenously, while avoided the accumulation in normal organs. This supramolecular chemistry technique may serve as a novel approach for designing delivery systems for AAVs as biomolecules. Here, we examined the delivery ability of AAV coated with TA and block copolymers, and checked its genetic transfection activity in in vitro and in vivo.

The coated AAV was constructed by mixing of AAV9-luciferase (luc), TA and phenylboronic acid-conjugated polymers in aqueous solution through sequential 'self-assembly'. The size of AAV and coated AAV were measured by Zetasizer (29 nm and 46 nm in diameter). The spherical shape of the coated AAV was observed using transmission electron microscope. At first, we evaluated the ability of the coated AAV to escape neutralizing antibodies in vitro.

Thus, the coated AAV9-luc showed comparable luciferase activity both in the presence and absence of AAV9-neutralizing antibodies (ADK9 mouse monoclonal antibody), but non-coated AAV9-luc were completely inhibited in the presence of the neutralized antibodies. Next, we verified the effect of suppressing the accumulation of coated AAV in the liver and kidney, which are the sources of hepato- and nephrotoxicity, respectively. In mouse models of CT26 colon cancer and orthotopic GL261 glioblastoma model, intravenously injected AAV9-luc or coated AAV9-luc achieved high luciferase activity in both tumors. Our data demonstrate successful protection of the AAV vector from antibody neutralization and reduction of the accumulation in normal tissues, increasing the safety and efficiency of therapeutic gene delivery.

Encapsidation of PCR-amplified transgene fragments into recombinant adeno-associated virus capsid

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Background: Recombinant adeno-associated virus (rAAV) is widely used as a transgene delivery vehicle in basic research and treatment due to its high transduction efficiency and safety. However, the heterogeneity of rAAV viral genome is a significant limitation when it applies to the clinical trials. rAAV vector genome contains two inverted terminal repeats (ITR), each of which consists of three palindromic regions (A, B, and C) and a nonpalindromic region (D). ITRs are involved in packaging not only the vector genome but also the non-vector genome into rAAV capsid. Here, we demonstrate that PCR-amplified transgene cassettes linked by a single copy of AD sequence (L-AD, AD: packaging sequence), which is a truncated form of ITR, can be encapsidated into rAAV capsid without including vector plasmid backbones.

Methods: To evaluate whether the rAAV vector genome with a minimal component of ITR can be packaged into rAAV capsid, an AAV vector plasmid with a single copy of the AD sequence (pAD) was engineered by inserting the synthetic AD oligonucleotides into an AcGFP expression plasmid and packaged into rAAV capsid (rAAV-pAD). PCR-amplified rAAV vector genomes encoding AcGFP that flanked a single copy of the AD sequence (L-AD) were also engineered to evaluate the packaging into rAAV capsid (rAAV-L-AD). The packaging and transduction efficiencies were compared with single-stranded and self-complementary AAV vectors (ssAAV and scAAV). To examine whether the L-AD system works, L-ADs harboring shRNA expression cassettes targeting human ATP5B or LacZ gene (L-AD-shATP5B/shLacZ) were generated and encapsidated into rAAV capsid (rAAV-L-AD-shATP5B/shLacZ). The rAAV-L-AD-shATP5B-mediated knockdown of ATP5B expression was confirmed by quantitative RT-PCR analysis.

Results: The packaging efficiency of rAAV-pAD/L-AD was comparable to that of scAAV until the size of L-AD is 2kb, although packaging efficiency decreased when L-AD size was longer than 2 kb. The transduction efficiency of rAAV-pAD/L-AD was inferior to ss/scAAV. However, notably, rAAV-L-AD had significantly fewer plasmid backbones in comparison with ss/scAAV. Moreover, we found that rAAV-L-AD-shATP5B transduction led approximately 80% knockdown in ATP5B mRNA, which was comparable to ATP5B-targeted siRNA transfection.

Conclusion: Our results demonstrate the potential of the L-AD system as a novel gene delivery platform that does not include DNA impurity.

Large-scale purification of functional recombinant adeno-associated virus with short-term zonal ultracentrifugation

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Background: Purification of recombinant adeno-associated virus (rAAV) is required for the safe and efficient transduction in rAAV-based in vivo gene therapies, because rAAV products contain full-genome (functional), intermediate, and empty rAAV particles as well as host-cell proteins. However, the current standard method for rAAV purification at the laboratory scale (up to 180mL) is based on two-day ultracentrifugation with a cesium chloride (CsCl) density-gradient protocol, limiting large-scale purification of functional rAAV for clinical usage. In addition, the infectivity decreases when the rAAV is incubated in CsCl over time. Therefore, in this study, we sought to develop a short-term purification method for large-scale rAAV production at high-purity by using a zonal ultracentrifuge.

Methods: rAAV9 encoding ZsGreen1 was collected from culture supernatant (without cell lysis) 5 days after plasmid transfection. Large-volume rAAV-containing culture media (up to 1,000mL) and escalating densities (2 or 4 steps) of CsCl solutions were separately placed in the zonal rotor, respectively. Following large-scale density-gradient ultracentrifugation of rAAV, the samples were fractionated and evaluated by rAAV genome copies in quantitative PCR, rAAV capsid protein amounts in western blotting, and ZsGreen1 transduction efficiency in flow cytometry. Purity of rAAV was analyzed by analytical ultracentrifuge (AUC) and transmission electron microscope (TEM) with phosphotungstic acid stain. The genomic DNA regions packaged in rAAV particles were detected by droplet digital PCR (ddPCR) using various primers and probes targeting the whole vector genome.

Results: We performed 2-step CsCl density-gradient ultracentrifugation with a zonal rotor, allowing for a large volume (900-1000mL) of rAAV purification as well as shorter ultracentrifugation for 4-5 hours with a steeper density-gradient in the area of rAAV fractions, as compared to our control method of 4-step density-gradient (300mL) for 10 hours. rAAV capsid proteins were detected in 2 of the fractions, one of which also contained the peaks of rAAV capsid, genome copies, and ZsGreen1 transduction, demonstrating a separation between functional full-genome and non-functional empty rAAV particles. The intermediate particles would be included between these fractions. We detected single AUC peak with separate sedimentation coefficients for fractions of full-genome (about 90S) and empty (about 60S) vectors, demonstrating a high-purity separation of rAAV. Interestingly, the whole genome regions were detected at similar levels in full-genome rAAV by ddPCR; however, ITR signals were 2.5-2.9 fold higher than the ZsGreen1 signal in the empty particles, suggesting that ITR fragments can be packaged in 'empty' particles.

Conclusion: We developed a 2-step density-gradient ultracentrifugation method with a zonal rotor in 4-5 hours, allowing for large-scale purification of functional full-genome rAAV. Our large-scale rAAV purification method would be applicable for considerable in vivo experimentation and clinical investigation.

Development of Novel Impurities Removal Technology and New Affinity matrix for Advanced Purification Platform Process of Adeno-associated Virus Vector

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Adeno-associated virus (AAV) vectors are promising tools for gene therapy, and some products have already been approved as commercial drugs. However, a standardized and efficient AAV vector manufacturing process does not exist like the platform for manufacturing monoclonal antibodies. The downstream process (DSP) for AAV vectors typically consists of four steps: (1) harvest and clarification, (2) capture, (3) polishing, and (4) concentration and formulation. We focused on improving the first two steps: (1) and (2), to simplify and intensify the whole process. The harvesting and clarification step usually includes cell lysis, DNA fragmentation by endonuclease, clarification by depth filter, and impurity removal and concentration by tangential flow filtration (TFF). The TFF step is generally essential for the capture step to reduce the high impurity burden and minimize the loading time because of the limitation of capture chromatography resins. We are developing a new affinity chromatography matrix capable of loading unconcentrated clarified solution at high flow rates to eliminate the TFF step. It is essential to reduce impurities during the harvesting and clarification steps because host cell proteins (HCPs) and DNA inhibit the adsorption of AAV vectors and an affinity matrix. Thus, we also investigated a novel technology to remove impurities during clarification.

A new harvest and clarification step using novel impurity removal technology was evaluated in terms of process simplification and DSP's productivity compared with the conventional process using depth filtration and TFF. Our new method was based on the unique characteristics of “Additive D” to adsorb process-related impurities (e.g., HCPs, DNA). The addition of “Additive D” to the endonuclease treated cell lysate of the AAV vector expressing HEK293 cells followed by filtering the mixture after agitation without TFF resulted in efficient removal of impurities to the same degree as the conventional process. The amount of the expensive endonuclease could be reduced to less than 1/10 in the new process. We also evaluated the affinity purification step using a commercial beads resin with the clarified solution and achieved a higher purification yield than the conventional process. In addition, we introduce a new membrane-type affinity matrix that can process the unconcentrated clarified solution at a high flow rate. Combining the novel impurity removal technology and the affinity matrix should be a powerful tool to construct an intensified AAV purification platform process.

CD38 silencing and inhibition of tyrosine kinase contribute to improving anti-CD38-CAR-T cells quality

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CAR-T cell therapy has highly effective clinical results in several diseases, but further improvement is necessary to target a wider range of antigens and tumors. In particular, excessive activation of CAR-T cells leads to cell exhaustion and reduction of naive/memory T cells' population, which are important for long-term immune response. Therefore, suppressing non-antigen-specific activity is necessary for CAR-T cell production. However, when targeting tumor-related antigens that are also expressed on T cells, CAR-T cells recognize the antigens on the T cells, resulting in cell death, poor cell growth, differentiation, and exhaustion during cell production. In order to overcome these problems, various approaches have been tried, such as blocking the target antigen using antibodies and disrupting the antigen gene by genome editing technology.

In this study, we investigated a method for producing CAR-T cells targeting CD38 antigen that is common to T cells and tumor cells. CD38 is a suitable target antigen for CAR-T cell therapy because it is highly expressed in lymphocyte malignant tumors including B-cell non-Hodgkin's lymphoma and multiple myeloma. However, as it is also intermediately expressed in normal blood cells, unwanted activation of CAR-T cells may be caused. Therefore, we tried to suppress the expression of CD38 in CAR-T cells by co-expressing CD38 siRNAs, and prevent the activation during cell production by modifying the signal domain of anti-CD38-CAR to the newly developed JAK/STAT-CAR. JAK/STAT-CAR contains the intracellular domain of the IL-2 receptor β chain and the STAT3 binding motif, which have been shown to improve the proliferation of CAR-T cells and suppresses differentiation compared to conventional second-generation CAR-T cells. CD38 siRNA co-expressing CAR-T cells showed decreased expression of CD38 and exhaustion markers, and the further decrease of exhaustion marker expression was observed in JAK/STAT CAR-T cells. However, compared to CAR-T cells targeting other antigens, CD38-CAR-T cells tended to be exhausted and differentiated.

Next, CAR-T cells were prepared in the presence of the tyrosine kinase inhibitor dasatinib to suppress activation during the cell manufacturing process. Dasatinib treatment maintained a high proportion of naive/memory T cells and was able to suppress exhaustion. Furthermore, combination of these approaches (CD38 siRNA-expressing CD38-JAK/STAT CAR-T cells with dasatinib treatment) showed long-term persistence of antitumor activity in in vitro re-challenge assay.

From the above results, CD38 siRNA co-expressing CD38-JAK/STAT CAR-T cells produced in the presence of a tyrosine kinase inhibitor are expected to be suppressed excessive activation and maintain long-term antigen-specific activity. This approach is also expected to be applied to other CAR-T cell therapies targeting tumor-related antigens expressed on T cells.

Conditionally Replicative Adenovirus Controlled by the Stabilization System of AU-rich Elements-Containing mRNA

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AU-rich elements (ARE) are RNA elements commonly present in the 3'-UTR of certain mRNAs that encode many early response genes or growth-related genes such as proto-oncogenes, and targets ARE-mRNA for rapid degradation. HuR, a member of the embryonic lethal abnormal vision (ELAV) family of RNA-binding proteins, binds to ARE in order to protect ARE-mRNA from rapid degradation. Although HuR is predominantly localized in the nucleus, it has the ability to shuttle between the nucleus and cytoplasm, and the stabilization of ARE-mRNA by HuR has been linked to its localization in the cytoplasm. The cytoplasmic expression of HuR has been implicated in the malignancy of several types of carcinomas, such as colon cancer, and has also been suggested to contribute to the cancerous malignant phenotype. Increased cytoplasmic HuR level was recently identified as an important prognostic marker in several cancers.

In this study, we developed the oncolytic adenoviruses AdARET and AdAREF, possessing the ARE of the TNF・ and c-fos genes in the 3'-UTR of the E1A gene, respectively. Since ARE-mRNA is degraded under normal conditions, but is stabilized in cancer cells, viral E1A expression was expected to be higher in cancer cells than in normal cells. Additionally, these viruses fail to express E1B55k, as the E1B gene was interrupted.

As expected, the expression of the E1A protein was higher in cancer cells than in normal cells and viral late gene product hexon was also expressed in high level in cancer cells, whereas there was no expression of E1B55k. With the expression of these viral proteins, the replication ability of these viruses was significantly higher in cancer cells than in normal cells. The inhibition of ARE-mRNA stabilization resulted in a reduction in viral replication, conversely promotion of ARE-mRNA stabilization activated it, demonstrating that the stabilization system was required for production of the virus. Virus production and cytolytic activities were also higher in many types of cancer cells. The growth of human tumors that formed in nude mice was inhibited by an intratumoral injection of AdARET and AdAREF.

These results indicate that these viruses have potential as oncolytic adenoviruses in the vast majority of cancers in which ARE-mRNA is stabilized. To the best of our knowledge, there are few reports describing oncolytic viruses with tumor selectivity based on the level of mRNA stability. As AdARET and AdAREF failed to express E1B55k, this feature is the same with the E1B-55k gene deleted-adenoviruses like Onyx-015 or H101, which have already been applied clinically.

A recombinant adenovirus vector containing the synNotch gene inhibited tumor growth of triple-negative breast cancer in mice

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Introduction: Triple-negative breast cancer (TNBC) is one of the most difficult molecular subtypes of breast cancer to treat. We developed a recombinant replication-deficient adenoviral vector (Ad-CD44-N-HIF3alpha4) containing a gene encoding a synthetic Notch (synNotch) receptor composed of the extracellular domain of CD44 (CD44-ECD) and the hypoxia-inducible factor (HIF)-3alpha4 connected by the Notch core regulatory region. CD44 is a cell surface adhesion receptor and a known cancer stem cell marker in breast cancer and other malignancies, and HIF-3alpha4 is a dominant-negative regulator of HIF-1alpha. The CD44-ECD in the synNotch receptor acts as a CD44 decoy receptor, and after a ligand binds to the CD44-ECD, HIF-3alpha4 is released, resulting in the inhibition of both CD44 and HIF-1alpha signaling pathway in cancer cells.

Methods: In this study, we employed a human TNBC cell line, MDA-MB-231. Gene transduction with recombinant adenoviral vector containing CD44-N-HIF3alpha4 (Ad-CD44-N-HIF3alpha4) and the gene expressions were determined. MDA-MB-231 cells were cultured under conditions of hypoxia (≤1% O2) with hyaluronic acid (HA). Ad-CD44-N-HIF3alpha4 and Ad-LacZ were infected into cells at 40 multiplicities of infection (MOIs), respectively. In vivo study, MDA-MB-231 cells were subcutaneously inoculated into mice. Intratumoral injections were performed with 1×109 PFU of adenoviral vectors Ad-CD44-N-HIF-3α4, Ad-LacZ, and PBS control.

Results: The expressions of the HIF-3alpha4 gene and surface expression of CD44 were significantly increased in MDA-MB-231 cells by infection with Ad-CD44-N-HIF3alpha4. In addition, the relative gene expressions of survivin and CCL2, downstream genes of CD44, in the cells infected with Ad-CD44-N-HIF3alpha4 were significantly lower than in cells infected with Ad-LacZ and control cells under culture conditions of hypoxia with HA. The relative gene expression of vascular endothelial growth factor (VEGF), a hypoxia target gene, was significantly decreased in the cells infected with Ad-CD44-N-HIF3alpha4 than that in control cells under culture conditions of hypoxia with HA. Ad-CD44-N-HIF3alpha4 significantly suppressed tumor growth compared to Ad-LacZ or PBS control in mice. The expression of CD44 was remarkably increased in mice tumors with the treatment of Ad-CD44-N-HIF3alpha4.

Conclusion: Ad-CD44-N-HIF3alpha4 inhibited the both signaling pathways of CD44 and HIF-1alpha in MDA-MB-231 cells under conditions of hypoxia with HA in vitro, and significantly suppressed tumor growth in vivo. These findings indicated that Ad-CD44-N-HIF3alpha4 has a high clinical applicability for invasive types of TNBC.

AAP requirement for AAV capsid assembly is determined by the degree of structural disorder in the C-terminus of VP

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Although Assembly-activating protein (AAP) has emerged to be an important regulator of AAV capsid assembly; it remains unclear why AAV serotypes 4,5, 11 rh32.33 are capable of assembling capsids without AAP. Here we demonstrate that AAV variants with enhanced intrinsic disorder in regions of the C-terminus of VP are capable of AAP-independent capsid assembly. Despite being close phylogenetic neighbors, AAV11 and 12 diverge in their AAP requirement for capsid assembly; presenting an ideal case to study mechanisms dictating AAP requirement. Upon mutational analysis, we found that a single amino acid in the C-terminus of VP3 could alter AAP requirement in AAV 4, 11 and 12. In AAV4 and AAV11, a P-to-L mutation at the corresponding positions 722 and 721 respectively, can convert AAV4 and 11 from being AAP-independent to AAP-dependent. Conversely, in AAV12, a L-to-P mutation at the corresponding position 730 can convert AAV12 from being AAP-dependent to AAP-independent. We designated this position (722 in AAV4, 721 in AAV11 and 730 in AAV12) as the "critical position" or CP. The CP lies in the C-terminus of VP, close to the VR-IX loop that is proximal to the 2-fold axis of VP. In the AAV5 VP context, we found that replacing 12 amino acids (dodecapeptide) of AAV5 at position 648 and 650 with each corresponding dodecapeptide from AAV2 VP abrogates AAV5's AAP independency. In AAV5, position 648 and 650 are in the HI loop, close to the 5-fold axis. Thus, for AAV4, 5, 11 and 12; we have variants that are either AAP-dependent or independent. For each of these serotypes and variants, we determined the degree of VP protein disorder using the Predictor of Natural Disordered Regions (PONDR) and compared the PONDR values between VPs showing distinct degrees of AAP dependency. We found that the disorder near the mutated region in the AAP-independent VPs always tends to be higher than that in the AAP-dependent VPs. The mutated regions are either near the 2-fold axis (for AAV4,11 and 12) or the 5-fold axis (for AAV5). Thus, AAP-independent VPs tend to have higher protein disorder than AAP-dependent VPs in these two regions. VP subunit interdigitation at the 2-fold and 5-fold axes is weaker relative to the 3-fold axis. Enhanced flexibility at the 2-fold and 5-fold axes likely promotes VP subunit interdigitations at VP-VP interfaces, leading to more stable VP oligomerization and subsequent capsid assembly. Conversely, VPs with reduced flexibility at the 2-fold or 5-fold axis don't autonomously oligomerize making them susceptible to degradation, necessitating AAP for VP oligomerization and subsequent capsid assembly. Our data suggest that enhanced disorder in the C-terminus of VP promotes structural flexibility in the 2-fold and 5-fold axis regions conferring VP the ability to undergo AAP-independent capsid assembly.

Suicide gene therapy for ovarian cancer using retroviral replicating vector

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Introduction: Retroviral replicating vectors (RRVs) have been shown to achieve tumor-selective replication, efficient tumor transduction and enhanced therapeutic benefit in a wide variety of cancer models. Here, we evaluated two different RRVs derived from amphotropic murine leukemia virus (AMLV) and gibbon ape leukemia virus (GALV), which utilize different cellular receptors (PiT-2 and PiT-1, respectively) for viral entry, in human ovarian cancer cells.

Materials & Methods: Expression levels the cellular receptors for AMLV (PiT-2) and GALV (PiT-1) in ovarian cancer cell lines (A2780, Caov3, RMG-1, SKOV-3) and fibroblast were evaluated by quantitative RT-PCR. In vitro RRV-GFP replication was monitored by flow cytometry, and cytotoxicity quantitated by AlamarBlue assay after 5-FC treatment of RRV-cytosine deaminase (CD) -transduced ovarian cancer cells. In vivo antitumor effect of RRV-mediated suicide gene therapy was investigated in an SKOV-3 subcutaneous tumor model.

Results: Quantitative RT-PCR analysis showed that high levels of expression of both receptors were observed in RMG-1 and SKOV-3 ovarian cancer cell lines compared to normal and non-malignant cells. Efficient RRV replication and spread was observed in vitro in RMG-1 and SKOV-3 cells with >90% transduction achieved by Day 10-13. Furthermore, after transduction of these cells with RRV-CD, significant reduction of cell viability was observed in a 5-FC prodrug dose-dependent manner.

Conclusion: These data indicate the potential utility of RRV vector-mediated suicide gene therapy in the treatment of human ovarian cancer.

Ultra-rapid Quantitative Detection of MMR- or HR-deficient Cancers via Transient Linear DNA Transfection

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Defects in DNA mismatch repair (MMR) or homologous recombination (HR) are causative of two major familial tumors (Lynch syndrome and hereditary breast and ovarian cancer (HBOC), respectively) as well as a wide range of sporadic cancers. Additionally, a recent report suggested a significant decrease in both MMR and HR activity in BRAFi/EGFRi-treated colorectal cancers, leading to an increase in drug-resistant cell population (Science, 2019). In clinical settings, MMR- and HR-deficient tumors can be successfully treated with ICI (immune checkpoint inhibitor; e.g., anti-PD-1 Ab) or PARPi, respectively, on the basis of outcomes associated with defective DNA repair. However, highly reliable diagnostic strategies to rapidly assess the cellular status of MMR/HR are yet to be established, narrowing proper application of targeted cancer therapies. Here we designed and developed luciferase-based DNA constructs, which, when transiently transfected after linearization, allow for quantitative detection of MMR- or HR-deficient cells in a highly sensitive (~30-100-fold difference between repair-proficient and -deficient cells) and rapid (measurable within ~1-4 hours after transfection) manner. We anticipate that this technique will pave the way for detecting thoroughly all types of cancers with defects (or significant reduction) in MMR or HR. Furthermore, the use of similar DNA constructs harboring a suicide gene instead of luciferase may make it feasible to specifically kill MMR-deficient cells as well as BRCA1/2-deficient tumors with acquired PARPi resistance through HR restoration.

Large-scale PCR for the production of DNA as source material for gene therapy

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In the corona virus pandemic era, mRNA and virus vector vaccines are rapidly developed and applied in the world. The world-wide successful application of these vaccines suggests that these relatively longer RNA/DNA will become effective next-generation nucleic-acid-based drugs. The longer nucleic-acid drugs cannot be produced by chemical synthesis but produced based on E. coli plasmids without exception. mRNA vaccines are produced by an RNA polymerase using linearized plasmid DNA as a template and virus-vector vaccines are produced by the transfection of virus vector plasmids to mammalian cells. Commonly, plasmid DNA is amplified in E. coli by cultivation, which is extracted from E. coli cells and extensively purified for gene therapy. The E. coli cultivation, extraction, and purification processes require specific facilities and equipment. In addition, endotoxin of E. coli, which elicits immunoreaction, must be completely removed by purification. Therefore, plasmid preparation is time-consuming laborious work. However, this method is still standard for DNA manipulation and production, may be due to the absence of alternative method of DNA production in large scale. Because of the long history of plasmid preparation, E. coli plasmid is believed to be safe even it usually contained an antibiotic resistance gene, which may have a risk of contamination to other microorganisms, and also is known to be attacked by transposons. We confirmed transposon insertion to a plasmid bearing a therapeutic gene prepared by normal LB medium culture by next-generation sequencing. One of which, IS1, was identified by PCR and sanger sequencing. This and other results indicated that high quality control is required for plasmid preparation and purification. Instead, PCR is a well-known method for DNA amplification but it is restricted to a small-scale reaction volume. This μl-scale reaction is the limitation for industrial-scale DNA production by PCR. The small-scale reaction of PCR may be due to strict requirement of a precise temperature control cycle consisting of denaturation, annealing, and extension. If PCR reaction can be performed in large scale, this would become alternative industrial production process of DNA. Moreover, in vitro PCR is an enzymatic reaction, thus eliminating laborious DNA purification processes. In order to achieve large-scale PCR, we improved a present thermal cycler. The PCR reaction mixture is set in an autoclavable plastic bag and it was placed between two temperature-controllable plates equipped with Peltier elements, which we call sandwich PCR. Using No. 3 prototype sandwich cycler, 200 mL-scale PCR was performed, and 3-kb DNA fragment encompassing mammalian CMV promoter, a gene, and a terminator region was efficiently produced from DNA template by 30 cycles of two-step temperature control. The large-scale PCR will become a next-generation DNA production procedure without time-consuming cultivation, extraction and purification processes.

Engraftability of gene-edited CD34+ cells at the sickle cell disease locus in a non-human primate transplantation model

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Background: Sickle cell disease (SCD) is caused by a 20A>T mutation in the β-globin gene (βs-globin). Genome editing technologies can potentially correct the SCD mutation in hematopoietic stem cells (HSCs), producing adult hemoglobin (Hb) with eliminating sickle Hb. We have previously demonstrated efficient gene correction in SCD CD34+ cells with SCD mutation-specific guide RNA, Cas9 mRNA/protein, and single-stranded donor DNA, resulting in ~30% gene correction and ~50% indels at the DNA level, and ~60% normal β-globin production at the protein level in in vitro erythroid differentiation, with engraftment in xenograft mice. Here, we evaluated engraftment of gene-edited CD34+ HSCs in a non-human primate transplantation model.

Methods: To model SCD gene correction in rhesus macaques, we designed normal β-globin to βs-globin gene conversion in the rhesus genome (n=2, 13U005 and 12U011). Rhesus CD34+ cells were electroporated by the GMP-compliant, FDA Master File-supported, and scalable MaxCyte GT System to deliver editing tools as well as an adjuvant to improve gene conversion efficiency. Mobilized rhesus CD34+ cells (3.4-3.8e7) were pre-stimulated for 2 days, electroporated to deliver rhesus β-globin-targeting guide RNA, Cas9 protein, and single-stranded donor DNA including the SCD mutation (20A>T), and cryopreserved after electroporation.

Results: Small aliquots of edited cells (before and after cryopreservation) were differentiated into erythroid cells in vitro, resulting in 17-26% gene conversion and 57-71% indels at the DNA level, and 50-100% β-globin production at the protein level, with no difference between aliquots taken before and after cryopreservation. Following 9.5 Gy total body irradiation, the frozen edited CD34+ cells (1.6-2.2e7) were injected into autologous macaques. Robust recovery of blood counts in 13U005 was observed, while peripheral blood recovery was delayed in 12U011, who was supported by serial whole blood transfusion. In 13U005 and 12U011, 7-11% and 1-2% of β-globin gene conversion were observed at the early timepoint (3 weeks) post-transplant, respectively, and the gene conversion ratios plateaued at ~1% in both animals 8-10 months post-transplant. At the early timepoint, 44-54% and 45-51% of indels were detected, but these levels plateaued to 10-12% and 7-11%, respectively. Sickle Hb production in red blood cells was ~17% and ~8% at the early timepoint in 13U005 and 12U011, plateauing at ~10% and ~3% 10-12 months post-transplant, respectively. Interestingly, 10-30% of fetal Hb production was observed at early timepoints post-transplant in 12U011, likely due to stress hematopoiesis during blood recovery.

Conclusion: We developed a rhesus transplantation model for HSC-targeted genome editing. The gene-edited CD34+ cells were engraftable for 8-10 months post-transplant, while gene conversion levels gradually decreased over the follow-up period. These findings will be helpful in the design of future HSC-targeted gene correction trials.

Disclosures: Linhong Li was an employee in the MaxCyte, Inc.

Gene therapy for congenital adrenal hyperplasia with AAV vectors into fibroblasts and model mice

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Background: Congenital adrenal hyperplasia (CAH) is due to defects of steroid synthetic enzymes, which includes microsomal P450s and mitochondrial P450s. 21-hydroxylase is a microsomal P450 and the defect (21-OHD), in which CYP21A2 is mutated or deleted, is the most common cause of CAH and result in underproduction of glucocorticoids and mineralocorticoids, and overproduction of androgens. 11β-hydroxylase deficiency, which is a mitochondrial P450, is the second common in CAH. Patients with CAH are treated with oral steroid supplementation, but optimal control of blood steroid levels remains difficult. Thus, new therapeutic approaches are still needed. Previously, adenovirus-mediated administration of human CYP21A2 into adrenal glands rescued the phenotype of a mouse model of 21-OHD. In this study, we examined the effects of induction of causative genes with an AAV vector into model mice and fibroblasts from patients.

Methods: A naturally occurring mouse model of 21-OHD was obtained by mating heterozygous pairs. A serotype-2 AAV vector (AAV2) containing Cyp21a1 cDNA was constructed and injected into the thigh muscles of the homozygote mice. Serum progesterone and DOC levels were measured before and every 4 weeks after injection. Fibroblasts from CAH patients were subjected to primary culture. The cells were infected with AAV2 containing CYP21A2 or CYP11B1 cDNA and cultured in steroid substrate-containing medium for 24 hours. We measured steroid metabolites in the medium by liquid chromatography tandem mass spectrometry and evaluated gene expressions by RT-PCR. In addition, iPS cells were established from fibroblasts of 11β-OHD and differentiated into adrenocortical cells with retinoic acid and cAMP. They were infected with a serotype-9 AAV vector (AAV9) containing CYP11B1 cDNA and measured steroid metabolites and gene expression. 11β-OHD model mice were made by a gene-editing method. We injected AAV9 containing Cyp11b1 cDNA into the adrenal gland and measured serum DOC and corticosterone levels before and every 4 weeks after injection.

Results: Serum progesterone/DOC ratios were markedly reduced at 4 weeks and remained low until 8 months after injection into 21-OHD mice. AAV2 infected fibroblasts from 21-OHD can metabolite 17-OHP to DOF, but failed to convert DOC to corticosterone in 11β-OHD. Differentiation into adrenocortical cells from iPS cells of 11β-OHD was confirmed by expression of CYP21A2. AAV9 gene induction of adrenal cortical cells successfully induced 11β-hydroxylase activity. 11β-OHD mice also showed improved steroid synthesis after AAV9 adrenal induction.

Conclusion: These results indicate that extra-adrenal induction of CYP21A2 may ameliorates steroid metabolism in 21-OHD patients. In contrast, 11β-OHD needs AAV9 adrenal induction to treat the steroid abnormality. Our results suggest that specific gene therapeutic strategies are needed needs to be adapted for each type of CAH congenital adrenal hyperplasia.

Development of the T cell gene therapy using the CRISPR/Cas9 system for the X-linked hyper IgM syndrome

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Background: X-linked hyper IgM syndrome (XHIM) is a combined immunodeficiency caused by mutations in the CD40 ligand (CD40LG) gene that typically results in decreased or absent CD40LG expression on activated T cells, leading to defective class switching and somatic hypermutation, and the patients therefore, have the recurrent bacterial infections during infancy. In the initiation of immune response, CD40LG expression is intricately regulated and therefore, a physiological expression of CD40LG is highly desirable for ensuring the safety and efficacy. We are now developing the CRISPR-Cas9-mediated site-specific gene editing to correct disease-causing mutations which enable the use of endogenous promoter for CD40LG expression.

Materials and Methods: Under approval of the institutional ethical committee, PBMCs were collected from a XHIM patient and healthy volunteers and processed by MACS cell separation. Preactivated T cells were edited with precomplexed SpCas9 ribonucleoprotein (RNP) and single-stranded oligodeoxyribonucleotides (ssODNs) delivered by electroporation (MaxCyte ATx). The replace endogenous CD40LG expression of were confirmed by FACS analysis following stimulation with PMA and Ionomycin activation. An in-vitro IgG and IgA class-switching assay to test the ability of edited T cells to induce class-switch recombination (CSR) in naïve B cells were evaluated by ELISA and RT-PCR.

Results: We designed single-guide RNA (sgRNA) and short ssODN (100bp) to make the correction of CD40LG mutation (c.420G>T) in a XHIM patient. We attempted that restored CD40L expression in T cells of a XHIM patient by CRISPR-Cas9-induced precise gene editing. After the treatment, CD40LG expression became detectable in edited T cells and the expression level improved 35-40% in CD4 positive T cells of a XHIM patient compared with healthy donors at almost 80%. Additionally, IgG and IgA class switching of naïve B cells co-cultured with activated edited XHIM T cells is a significant increase in the concentration compared with non-edited XHIM T cells in vitro.

Conclusions: In this study, we developed a strategy for the correction of the defective CD40LG gene in T cells from XHIM patient. This study method of non-viral genome targeting should prepare for short donor DNA and gRNA for every mutation of patient, but it is very cheaper and simpler of process than virus production for viral genome targeting. Furthermore, this study demonstrate restoration of functional CD40LG in edited XHIM T cells and the potential of T cell therapy for XHIM syndrome.

AAV vector-mediated pancreatic cell transduction in non-human primates by real-time image-guided intra-pancreatic duct injection

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Type 1 diabetes (T1D), a chronic disease caused by autoimmune destruction of insulin-producing beta cells in the pancreatic islets, remains one of the major causes of blindness, kidney failure and cardiovascular diseases. Patients with T1D are treated with a combination of diet control and lifelong exogenous insulin administration; however, progressive morbidity remains as a significant unmet medical need. Therefore, new approaches that effectively preserve and restore functional beta cells are needed. Recent studies have demonstrated successful conversion of non-beta cells into insulin-producing beta cell-like cells in human islets and rodent models by viral vector-mediated delivery of key transcription factors. This has brought patients a new hope on curative gene therapy for T1D.

Here, we report successful establishment of a real-time image-guided retrograde pancreatic duct (PD) injection method for AAV vector delivery to rhesus macaque pancreas, and demonstrate its safety and successful transduction in both pancreatic acinar cells and islet cells with an AAV9 vector. In the study, using two rhesus macaques negative for anti-AAV9 neutralizing antibodies, we performed laparotomy, made a small incision of the duodenum wall to directly visualize the ampulla of Vater, and inserted under fluoroscopy a catheter into PD with the catheter tip wedged at the proximal portion of the pancreatic body. Backflow of PD-injected agents to the duodenum could be prevented by selecting an appropriate size of the catheter. We then injected 1.4×10^{13} vector genome of AAV9-CAG-tdTomato over a period of 2 min followed by a 20-min dwelling of the injected agent in the pancreas. Blood samples were collected multiple times pre- and post-injection to assess safety and determine pharmacokinetic profiles of the PD-injected AAV9 vector. Tissues including the pancreas and the liver were harvested 4 weeks post-injection for downstream molecular and histological analyses. Although serum amylase and lipase levels were elevated following the injection, the levels went down to the normal ranges within 3 days post-injection and no clinical signs suggesting post-procedure pancreatitis were developed. The AAV9 vector leaked into the systemic circulation with the vector concentrations in blood reaching a peak at 8 h post-injection, resulting in vector genome dissemination to non-pancreatic organs to some degree. In the pancreas, the AAV9 vector transduced many acinar cells and some islets with a few showing good endocrine cell transduction. In summary, our study demonstrated that the PD injection is safe and transduces both acinar and islet cells with an AAV9 vector. However, this local approach with AAV9 still allows vector spillover in other non-target organs. We are currently searching and developing AAV capsids that can transduce pancreatic cells much more effectively than AAV9 via the PD delivery by employing our AAV Barcode-Seq and TRADE technologies to non-human primates.

Modulation of neurodegeneration by immune system in Niemann-Pick disease type C

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Niemann-Pick disease type C (NPC) is an autosomal-recessively inherited lysosomal storage disorder affecting an estimated 1 in 120,000 live births worldwide. Mutations in NPC1 or NPC2 gene represent approximately 95% or 5% of total patients with NPC, respectively. Their gene products, NPC1 and NPC2 proteins, function cooperatively in late endosomes and lysosomes to transport unesterifed cholesterol to the plasma membranes. Typical clinical feature of the disease is neurovisceral accumulation of unesterified cholesterol and several forms of glycosphingolipids. NPC can present with a broad range of clinical manifestation from a neonatal acute fatality to an adult-onset chronic disease associated with neurodegeneration. The development of neurological symptoms, including cerebellar ataxia, laughter-induced cataplexy, dystonia, and progressive dementia, affects quality of life of the patients drastically. Hence, it is essential to explore the pathogenic events that trigger and/or promote the neurodegenerative process for future clinical interventions. In this study, we addressed an involvement of immune system in neuropathogenic process using a murine model of NPC, npc1 mutant mice. Breaching of blood-brain barrier and infiltration of monocyte-derived macrophages correlated spatially and temporally with the loss of cerebellar Purkinje cells, which is a hallmark of neurodegeneration in NPC. Reduction of circulating monocytes using CD11b-diphtheria toxin receptor transgenic mice ameliorated Purkinje cell degeneration. Moreover, involvement of acquired immune system in the Purkinje cell loss was investigated by crossing npc1 mutant mice with Rag1 knockout mice. The npc1 mutant mice that lack Rag1 exhibited enhanced cerebellar ataxic phenotype. These results imply that delivery of lymphocytes may be effective for the treatment of neurodegeneration in NPC. Importantly, we confirmed that peripheral delivery of CD4 and CD25-double-positive regulatory T lymphocytes ameliorated cerebellar ataxia and neurodegeneration of Purkinje cells in npc1 mutant mice. Depletion of regulatory T lymphocytes enhanced cerebellar ataxic phenotype. Moreover, neonatal bone marrow transplantation ameliorated cerebellar ataxia and neuronal degeneration. Our results disclose a previously unrecognized neuropathogenicity of immune system in NPC and would benefit future remedies for devastating neurological diseases.

Neoadjuvant Use of Oncolytic Herpes Virus G47Δ Prevents Stage Advancement of Tongue Cancer

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Surgical resection remains the first treatment option for tongue cancer. One of the main issues is the waiting time for patients until surgery, often more than one month, which may cause a tumor growth and/or metastasis leading to an advancement of the cancer stage. G47Δ, a third-generation oncolytic herpes simplex virus type 1, when administered intratumorally, has been shown to exhibit a robust antitumor effect via direct cytopathic effect accompanied by systemic antitumor immunity in a variety of cancer. In this study, we investigated the efficacy of G47Δ as a neoadjuvant therapy for tongue cancer aiming to suppress the tumor growth and metastases before surgery. G47Δ exhibited an antitumor effect on a murine squamous cell carcinoma cell line SCCVII in vitro and in its subcutaneous tumor model. We further generated an orthotopic tongue tumor model using SCCVII in which cervical lymph node metastases occurs that mimic tongue cancer patients. G47Δ when used as a neoadjuvant therapy significantly prolonged the survival of tumor-bearing mice compared with primary tumor resection alone. Analyses of infiltrating immune cells in tumors and cervical lymph nodes showed increased CD8+ T cells, which indicated an efficient induction of antitumor immune responses caused by the intratumoral administration of G47Δ. These findings indicate that an application of G47Δ as a neoadjuvant therapy is a promising strategy for treating tongue cancer, which may prevent the stage advancement of the disease and the need for an extensive resection.

Efficient antitumor effects of a novel oncolytic adenovirus fully composed of species B2 adenovirus serotype 35

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Oncolytic viruses are attracting attention as novel cancer therapeutics. Among oncolytic viruses, an oncolytic adenovirus (OAd) is one of the most promising oncolytic viruses. Almost all OAds are composed of human adenovirus (Ad) serotype 5 (Ad5), which belongs to species C. The OAd composed of Ad5 (OAd5) showed significant antitumor effects. However, it has two drawbacks. First, expression levels of the primary infection receptor, coxsackievirus and adenovirus receptor (CAR), often declines on malignant tumor cells, leading to inefficient infection of CAR-low/negative tumor cells with OAd5. Second, more than 80% of adults have neutralizing antibodies against Ad5. Anti-Ad5 neutralizing antibodies significantly inhibit infection of the tumor cells with an OAd5, leading to reduction in antitumor effects. In this study, in order to overcome these drawbacks of OAd5, we developed a novel OAd fully composed of human Ad serotype 35 (Ad35) (OAd35), which belongs to species B2. Ad35 recognizes CD46, which is ubiquitously expressed on almost all human cells except erythrocytes and is often upregulated on malignant tumor cells, as an infection receptor. Therefore, OAd35 can infect a wider variety of tumor cells, including malignant tumor cells, than conventional OAd5. Moreover, only 20% or fewer adults have neutralizing antibodies against Ad35. Hence, an OAd35 is unlikely to be inhibited by preexisting neutralizing antibodies. OAd35 expressed E1A gene, which is essential for viral replication, by a human telomerase reverse transcriptase (hTERT) promoter. OAd35 was propagated in HEK293 cells. The titers of OAd35 were slightly lower than those of OAd5. First, in order to examine tumor cell lysis activities of OAds, crystal violet staining was performed. OAd35 mediated efficient cell lysis activities at levels similar to OAd5 in CAR-positive tumor cells, while OAd35 showed higher levels of cell lysis activities than OAd5 in CAR-negative tumor cells. Next, in order to examine the tumor cell lysis activities of OAds in the presence of anti-Ad5 neutralizing antibodies, OAds were pre-incubated with mouse anti-Ad5 serum. Anti-Ad5 serum significantly inhibited tumor cell lysis activities of OAd5, whereas OAd35 exhibited comparable levels of tumor cell lysis activities in the presence of anti-Ad5 serum and naïve serum. Finally, in order to examine the in vivo antitumor effects of OAds, OAds were intratumorally administered in the mice bearing subcutaneous H1299 tumors. Both OAds mediated similar levels of growth inhibition of the subcutaneous xenograft tumors following intratumoral administration. These results indicated that OAd35 can overcome the drawbacks of a conventional OAd5 and become a promising alternative oncolytic virus.

Oncolytic vaccinia virus carrying IL-7 and IL-12 alters intratumoral TCR repertoire, systemically sensitizing tumors to immune checkpoint blockade

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Immune checkpoint blockade using an anti-PD-1 or anti-CTLA4 antibody induces durable clinical responses in various cancers, however, the benefits are limited to small subsets of patients. As a solution, various immunotherapies alone and in combination have been investigated, among which oncolytic virotherapy is emerging as a promising approach. Oncolytic viruses can upregulate immune responses to tumor antigens following direct killing of cancer cells, resulting in in situ cancer vaccination. We have explored this approach using tumor-selective oncolytic vaccinia viruses (VVs), and recently demonstrated that intratumoral treatment with a VV carrying human IL-7 and murine IL-12 genes (hIL-7/mIL-12-VV) activates the inflammatory immune status of treated tumors and non-treated distant tumors, and markedly improves the sensitivity of both tumors to anti-PD-1 antibody treatment. The rate of complete regression of poorly immunogenic tumors is correlated with the number of tumor infiltrating T cells, which is higher when both IL-7 and IL-12 are expressed in tumors than either alone. Expecting that this combination may contribute to a more preferable T-cell architecture within tumors, in this study, we examined the activation status of intratumoral T cells and intratumoral TCR clonality after treatment with hIL-7/mIL-12-VV or its component viruses, hIL-7-VV and mIL-12-VV. In poorly immunogenic LLC tumors, the number of activated CD8+ T cells expressing granzyme B or IFN-gamma was significantly higher following treatment with hIL-7/mIL-12-VV than mIL-12-VV, with no apparent difference in the number of $PD\text{-}1^{+}Tim3^{+}$ exhausted $CD8^{+}$ T cells. Whereas IL-7 alone increased the diversity of intratumoral $CD8^{+}$ T cells, IL-12 alone increased clonality with a unique high frequency clone. IL-12 combined with IL-7 further increased clonality with expansion of multiple high frequency clones. Our findings provide additional insight into the wide ranging roles of IL-7 in cancer immunology and a scientific rationale for the combined expression of IL-7 and IL-12, supporting the clinical development of this virotherapy.

p53-armed Telomerase-Specific Oncolytic Adenovirus Modulates Long-Lived Memory CD8 T Cells in Neoadjuvant Therapy for Pancreatic Cancer

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Pancreatic cancer is one of the most aggressive cancers, and the prognosis is still extremely poor despite the developments of various multimodal treatment strategies because of early metastasis. Neoadjuvant chemotherapy has currently attracted much attention as an effective treatment strategy to improve the prognosis. We developed telomerase-specific oncolytic adenoviruses (OAs), including OBP-301 which is now tested in a clinical trial of combination with anti-PD-1 antibody, and a p53-armed variant of OBP-301 (OBP-702). OAs have immune-modulate functions and induce CD8 T cells into tumors via release of immunogenic cell death markers such as ATP. However, immunotherapeutic effects of OBP-702, especially, long-term immune effects which is considered important in neoadjuvant therapy or metastatic site, are still unknown. The memory precursor effector cells (MPECs), which exist in effector T cells at early phase, differentiate into tissue-resident (T_{RM}), effector (T_{EM}) or central (T_{CM}) memory T cells, particularly T_{RM} and T_{EM} are considered to have a high effector function for long-term anti-tumor immunity. Here we investigated the effectiveness of OBP-702 in treatments for pancreatic cancer, focusing on the influence on long-lived memory CD8 T cells.

We used a PANC-1 human pancreatic cancer cell line and a PAN02 murine pancreatic cancer cell line. OBP-702 showed strong anti-tumor effects for both cell lines following infection via coxsackie adenovirus receptors and integrin alpha v beta 5 receptors. OBP-702 strongly induced extracellular ATP which is considered important for the establishment of MPECs. In a PAN02 subcutaneous mouse model, local treatment of OBP-702 strongly induced MPECs, especially CD62L negative MPECs which differentiate into T_{RM} and T_{EM}. Combined therapy of OBP-702 and gemcitabine plus nab-paclitaxel (GN), which is a primary regimen for pancreatic cancer, showed synergistic effects in vitro and in vivo. Combined therapy of OBP-702 and GN significantly suppressed the growth of not only OAs-injected tumors but also un-injected tumors, compared with the monotherapy of OBP-702 or GN in a PAN02 bilateral subcutaneous tumor model, in which OBP-702 and combined therapy significantly increased T_{RM} in both tumors, and increased T_{EM} in the spleen as well. Finally, we performed a re-challenge experiment assuming a neoadjuvant therapy, in which PAN02 tumors implanted on the left flank of mice were resected after three times treatments, and PAN02 tumors re-inoculated on the right flank after 30 days after resection were observed with no further treatment to check long-term immune effects. In this re-challenge model, neoadjuvant combined therapy strongly increased T_{EM} in the spleen, and significantly suppressed tumor growth compared with other control treatments.

In conclusion, OBP-702 local treatment has the potential to increase anti-tumor immunity via modulation of long-lived memory CD8 T cells in pancreatic cancer, leading to suppressing tumor growth of metastatic lesion and inhibition of tumor recurrence after neoadjuvant therapy.

Establishment of a Pancreatic Cancer Animal Model using the Pancreas-Targeted Hydrodynamic Gene Delivery method

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Pancreatic cancer is a leading cause of cancer-related death worldwide and the development of the effective therapeutic option is an unmet clinical need. However, due to the lack of useful pancreatic cancer animal model is the major drawback to solve the need. Therefore, the establishment of the animal model of the pancreatic cancer is essential and the analyses of the molecular mechanisms and histological characteristics will be the milestone for the issue. For this purpose, we have conducted this study aiming at establishing a pancreatic cancer animal model in wild type rat model utilizing the non-viral gene delivery method of hydrodynamic gene delivery (HGD). The procedure involves the insertion of a catheter into the superior mesenteric vein with temporary blood flow occlusion at the portal vein followed by the hydrodynamic injection of DNA solution to the rats. The efficiency and specificity of the procedure on gene expression in the pancreas was evaluated using a pCMV-Luc reporter plasmid. We demonstrated that our procedure achieved effective pancreas-specific gene expression which was 2,000-fold higher than that was seen in the liver, and no detectable level of luciferase activity was seen in the other organs, including the brain, heart, lungs, spleen, and kidneys. The highest level of gene expression was achieved with the HGD of plasmid DNA (5 μg/mL) at the optimal injection parameters of injection volume of 2% body weight and flow rate of 1 mL/s. Then, the parameters were utilized to deliver the plasmid, expressing human cancer related genes of KRAS wild-type, $KRAS^{G12D}$, NRAS, MYC, and YAP genes under the control of a chicken β-actin promoter and cytomegalovirus enhancer. The pancreas-targeted HGD of the $KRAS^{G12D}$ alone caused development of the pancreatic tumor in 33% of rats at 5 weeks after the HGD, while the combination of $KRAS^{G12D}$+YAP caused higher incidence of pancreatic tumors in 80% and metastasis in 33% of plasmid delivered rats. Histological evaluation of the tumor revealed high-grade atypical epithelia of the pancreatic duct cells with interstitial fibrosis and the metastatic lesions showed higher grade malignancy with the suspicious of pancreatic cancer origin. Immunohistochemical examination revealed the large number of Ki-67 positive tumor cells, stained positive for CK7 and CK20. Furthermore, when delivered $KRAS^{G12D}$ and YAP simultaneously, the epithelial-mesenchymal transition was evidenced by the reduction of E-cadherin and increase of N-cadherin expression which contributed for the aggressive tumor behaviors. These phenotypes were confirmed by the molecular analyses of the KRAS-related pathways including Erk, Akt, Ctgf, and Tgf-β and the time-dependent assessment of the serum tumor markers. These results suggest the efficacy of HGD for developing the pancreatic cancer animal model which could be used to develop the therapeutic options.

A novel Echovirus 4 shows noticeable oncolytic activity against esophageal cancer

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Recently, esophageal cancer is one of the frequent cause of cancer death in the world. The standard therapeutic approaches including surgery, chemotherapy and radiotherapy are currently used. There are, however, still many patients suffered from recurrent or metastatic tumors refractory to these therapies. Development of novel therapies is definitely required to overcome such situation. Oncolytic viral therapy is one of the promising anticancer candidates for refractory esophageal cancers. A small number of enteroviruses, such as coxsackievirus A (CVA) and echovirus, were reported to have lytic activities for various human malignancies. We found that coxsackievirus B3 (CVB3) displayed a high level of tropism and lytic activities for human non-small-cell lung cancer cells after screening of representative human enteroviruses. However, wild type CVB3 caused both pancreatitis and myocarditis, so we focused on echovirus 4 (EV4) because of its low toxicity.

EV4 targeted and destroyed esophageal cancer cells (ESCC) more than any other solid cancer cells. Especially, EV4 showed the potent oncolytic activities in human esophageal cancer cell line of TE-1, TE-6 and TE-8 at a low multiplicity of infection (MOI) of 0.001 in crystal violet assay. On the other hand, EV4 did not destroy normal human cell line at a MOI of 0.1. We also found that the caspase-dependent apoptotic pathway was involved in oncolytic activity of EV4 because flow cytometric Annexin V and PI co-staining induced apoptosis in initial EV4 infection in ESCC, and the addition of pan-caspase inhibitor z-VADfmk significantly reduce its cytotoxicity. Furthermore, in vivo study using human tumor-bearing nude mice showed that the intratumor injections of EV4 inhibited substantial growth of subcutaneously pre-established esophageal tumors.

In conclusion, our findings suggested that EV4 could be a novel therapeutic modality for human esophageal cancer.

p53-armed oncolytic adenovirus reverses immunosuppressive tumor microenvironment in gemcitabine-resistant pancreatic cancer

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Background: Pancreatic ductal adenocarcinoma (PDAC) is the most lethal disease with a 5-year survival rate of less than 10%. Although gemcitabine (GEM) is widely used as a key drug for the treatment of PDAC, the resistance to GEM contributes to poor prognosis in PDAC patients. Moreover, GEM treatment induces an immunosuppressive tumor microenvironment (TME). Thus, new therapeutic strategies for improving therapeutic response are critically needed to improve the clinical outcomes in GEM-resistant PDAC patients. Oncolytic virotherapy has recently emerged as a novel antitumor therapy against PDAC. We generated a telomerase-specific oncolytic adenovirus (OBP-702) that induces the tumor suppressor p53 gene. OBP-702 exhibited profound antitumor effect against human PDAC cells. In this study, we investigated the therapeutic effect of OBP-702 in GEM-resistant PDAC tumors with immunosuppressive TME.

Methods: We established a mouse GEM-resistant PDAC cell line PAN02 by sequential exposure to GEM for 3 months. To investigate the immunosuppressive TME, parental and GEM-resistant PAN02 cells were subcutaneously implanted into immunocompetent C57BL/6J mice. Tumor-infiltrating immune cells were analyzed by immunohistochemistry. To determine whether GEM-resistant PAN02 cells induce the differentiation of immunosuppressive cells, bone marrow-derived cells were stimulated with conditioned media (CM) from parental and GEM-resistant PAN02 cells. To explore the underlying mechanism of PDAC-induced immunosuppressive TME, we analyzed the secretory protein levels using cytokine array and ELISA assay.

Results: GEM-resistant PAN02 cells were 3-fold more resistant to GEM compared with parental cells. OBP-702 suppressed the viability of GEM-resistant PAN02 cells as similar with parental cells. In the subcutaneous tumor model, the infiltration of immunosuppressive cells, including Gr1-positive myeloid-derived suppressor cells (MDSCs) and FoxP3-positive regulatory T cells (Treg), were significantly increased in GEM-resistant PAN02 tumors compared with parental tumors. Intratumoral injection of OBP-702 resulted in a significant decrease in the growth of GEM-resistant PAN02 tumors and infiltration of MDSCs and Tregs. The CM from GEM-resistant PAN02 cells significantly induced larger proportion of MDSCs than parental cells. Cytokine array and ELISA assay revealed that the amount of GM-CSF was significantly increased in the CM from GEM-resistant PAN02 cells compared with parental cells. OBP-702 significantly decreased the proportion of MDSCs stimulated by CM from GEM-resistant PAN02 cells via suppression of GM-CSF secretion.

Conclusions: Our results suggest that GEM-resistant PDAC cells induce the immunosuppressive TME with infiltration of MDSCs and Tregs via secretion of GM-CSF. Oncolytic virotherapy with OBP-702 may be a promising antitumor strategy to reverse the immunosuppressive TME on GEM-resistant PDAC.

AAV5 preferentially transduces neural stem cells of gerbil dentate gyrus compared to AAV2 and AAVrh10

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Objective: Adeno-associated virus (AAV) are now widely used for basic and clinical research. Tropism of AAVs are considered to be determined by the viral capsids. The precise tropism to the neural stem cells has not been determined. In this study, we show the tropism to the neural stem cells of gerbil dentate gyrus using three different serotypes, AAV2, 5, and rh10.

Methods: AAV2, 5, and rh10 vector solution carrying green fluorescent protein (GFP) driven by cytomegalovirus promoter was made. Twelve male gerbils (4-week-old) were stereotaxically injected with 1.5×10^{10} viral genomes of AAV solution or phosphate-buffered saline into the right dentate gyrus. One week later, brains were taken, and coronal sections were made for immunohistochemical analysis. Average count of three sections of whole dentate gyrus was defined as a result.

Results: AAV5 showed the largest number of double positive cells for GFP and Sox2, a marker for neural stem cells, compared to the AAV 2 and rh10 (AAV2: 0.0 ± 0.0, AAV5: 24.4 ± 1.6, AAVrh10: 15.4 ± 3.5, $p < 0.001$ and $p < 0.05$ for AAV5 compared to the control and AAVrh10, each). On the contrary, AAVrh10 showed the largest number of double positive cells for GFP and NeuN, a marker for mature neurons, compared to AAV2 and 5 groups (AAV2: 6.4 ± 3.3, AAV5: 13.6 ± 5.9, AAVrh10: 143.4 ± 20.0, $p < 0.001$ for AAVrh10 compared to the control).

Conclusions: AAV5 showed higher preference to neural stem cells, compared to AAVrh10. On the other hand, AAVrh10 showed robust transgene expression, with preference to both neural stem cells and mature neurons. We should note that appropriate choice of AAVs could be important, which depends on what the target is in the experiment.

Examination of a novel therapy for amyotrophic lateral sclerosis by enhanced expression of neuroprotective factor Necdin

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Background: Necdin, a growth suppressor expressed predominantly in postmitotic neurons, interacts with transcription factor p53. Mitochondrial dysfunction plays central roles in the pathophysiology of neurodegenerative disease. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-la) is master regulator in mitochondrial biogenesis. Necdin promotes neuronal mitochondrial biogenesis induced by enhanced PGC-la expression. Necdin binds and strongly stabilizes PGC-la by inhibiting its ubiquitin-proteasomal degradation. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease in which degeneration of motor neurons causes systemic muscle weakness, and no radical therapeutic drug has been developed yet. In this study, we investigated the therapeutic effect of ALS model animals by increasing the expression of the necdin gene using adeno-associated virus (AAV).

Methods: We generated AAV1-eGFP and AAV1-Necdin. Female 8-week-old SOD1 G93A mice were intramuscularly administered with AAV1-eGFP (control group) or AAV1-Necdin to the left quadriceps muscle. AAV viral titers are 1x1010 (low titer group) or 1x1011 (high titer group) viral genomes / injection. We examined the weight, survival of those mice, and neurological functions. In in vitro, AAV1-eGFP or AAV1-necdin was administered to the differentiated NSC-34, and the expression level of necdin and survival rate using LDH assay were examined.

Results: The AAV1-necdin expression vector was administered into the hind limb muscle of mutant SOD1-expressing ALS model mice, which was suggested to have an onset-suppressing effect in the studies up to last year, with multiple viral titers, and its effect on motor symptoms was examined. High titer administration did not change either onset or longevity, while low titer administration delayed onset but not extended longevity. When the AAV1-necdin expression vector was administered to NSC34 cells, which are motor neuron-like cultured cells, at multiple titers, dose-dependent cytotoxicity was observed.

Conclusion: These findings suggest that intramuscular administration of the AAV1-necdin to ALS model mice showed an inhibitory effect on the onset at low titers, but the effect was not clear at high titers.

Biological and functional characterization of human mesenchymal stem cells derived from different sources for cancer gene therapy

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Background: Mesenchymal stem cells (MSCs) hold great promise as virus carrier cells due to their ability to home to inflammatory tissues including tumors, immunomodulatory function, and immunological tolerance. For cancer gene therapy, MSCs can be loaded with oncolytic viruses and deliver them to tumors effectively. MSCs are originated from various tissues and known to have different characteristics, such as expression of surface markers, gene expression, and growth rate. The impact of MSC origin on cancer gene therapy remains elusive. Therefore, here we compared the biological and functional properties of human MSCs (hMSCs) derived from different tissues as oncolytic virus delivery vehicles.

Methods: Immortalized hMSCs generated from different tissues, including human bone marrow (BMMSCs), adipose tissue (ADMSCs), umbilical cord blood (UCBMSCs), and endometrium (EPCMSCs) were labeled by transduction of a lentivirus vector encoding AcGFP gene for live imaging analysis. The tumor-specific migration and invasion ability of hMSCs were monitored by co-culture of hMSCs with a human pancreas cancer cell line PANC-1 spheroids and cholangiocarcinoma organoids in two- and three-dimensional (2D and 3D) culture system. To assess the capacity of hMSCs as carrier cells of oncolytic HSV (oHSV), hMSCs were infected with an oHSV C-REV (canerpaturev) at different multiplicity of infection (MOI), and the cell viability and the cellular tropism of oHSV were investigated.

Results: In co-culture of hMSCs and cancer spheroids or organoids in 3D culture, hMSCs preferentially accumulate to cancer spheroids or organoids in a time-dependent manner, but not to spheroids of human fibroblast, suggesting that the migration/invasion is tumor-specific. The directed migration and invasion of hMSCs to cancer spheroids and organoids were observed in all hMSCs tested whereas the efficiency depended on the origins. Cell viability assay of hMSCs infected with oHSV showed that all hMSCs were effectively infected by oHSV while ADMSCs had the highest survival rate after oHSV infection.

Conclusion: Collectively, our results demonstrate that the homing ability of hMSCs to cancer spheroids and organoids and the susceptibility of hMSCs to oHSV infection depended on the tissue origins and provides useful information for hMSC-mediated delivery of oHSV.

Enhanced cell survival and therapeutic benefits of IL-10-expressing multipotent mesenchymal stromal cells for Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is an incurable genetic disease with early mortality that exhibits skeletal muscle weakness with chronic inflammation. Multipotent mesenchymal stromal cells (MSCs) are potentially therapeutic for muscle disease because they can accumulate at the sites of injury and act as immunosuppressant. To improve the functionality of MSCs as a cell source, we focused on the anti-inflammatory properties of IL-10 and that IL-10 could improve the typically low survival of MSCs by exerting a paracrine effect after transplantation. We designed and examined the strategies for safe and effective ex vivo treatment of IL-10 producing MSCs using DMD models for functional recovery of the skeletal muscles, as well as focusing on factors related to the effects of MSC treatment.

Methods: We developed a continuous IL-10 producing system of MSCs derived from dental pulp using an adeno-associated virus (AAV) vector. IL-10/AAV-transduced MSCs (IL-10-MSCs) or MSCs were intravenously injected into canine X-linked muscular dystrophy in Japan (CXMDJ) in acute phase. To assess the safety and effects of therapeutic interventions, treated CXMDJ was comprehensively analyzed such as blood tests, growth, spontaneous activity, tetanic force, running function, MRI and histological examination.

Results: Repeated systemic administration of IL-10-MSCs into the CXMDJ resulted in long-term engraftment of cells and slightly increased the serum levels of IL-10. IL-10-MSCs showed significantly reduced expression of pro-inflammatory MCP-1 and upregulation of stromal-derived factor-1 (SDF-1). MRI and histopathology of the IL-10-MSCs-treated CXMDJ indicated the regulation of inflammation in the muscles, but not myogenic differentiation from treated cells. IL-10-MSCs-treated CXMDJ showed improved running capability and recovery in tetanic force with concomitant increase in physical activity. While no significant difference was observed in both MSC- and IL-10-MSC treated CXMDJ on the maintaining apparent function, we confirmed the weight of each muscle obtained from IL-10-hDPSC-treated CXMDJ increased compared to that in the MSC-treated CXMDJ indicating morphological improvement. Furthermore, the increased serum creatine kinase levels after running exercise were suppressed in IL-10-MSC-treated CXMDJ, but not in MSC-treated CXMDJ, suggesting a protective effect against dystrophic damage caused by exercise.

Discussion: IL-10-MSCs facilitated the long-term retention of the cells in the skeletal muscle and also protected muscles from physical damage-induced injury, which improved muscle dysfunction in DMD. We can suggest that the systemic administration of IL-10-producing MSCs offers potential benefits for DMD therapy through the beneficial paracrine effects of IL-10 involving SDF-1. This study would help us understanding the mechanisms of MSC function.

Development of safety assessment system using non-human primate model for practical use of CAR-T cell products

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Purpose: Chimeric antigen receptor T (CAR-T) cell therapy possesses the potential to cause unexpected off-tumor/off-target toxicity that may sometimes be life-threatening. Therefore, various non-clinical safety studies need to be conducted, and animal models for evaluating the toxicity of newly developed immune-cell based products should be carefully selected according to the toxicity profiles and endpoints. Although rodent models are suitable for assessing the antitumor efficacy and pharmacodynamics, they are inadequate for assessing toxicity because of the interspecies divergence between humans and rodents. To address this issue, we have established a system and facilities to evaluate the safety of CAR-T cell products using non-human primate (NHP) models, in which most protein structures are highly conserved and exhibited high cross-reactivity with humans. Here, we introduce the facilities dedicated to the toxicity evaluation of gene and cell therapy products, and report on the recent non-clinical safety studies conducted using two different NHP models to evaluate the on-target and off-tumor toxicities of newly developed CAR-T cell products.

Methods: We established piggyBac-mediated CAR-T cells using cynomolgus peripheral blood mononuclear cells, and transferred them into autologous individuals, and evaluated the potential toxicity related to CAR-T cells (Autologous transplantation model). Alternatively, we have also developed a lymphodepleted NHP model by conditioning animals with immunosuppressive chemotherapy designed to simulate clinical practice conditions, to induce transient mixed chimerism before the administration of human CAR-T cells (Xenotransplantation model). All macaques were closely monitored for 14 days (autologous transplantation model) or 7 days (Xenotransplantation model) to assess the potential toxicity.

Results: In the autologous transplantation model, 3×10^5/kg cynomolgus CAR-T cells redirected to human granulocyte-colony stimulating factor receptor (hGMR) were infused to autologous individuals. Cynomolgus hGMR-CAR-T cells recognized and killed cynomolgus monocytes and macrophages in vitro, but showed no overt organ toxicities such as bone marrow suppression, monocytopenia, and vasculitis. In the xenotransplantation model, lymphodepletion was performed in cynomolgus macaques by intravenous administration of fludarabine for 4 days and cyclophosphamide for 2 days, followed by 3.3×10^6/kg of human PBMC derived CAR-T cells redirected to EPHB4 receptor. Lymphodepletion was successfully achieved on Day -1 of T cell infusion, and persisted until day7 without severe organ toxicity. A single administration of human EPHB4-CAR-T cells did not induce overt organ toxicities, although EPHB4-CAR-T cells were activated in vivo as evidenced by the elevation in copy numbers of the CAR transgene 24 h after infusion.

Conclusion: Although these NHP models still have limitations and need to be further optimized to fully assess the toxicity of human CAR-T cells, the NHP model may be useful in the assessing the on-target/off-tumor toxicities of CAR-T cells.

Liver-specific overexpression of lipoprotein lipase improves glucose metabolism in mice fed a high-fat diet

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Ectopic fat is defined by excess adipose tissue in locations not classically associated with adipose tissue storage. Ectopic fat deposition can be observed in several organs such as muscle, liver, and pancreas. The liver is the main organ controlling glucose metabolism. Excess lipid accumulation in the liver impairs insulin sensitivity and glucose metabolism, leading to type 2 diabetes mellitus (T2DM). Lipoprotein lipase (LPL) plays a major role in lipid metabolism by hydrolysis of triglyceride (TG)-rich lipoproteins such as chylomicrons and very-low-density lipoproteins. LPL converts TG to glycerol and fatty acid. One of the main components of lipid droplets is TG. Therefore, we hypothesized that attenuation of lipid accumulation in the liver may improve glucose metabolism, leading to treatment of non-alcoholic fatty liver disease (NAFLD) and T2DM. In this study, we investigated the effect of LPL overexpression in the liver using a modified adenovirus (Ad) vector on a high-fat diet (HFD)-induced lipid accumulation and glucose metabolism.

To overexpress LPL in the mouse liver, we generated an LPL-expressing Ad vector (Ad-LPL) containing the target sequences of liver-specific miR-122a at the 3'-untranslated region of the E4 gene for suppression of hepatotoxicity (Ad-E4-122aT ref1,2). C57BL/6 mice were transduced with Ad vectors and simultaneously started to be fed HFD for two weeks. To investigate the effect of LPL on lipid accumulation in the liver, we performed a histopathological examination of liver sections using hematoxylin-eosin staining. Much lipid droplets were observed in the liver of mice treated with the control Ad vector. The lipid-droplet formation was decreased in Ad-LPL-treated mice relative to the levels in the control mice. To explore the effect of LPL on glucose metabolism, glucose tolerance tests were performed after intraperitoneally injecting glucose in HFD-fed mice that had fasted for 16 h. Glucose levels after glucose injection among Ad-LPL-treated mice were significantly lower than those of control Ad-treated mice, indicating that glucose tolerance was significantly higher in Ad-LPL-treated mice than in control Ad-treated mice. Furthermore, fasting blood glucose and insulin levels were significantly lower in Ad-LPL-treated mice than in control mice.

In summary, these results suggest that LPL overexpression in the HFD-fed mouse liver attenuates the accumulation of lipid droplets in the liver, and improves glucose metabolism. Thus, this approach could be helpful in the development of new drugs to treat metabolic syndrome, including NAFLD and T2DM.

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Development of gene modified oncolytic coxsackievirus for clinical trial

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Recently, oncolytic viruses have emerged as new modalities for cancer treatment because of their tumor cell lysis via preferential replication of oncolytic viruses in tumor cells, followed by the activation of ability host's antitumor immunity. Our group previously reported that coxsackievirus B3 (CVB3) is a novel oncolytic viruse with a strong ability to lyse human non-small cell lung cancer cells. And two organ-specific (enriched in muscle and pancreas) miRNAs target sequences were constructed into the 3' untranslated region (3'UTR) of the CVB3 genome (CVB3-HP) resulting in the markedly reduced occurrence of CVB3-induced pancreatitis and myocarditis. However, non-clinical acute toxicity studies of CVB3-HP in mice and monkeys showed mild hematological and histopathological abnormalities in the highest dose group.

To further suppress replication of virus in normal organs, target sequence complementary to miR-217 (enriched in pancreas) and miR-34a (enriched in normal organs) were inserted into both of the 5'UTR and 3'UTR of CVB3 genome (CVB3-BHP). The intratumoral CVB3-BHP administrations into xenograft tumor mice model showed dramatically decreased side effects. And, mice treated with CVB3-WT showed significantly increased serum levels of AST, LDH and amylase, and histological findings of pancreatitis. In contract, CVB3-BHP showed neither notably increased serum levels of any enzymes nor pancreatic injury.

Next, to conduct non-clinical and clinical study, we have developed a closed-system GMP manufacturing method for CVB3-BHP. Thereafter, we are going to consult the Pharmaceuticals and Medical Devices Agency (PMDA) about sufficiency and validity of the above items, and conduct non-clinical study.

Safe and low-dose but therapeutically effective adenovirus-mediated hepatocyte growth factor gene therapy for type 1 diabetes: Important implications for developing strategies for clinical application of in vivo gene therapy

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Type 1 diabetes (T1D) is an autoimmune disease resulting from the destruction of insulin-producing β-cells. Exogenous insulin supplementation remains the mainstay of therapy, innovative therapies are urgently required. Hepatocyte growth factor (HGF) is a multifunctional cytokine that plays important roles in pancreatic physiology. The potential of HGF gene therapy against T1D was examined in only three previous studies, all of which used the most popular cytomegalovirus (CMV) promoter to express the exogenous HGF gene.

In two of the three studies, however, the mice were pre-treated before the onset of T1D, which is not clinically practical. In the third study, the authors administered an intravenous injection of a high dose of adenoviral vector (Ad) expressing HGF after the onset of T1D, resulting in partial efficacy. However, intravenous injection of high-dose Ad limited the clinical application due to the critical risk of lethal toxicity observed in previous clinical studies. Moreover, the very recent report of a death in a clinical trial using an adeno-associated viral vector, long believed to be non-pathogenic and safe, has revealed a very serious problem: high-dose vector administration, regardless of vector type, can cause serious adverse events including dangerous liver toxicity following intravenous injection. The present study aimed to develop a safe and low-dose but therapeutically effective adenovirus-mediated HGF gene therapy for streptozotocin (STZ)-induced T1D in mice.

A single intravenous injection of a low dose (3 × 10E8 plaque forming units) of adenoviral vector expressing the HGF gene under the transcriptional control of a strong promoter, i.e., the cytomegalovirus immediate-early enhancer and a modified chicken β-actin promoter (Ad.CA-HGF), was given to T1D mice. Low-dose HGF gene therapy significantly attenuated the elevation of blood glucose concentrations at the acute phase of T1D, and this effect persisted for several weeks. Temporal upregulation of plasma insulin at the acute phase was maintained at a normal level in Ad.CA-HGF-treated mice, suggesting that the therapeutic mechanism may involve protection of the remaining β-cells by HGF. Liver enzymes in plasma were not elevated in any of the mice, including the Ad.CA-HGF-treated animals, all of which looked healthy, suggesting the absence of lethal adverse effects observed in patients receiving high intravenous doses of viral vectors. In conclusion, a low dose of intravenous Ad-mediated HGF gene therapy is clinically feasible and safe, and thus represents a new therapeutic strategy for treating T1D.

Bone morphometric analyses for alveolar bone regeneration by bmp-2 gene therapy

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Alveolar bone is not spontaneously regenerated following trauma and periodontitis. We previously proposed an animal model for new alveolar bone regeneration therapy based on the non- viral BMP-2/7 gene expression vector and in vivo electroporation, which induced the formation of new alveolar bone over the course of a week. In general, BMP-2 also has a strong potential to induce new bone formation. Here, we analyzed alveolar bone following BMP-2 gene transfer to periodontal tissue. Non-viral plasmid vector pCAGGS-BMP-2 or pCAGGS control was injected into palatal periodontal tissue of the first molar of the rat maxilla and immediately electroporated with 32 pulses of 50 V for 50 msec. Over the following three weeks, rats were double bone-stained by calcein and tetracycline every three days and mineral apposition rates (MAR) were measured. Double bone-staining revealed that MAR of alveolar bone was as similar level three days before BMP-2 gene transfer as three days after gene transfer. However, from 3 to 6 days after, MARs were significantly higher than prior to gene transfer. Our proposed gene therapy for alveolar bone regeneration combining non-viral BMP-2 gene expression vector and in vivo electroporation could increase alveolar bone regeneration potential in the targeted area for up to three weeks.

Development of a DNA-based vaccine for SARS-CoV-2

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The development of an effective and safe vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), casing coronavirus disease 2019 (COVID19) pandemic, is urgent.

In this study, we designed and develop a DNA-based vaccine using plasmid DNA encoding spike glycoprotein of SARS-CoV-2. To evaluate the effectiveness of DNA vaccine, Jcl:SD rats were intramuscularly injected DNA vaccine with adjuvant three times at 2 weeks interval, and analyzed for the humoral and cellular responses. After immunization, antibody titer for spike protein was induced from 2 weeks after 1st immunization. And IgG2b titer was higher, compared to IgG1. ELISPOT assay using immunized rat splenocytes at 7 weeks after 1st immunization showed that the cellular immune-response was strongly activated against SARS-CoV-2 spike glycoprotein. Next, neutralizing activity of DNA vaccine-induced antibody was assessed by ACE2, angiotensin-converting-enzyme 2, binding assay, and infection assay using VSV (vesicular stomatitis virus) based-pseudo-typed virus containing SARS-CoV-2 spike glycoprotein. As the result, immunized rat serum suppressed the binding of spike glycoprotein recombinant with ACE2 protein recombinant, and infection of pseudo-typed virus. Finally, DNA vaccine also suppressed live SARS-CoV-2 infection in hamsters.

In conclusion, DNA-based vaccine targeting SARS-CoV-2 spike glycoprotein can be an effective approach to fight against COVID-19 pandemic.

Functional recovery of brain networks after AADC gene therapy

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Aromatic L-amino acid decarboxylase (AADC) is an essential enzyme required for synthesizing dopamine, which modulates cortical inputs in the putamen. Adeno-associated virus (AAV) vector-mediated delivery of DDC into the putamen was shown to provide transformative medical benefits, including cognitive and motor improvements and the disappearance of dystonia. The putamen targeted by AADC gene therapy is a hub for the cortico-basal ganglia network. However, it remained to be elucidated whether the dopaminergic restoration in the putamen induces the recovery of the cortical and subcortical network after the gene therapy. Combining the high-resolution positron emission tomography with a specific AADC tracer, 6-[18F] fluoro-L-m-tyrosine (FMT) and the structural connectivity-based parcellation by diffusion tensor imaging, we confirmed the transduced putaminal areas robustly connected to various cortical networks, especially frontoparietal control network and sensorimotor network over the years. In addition, the resting-state functional magnetic resonance imaging data demonstrated the recovery of functional connectivity in the frontoparietal control network and sensorimotor network as well as the putamen after the post-treatment. These results demonstrated the functional recovery of cortical and putaminal networks after the local replacement of dopamine in the putamen.

A phase I/II clinical trial of piggyBac-modified GMR CAR-T cell therapy for CD116 positive relapsed/ refractory myeloid malignancies

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Background: The prognosis of relapsed/refractory (R/R) myeloid malignancies remains poor, and the development of novel treatment strategies is crucial. We previously developed the piggyBac-modified chimeric antigen receptor (CAR)-T cells targeting CD116, also known as GM-CSF receptor alpha chain (GMR). GMR CAR-T cells showed substantial antitumor effects against both acute myeloid leukemia and juvenile myelomonocytic leukemia. Moreover, modulation of the spacer and antigen recognition site of the CAR vector further enhanced the anti-tumor effects of GMR CAR-T cells. GMR CAR-T cells showed an acceptable safety profile with limited cytotoxicity on normal hematopoietic cells except monocytes. Based on these promising results, a first-in-human clinical trial of GMR CAR-T cells has been planned.

Methods/Design: The study is a phase I/II, single-center, dose-escalation study with a traditional 3+3 dose-escalation design. The patients aged more than 1 year with myeloid malignancies who experienced an induction failure or a relapse after hematopoietic stem cell transplantation (HSCT) will be recruited. Peripheral blood mononuclear cells will be harvested from the patient by leukapheresis and then will be transduced with GMR CAR vector by piggyBac transposon system. All manufacturing process of GMR CAR-T cells is performed in Cell Processing Center in Shinshu University Hospital under good manufacturing practice conditions. The patient will be treated with lymphodepleting chemotherapy consisting of fludarabine and cyclophosphamide, followed by CAR-T cell infusion. The dose of CAR-T cells will be 3×10^5 and 1×10^6 in cohorts 1 & 2 and cohort 3, respectively. All the patients will be required to receive HSCT by day 56 following CAR-T cell infusion.

Discussion: The primary endpoint of the study is the safety, pharmacokinetics, and efficacy of GMR CAR-T therapy. GMR CAR-T therapy could be a promising alternative for the relapsed/refractory CD116+ myeloid malignancies.

An “all-in-one university R&D” environment towards clinical studies: promoting clinical application of GMR CAR-T cells

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Drug development requires a regulatory and technical environment to ensure quality and manufacturing standards of drug candidates. However, in a university setting, these prerequisites are usually not fulfilled, because academic research differs from clinical research in pharmaceuticals. Outsourcing may be an option to obtain reliable data that meet the regulatory requirements, but it is costly and time-consuming. Many basic scientific discoveries remain in the early development phase and fail to get into the development process.

Our CAR-T cell project team has been working to develop novel ligand-based CAR-T cells (GMR CAR-T cells) against acute myeloid leukemia and juvenile myelomonocytic leukemia. In order to drive and accelerate the translation of our discoveries into clinical practice, we have created a regulatory environment within our university. We started with identifying the seed compound by repeating basic research including gene editing and created preclinical candidates with optimized potency and safety. We then assessed the function of the candidates in preclinical efficacy and toxicity studies, such as in vitro, in vivo, and tumorigenicity assays. In addition, we established an approach to control of the manufacturing process that is capable of consistently delivering quality products. As a result, we were able to establish a robust GMP-manufacturing process platform to develop clinical-grade CAR-T cells within a university setting. Currently, a first-in-human, investigator-led clinical trial is being conducted in order to determine the efficacy and safety of GMR CAR-T cells. This translation from research to the clinical trial was achieved by gaining knowledge of drug development regulations, educating researchers and staff, and installing necessary equipment and utilities to comply with all regulatory approval requirements. We also utilized PMDA's regulatory science strategy consultation service. Because we were able to gain a high degree of assurance in the performance of the manufacturing process and regulatory requirements within our university, we could accelerate our research and shorten development timelines of GMR CAR-T cells. Drug development in academia is not an easy undertaking. Nevertheless, this manufacturing process platform, ""all-in-one” university R&D, is a major step forward that will lead to the practical application of the research discoveries in academia.

Intensification of viral vector yield using HEK293 suspension cells in perfusion mode process

Rachel Legmann, Rene Gantier

Repligen Corp.

Gene Therapies represent an important new frontier in therapeutic development as they have the potential to treat diseases that have previously been difficult to manage. The complexity of the vector products and their production processes using HEK293 cell line and transient transfection represent the main limitation of current processes to meet the demand. With this comes the requirement to ensure that there are robust manufacturing processes in place to scale with demand and to make these therapies readily accessible to those who need them. However, current manufacturing processes for gene therapies have often been developed with limited scalability in mind and large shifts in technology must take place to enable industrialization. We used process intensification and integration to overcome this challenge.

We will present two case studies which illustrate the next generation manufacturing process for viral vector intensification using HEK293 cells in suspension. The first case study will demonstrate how intensify cell density in perfusion mode from 2 X 106 cells/mL to 10 X 106 cell/mL at time of transfection led to 2-4 fold increase of total rAAV produced This case study will also show how the clarification of the lysate through the cell retention filter used for perfusion enables an integrated upstream/downstream process.

The second case study will show how the tangential flow depth filtration (TFDF) technique was applied to increase lentivirus (LV) yield through high productivity harvest. The suspension cell culture was performed in batch mode, then, on one hand a standard harvest using depth filtration was conducted while on the other hand the harvest was done with the TFDF technique. The cell retention filters enabled the continuous harvest clarification of LV particles present in the media during the virus production, demonstrating the potential for continuous upstream-downstream processing of secreted LV vectors. The depth filtration process led to ~70% yield while the TFDF harvest was at 100%, but more importantly, the TFDF harvest kept the cells intact in the bioreactor and after having replenished the bioreactor with fresh media the cells continued to produce virus and a second harvest was possible leading to a total yield of ~200% compared to the depth filtration process.

For both case studies, we will show the cost benefit of process intensification for viral vector manufacturing.

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より良い治療法の実現

アミカスは、希少疾患とともに
生きる患者さんの生活の質を
より良くすることを使命としています。

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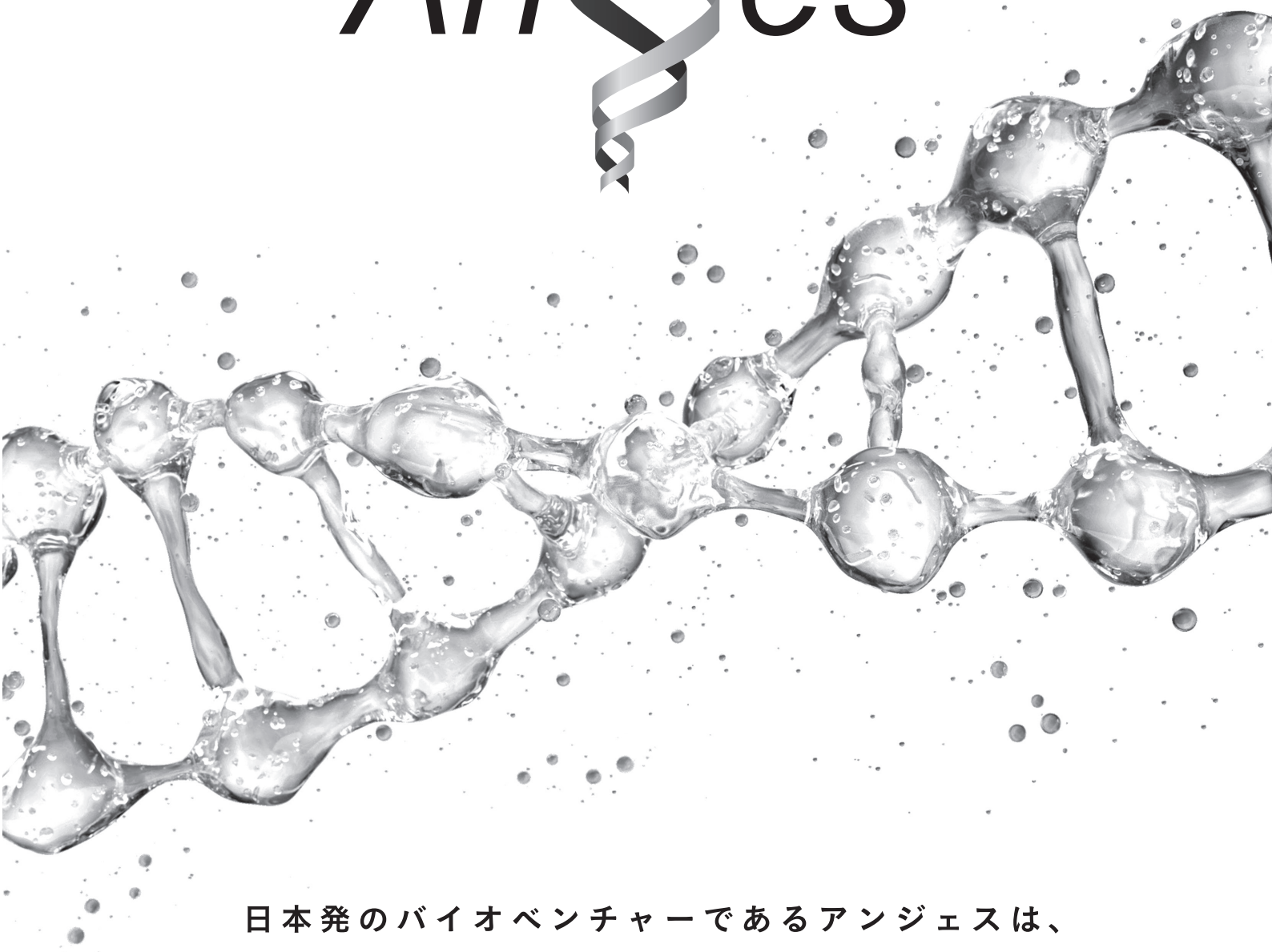
アミカス・セラピューティクス株式会社

NP-NN-JP-00010221
2021年4月作成

Anges



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【警　告】
本剤投与により重篤なアナフィラキシーが発現する可能性があるので、本剤は、緊急時に十分な対応のできる準備をした上で投与を開始し、投与終了後も十分な観察を行うこと。また、重篤なinfusion related reactionが発現した場合には、本剤の投与を中止し、適切な処置を行うこと。〔「重要な基本的注意」の項参照〕

【禁　忌】（次の患者には投与しないこと）
本剤の成分又はα-ガラクトシダーゼ製剤に対するアナフィラキシーショックの既往歴のある患者〔「重要な基本的注意」の項参照〕

効能・効果
ファブリー病

効能・効果に関連する使用上の注意
本剤はファブリー病と確定診断された患者にのみ使用すること。

用法・用量
通常、アガルシダーゼ　アルファ（遺伝子組換え）として、1回体重1kgあたり0.2mgを隔週、点滴静注する。

用法・用量に関連する使用上の注意
（1）投与速度：投与速度が速いとinfusion related reactionが発現しやすいので、投与は40分以上かけて行うこと。
（2）希釈方法：患者の体重あたりで計算した本剤〔アガルシダーゼ　アルファ（遺伝子組換え）として1mg/mLの溶液〕の必要量を用時にバイアルから採取し、100mLの日局生理食塩液に加えて希釈する。
（3）本剤は保存中に少量の微粒子を生じることがあるため、本剤投与時には0.2μmのインラインフィルターを通して投与すること。

使用上の注意（抜粋）

1. 慎重投与（次の患者には慎重に投与すること）
本剤の成分又はα-ガラクトシダーゼ製剤に対する過敏症の既往歴のある患者〔「重要な基本的注意」の項参照〕

2. 重要な基本的注意
（1）本剤はたん白質製剤であるため、アナフィラキシーショックが起きる可能性は否定できないので、観察を十分に行い、このような症状が認められた場合には直ちに投与を中止し、適切な処置を行うこと。また、このような症状の発現に備え、緊急処置を取れる準備をしておくこと。
（2）本剤の投与中又は投与終了後1時間以内にinfusion related reactionがあらわれることがある。主な症状は発熱、倦怠感、四肢疼痛、胸部不快感、悪寒、顔面潮紅であり、頭痛、呼吸困難、腹痛、嘔気、胸痛、瘙痒、浮腫、蕁麻疹等のアレルギー反応を伴うこともある。Infusion related reactionは、通常本剤による治療開始2～4ヵ月で発現するが、1年以降に発現する例も報告されている。本剤投与中にinfusion related reactionがあらわれた場合には、必要に応じて投与を中断し、適切な処置（抗ヒスタミン剤、副腎皮質ホルモン剤投与等）を行うこと。処置後は経過を観察し、投与再開に際しては以下を考慮すること。
1）Infusion related reactionが不変又は悪化した場合には、投与を再開しないこと。Infusion related reactionに対する追加処置を考慮すること。
2）Infusion related reactionが軽快又は消失した場合、投与再開を考慮すること。再開の場合、必要に応じ、投与速度を中断前の1/2を目安として下げること。
（3）Infusion related reactionが発現した患者への次回投与に際しては、以下を考慮すること。
1）前投薬（抗ヒスタミン剤、副腎皮質ホルモン剤等を本剤投与1～3時間前に投与）の処置を行うこと。
2）前投薬等の処置を行ってもinfusion related reactionが軽減しない症例において、同処置を実施した上で本剤を1～5分間投与して中断し、約5分後に投与を再開することによりinfusion related reactionが軽減された例がある。
（4）心臓にファブリー病の病変が認められる患者において、本剤の投与中又は投与終了後24時間以内に、infusion related reactionに関連して、心房細動、心室性期外収縮、頻脈性不整脈、心筋虚血、心不全等があらわれたとの報告がある。このような症状があらわれた場合には、投与を中断し、適切な処置を行うこと。
（5）本剤の投与により、アガルシダーゼ　アルファ（遺伝子組換え）に対するIgG抗体が産生し、効果が減弱した例が報告されている。これらの大部分では、本剤の投与を継続することにより効果が回復したが、回復がみられない例もあった。本剤投与中に、疼痛の悪化など効果の減弱がみられた患者では他の治療法に切り替えることも考慮すること。〔添付文書の「臨床成績」の項参照〕

3. 相互作用
併用注意（併用に注意すること）

薬剤名等	臨床症状・措置方法	機序・危険因子
ヒドロキシクロロキン硫酸塩	本剤の作用が減弱する可能性がある。	細胞内α-ガラクトシダーゼの活性を阻害する可能性がある。

4. 副作用
国内での臨床試験において、12例中10例に臨床検査値異常を含む副作用が認められた。主な副作用は、発熱6例、悪寒及び倦怠感が各4例、四肢疼痛、熱感、CK（CPK）上昇及び呼吸困難が各2例であった。（承認時）
特定使用成績調査（長期使用に関する調査）において、493例中121例（24.5％）に臨床検査値異常を含む副作用が認められた。主な副作用は発熱18例（3.7％）、倦怠感14例（2.8％）、悪心11例（2.2％）、四肢疼痛10例（2.0％）等であった。（再審査終了時）
外国での臨床試験において、65例中41例（63.1％）に副作用が認められた。主な副作用は、潮紅14例（21.5％）、悪寒12例（18.5％）、発熱9例（13.8％）、嘔気8例（12.3％）及び頭痛7例（10.8％）であった。（承認時）

（1）重大な副作用
アナフィラキシー（頻度不明）
観察を十分に行い、異常が認められた場合には投与を中止し、適切な処置を行うこと。

●使用上の注意等の詳細は製品添付文書をご参照ください。

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提携 Shire

2019.6作成

年をとっても健康のまま活躍し、社会にも貢献を続ける生涯現役人生“プロダクティブ・エイジング”を支援！！

現在、AI（人工頭脳）の進化によりビックデータの解析が可能になり、遺伝子細胞治療、iPS 細胞治療の可能性はすぐ目の前に来ています。例えば 2025 年頃には癌は完全に根治するという研究者が多くなって来ました。このような革新的な変化の中でも、現在世界中で人類を脅かす感染症（COVID-19）のパンデミックが発生しています。今後も大きな問題点となるのが感染症です。感染症は毎年新たな感染症原因菌が出てきて多くの人命を奪ってゆきます。また非結核性抗酸菌症に感染する高齢者方々も毎年増えてきています。

我々は IgG 抗体、s-IgA 抗体を高濃度含有し（IgG 抗体 30%以上、s-IgA 抗体 8%）20 種類の細菌、真菌の感染を防御する生乳由来の濃縮乳清たんぱくを開発し一昨年秋から上市しました。今年新たに順天堂大学医学部によりコロナウィルスのスパイクタンパクを抑制することがわかり、学会発表、科学雑誌に投稿しました。

ニュージーランド産 IgG 含有ウシ生乳由来乳清タンパクに存在する新型コロナウイルス SARS-CoV-2 スパイクタンパク抗体

International Dairy Journal
2021

https://www.heimat-ltd.com/academy/international-dairy-journal-2021.html

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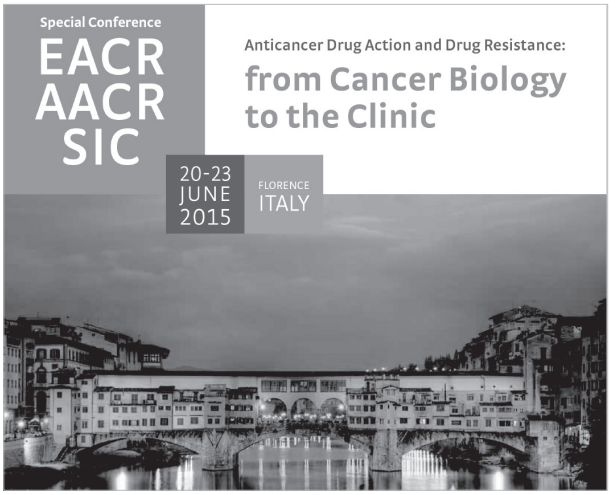
IgG 抗体、s-IgA 抗体、ラクトフェリン含有
ニュージーランド免疫ミルクの魅力

https://www.heimat-ltd.com/academy/igg25-2019-infection.html

ノコギリヤシ色素の血管新生抑制効果



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血管新生阻害活性を有するサメ抽出脂質のメカニズムと臨床への応用

https://www.heimat-ltd.com/academy/20060309angiogenesisresearch.html

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2005
Annual Meeting of
Korean Sciety of Cancer Prevention

https://www.heimat-ltd.com/academy/20051202kscp.html

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効能又は効果、用法及び用量、警告、禁忌を含む使用上の注意等については最新の添付文書をご参照ください。

（文献請求先及び問い合わせ先）
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HTR210613
2021年6月作成

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PackGeneではCDMO（Contract Development and Manufacturing Organization）として、AAV（adeno-associated virus vector）の関連受託サービスに集中し、
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PackGene Biotechは高収量、短納期、高品質及び経済的な製品を提供し、遺伝子治療の研究者及び製薬会社の関連実験をサポートいたします。

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現時点弊社がBiogen, A-star, ハーバード大学Agency for Science, Technology and Research(A*STAR)、UBC（University of British Columbia）、UF（University of Florida）、EMORY（Emory University）、Johns Hopkins（Johns Hopkins University）とChinese Academy of Sciencesなどの研究機構や医薬品企業と長期の共同研究及び受託サービスを展開しております。

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