APASL Single Topic Conference on Liver Immunology and Genetics

Program & Abstracts

Term: April 18-20, 2019
City: Tokyo, Japan
Venue: Keio Plaza Hotel
President: Atsushi Tanaka, MD., PhD.
Professor, Department of Medicine, Teikyo University School of Medicine
APASL Single Topic Conference
in Tokyo

“Liver Immunology and Genetics”

April 18-20, 2019

Tokyo, Japan

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Welcome Message

Dear Colleagues,

On behalf of the Organizing Committee, it gives us great pleasure to invite you to Asian Pacific Association for the Study of the Liver Single Topic Conference (APASL STC), which will be held on April 18-20, 2019 in Tokyo, Japan. We are delighted to welcome you to the attractive cosmopolitan city, Tokyo.

Progress of hepatology is remarkable worldwide, and of course in Asia-Pacific region as well, thanks to evolution of basic science, especially of genetics and immunology. Now we can easily find and enjoy the fruits of outstanding progression in every field of the hepatology – HBV, HCV, HCC, autoimmune, and so on. Therefore, we definitely believe the time has come for APASL STC to take a panoramic perspective of hepatology not from a single disease-basis, but from a cross-sectorial manner. We should learn how genetics and immunology contribute to understanding of etiology and development of novel treatments and should overview which way and how to proceed from now on. Thus, we have proposed the APASL STC 2019 Tokyo under the theme of “Liver Immunology and Genetics”. The conference program will present high-quality content based on up-to-date information and cutting-edge lectures by eminent researchers, in order to reconfirm the achievement of better basic and clinical practice from the view of Liver Immunology and Genetics.

The scientific program consists of 3 State-of-the-Art lectures, 4 keynote lectures, 36 invited lectures, 7 sponsored-lectures, as well as 39 oral and 107 poster free papers on significant developments on the theme of “Liver Immunology and Genetics”. The program also provides the latest information and fresh ideas for hepatologists. The delegates of experts from all over the world are expected to attend this conference. We are sure that this will provide an excellent opportunity for those of us in the Asian Pacific region to share the latest views, values, experience and practice, and greatly contribute to this field. We look forward to welcoming you in Tokyo.

With warmest regards,

Atsushi Tanaka, MD., PhD.
President of APASL STC 2019 Tokyo on Liver Immunology and Genetics
Professor, Department of Medicine, Teikyo University School of Medicine
Organizing Committee

Scientific Committee/Speakers/Chairs from Overseas

Dr. Oidov Baatarkhuu (Mongolia)  Dr. Sook-Hyang Jeong (Korea)  Dr. Chloie M. Mak (Hong Kong)
Dr. Chao-Long Chen (Taiwan)  Dr. Jidong Jia (China)  Dr. Yen Hsuan Ni (Taiwan)
Dr. Ann-Lii Cheng (Taiwan)  Dr. Jia-Horng Kao (Taiwan)  Dr. Joong Won Park (Korea)
Dr. A. Kadir Dokmeci (Turkey)  Dr. George Lau (Hong Kong)  Dr. Seng Hock Quak (Singapore)
Dr. Cumali Efe (Turkey)  Dr. Zhe-Xiong Lian (China)  Dr. Eui-Cheol Shin (Singapore)
Dr. M. Eric Gershwin (USA)  Dr. Qianjin Lu (China)  Dr. Anthony Tan (Singapore)
Dr. Bettina E. Hansen (Canada)  Dr. Xiong Ma (China)  Dr. Lai Wei (China)
Dr. Pietro Invernizzi (Italy)  Dr. Nancy K. Man (Hong Kong)  Dr. Jin Mo Yang (Korea)

In alphabetical order

Scientific Committee/Speakers/Chairs from Japan

Dr. Masanori Abe  Dr. Norifumi Kawada  Dr. Motoko Sasaki
Dr. Nobuhisa Akamatsu  Dr. Kiminori Kimura  Dr. Shuichihi Shiina
Dr. Yasuhiro Asahina  Dr. Kazuhiko Koike  Dr. Masahito Shimizu
Dr. Hirotoshi Ebinuma  Dr. Atsumasa Komori  Dr. Tsuyoshi Sogo
Dr. Yuichiro Eguchi  Dr. Yasuteru Kondo  Dr. Fuminaka Suzuki
Dr. Nobuyuki Enomoto  Dr. Masatoshi Kudo  Dr. Koichi Takaguchi
Dr. Takuya Genda  Dr. Akihiro Matsumoto  Dr. Akinobu Takaki
Dr. Kenichi Harada  Dr. Eishiro Mizukoshi  Dr. Tetsuo Takehara
Dr. Hisamitsu Hayashi  Dr. Satoshi Mochida  Dr. Yasuhiro Takikawa
Dr. Naoki Hiramatsu  Dr. Mitsuhiko Moriyama  Dr. Shinji Tanaka
Dr. Akira Honda  Dr. Kazumoto Murata  Dr. Yasuhiro Tanaka
Dr. Masao Honda  Dr. Hidewaki Kakagawa  Dr. Makiko Taniai
Dr. Akio Ido  Dr. Mina Nakaagawa  Dr. Nobuhiko Taniai
Dr. Masafumi Ikeda  Dr. Nobuhiro Nakamoto  Dr. Ryoosuke Tateishi
Dr. Kenichi Ikejima  Dr. Yasunari Nakamoto  Dr. Katsutoshi Tokushige
Dr. Ayano Inui  Dr. Minoru Nakamura  Dr. Kaoru Tsuchiya
Dr. Tetsuya Ishikawa  Dr. Nobuaki Nakayama  Dr. Yoshihide Ueda
Dr. Masanori Isogawa  Dr. Naoshi Nishida  Dr. Takeji Umeda
Dr. Namiki Izumi  Dr. Shueji Nishiguchi  Dr. Yoshiyuki Wada
Dr. Tatehiro Kagawa  Dr. Shuntaro Obi  Dr. Hiroshi Yatsushashi
Dr. Masayoshi Kage  Dr. Sadahisa Ogasawara  Dr. Osamu Yokosuka
Dr. Toshimi Kaido  Dr. Hideki Ohdan  Dr. Hideo Yoshida
Dr. Tatsuo Kanda  Dr. Hiromasa Ohira  Dr. Hitoshi Yoshiji
Dr. Shuichi Kaneko  Dr. Masao Omata  Dr. Hiroshi Yotsuyanagi
Dr. Jong-Hon Kang  Dr. Naoya Sakamoto  
Dr. Naoya Kato  Dr. Hideyuki Sasaki  

In alphabetical order

Local Organizing Committee

Honorary President: Dr. Hajime Takikawa  Treasurer: Dr. Masayuki Kurosaki
President: Dr. Atsushi Tanaka  Vice-Treasurer: Dr. Shinji Shimoda
Vice-President: Dr. Tatsuya Kanto  Secretary General: Dr. Yoshinari Asaoka
APASL Steering Committee

Chairman of Steering Committee
Dr. Shiv Kumar Sarin (India)
President
Dr. Rino Gani (Indonesia)
Immediate Past President
Dr. Diana A. Payawal (Philippines)
President Elect
Dr. Tawesak Tanwandee (Thailand)
Secretary General-cum-Treasurer
Dr. Lai Wei (China)
Past Presidents
Dr. Laurentius A. Lesmana (Indonesia)
Dr. Jose Sollano (Philippines)
Dr. Masao Omata (Japan)
Dr. Dong Jin Suh (Korea)
Dr. George Lau (Hong Kong)
Dr. Jidong Jia (China)
Dr. Teerha Piratvisuth (Thailand)
Dr. Jia-Horning Kao (Taiwan)
Dr. Darrell Crawford (Australia)
Dr. A. Kadir Dokmeci (Turkey)
Dr. Osamu Yokosuka (Japan)
Dr. Jinlin Hou (China)
Dr. Barjesh Chander Sharma (India)

APASL Executive Council

President
Dr. Rino Gani (Indonesia)
Immediate Past President
Dr. Diana A. Payawal (Philippines)
President Elect
Dr. Tawesak Tanwandee (Thailand)
Secretary General-cum-Treasurer
Dr. Lai Wei (China)
Assistant Secretary
Dr. Manoj Kumar Sharma (India)
Executive Council
Dr. Hasmik Ghazinyan (Armenia)
Dr. Han-Chieh Lin (Taiwan)
Dr. Rosmawati Mohamed (Malaysia)
Dr. David Handojo Muljono (Indonesia)
Dr. Shuichiro Shiina (Japan)
Dr. Fu-Sheng Wang (China)
Dr. Simone Strasser (Australia)
Dr. Yaman Tokat (Turkey)
Dr. Jin Mo Yang (Korea)
Dr. Hong You (China)
Conference Information

Registration Fee and Category

<table>
<thead>
<tr>
<th></th>
<th>Until January 31, 2019</th>
<th>Until March 31, 2019</th>
<th>On-site</th>
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<tbody>
<tr>
<td>APASL Member*</td>
<td>JPY15,000</td>
<td>JPY20,000</td>
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<tr>
<td>Non-Member</td>
<td>JPY20,000</td>
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<tr>
<td>Trainee / Resident**</td>
<td>JPY10,000</td>
<td>JPY15,000</td>
<td>JPY20,000</td>
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<td>Presenting Author</td>
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<td>JPY25,000</td>
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<tr>
<td>Accompanying Person</td>
<td>JPY5,000</td>
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JPY=Japanese Yen

*APASL Members who have paid 2019 Membership Fee can apply for discounted registration fee.

**Proof of status is required.

Registration Fee includes

APASL Member, Non-Member, Trainee/Resident, Presenting Author:

- Attendance to all scientific sessions
- Entrance to exhibition area
- Congress envelop and printed materials
- Coffee breaks to be served during the scientific program
- Welcome Reception on April 19 (Friday) 2019

Accompanying Person:

- Entrance to exhibition area
- Welcome Reception on April 19 (Friday) 2019

Registration Hours

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
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<tbody>
<tr>
<td>April 18 (Thursday)</td>
<td>7:30-18:00</td>
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<tr>
<td>April 19 (Friday)</td>
<td>7:00-18:00</td>
</tr>
<tr>
<td>April 20 (Saturday)</td>
<td>7:00-11:00</td>
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</tbody>
</table>

Exhibition Hours

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
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<tbody>
<tr>
<td>April 18 (Thursday)</td>
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</tr>
<tr>
<td>April 19 (Friday)</td>
<td>7:30-18:00</td>
</tr>
<tr>
<td>April 20 (Saturday)</td>
<td>7:30-12:00</td>
</tr>
</tbody>
</table>

PC Preview Desk Hours

<table>
<thead>
<tr>
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<tr>
<td>April 19 (Friday)</td>
<td>7:00-18:00</td>
</tr>
<tr>
<td>April 20 (Saturday)</td>
<td>7:00-11:00</td>
</tr>
</tbody>
</table>

Welcome Reception

Date and Time: April 19 (Friday) 19:30-21:30
Place: Room 2 “Ohgi”, 4th Floor,
The South Tower, Keio Plaza Hotel

*Welcome Reception is included in Registration Fee.
*Awarding Ceremony will be presented during Welcome Reception.
Transportation

Venue: Keio Plaza Hotel Tokyo
Address: 2-2-1 Nishi-Shinjuku,
Shinjuku-Ku, Tokyo, 160-8330, Japan
Tel: +81-3-3344-0111
Website: http://www.keioplaza.com/

From/To Narita International Airport

By Airport Limousine Bus       Duration: approx. 120 minutes
By Narita Express Train        Duration: approx. 80 minutes
By Taxi                        Duration: approx. 90 minutes*

*The travel time is depending on the traffic.

From/To Haneda International Airport

By Airport Limousine Bus       Duration: approx. 70 minutes
By Train                       Duration: approx. 40 minutes

Take Keikyu Line to Shinagawa Station and change to JR Yamanote Line. Get off at Shinjuku Station. 5 minute walk from the west exit of Shinjuku Station to the venue “Keio Plaza Hotel”.

By Taxi                        Duration: approx. 40 minutes*

*The travel time is depending on the traffic.
Venue
Keio Plaza Hotel Tokyo
Room 1: “Eminence Hall”, 5th Floor, South Tower
Room 2: “Ohgi”, 4th Floor, South Tower
Registration, PC Preview Desk: In front of “Eminence Hall”, 5th Floor, South Tower
Secretariat Room: “Dahlia B”, 5th Floor, South Tower
Cloak: Near Front Desk, 3rd Floor, Main Tower

Eminence Hall
5F
Registration
Room 1
Exhibition Area
Dahlia A (Speakers’ Ready Room)
Dahlia B (Secretariat Room)

4F
Poster Session (April 18)
Oral Session (April 19)
Welcome Reception (April 19 19:30-21:30)
Instruction for Oral Presentation

* All Oral Presentation is performed with PowerPoint data on Windows PC.
* Please complete your PC registration until 30 minutes ahead of your presentation time.
* PC Data Registration Desk is located in front of Room 1 “Eminence Hall” 5th Floor, South Tower, Keio Plaza Hotel.
* The open hours is as follows.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
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<tbody>
<tr>
<td>April 18 (Thurs.)</td>
<td>7:30-18:00</td>
</tr>
<tr>
<td>April 19 (Fri.)</td>
<td>7:00-18:00</td>
</tr>
<tr>
<td>April 20 (Sat.)</td>
<td>7:00-11:00</td>
</tr>
</tbody>
</table>

<If you bring your own PC>
* Windows PC will be set at the conference room for your presentation.
* Please make sure that your PC has D-Sub 15 pin mini terminal for monitor output. (Some compact PC needs another connector. In case of that, please carry your own connector.)
* Macintosh and Key Note are acceptable only if you will bring your own PC (Please carry your own connector).
* Please bring battery adapter to avoid battery off. Because sometimes screen saver or power saving system could be a reason of battery off, please set your PC appropriately.
* Please operate your PPT data by yourself at the podium.

<If you bring your data by portable media>
* To avoid garbled characters, please use standard font which is originally installed by OS.
* Please put your name on your data file.
* Please bring your data by USB memory stick, CDR, or DVDR (Disk at Once).
* Backup data by another media should be kept by presenter.
* If you bring your movies by data file, please prepare the file which can be played by standard Windows Media Player.
* The projector’s screen resolution is set at 4:3 XGA. Please make your PPT data the necessary preparation if needed (16:9 XGA is also projectable with a size smaller, black flamed at the top and bottom).

If you have any question, please contact the congress secretariat.
APASL STC Tokyo Congress Secretariat
Tel: +81-3-6380-0102   Fax: +81-3-6380-0103
Email: info@apaslstc2019tokyo.org

APASL STC Tokyo URL: http://www.apaslstc2019tokyo.org/index.html

Instruction for Chairs

Session Chairs are requested to come to the next chair’s seat until 10 minutes before their sessions.
**Instruction for Poster Presentation**

* A panel width 90cm×length 210cm will be provided for each poster as following sample.
* Poster number will be prepared by secretariat.
* Title and author’s name are required to be prepared by each presenter.
* Pins for display will be provided at each poster panel.
* Location: Poster Session will be located in (or foyer of) the Room 2 “Ohgi” 4F, South Tower, Keio Plaza Hotel.

* Schedule:
  
  - Poster Set up: 7:30-11:00 on April 18 (Thursday)
  - Poster Presentation: 11:00-18:00 on April 18 (Thursday)
  - Poster Removal: 18:00-19:00 on April 18 (Thursday)

  *For those who have not removed posters until above removal time, please accept that the secretariat will discard any posters that have remained.

Awarding Ceremony: The Awardees will be presented at Welcome Reception during 19:30-21:00 on April 19 (Friday).

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**Poster Panel**

![Poster Panel Diagram]

- Poster No. (Prepared by Secretariat)
- Title and Author(s)

Dimensions:
- Width: 20cm
- Height: 210cm
- Total Width: 90cm (including margins)
- Total Height: 210cm (including margins)

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Awards

Excellent papers will be awarded as “Presidential Award” or “Young Investigator Award”. Awarding Ceremony will be held at the Welcome Reception during 19:30-21:30 on April 19 (Friday).

Presidential Award

“APASL STC Tokyo Presidential Award” will be awarded to whom performed the most excellent presentation in APASL STC Tokyo to encourage to further their research and progress.

Young Investigator Award (Under 40 years old)

The purpose of the “APASL STC Tokyo Young Investigator Award” is to praise outstanding examples of excellence amongst those involved in research training in the early stages of their career.

Contact

APASL STC 2019 Tokyo Scientific Secretariat
Department of Medicine, Teikyo University School of Medicine
2-11-1 Kaga, Itabashi-Ku, Tokyo 173-8606, Japan

APASL STC 2019 Tokyo Congress Secretariat
c/o Academia Support Japan
Email: info@apaslstc2019tokyo.org
Tel: +81-3-6380-0102 Fax: +81-3-6380-0103

APASL Central Office (APASL Secretariat-Tokyo)
Asian Pacific Association for the Study of the Liver [APASL]
1-24-7-920, Shinjuku, Shinjuku-ku, Tokyo, 160-0022 Japan
Email: apasl_secretariat@apasl.info
Tel: +81-3-5312-7686 Fax: +81-3-5312-7687
Sponsors and Support Organization

Platinum Sponsor

AbbVie GK

Gold Sponsors

Eisai Co., Ltd.

Gilead Sciences K.K.

MSD K.K.

Sysmex Corporation

Silver Sponsors

EA Pharma Co., Ltd.

Institute of Immunology Co., Ltd.

Miyarisan Pharmaceutical Co., Ltd.

Otsuka Pharmaceutical Co., Ltd.

Shionogi & Co., Ltd.
Bronze Sponsors

Ageo Central General Hospital
ASKA Pharmaceutical Co., Ltd.
Bayer Yakuhin, Ltd.
Bristol-Myers Squibb
Covidien Japan Inc.
Daiichi Sankyo Company, Limited
Eli Lilly Japan K.K.
Fuji Chemical Industries Co., Ltd.
FUJIREBIO Inc.
Higashi-Saitama Hospital
InBody Japan Inc.
Integral Corporation
KAN Research Institute, Inc.
Mitsubishi Tanabe Pharma Corporation
Mochida Pharmaceutical Co., Ltd.
Mylan N.V.
Nittobo Medical Co., Ltd.
Novartis Pharma K.K.
Ono Pharmaceutical Co., Ltd.
Sumitomo Dainippon Pharma Co., Ltd.
Takeda Pharmaceutical Company Limited
Urayasu-Takayanagi Hospital

Support Organizations

The Japan Society of Hepatology
Tokyo Convention & Visitors Bureau
Tokyo Metropolitan Government

*In alphabetical order

The Organizing Committee of the APASL Single Topic Conference 2019 Tokyo would like to express sincere gratitude to the sponsors and organizations for supporting this conference.
# Program at a Glance

## Day 1: April 18 (Thursday) 2019

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AM</strong></td>
<td><strong>Room 1</strong></td>
</tr>
<tr>
<td>7:00-7:30</td>
<td>Registration</td>
</tr>
<tr>
<td>8:00-8:10</td>
<td>Opening Ceremony</td>
</tr>
<tr>
<td>8:10-8:25</td>
<td>Opening Lecture: Masao Omata</td>
</tr>
<tr>
<td>8:25-9:35</td>
<td>HBV/HCV (1)</td>
</tr>
<tr>
<td>9:40-10:50</td>
<td>HBV/HCV (2)</td>
</tr>
<tr>
<td>11:00-11:40</td>
<td>State-of-the Art Lecture (1): Tetsuo Takehara</td>
</tr>
<tr>
<td><strong>Room 2</strong></td>
<td><strong>Room 2</strong></td>
</tr>
<tr>
<td>7:30-11:00</td>
<td>Poster Set up</td>
</tr>
<tr>
<td><strong>Noon</strong></td>
<td><strong>Room 1</strong></td>
</tr>
<tr>
<td>11:50-12:50</td>
<td>Luncheon Seminar (1) Presented by AbbVie G.K.</td>
</tr>
<tr>
<td><strong>PM</strong></td>
<td><strong>Room 1</strong></td>
</tr>
<tr>
<td>12:55-14:00</td>
<td>Pediatric/Metabolic (1)</td>
</tr>
<tr>
<td>14:05-15:05</td>
<td>Educational Seminar (1) Presented by MSD K.K.</td>
</tr>
<tr>
<td>15:15-16:40</td>
<td>ALF/ACLF</td>
</tr>
<tr>
<td>16:40-17:50</td>
<td>HBV/HCV (3)</td>
</tr>
<tr>
<td><strong>Room 2</strong></td>
<td><strong>Room 2</strong></td>
</tr>
<tr>
<td>11:00-18:00</td>
<td>Poster Free Papers Presentation</td>
</tr>
<tr>
<td><strong>Night</strong></td>
<td><strong>Room 1</strong></td>
</tr>
<tr>
<td>17:55-18:55</td>
<td>Evening Seminar (1) Presented by Sysmex Corporation</td>
</tr>
<tr>
<td><strong>Room 2</strong></td>
<td><strong>Room 2</strong></td>
</tr>
<tr>
<td>18:00-19:00</td>
<td>Poster Removal</td>
</tr>
</tbody>
</table>

(Presidential Dinner by Invitation)
Day 2: April 19 (Friday) 2019

<table>
<thead>
<tr>
<th>Room</th>
<th>Morning</th>
<th>AM</th>
<th>Noon</th>
</tr>
</thead>
</table>
| Room 1 | 7:30-8:30  Morning Seminar (1)  
Presented by EA Pharma Co., Ltd. | 8:35-9:50  Autoimmunity (1)  
9:55-11:10  Autoimmunity (2) | 12:10-13:10  Luncheon Seminar (2)  
Presented by Eisai Co., Ltd. / MSD K.K. |
| Room 2 | | 8:00-9:35  Pediatric/Metabolic (2)  
9:40-11:05  Transplantation  
11:10-11:30  HBV/HCV (4) | 12:05-13:05  Luncheon Seminar (3)  
Presented by Gilead Sciences K.K. |
| | 11:20-12:00  State-of-the Art Lecture (2): M. Eric Gershwin  
Supported by Institute of Immunology | | |
| | | 11:30-11:51  Oral Free Papers | |
| Noon | 12:10-13:10  Luncheon Seminar (2)  
Presented by Eisai Co., Ltd. / MSD K.K. | | |
| PM | 13:15-14:30  Autoimmunity (3) | 13:10-16:31  Oral Free Papers | |
| | 14:30-15:30  Educational Seminar (2)  
Presented by Miyarisan Pharmaceutical Co., Ltd. | | |
| | 15:40-16:30  Autoimmunity (4) | | |
| | 16:40-17:36  Oral Free Papers | | |
| | 17:40-18:00  HCC (1) | | |
| Night | Welcome Reception | Awarding Ceremony | |

Day 3: April 20 (Saturday) 2019

<table>
<thead>
<tr>
<th>Day</th>
<th>Room</th>
<th>AM</th>
<th>Noon</th>
</tr>
</thead>
</table>
| Room 1 | 7:30-8:30  Morning Seminar (2)  
Presented by Shionogi & Co., Ltd. | | 12:00-12:10  Closing Ceremony |
| Morning | | 8:35-10:00  HCC (2)  
10:05-11:20  HCC (3)  
11:20-12:00  State-of-the Art Lecture (3): Ann-Lii Cheng | |
| AM | | | |
| Noon | | | |
## Scientific Program

### Day 1: April 18 (Thursday) 2019

**Room 1 (Eminence Hall, 5F, South Tower, Keio Plaza Hotel)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>8:00-8:10</td>
<td>Opening Ceremony</td>
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<tr>
<td></td>
<td><strong>Opening Remarks</strong> Atsushi Tanaka, President of APASL STC 2019 Tokyo</td>
</tr>
<tr>
<td>8:10-8:25</td>
<td>Opening Lecture</td>
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<tr>
<td></td>
<td><strong>Mission of APASL; Eradication of Virus and Cure of HCC</strong></td>
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<tr>
<td></td>
<td><strong>Masao Omata (Japan)</strong></td>
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<tr>
<td>8:25-9:35</td>
<td>HBV / HCV (1)</td>
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<tr>
<td></td>
<td><strong>Chairs: George Lau (Hong Kong), Nobuyuki Enomoto (Japan)</strong></td>
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<tr>
<td>8:25-8:45</td>
<td>The Impact of Hepatic Antigen Presentation on HBV-Specific CD8+ T Cell</td>
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<tr>
<td></td>
<td><strong>Masanori Isogawa (Japan)</strong></td>
</tr>
<tr>
<td>8:45-9:05</td>
<td>TNF-Producing Regulatory T Cells in Viral Hepatitis</td>
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<tr>
<td></td>
<td><strong>Eui-Cheol Shin (Korea)</strong></td>
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<tr>
<td>9:05-9:20</td>
<td>HCV and Lymphomagenesis</td>
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<tr>
<td></td>
<td><strong>A. Kadir Dokmeci (Turkey)</strong></td>
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<tr>
<td>9:20-9:35</td>
<td>Panel Discussion</td>
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<tr>
<td>9:35-9:40</td>
<td>Break</td>
</tr>
<tr>
<td>9:40-10:50</td>
<td>HBV / HCV (2)</td>
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<td><strong>Chairs: A. Kadir Dokmeci (Turkey), Fumitaka Suzuki (Japan)</strong></td>
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<tr>
<td>9:40-10:00</td>
<td>Hepatitis B and C Virus Infection and Risk Factors for Developing Hepatocellular Carcinoma</td>
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<td><strong>Hiroshi Yatsuhashi (Japan)</strong></td>
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<td>10:00-10:15</td>
<td>HCV and Co-infections in Mongolia</td>
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<td><strong>Oidov Baatarkhuu (Mongolia)</strong></td>
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<td>10:15-10:35</td>
<td>Tailoring Immunity in Patients with HBV or HCV Infection – Aiming at the Virus Elimination by 2030</td>
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<td><strong>Tatsuya Kanto (Japan)</strong></td>
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<td>10:35-10:50</td>
<td>Panel Discussion</td>
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<td>10:50-11:00</td>
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<td>11:00-11:40</td>
<td>State-of-the Art Lecture (1)</td>
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<td><strong>Chair: Hajime Takikawa (Japan)</strong></td>
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<td>Translational Research in Hepatology Using Genetically-Modified Mice</td>
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<td><strong>Tetsuo Takehara (Japan)</strong></td>
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<td>11:40-11:50</td>
<td>Break</td>
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<td>11:50-12:50</td>
<td>Luncheon Seminar (1)</td>
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<tr>
<td>Chair: Norifumi Kawada (Japan) Presented by AbbVie GK</td>
<td><strong>Treatment of Hepatitis C: So Far and from Now on</strong></td>
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<td>Naoya Kato (Japan)</td>
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<td>12:50-12:55</td>
<td>Break</td>
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<tr>
<td>12:55-14:00</td>
<td>Pediatric / Metabolic (1)</td>
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<tr>
<td>Chairs: Akio Ido (Japan), Ayano Inui (Japan)</td>
<td><strong>Genetics of Metabolic Liver Disease (Keynote Lecture)</strong></td>
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<td>Chloe M. Mak (Hong Kong)</td>
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<td>12:55-13:25</td>
<td><strong>Advance of Genetic Diagnosis of Cholestasis in Pediatric Patients</strong></td>
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<td>Yen Hsuan Ni (Taiwan)</td>
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<td>13:25-13:45</td>
<td><strong>Panel Discussion</strong></td>
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<td>13:45-14:00</td>
<td><strong>Panel Discussion</strong></td>
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<td>14:05-15:05</td>
<td>Educational Seminar (1)</td>
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<td>Chair: Yasuhito Tanaka (Japan) Presented by MSD K.K.</td>
<td><strong>The Real-World Experience of DAA for Hepatitis C: Benefits and Challenges after Virological Cure Revealed by Nation-Wide Cohort</strong></td>
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<td>Masayuki Kurosaki (Japan)</td>
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<td>15:05-15:15</td>
<td>Break</td>
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<td>15:15-15:45</td>
<td><strong>Acute Liver Failure and Acute-on-Chronic Liver Failure in Japan (Keynote Lecture)</strong></td>
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<td>Chairs: Jidong Jia (China), Naoya Sakamoto (Japan)</td>
<td>Satoshi Mochida (Japan)</td>
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<td>15:45-16:05</td>
<td><strong>Early Prediction of ALF in Patients with Acute Liver Injury</strong></td>
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<td>Yasuhiro Takikawa (Japan)</td>
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<td>16:05-16:25</td>
<td><strong>ACLF -Korean Experiences-</strong></td>
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<td>Jin Mo Yang (Korea)</td>
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<td>16:25-16:40</td>
<td><strong>Panel Discussion</strong></td>
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<td>16:40-17:50</td>
<td>HBV / HCV (3)</td>
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<tr>
<td>Chairs: Oidov Baatarkhuu (Mongolia), Hiroshi Yotsuyanagi (Japan)</td>
<td><strong>HBV Reactivation after DAAs Therapy in CHC in HBV Endemic Area</strong></td>
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<td>George Lau (Hong Kong)</td>
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<td>16:40-16:55</td>
<td><strong>HCC Occurrence/Recurrence after HCV Eradication</strong></td>
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<td>Mina Nakagawa (Japan)</td>
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<td>16:55-17:15</td>
<td><strong>Anti-HCV Therapy after Liver Transplantation</strong></td>
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<td>Yoshihide Ueda (Japan)</td>
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</tbody>
</table>
17:35-17:50  
Panel Discussion

17:50-17:55  
Break

17:55-18:55  
Evening Seminar (1)

Chair: Osamu Yokosuka (Japan)  
Presented by Sysmex Corporation

Impact of Serum M2BPGi Measurement on Clinical Practice of Chronic Liver Disease  
Masayuki Kurosaki (Japan)

Clinical Significance of M2BPGi in Patients with Autoimmune Liver Disease  
Takeji Umemura (Japan)
Scientific Program

Day 2: April 19 (Friday) 2019
Room 1 (Eminence Hall, 5F, South Tower, Keio Plaza Hotel)

7:30-8:30 Morning Seminar (1)
Chair: Shuichi Kaneko (Japan) Presented by EA Pharma Co., Ltd.
Survival Benefit of BCAA for Patients with Liver Cirrhosis - Prevention of Hepatic Failure and HCC-
Masahito Shimizu (Japan)

8:30-8:35 Break

8:35-9:50 Autoimmunity (1)
Chairs: M. Eric Gershwin (USA), Atsumasa Komori (Japan)
8:35-8:55 Liver-resident NK Cells Suppress Autoimmune Cholangitis and Limit the Proliferation of CD4+ T Cells
Zhe-Xiong Lian (China)
8:55-9:15 Epigenetic Changes in Autoimmune Diseases
Qianjin Lu (China)
9:15-9:35 Autoimmunity against Biliary Epithelial Cells
Shinji Shimoda (Japan)
9:35-9:50 Panel Discussion

9:50-9:55 Break

9:55-11:10 Autoimmunity (2)
Chairs: Xiong Ma (China), Yasunari Nakamoto (Japan)
9:55-10:15 Genetics of Autoimmune Liver Disease
Minoru Nakamura (Japan)
10:15-10:35 Female Predominance in Autoimmune Liver Diseases
Pietro Invernizzi (Italy)
10:35-10:55 Geoepidemiology of Primary Biliary Cholangitis and Autoimmune Hepatitis
Sook-Hyang Jeong (Korea)
10:55-11:10 Panel Discussion

11:10-11:20 Break

11:20-12:00 State-of-the Art Lecture (2)
Chair: Atsushi Tanaka (Japan) Supported by Institute of Immunology
UNMET NEEDS and the Paradox of Primary Biliary Cholangitis
M. Eric Gershwin (USA)
12:00-12:10 Break

12:10-13:10 Luncheon Seminar (2)
Chair: Ryosuke Tateishi (Japan)  Presented by Eisai Co., Ltd. / MSD K.K.
Reconsideration of Treatment Strategy in the Era of Multi-TKIs
Sadahisa Ogasawara (Japan)
Diversity of Lenvatinib Treatment Strategy and Management of Lenvatinib Treatment for Unresectable Hepatocellular Carcinoma
Yoshiyuki Wada (Japan)
13:10-13:15 Break

13:15-13:40 Autoimmunity (3)
Chairs: Bettina E. Hansen (Canada), Masanori Abe (Japan)
13:15-13:35 Noninvasive Prediction of Liver Fibrosis in Autoimmune Liver Disease
Takeji Umemura (Japan)
Xiong Ma (China)
13:55-14:15 Treatment of Autoimmune Hepatitis and Overlap Syndromes
Cumali Efe (Turkey)
14:15-14:30 Panel Discussion

14:30-14:50 Break

14:40-16:30 Autoimmunity (4)
Chairs: Sook-Hyang Jeong (Korea), Atsushi Tanaka (Japan)
14:40-15:00 APASL Guidance on the Management of PBC
Jidong Jia (China)
15:00-15:20 Global PBC - What Can We Learn from Global Collaboration?
Bettina E. Hansen (Canada)
15:20-15:30 Panel Discussion
15:30-15:40 Break

15:40-16:00 APASL Guidance on the Management of PBC
Jidong Jia (China)
16:00-16:20 Global PBC - What Can We Learn from Global Collaboration?
Bettina E. Hansen (Canada)
16:20-16:30 Panel Discussion
16:30-16:40 Break
Oral Free Papers (1) “Autoimmune”

Chairs: Zhe-Xiong Lian (China), Hiromasa Ohira (Japan)

16:40-16:47  O-01  Pathway-analysis Using Datasets of GWAS and mRNA Expression Array Identified IFNG as the Most Significant Upstream-regulator in Primary Biliary Cholangitis
Kazuko Ueno (Japan)

16:47-16:54  O-02  Altered Expression of Various Inflammation and Immune-related Genes in Senescent Biliary Epithelial Cells Relating to Primary Biliary Cholangitis
Motoko Sasaki (Japan)

16:54-17:01  O-03  Validation of Risk Scoring Systems in Ursodeoxycholic Acid Treated Patients with Primary Biliary Cholangitis
Cumali Efe (Turkey)

17:01-17:08  O-04  Access to PBC Foundation Services Improves Patient Self-Management and Mental Well-being
Robert J. Mitchell-Thain (UK)

Oral Free Papers (2) “HCC”

Chairs: Anthony Tan (Singapore), Masao Honda (Japan)

17:08-17:15  O-05  Specific Inhibition of Cancer Cell Glycolysis Enhances Antitumor Immunity in Hepatocellular Carcinoma
Kyo Sasaki (Japan)

17:15-17:22  O-06  Structure Guided Design of a Therapeutic Inhibitor of SALL4 Positive Advanced Liver Cancer
Bee Hui Liu (Singapore)

17:22-17:29  O-07  A Hepatocyte Differentiation Model Reveals Two Subtypes of Liver Cancer with Different Oncofetal Properties
Ming Liu (China)

17:29-17:36  O-08  A Subgroup of Hepatocellular Carcinoma Rich in Druggable Immune Checkpoints
Anthony W. H. Chan (Hong Kong)

17:36-17:40  Break

17:40-18:00  HCC (1)
Chair: Tatsuo Kanda (Japan)

Whole Genome and Immuno-genome Landscape of Liver Cancer
Hidewaki Nakagawa (Japan)

18:00-18:05  Break

18:05-19:05  Evening Seminar (2)
Chair: Naoya Kato (Japan)
Presented by Otsuka Pharmaceutical Co., Ltd.

Sarcopenia and Hand Grip Strength (HGS) in the Patients with Chronic Liver Disease
Hiroshi Yatsuhashi (Japan)
Scientific Program

Day 2: April 19 (Friday) 2019
Room 2 (Ohgi, 4F, South Tower, Keio Plaza Hotel)

8:00-9:35 Pediatric/Metabolic (2)

Chairs: Jong-Hon Kang (Japan), Yuichiro Eguchi (Japan)
8:00-8:20 Autoimmune Liver Diseases in Children
Tsuyoshi Sogo (Japan)
8:20-8:40 Biliary Atresia
Hideyuki Sasaki (Japan)
8:40-9:00 Development of Novel Medical Therapy for Pediatric Liver Diseases with Intrahepatic Cholestasis
Hisamitsu Hayashi (Japan)
9:00-9:20 Liver Transplant
Seng Hock Quak (Singapore)
9:20-9:35 Panel Discussion

9:35-9:40 Break

9:40-11:05 Transplantation

Chairs: Nobuhisa Akamatsu (Japan), Akinobu Takaki (Japan)
9:40-10:10 Liver Transplantation – Graft Injury and Cancer Recurrence (Keynote Lecture)
Nancy K. Man (Hong Kong)
10:10-10:30 Living-donor Liver Transplantation for PBC and PSC in Japan
Nobuhisa Akamatsu (Japan)
10:30-10:50 Long-Term Outcomes of Patients with Pre-transplant Portal Vein Thrombosis after Living Donor Liver Transplantation
Chao-Long Chen (Taiwan)
10:50-11:05 Panel Discussion

11:05-11:10 Break

11:10-11:30 HBV/HCV (4)

Chair: Yasuhiro Asahina (Japan)
Immune and Genetic Markers Associated with Hepatitis B Viral Relapse after Discontinuation of Oral Antivirals
Jia-Horng Kao (Taiwan)
11:30-11:51 Oral Free Papers (3) “HBV/HCV”

Chairs: Jia Horng Kao (Taiwan), Yasuhiro Asahina (Japan)

11:30-11:37 O-09 Ex Vivo Detection and Characterization of Hepatitis B Virus-specific CD8+ T Cells in Patients Considered Immune Tolerant
Pil Soo Sung (Korea)

11:37-11:44 O-10 mTORC2-related Protein Kinase B Phosphorylation is Associated with Nucleotide Analogues Treatment for Chronic Hepatitis B
Kazunori Kawaguchi (Japan)

11:44-11:51 O-11 The Risk Factor of Developing Hepatocellular Carcinoma in Chronic Hepatitis B Patients with Long-term Administration of Oral Antiviral Therapy
Shun Kaneko (Japan)

11:51-12:05 Break

12:05-13:05 Luncheon Seminar (3)
Chair: Namiki Izumi (Japan) Presented by Gilead Sciences K.K.

Hepatitis Action Plan and Changing Trend of Liver Disease in Japan
Tatsuya Kanto (Japan)

13:05-13:10 Break

13:10-13:52 Oral Free Papers (4)

Chairs: Tatehiro Kagawa (Japan), Yoshinari Asaoka (Japan)

Han Yue (China)

Qian Zhu (China)

13:24-13:31 O-14 Usefulness of Serum Mac-2 Binding Protein Glycosylation Isomer in Children with Primary Sclerosing Cholangitis
Shuichiro Umetsu (Japan)

13:31-13:38 O-15 Autoimmune Hepatitis with Acute Presentation; are There Clinical Characteristics could Contribute its Early Diagnosis?
Jong-Hon Kang (Japan)

13:38-13:45 O-16 Clinical Course and Correlation with Ulcerative Colitis in Japanese Patients with Primary Sclerosing Cholangitis
Junichiro Kumagai (Japan)

13:45-13:52 O-17 Characterization and Identification of Differentially Regulated Proteins may Identify Biomarkers for Early Diagnosing and Prognosis Drug-induced Liver Injury (DILI)
Mohammad Shabir Hussain (India)
13:52-14:34 Oral Free Papers (5)

Chairs: Oidov Baatarkhuu (Mongolia), Tetsuya Ishikawa (Japan)

13:52-13:59 O-18 Cytomegalovirus-based HBsAg Vaccine Induces Robust T Cell Responses and Results in Viral Clearance in HBV Persistent Mice

Hongming Huang (China)

13:59-14:06 O-19 The Role of Intrahepatic Type I Interferon Signaling in HBV-specific T Cell Responses

Keigo Kawashima (Japan)

14:06-14:13 O-20 CHI3L1 is a Non-invasive Surrogate Serum Marker for Effectively Identifying Chronic HBV Patient with Normal ALT Levels but with Advanced Liver Fibrosis for Treatments

Biaoyang Lin (USA)

14:13-14:20 O-21 Analysis of Factors Associated with Reduction of HBs Antigen Levels in Chronic Hepatitis B Patients Treated with TDF + Peg-IFN Combination Therapy

Akihiro Matsumoto (Japan)

14:20-14:27 O-22 High Antibody Response to Standard and Double Dose of Hepatitis B Vaccine in Children after Liver Transplantation: A Randomized Controlled Trial

Palittiya Sintusek (Thailand)

14:27-14:34 O-23 Genetic Polymorphism and Reduced mRNA Expression of HLA class II DP Genes are Associated with Hepatitis B virus Reactivation in Japanese Patients Treated with Immunomodulatory Agents

Hidetaka Matsuda (Japan)

14:34-14:39 Break

14:39-15:14 Oral Free Papers (6)

Chairs: A. Kadir Dokmeci (Turkey), Katsutoshi Tokushige (Japan)

14:39-14:46 O-24 Lipoprotein-apolipoprotein Changes in Chronic Hepatitis C Patients Treated with Direct-acting Antivirals

Satoru Joshita (Japan)

14:46-14:53 O-25 Albumin-bilirubin Score Indicates Liver Fibrosis Staging and Prognosis in Chronic Hepatitis C Patients

Koji Fujita (Japan)

14:53-15:00 O-26 High Burden of Hepatitis C Infection and Increase Risk of Liver Fibrosis on Chronic Kidney Disease Underwent Hemodialysis Patients in Hasan Sadikin General Hospital, Bandung, Indonesia: A Preliminary Study Prior of Hepatitis C Eradication Program in Hemodialysis Patients

Eka Surya Nugraha (Indonesia)

15:00-15:07 O-27 Th17 Cell Activation of T Lymphocytes in Peripheral Blood in Patients with Chronic Hepatitis Delta

Bibigul Saparbekovna Ilyassova (Kazakhstan)

15:07-15:14 O-28 Soluble Siglec-7 as a Macrophage Activation Marker in Patients with Non-alcoholic Fatty Liver Disease

Yuzuru Sakamoto (Japan)
15:14-15:56 Oral Free Papers (7)

Chairs: Joong Won Park (Korea), Kaoru Tsuchiya (Japan)

Haruhiko Takeda (Japan)

Thuy Thi Minh Ngo (USA)

15:28-15:35 O-31 Increased Expression of a Disintegrin and Metalloproteinase 9 (ADAM9) in Advanced Hepatocellular Carcinoma (HCC) and its Role of HCC
Jooho Lee (Korea)

15:35-15:42 O-32 Milk Fat Globule-EGF Factor 8 (MFG-E8) as an Early Diagnostic and Postoperative Prognostic Biomarker in Patients with Hepatocellular Carcinoma
Tomonari Shimagaki (Japan)

15:42-15:49 O-33 SIRveNIB: Selective Internal Radiation Therapy (SIRT) Sorafenib in Mongolian Patients with Hepatocellular Carcinoma
Ariunaa Khasbazar (Mongolia)

15:49-15:56 O-34 Prospective Validation of New Selection Criteria Considering Pre-transplant Body Compositions in Living Donor Liver Transplantation
Toshimi Kaido (Japan)

15:56-16:31 Oral Free Papers (8)

Chairs: Jin Mo Yang (Korea), Toshimi Kaido (Japan)

15:56-16:03 O-35 Serum GPNMB Level Increases in Patients with Acute Liver Failure
Kotaro Kumagai (Japan)

16:03-16:10 O-36 Hepatic Intracellular Stress Responsible for the Development of HBV-related Fulminant Hepatitis
Eri Nanizawa (Japan)

16:10-16:17 O-37 Antibiotics-mediated Intestinal Microbiome Perturbation Aggravates Tacrolimus-induced Glucose Disorders in Mice
Baohong Wang (China)

16:17-16:24 O-38 H2-Bl/HLA-G as a Regulator of the Inflammatory Process Following Partial Hepatectomy
Johan Jaime Medina (Australia)

16:24-16:31 O-39 Bone Marrow Monocytes Dysfunction in Chronic Liver Disease
Dhananjay Kumar (India)
# Scientific Program

**Day 3: April 20 (Saturday) 2019**

Room 1 (Eminence Hall, 5F, South Tower, Keio Plaza Hotel)

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<td>7:30-8:30</td>
<td>Morning Seminar (2)</td>
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<tr>
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<td>Chair: Satoshi Mochida (Japan)</td>
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<td>Presented by Shionogi &amp; Co., Ltd.</td>
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<td>Safe Radiofrequency Ablation for Low Platelet Count Patients with Thrombopoietin (TPO) Receptor Agonist (Lusutrombopag)</td>
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<td>Hideo Yoshida (Japan)</td>
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<td>The Role of Lusutrombopag for the Multidisciplinary Treatment of Hepatocellular Carcinoma</td>
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<td>Yasuteru Kondo (Japan)</td>
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<td>8:30-8:35</td>
<td>Break</td>
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<td>8:35-10:00</td>
<td>HCC (2)</td>
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<td>Chairs: George Lau (Hong Kong), Mitsuhiko Moriyama (Japan)</td>
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<td>8:35-9:05</td>
<td>Adaptive Immunotherapy against HCC Recurrence after Transplantation (Keynote Lecture)</td>
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<td>Hideki Ohdan (Japan)</td>
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<td>9:05-9:25</td>
<td>Systemic Therapy of Hepatocellular Carcinoma: Korean Experience (Keynote Lecture)</td>
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<td>Joong Won Park (Korea)</td>
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<td>9:25-9:45</td>
<td>Personalised TCR T-Cell Immunotherapy for HBV-HCC</td>
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<td>Anthony Tan (Singapore)</td>
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<td>9:45-10:00</td>
<td>Panel Discussion</td>
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<td>10:00-10:05</td>
<td>Break</td>
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<td>10:05-11:20</td>
<td>HCC (3)</td>
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<td>Chairs: Joong Won Park (Korea), Shuntaro Obi (Japan)</td>
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<tr>
<td>10:05-10:25</td>
<td>Recent Advancement of Molecular Targeted Therapy in Hepatocellular Carcinoma</td>
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<td>Naoshi Nishida (Japan)</td>
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<td>10:25-10:45</td>
<td>Recent Advance of Immune-Therapy for Hepatocellular Carcinoma</td>
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<td>Masafumi Ikeda (Japan)</td>
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<td>10:45-11:05</td>
<td>Liver Function Disorder as irAE by Immune Checkpoint Inhibitor</td>
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<td>Eishiro Mizukoshi (Japan)</td>
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<td>11:05-11:20</td>
<td>Panel Discussion</td>
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<td>11:20-12:00</td>
<td>State-of-the Art Lecture (3)</td>
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<td>Chair: Osamu Yokosuka (Japan)</td>
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<td>Systemic Therapy of Hepatocellular Carcinoma</td>
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<td>Ann-Lii Cheng (Taiwan)</td>
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<td>12:00-12:10</td>
<td>Closing Ceremony</td>
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**Closing Remarks**

Atsushi Tanaka, President of APASL STC 2019 Tokyo
Poster Session Program

Day 1: April 18 (Thursday) 2019
Room 2 (Ohgi, 4F, South Tower, Keio Plaza Hotel)
Presentation Hours: 11:00-18:00 on April 18 (Thursday)

P-001  Hepatic Arterial Infusion Chemotherapy with Reservoir for Advanced Hepatocellular Carcinoma, Results of under Indication Limit
Shinpei Sato (Japan)

P-002  The Analysis of Genetic Mutations related to Expression of Receptor Tyrosine Kinases of Hepatocellular Carcinoma from TCGA Data
Yoshinari Asaoka (Japan)

P-003  Study on Validity of Biomarkers DKK1 and HBx-LINE1 in Diagnosis and Posttreatment Monitoring of Hepatocellular Carcinoma
Hieu Trung LE (Vietnam)

P-004  Spiral CT in the Clinical Significance of Portal Cavernous Change in Hepatocellular Carcinoma
Yi Yu (China)

P-005  Association between Type-2 Diabetes Mellitus and Platelet Distribution width in Patients with Primary Liver Cancer
Yi Yu (China)

P-006  The Role of Interferon-Induced Transmembrane Protein-3 in Progression of Hepatitis B Virus Related HCC in China
Yan Zhao (China)

P-007  The Characterization of Genetic Alterations Using Whole-Genome Sequencing Data in HBV-Related Hepatoma Cell Line
Ryosuke Muroyama (Japan)

P-008  Sorafenib-Regorafenib Sequential Therapy in Japanese Patients with Hepatocellular Carcinoma in Real World Practice Including the Early Experiences of Lenvatinib as a 3rd-Line Agent
Wan Wang (Japan)

P-009  Immunomodulation after Radiofrequency Ablation of Hepatocellular Carcinoma
Kaiwen Huang (Taiwan)

P-010  Sensitive Detection of Circulating Tumor Cells in Patients with Chronic Liver Disease and Hepatocellular Carcinoma Using a Microcavity Array
Kazuto Takahashi (Japan)

P-011  A Case of Multiple Tumors with Individual Histopathologic Features in Hepatitis C Cirrhotic Liver
Yoshie Kadota (Japan)

P-012  Melatonin Inhibits Liver Cancer Progression by Downregulating the Long Noncoding RNA CCAT1/let7i/RAF1 Axis
Tong-Hong Wang (Taiwan)

P-013  Investigation of ARID Family Gene Mutation in Hepatocellular Carcinoma
Kengo Kanayama (Japan)

P-014  Autophagy of Hepatic Stellate Cells Promotes HCC Progression
Hayato Hikita (Japan)

P-015  EpCAM-High Liver Cancer Stem Cells Show Resistance to Natural Killer Cell-Mediated Cytotoxicity via CEACAM1 in both in Vitro and in Vivo Models of Hepatocellular Carcinoma


Carcinoma
P-016  Massive Hemothorax Caused by Diaphragmatic Vessel Injury after Radiofrequency Ablation for Hepatocellular Carcinoma  Ming-Jeng Kuo (Taiwan)

P-017  Effects of Regorafenib on the Toll-Like Receptor Signaling Pathways in HCC  Tatsuo Kanda (Japan)

P-018  Alpha-Interferon Increases Methylation of Hepatitis B Virus DNA in Human Hepatoma Cells  Jin-Wook Kim (Korea)

P-019  Quantitative Analysis of TERT Promoter Mutation with Blood during HCC Treatment  Masaru Muraoka (Japan)

P-020  The Association between HMG-CoA Inhibitors and Cholangiocarcinoma  Mohamed M Gad (Egypt)

P-021  MicroRNA-30b-5p Promotes Hepatitis B Virus-Induced Hepatocarcinogenesis through Modulation of Proliferation and Metastasis  Jingjing Jiang (China)

P-022  Macrophage Colony-Stimulating Factor (M-CSF) Receptor Antagonist Inhibits Progression of Hepatocellular Carcinoma in Vivo  Hiroshi Kono (Japan)

P-023  The Real World Practice of Lenvatinib in Patients with Advanced Hepatocellular Carcinoma in Japan  Susumu Maruta (Japan)

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APASL Single Topic Conference 2019 in Tokyo

“Liver Immunology and Genetics”

Abstracts
State-of-the Art Lectures
Invited Lectures
Mission of APASL; Eradication of Virus and Cure of HCC

Since inauguration of APASL in 1978, we have organized bi- and annual meetings for 26 times for the last 40 years. Furthermore, we started STC (Single Topic Conference) from 2006 and held 29 times for the last 13 years.

Principal aims of these meeting are to promote the latest scientific advancement, exchange of information and the development of consensus in the Hepatology, particularly in the topics of infections of HBV and HCV infection. Because of recent past effort, treatment for the patients with these infection have been much improved. In fact what we have accomplished for the last 40 years are to promote HBV vaccination program and the development of nearly 100% cure of HCV infection.

On the contrary, care for patients with advanced HCC, alcoholic liver disease, NASH, autoimmune liver disease and advanced H HCC need much to be improved.

In this presentation, I will present the problems which we encounter in daily clinical practice and address the future perspectives based upon my own 40-year experience (3 Yrs at Yale, 3 Yrs at USC, 16 Yrs at Chiba U, 17 Yrs at U of Tokyo and 10 Yrs at Yama-nashi).
Translational Research in Hepatology Using Genetically Modified Mice

Hepatitis C virus (HCV) infects only humans and chimpanzees. However, small-animal models are needed for studying antiviral therapy and HCV infection. TK-NOG mice are immunodeficient and express thymidine kinase-transgene specifically in the liver. Ganciclovir ablates murine liver cells in these mice and subsequent injection of human hepatocytes enable repopulation in the liver. Humanized-liver TK-NOG mice are susceptible to HCV infection after either intravenous injection with sera from HCV patients or intrahepatic injection with replication-competent, full-length HCV RNA. The former model replicates HCV infection in patients; the latter provides a unique HCV infection model from a single clone.

Direct-acting antiviral (DAA) therapy has enabled a sustained virologic response (SVR) in most patients, but some experience virological failure and acquire HCV with resistance-associated substitutions (RAS). HCV exists as a diverse quasispecies in the body and pre-existing mutant viruses may contribute to the emergence of resistant viruses. However, their origins have not been clarified in detail. Among 11 patients with HCV 1b genotypes and virological failure with asunaprevir plus daclatasvir, 10 had NS5A-Y93H variants and 1 had NS5A-P32 deletion after treatment. Phylogenetic tree analysis suggested selection from baseline quasispecies in 4 patients. De novo mutations seemed to occur in the remaining 7 but we could not exclude the possibility of selection from minor clones that were under the detection threshold in deep sequencing. To overcome this limitation, we developed an infection model from a single HCV clone by intrahepatic injections of wild-type HCV RNA into humanized-liver TK-NOG mice. A new Y93H mutation occurred after 4 weeks of ledipasvir monotherapy, indicating that novel mutations can occur during therapy.

Second-generation NS5A inhibitors have enabled DAA retreatment for non-SVR patients with signature RAS, such as NS5A-Y93H. However, NS5A-P32 deletion is an emerging problem for retreatments. We analyzed 10 non-SVR patients after ledipasvir/sofosbuvir therapy and found NS5A-P32 deletion in 1. Humanized-liver TK-NOG mice developed persistent infection when administered with this patient’s serum, and were highly resistant to ledipasvir and elbasvir, a second-generation NS5A inhibitor. Although they did not respond to a combination of ledipasvir/sofosbuvir, they did respond to sofosbuvir plus either simeprevir or interferon. These results suggest that P32 deletions should be treated with a combination of at least two classes of antivirals from among NS3/4A protease inhibitors, NS5B polymerase inhibitors, and non-selective antivirals (e.g., interferon and ribavirin).

Humanized-liver TK-NOG is a useful mouse model for investigating the mechanisms and treatment strategies for treatment-emergent HCV RAS.
UNMET NEEDS and the Paradox of Primary Biliary Cholangitis

Primary Biliary Cholangitis (PBC) is a female predominant disease and illustrates the gender bias that occurs in the majority of autoimmune diseases. PBC considered a model autoimmune disease and antimitochondrial antibodies (AMA) are unique among autoimmune serologic reactants because of their extremely high association with PBC. The AMA is not only highly directed, but also very specific to the lipoyl domain of PDC-E2, the major mitochondrial autoantigen. Modification of this lipoyl domain, with specific small molecular mimics of lipoic acid, results in structures that react as well as and often better to PBC sera than the native molecule. Mice, immunized with xenobiotic-BSA, without any PDC-E2 in the immunogen, develop self-reacting AMA and biliary specific pathology. We propose that PBC is caused by a highly directed multi-cell lineage response to PDC-E2 and that chemical modification of PDC-E2 in genetically susceptible hosts begins the long natural history of disease. One major unanswered question is why there is an overwhelming female gender bias. Our current work has provided several clues regarding this issue, including the identification of chemicals as potential modifiers of PDC-E2 and triggers of PBC and the role of the interferon pathway. To address this issue we have defined the molecular recognition requirements of AMA binding of both our peptide and xenobiotic conjugates using a series of synthetic analogues. The β-sheet structure of PDC-E2 appears essential for AMA specificity and we have defined the critical amino acids in this region. We also recognize the possibility that peptides other than PDC-E2 may be involved in the initial AMA eliciting event and we have taken advantage of our expertise in xenobiotic combinatorial peptide technology to study epitope diversity. Importantly we will also discuss the role of the interferon pathway in a unique murine strain that involves altered interferon metabolism and which illustrates putative activation pathways that are central to female bias. We submit that the results of our work will prove our thesis that PBC is induced by a xenobiotic chemical or bacterial exposure that breaks tolerance and that the multi-cell lineage response to PDC-E2 will be the pathologic effector mechanism of this disease modulated by estrogens and interferon pathways.
Systemic Therapy of Hepatocellular Carcinoma

The past few years has witnessed a booming development of new drugs for the treatment of hepatocellular carcinoma (HCC). The paradigms have rapidly shifted from chemotherapy to molecular targeted therapy, and now immunotherapy. First-line treatment includes sorafenib and lenvatinib; second-line treatment includes regorafenib, cabozantinib, ramucirumab, and two anti-PD1 checkpoint inhibitors, nivolumab and pembrolizumab. Some practice guidelines suggest the use of anti-PD1 in the first-line setting, but this remains to be confirmed.

Although the tumor response rate of single-agent anti-PD1 is only around 15-20%, the quality of response appears to be superior, with many remitted metastatic tumors remain under control for a long time. This observation has encouraged resection or ablation for the residual hepatic tumors. Further, peri-operative administration of immune checkpoint inhibitors (ICIs) may further enhance the host immunity against tumors. Therefore, several ongoing trials are enthusiastically testing the efficacy of adjuvant or neoadjuvant ICIs in HCC.

Combinations of ICIs with multi-target TKIs or selected VEGF/VEGFR inhibitors may further improve tumor response to 30-40%. Final results of these trials are eagerly awaited. On the other hand, the progress of combinations of ICIs with other immunotherapy has been relatively faltered. Recently, breakthrough in exploring pharmacological measures to enhance innate immunity has shed light on this issue. Whether these discoveries may benefit HCC patients remains to be seen.
The Impact of Hepatic Antigen Presentation on HBV-Specific CD8+ T cell Responses

The hepatitis B virus (HBV)-specific CD8+ T-cell response plays a central role in purging the virus from the liver. However, little is known about the mechanisms responsible for the induction of functional HBV-specific CD8+ T cell responses. By adoptively transferring HBV-specific naive CD8+ T cells into HBV transgenic mice that replicate HBV at a high level, we have shown previously that intrahepatic antigen presentation induces functionally defective HBV-specific CD8+ T cells. We also showed that this functional defect of HBV-specific CD8+ T cells was reversed by activating dendritic cells (DCs) with an agonistic anti-CD40 antibody (αCD40). Building upon these finding, we here examined the importance of priming location and the relative contribution of endogenous antigen presentation by hepatocytes versus cross-presentation by bone marrow-derived cells to the induction of functional HBV-specific CD8+ T cell responses. Functional HBV-specific CD8+ T cell responses could be induced to intrahepatically expressed HBV even when T cell homing to the lymphoid tissues was severely suppressed, suggesting that functional priming could occur in the liver. Intravital and electron microscopic imaging analyses revealed that circulating HBV-specific CD8+ T cells arrest within liver sinusoids and probe sub-sinusoidal hepatocytes for the presence of antigens by extending cytoplasmic protrusions through endothelial fenestrae. These results indicate that HBV-specific CD8+ T cells directly interact with hepatocytes without extravasating to hepatic parenchyma. As expected, the expansion of HBV-specific CD8+ T cells was significantly reduced in the mice whose major histocompatibility complex (MHC) class I expression was mostly restricted to nonhematopoietic cells, suggesting the importance of cross-presentation by hematopoietic cells in the induction of HBV-specific CD8+ T cells. Strikingly, the expansion and cytolytic differentiation of HBV-specific CD8+ T cells were reduced even more severely in the mice whose MHC class I expression was restricted to hematopoietic cells. Collectively, these results indicate that cross-presentation is required but relatively inefficient in terms of inducing the cytolytic differentiation of HBV-specific CD8+ T cells by itself. Instead, the expansion and functional differentiation of HBV-specific CD8+ T cells are primarily dependent on hepatocellular antigen presentation.
TNF-Producing Regulatory T Cells in Viral Hepatitis

CD4⁺CD25⁺Foxp3⁺ T-regulatory (Treg) cells control immune responses and maintain immune homeostasis. However, under inflammatory conditions, Treg cells produce cytokines that promote inflammation. We investigated production of tumor necrosis factor (TNF) by Treg cells in patients infected by hepatitis viruses including HAV, HBV and HCV, and examined the characteristics of these cells and association with clinical factors. We found that a higher proportion of CD4⁺CD25⁺Foxp3⁺ Treg cells from patients with acute hepatitis A, chronic hepatitis B, or chronic hepatitis C, compared with controls, produced TNF upon stimulation with anti-CD3 and anti-CD28. DNA methylation analysis confirmed the identity of the Treg cells. TNF-producing Treg cells had features of T-helper 17 cells, including upregulation of RORγt, which was required for TNF production. The Treg cells had reduced suppressive functions compared to Treg cells from controls. The frequency of TNF-producing Treg cells in HAV-infected patients’ blood correlated with their serum level of alanine aminotransferase.

Patients with chronic hepatitis C were treated with direct-acting antivirals (DAAs) (daclatasvir (DCV)/asunaprevir (ASV) or ledipasvir (LDV)/sofosbuvir (SOF)), and sustained virologic response was achieved in all the patients. At week 8 after starting DAA treatment, the frequency of TNF-producing Treg cells among peripheral blood Treg cells was transiently decreased in both DCV/ASV- and LDV/SOF-treated groups although the frequency of Treg cells among CD4⁺ T cells was not changed. However, the frequency of TNF-producing Treg cells was increased thereafter, and the frequency measured at week 12 after the cessation of DAA treatment was returned to the level prior to DAA treatment.

Conclusions: Treg cells from patients with viral hepatitis produce higher levels of TNF and gain features of T-helper 17 cells. This inflammatory change of Treg cells are not normalized even after successful treatment with DAAs in patients with chronic hepatitis C.
HCV and Lymphomagenesis

Although the HCV primarily affects the liver, extrahepatic manifestations are well recognized among patients with chronic HCV infection. HCV is a single, positive strand RNA hepatotrophic virus with marked genetic variability. HCV is the possible cause of B cell dysregulation diseases and conditions and B cell lymphoproliferative disorders that may progress to nonHodgkin lymphoma (1). Evidences from experimental studies suggest that several different mechanisms may be involved in HCV mediated B-cell transformation (2). Various microenvironmental signals, such as cytokines, viral antigenic external stimulation of lymphocyte receptors by HCV antigens, and intercellular interactions contribute to B cell proliferation. HCV bound to B cell surface receptors can induce lymphoproliferation, leading to DNA mutations and/or lower antigen response thresholds (3). HCV lymphotropism and chronic antigenic stimulation are involved in B lymphocyte expansion, as mixed cryoglobulinemia or monoclonal gammopathy of undetermined significance, which can progress to B cell non-Hodgkin's lymphoma (BCNHL). The presence of both HCV RNA and viral proteins in peripheral B cells of CHC patients were demonstrated (4). Chronic antigenic stimulation is thought to be important in the pathogenesis of HCV related BCNHL. The immune response that occurs in HCV positive patients against one HCV antigen, the E2 envelope glycoprotein. These studies implicate the specific immune response against the E2 antigen in the pathogenesis of B cell lymphoproliferative diseases and, potentially, in HCV associated lymphomas. Lymphomagenesis occurs when B cells experience somatic hypermutation and proliferate in response to an antigen (5). Human CD81 is the first identified necessary receptor for HCV cell entry. HCV exploits CD81 not only to invade hepatocytes but also to modulate the host immune responses (6). The different oncogenetic mechanisms may be integrated and cooperate in a multifactorial pathogenetic model of HCV-associated B cell lymphoproliferation (7). The mechanism or mechanisms of HCV related lymphomagenesis remain to be elucidated but emerging data are now reported regarding the genetic basis for lymphotropism of HCV (8). Kasawa et al proposed that the activation of both canonical and alternative NF-kB signalling pathways and down regulation of miR 26b contribute to the development of HCV associated BCNHL (9).

Molecular mechanisms of HCV-NHL development are still poorly understood. Peveling-Oberhag pointed out that three possible theories can explain the process of HCV transformation;

1) The external lymphocyte receptors are continuously stimulated by the viral antigen resulting in its proliferation
2) Replication of HCV occurs inside the B cells and then mediate their oncogenic effects through intracellular HCV proteins
3) “hit and run” theory which means permanent damage of B cell, caused by the intracellular virus (e.g., mutation of tumor suppressor genes) (10).

Epidemiological studies have clearly demonstrated a correlation between chronic HCV infection and development of BCNHL. The follow up of Asian patients infected with HCV established that their risk of developing a lymphoid neoplasm and especially NHL was 2 times higher than that of a group of HCV uninfected patients (11).

The best evidence of a direct relationship between HCV and BCNHL is the demonstration of regression of BCNHL with anti HCV therapy with interferon, which has antiproliferative properties (12). A role for the virus in lymphomagenesis is also suggested by the curative activity of antiviral therapy for patients with HCV-related low-grade B cell lymphomas, even when non interferon containing regimens are used (13).

The most frequent subtypes of lymphomas associated with HCV infection are marginal zone NHL, especially splenic marginal zone lymphomas, lymphoplasmacytic lymphoma, and diffuse large BCNHL (14). The clinicopathological characteristics of HCV positive follicular lymphoma was reported recently (15).

The clinical course of the disease is generally indolent.

Low grade malignant lymphomas can respond to antiviral therapy. HCV infected patients with indolent BCNHL who receive antiviral therapy can be potentially cured.

Direct acting antiviral drugs could be a solution for the patients who did not tolerate or respond to interferon, as they are safe and highly effective, according to recent findings (16).

Conclusion;

Although there is now good evidence linking HCV infection and NHL but mechanism of HCV induced lymphomagenesis are still poorly understood. Lymphomagenesis is a multifactorial process involving genetic, environmental, and infectious factors. The presence of HCV proteins is a potent trigger for lymphoproliferation and clonal expansion of B cells. Indolent non Hodgkin’s lymphomas were more frequently associated with HCV infection.

Antiviral therapy may have a significant role in the treatment and prevention of some HCV associated BCNHL disorders. Primary treatment of HCV infection may be an alternative to standard lymphoma therapy in some HCV-associated indolent lymphomas. The treatment of patients chronically infected with HCV and having BCNHL is complex and requires a multidisciplinary approach by hematologist and hepatologist.

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Hepatitis B and C Virus Infection and Risk Factors for Developing Hepatocellular Carcinoma

Hepatitis B and C are worldwide health problems that cause acute and chronic infections that can cause cirrhosis and hepatocellular carcinoma (HCC). These infections are the leading cause of HCC worldwide and are associated with significant mortality and account for over 1.3 million deaths annually. Due to its high incidence and resistance to treatment, liver cancer is the second leading cause of cancer-related death globally and HCC accounts for about 90% of all primary liver cancer cases. Many HCC patients with persistent hepatitis B and C infections complicate liver cirrhosis. However, hepatitis B virus infection may promote the development of HCC without end-stage liver disease. Therefore, understanding the role of hepatitis B and C virus infections in the development of HCC is important for the future design of therapies and treatments for HCC.

The success of direct-acting antiviral (DAA) agents in the treatment of chronic HCV infection is expected to reduce the incidence of HCV-associated HCC. In addition, removal of HCV is helpful in restoring liver function and recovery of mild fibrosis, but patients after SVR with liver cirrhosis progress are still at high risk of HCC. Identifying the risk factors and biomarkers of HCC after SVR is a very important research topic.

Accumulating evidence indicates that antiviral therapy with the current NAs entecavir or tenofovir prescribed to control hepatic inflammation and prevent or reverse liver fibrosis can also reduce the risk of HCC. Recently Korean nationwide cohort study of patients with CHB infection to compare the risk of HCC and death or liver transplant with entecavir vs tenofovir treatment in patients with CHB infection. Outcomes revealed a significantly lower risk of HCC and mortality in relation to tenofovir vs entecavir treatment.

My lecture summarizes the current knowledge of direct and indirect risk factors for developing HCC in the patients with chronic hepatitis B and C.
HCV and Co-infections in Mongolia

Viral hepatitis B and C are one of the major causes of liver cirrhosis and HCC in Mongolia. 92-95% of HCC patients in Mongolia are related with HCV and HBV co-infections and occurring in 115 cases per 100,000 people per year. However, HCV and co-infections are still one of the serious public health concerns in Mongolia. Today I will discuss HCV and co-infections based on several published studies conducted in the last decade.

We have completed the study named “Prevalence and genotype distribution of hepatitis B and C virus among apparently healthy populations in Mongolia: a population-based nationwide study. This study population was consisted of 1512 subjects from 13 provinces and Ulaanbaatar city which is the capital city of Mongolia, and the age ranged from 0 to 80 years. According to our study results, the prevalence of anti-HCV was 15.6%, and the HCV RNA was detected in 11%; HBsAg-11.8%. The most of HCV infection is caused by genotype 1b.

The second study is the baseline survey of a Nationwide Cancer Cohort Study. Population based national cross-sectional survey was conducted with multistage random cluster sampling. Population aged 10-64 years in Metropolitan area and 4 geographic regions were randomly selected. Prevalence of HBsAg, anti-HBc, anti-HBs, HBeAg, and anti-HBe-positivity were 10.6%, 46.3%, 42.1%, 6.1% and 40.1%, respectively among general population aged 10-64 years. HCV infection was observed in 9.9% and 0.8% were co-infected with HBV and HCV.

The third study is to investigate the population-based prevalence of HBV and HCV infection prevalence among apparently healthy population in Ulaanbaatar city of Mongolia. 2667 people who live in Ulaanbaatar city were included in this study. The anti-HCV prevalence was 9%, HBsAg positive was 8% and HBsAb positive was 32%.

We undertook time trend analyses between our initial study published in 2005 and present study between 2012-2014. We sought to investigate changes in the proportion of acute viral hepatitis types in Mongolia over the last decade. The cohort comprised 546 consecutive patients clinically diagnosed in 50.9%, 26.2%, and 6.0% of the cohort. Notably, 16.8% of the cohort had a dual infection.

This study population was consisted of 195 subjects from 4 hospitals in UB city of Mongolia and age ranged from 30 to 86 year-olds. The most common etiology for HCC in our patients was HCV infection which is 46%, HBV infection -34%, co-infection B and C -14% and alcohol which is 6.0%.
Tailoring Immunity in Patients with HBV or HCV Infection – Aiming at the Virus Elimination by 2030

Mild and pervasive immune cell dysfunction, but not fully compromised, is a hallmark of chronic HBV or HCV infection. HBV is recognized by macrophages, dendritic cells (DCs) and natural killer (NK) cells. Indoleamine-2, 3-dioxygenase (IDO) is an enforcer of sequential immune reactions in acute hepatitis B, and this molecule is induced by the interaction of HBV-infected hepatocytes, DCs, and NK cells (Yoshio S, Kanto T, Hepatology 2016). Functional cure of HBV, or HBsAg loss, from chronically-infected patients is a clinical target of reducing the risk of liver cancer. In cases of spontaneously-resolving acute hepatitis and chronic hepatitis on treatment, we demonstrated that sequential chemokine and cytokine activation, including CXCL13 and IL-21, is involved in HBsAg seroconversion (Yoshio S, Kanto T, Hepatology 2016). These cases shed light on an active role of DC, macrophages and follicular helper T cells in attaining functional HBV cure (Yoshio S, Kanto T, JCI insight 2018).

We reported that human DCs play unique and substantial roles in HCV infection (Kanto T, JI 1999, JID 2004, Yoshio S, Kanto T, Hepatology 2013). Disabled DCs potentially give negative impact on adjacent cells. However, lack of evidence for active viral replication in DCs imply the presence of undisclosed contrivances that are independent of infection. Real-world clinical data have proven that direct anti-viral agents (DAAs) successfully eradicate HCV from more than 95% of the infected patients. HCV eradication by DAAs are considered to restore host immunity either indirectly by reducing viral burden or directly by immune modulation. We showed that plasmacytoid DCs (pDCs), a main producer of Type-I IFNs, were numerically and functionally recovered in patients who attained SVR. The comparison of gene signature of pDCs before and after DAA revealed that apoptotic pathways play key roles in the fundamental regulation of DC population (Yoshio S, Kanto T, AASLD 2018). However, the risk of HCC could not be completely erased from aged patients with advanced fibrosis even after HCV clearance, the impact of DAA-mediated immune alteration on de novo or recurrent HCC is still undisclosed.

Here, we would like to discuss the current status of research on immune responses against HBV and HCV infection. A comprehensive study of clinical samples, based on cutting edge technologies, is urgently needed to improve our understanding of the immune mechanisms associated with viral control in the liver and thus to develop novel immune modulatory therapies to cure chronic HBV and HCV infection.
HBV Reactivation after DAAs Therapy in CHC in HBV Endemic Area

In the past few years, pan-oral direct-acting antiviral (DAAs) therapy has revolutionized and allowed complete cure of chronic hepatitis C infection with a finite duration of therapy, with little side effects. Due to the shared modes of transmission, coinfection with both hepatitis B virus (HBV) and hepatitis C virus (HCV) is not uncommon. This is especially so in high-risk populations such as intravenous drug abusers, patients on hemodialysis, patients who have received an organ transplant, human immunodeficiency virus-positive patients, and β-thalassemia patients. Similar to patients coinfected with HBV and HCV treated with interferon (IFN)-based therapy, hepatitis due to HBV reactivation in chronic hepatitis C (CHC) patients who are also hepatitis B surface antigen (HBsAg) positive has been reported after treatment with pan-oral direct-acting antiviral agents (DAAs). As HBsAg-positive individuals were excluded from clinical trials of DAAs, HBV reactivation after HCV clearance was only reported after DAAs entered clinical use. The severity of hepatitis ranged from HBV reactivation without hepatitis to fulminant hepatic failure, requiring liver transplantation. On the other hand, it is very rare among patients with resolved HBV infection. The underlying mechanism of HBV reactivation owing to HCV treatment is not entirely clear. This might be related to the induction of types I and III interferons by HCV, both of which are active against HBV. With DAAs, rapid HCV suppression leads to reduced activation of the interferon cascade, allowing for enhanced HBV replication as early as 4 weeks into therapy. The clinical outcome of increased HBV replication likely relates to the degree of immune control that predated therapy, which may explain why those with occult HBV had a much lower or negligible risk than HBsAg positive CHC patients treated with pan-oral DAAs. A better understanding of the mechanisms involved may aid in pretreatment risk stratification. Nonetheless, the occurrence of these events had prompted both the US Food and Drug Administration and the European Medicine Agency's Pharmacovigilance Risk Assessment Committee to confirm the risk of HBV reactivation in HBsAg positive CHC patients treated with pan-oral DAAs. Hence, all CHC planned for pan-oral DAAs therapy should be screened for HBsAg and pre-emptive anti-HBV nucleos(t)ide therapy should be considered before initiation of DAAs therapy.
HCC Occurrence/Recurrence after HCV Eradication

According to the report of WHO, an estimated 71 million people have chronic hepatitis C (CHC) infection globally and a significant number of those who are chronically infected will develop cirrhosis or liver cancer. Approximately 399,000 people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (HCC). Viral eradication after interferon (IFN)-based therapy has been associated with an improvement of liver function and a reduced risk of developing HCC in CHC patients. With the recent development of potent direct-acting antiviral agent (DDA) combinations, antiviral medicines can cure more than 95% of CHC patients, thereby hopeful of the reducing the risk of death from liver cancer and cirrhosis.

Nevertheless, in the early years of their use, there are some negative reports on the effectiveness of DAA therapy in the recurrence of HCC. Following the reports of larger prospective studies, the risk of de novo HCC occurrence has been proven clearly to be lower after the achievement of sustained virological response (SVR), regardless of antiviral treatment. On the other hand, the risk of HCC recurrence following treatment with DAAs is debatable. The possible alterations in the immune system after clearance of the virus with the DAAs and how they may affect the conventional cancer immunity cycle are still under discussion. The aim of this presentation is to review published data on the risk of HCC occurrence and recurrence after viral eradication and provide an update on the risk and benefit of DAAs treatment revealed by clinical and basic research so far.
Anti-HCV Therapy after Liver Transplantation

Liver cirrhosis and hepatocellular carcinoma caused by hepatitis C virus (HCV) infection are the leading indications for liver transplantation in many countries including Japan. Almost all HCV-positive recipients develop recurrent hepatitis C, and the progression of fibrosis in the transplanted liver is often accelerated, resulting in poor transplant recipient prognosis. Antiviral therapy to eradicate HCV is expected to be the most effective way to prevent the progression from recurrent hepatitis C to cirrhosis and to improve the prognosis after liver transplantation.

Treatment for recurrent hepatitis C after liver transplantation has dramatically changed from interferon-containing regimens to interferon-free therapies. Several regimens which have shown no or few clinically significant drug-drug interactions with immunosuppressive agents have improved the safety of treatment after liver transplantation. At present, the standard treatment for all HCV genotype after liver transplantation in Japan is a 12 week-regimen of sofosbuvir and ledipasvir without ribavirin, or an 8- and 12-week regimen of glecaprevir and pibrentasvir. In this presentation, the efficacy and safety of these treatment for recurrent hepatitis C after liver transplantation will be demonstrated and optimal strategy for the treatment in patients with prior direct acting antiviral failure, severe renal impairment, and cirrhosis and/or jaundice will be discussed.
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Immune and Genetic Markers Associated with Hepatitis B Viral Relapse after Discontinuation of Oral Antivirals

Chronic hepatitis B virus (HBV) infection is a global health problem, especially in the Asia-Pacific region. In clinical practice, spontaneous or treatment-induced hepatitis B surface antigen (HBsAg) seroclearance defines functional cure for chronic hepatitis B (CHB). However, prolonged nucleos(t)ide analogue (NUC) therapy is usually necessary because achieving seroconversion requires decades. Therefore, identification of host immune and genetic markers associated with HBV relapse after discontinuation of NUC therapy is clinically important. In a recent study, we prospectively enrolled 100 CHB patients who were discontinuing NUC therapy. Viral and host predictors of relapse were evaluated, including HBsAg level, anti–HBV core antibody level, and presence of single-nucleotide polymorphisms in the genes encoding the receptors NTCP (rs2296651) and CTLA4 (rs231775) and in the 3′ untranslated regions of the genes encoding HLA-DPA1 (rs3077) and HLA-DPB1 (rs9277535); posttherapy predictors of relapse were also investigated. Our data showed that patients discontinuing TDF exhibited significantly higher rates of virological relapse (VR) (52.9% vs 6.1%; \( P < .001 \)) and clinical relapse (CR) (15.2% vs. 1.5%, \( P = .007 \)) at 3 months than those discontinuing ETV, but relapse rates at 12 months were comparable. The end-of-therapy HBsAg levels predicted VR (hazard ratio [HR], 1.62; 95% confidence interval [CI], 1.19–2.21), CR (HR, 1.78; 95% CI, 1.13–2.81), and sustained clinical response (SCR) (OR, 0.57; 95% CI, .35–.94). The CTLA4 (rs231775) non-GG genotype predicted VR (HR, 1.74; 95% CI, 1.01–3.00) and CR (HR, 2.06; 95% CI, 1.04–4.11), while the HLA-DPA1 (rs3077) AA genotype predicted SCR (OR, 10.84; 95% CI, 1.12–105). In another collaborative immunological study, we aimed to define possible immune markers associated with HBV control or viral relapse upon NUC discontinuation, and characterized the functional profile of both antigen-specific T cells and global non–antigen-specific immunity in chronic HBV patients during NUC therapy. Both traditional immunological assays and methods for analysis of the global non–antigen-specific immune populations were used to define the frequency and phenotype of global T, NK, and B cell populations. Our data demonstrated an increased frequency of functional PD-1+ HBV polymerase/core-specific T cells correlate with the absence of hepatic flares upon NUC therapy withdrawal in 2 independent cohorts of CHB patients undergoing long-term NUC therapy. In summary, host genetic and immune factors may be associated with HBV relapse after discontinuation of NUC therapy and further studies are needed to validate these findings.
Genetics of Metabolic Liver Diseases

There are many inherited metabolic and genetic disorders that can affect the liver from conditions related to accumulation of metals or toxic metabolites such as hemochromatosis, Gilbert syndrome and Wilson’s disease to protein-misfolding disorders such as alpha1-antitrypsin deficiency, Gaucher disease and cystic fibrosis. Inherited liver diseases typically cause early chronic liver involvement and some can be associated with hepatic adenoma formation and transformation to hepatocellular carcinoma. In recent decades, there are significant and rapid developments in the understanding and clinical applications of genetics in many metabolic liver diseases. Such developments enlighten new understanding of the disease pathogenesis, natural history and bring new possibilities for the diagnosis of these genetic disorders through genetic testing. It is not uncommon nowadays that clinicians have to face new expectations and demands from patients and their family members for genetic testing. Clinicians are required to integrate the genetic information into their clinical practice in order to aid the diagnosis, predict prognosis, perform cascade screening and sometimes guide treatment plans. While there is a growing tendency in applying genetic data into the conventional clinical management, the importance of careful interpretation of genetic results together with the provision of adequate genetic counseling cannot be overemphasized to ensure proper utilization of genetic information.
Advance of Genetic Diagnosis of Cholestasis in Pediatric Patients

The advent of next generation sequencing (NGS) now is available to unravel the genetic causes in both rare genetic diseases and common but heterogeneous disorders. NGS is applied in both research and clinical settings, and there is a rapid transition of research findings to diagnostic applications. Now the whole-genome analysis can be performed in several days (or even hours?) at reasonable costs compared with gene-by-gene analysis based on Sanger sequencing. We applied this new technique to the diagnosis of cholestatic diseases.

Many hereditary diseases can cause pathological jaundice including: (1) hematological diseases, such as G6PD deficiency, (2) intrahepatic or extrahepatic cholestasis such as Dubin-Johnson syndrome, Alagille Syndrome and progressive family intrahepatic cholestasis (PFIC), (3) inborn errors of bile acid synthesis (4) mitochondria diseases (5) hereditary metabolic liver disease. We developed a capture-based target enrichment NGS jaundice panel containing 42 known disease-causing genes associated with jaundice or cholestasis and 10 pathway-related genes. Target enrichment strategy was used to cover the whole genomic regions of target genes related to aforementioned diseases. Libraries were paired-end sequenced by NGS system. Its high throughput and high detection rate shows the great potential to facilitate the clinical genetic testing for jaundice related diseases. However, there are still some limitations and hurdles. Proper definition of clinical phenotype, indications of the most appropriate subjects to be tested, optimization of both sensitivity and specificity of the test, and the economic, ethical and legal issues are vital in the final application of NGS diagnostic tests. Whole exome or genome sequence will be an alternative or complementary method for our panel.
Autoimmune liver diseases (AILD) include autoimmune hepatitis (AIH), primary sclerosing cholangitis and AIH/PSC overlap syndrome, also called autoimmune sclerosing cholangitis (ASC). Immunosuppressive therapy, including corticosteroids, is effective for AIH, but ineffective or partially effective for PSC and AIH/PSC overlap syndrome. In some cases, it is difficult to differentiate AIH from other AILD without endoscopic retrograde cholangiography (ERCP). To diagnose AIH, revised AIH diagnostic criteria and scoring system proposed by International AIH Group (IAIHG) in 1999 have been used for pediatric and adult AIH. However, some of children with PSC are classified into definite AIH by the revised criteria. The simplified AIH criteria was proposed for clinical use in 2008. However, the simplified criteria cannot differentiate between AIH and PSC in children, and diagnose acute hepatitis phase AIH. Therefore, the simplified criteria should not be used for children.

There are some differences between adult AIH and pediatric AIH. First, the proportion of female patients with AIH in children is less than that in adults. Second, acute hepatitis is more common in children. Third, the prevalence of patients with a family history or complications from autoimmune diseases was less in children. Growth disorder, an adverse effect of corticosteroids, is a major problem in pediatric AIH. To reduce the adverse effects of corticosteroids, methylprednisolone pulse therapy could be useful for AIH in children. The serum level of aminotransferase will normalize within 3 months after initiation of methylprednisolone pulse therapy in pediatric AIH. In pediatric PSC and AOH/PSC overlap syndrome, the serum level of aminotransferase won’t normalize, or it will relapse with tapering corticosteroids. To avoid unnecessary immunosuppressive therapy, ERCP and colonoscopy may be recommended before administering immunosuppressive agents including corticosteroid.

Some cases of AIH could present as fulminant hepatitis and may require artificial liver support. Patients with fulminant AIH will need liver transplantation if immunosuppressive therapy isn't initiated immediately. Continuous intravenous cyclosporine A may be effective for fulminant AIH to avoid liver transplantation. Serum level of soluble interleukin 2 receptor, which indicate activation of T-lymphocytes, may be useful to initiate appropriate and prompt treatment based on the pathogenesis before diagnosed as AIH.
Biliary Atresia

Biliary atresia (BA) is an important obstructive cholestatic disease in neonates and young infants; however, the etiology of this condition remains unclear. Hepatic portoenterostomy (HPE) and liver transplantation are important therapeutic options for this condition. Usually, HPE serves as first-line operative treatment for BA. Liver transplantation is necessary in patients with prolonged or recurrent obstructive jaundice after HPE. Based on the Japanese Biliary Atresia registry, the rate of disappearance of jaundice following HPE was approximately 60%, and the 20-year native liver survival and overall survival rates were 46% and 86%, respectively.

Several therapeutic advances have led to an increase in the number of long-term native liver survivors (LTNLS) of BA. However, previous reports have shown that 30–40% of LTNLS developed long-term sequelae such as cholangitis and portal hypertension, and appropriate management of them is controversial. Clinical practice guidelines for BA have been recently established in Japan. These guidelines consist of 25 clinical questions and clinical practice algorithms.

The HPE procedure needs to be performed promptly and appropriately to ensure long-term survival with a good quality of life.

Adding to proper antibiotics in treating cholangitis, mechanical obstruction of the biliary drainage route, and deformity of intrahepatic bile ducts should be evaluated in cases of intractable cholangitis with reserved hepatic function. In patients with portal hypertension, appropriate interventions such as endoscopic variceal treatment and partial splenic embolization can provide a good quality of life to patients with preserved hepatic function. The use of appropriate therapeutic modalities is recommended in a selected subset of patients with late complications.

Despite appropriate treatment for various conditions, > 50% of patients with BA need liver transplantation. In such cases, choosing an appropriate time course for liver transplantation is an essential factor to ensure optimal treatment of BA.
Development of Novel Medical Therapy for Pediatric Liver Diseases with Intrahepatic Cholestasis

Bile salt export pump (BSEP, encoded by \textit{ABCB11}), an ABC transporter localized on the canalicular membrane (CM) of hepatocytes, mediates biliary excretion of bile acids (BA). Its dysfunction impairs bile formation, a liver condition called intrahepatic cholestasis (IC). PFIC2, the most severe form of IC caused by mutation in \textit{ABCB11}, progresses to liver failure and death before adulthood. Currently, the only therapeutic approach for PFIC2 is liver transplantation.

We have shown that PFIC2-causing mutations predominantly affect expression of BSEP on the CM but not its transport activity (1) and then searched potential compounds to restore BSEP expression. Sodium 4-phenylbutyrate (NaPB), a drug approved for urea cycle disorder (UCD), was found as the candidate. Animal experiments and retrospective study in UCD patients indicated that treatment with NaPB increases BSEP expression on the CM and thereby its function (2–4). Clinical study in three PFIC2 patients showed that NaPB therapy markedly improved biochemical tests, clinical symptoms, and liver histology (5, 6).

Based on these facts, we have started clinical trial to obtain approval for new indications of NaPB for PFIC2 (UMIN000024753) and clinical study to investigate therapeutic potency of NaPB in patients with IC other than PFIC2 (UMIN000027666).

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Liver Transplant

To be announced.
Acute Liver Failure and Acute-on-Chronic Liver Failure in Japan

In Japan, patients manifesting prothrombin time INR of 1.5 or more caused by severe liver damage developing within 8 weeks of the onset of symptoms are diagnosed as having “acute liver failure (ALF)”, when the liver function prior to the current onset of liver damage being estimated to be normal. ALF is classified into “ALF without hepatic coma” and “ALF with hepatic coma,” depending on the severity of the hepatic encephalopathy; the latter is further classified into 2 types, the “acute type” and the “subacute type”, in which grade II or more severe hepatic coma develops within 10 days and between 11 and 56 days, respectively, after the disease symptoms onset. Also, patients showing grade II or more severe hepatic coma between 8 and 24 weeks of the onset of disease symptoms are diagnosed as having “late-onset hepatic failure (LOHF)”. According to these criteria, the Intractable Hepato-Biliary Diseases Study Group of Japan sponsored by Ministry of Health, Labour and Welfare have been conducted annually nationwide surveys for ALF and LOHF. In these surveys, a total of 1,121 patients with ALF or LOHF seen between 2010 and 2018 were enrolled; the subjects consisted of 1,126 patients without hepatic coma and 996 patients with hepatic coma, and the later patients were classified into 527 patients with acute type ALF, 402 patients with subacute type ALF and 66 patients with LOHF.

Moreover, the study group established Japanese criteria for acute-on-chronic liver failure (ACLF) in 2018; patients with cirrhosis and a Child-Pugh score of 5-9 should be diagnosed as having ACLF when a deterioration of liver function (serum bilirubin level of 5.0 mg/dL or more and prothrombin time value of 40% or less of the standardized values and/or international normalization rates [INRs] of 1.5 or more) caused by severe liver damage develops within 28 days after an acute insult including alcohol abuse, bacterial infection, gastrointestinal bleeding, and the exacerbation of underlying liver diseases. The severities of the patients can be classified into 4 grades depending on the extent of the deterioration in organ functions, including liver, kidney, cerebral, blood coagulation, circulatory and respiratory functions. The study group have also performed a nationwide survey, and 202 patients with ACLF including related diseases of ACLF seen in 2018 were enrolled.

In the present lecture, demographic and clinical features of patients with ALF and ACLF in Japan were discussed based on the nationwide surveys.
Early Prediction of ALF in Patients with Acute Liver Injury

In Japan, acute liver failure (ALF) is defined as acute liver injury (ALI) with a prolonged prothrombin time (PT; < 40% activity or > 1.5 INR), and is classified into ALF with hepatic encephalopathy (HE) and ALF without HE. Because the survival rate of ALF with HE (24.0%) is extremely poor in comparison to that of ALF without HE (86.6%), the prevention of HE-development is the most important therapeutic strategy in early stage of ALI. On the other hand, most patients with ALI spontaneously recover without any specific therapy. Thus, a method to accurately distinguish patients with an increased risk of developing HE from patients with conventional ALI is necessary to indicate patients who require HE-preemptive therapy. Based on this concept, we developed a prediction model to discriminate patients who will develop HE from severe acute hepatitis patients with a prolonged PT < 80%. In this model, the probability (%) of HE-development in each ALI patient was calculated based on age, etiology, PT (%) and total bilirubin level. We constructed a local hospital network for the early prediction of HE-development using this model and transferred high-risk patients (> 20% probability of HE-development) to a major liver center (Iwate Medical University Hospital) for early treatment. This network system has been verifying the efficiency of the prediction model and evaluated the efficacy of therapies using steroids, N-acetyl cysteine and nucleic analogs in preventing the development of HE, and the efficacy of precise examinations for the grading of liver damage, including galactosylated albumin (GSA) scintigraphy, ultrasonographic elastography (USE) and contrast-enhanced ultrasonography (CEUS). The estimated probability of HE-development predicted by the model was approximately 15% over the real rate of HE-development. With the hospital network system, which facilitated early transfer and the early initiation of treatment, the rates (%) of HE-development in transferred severe ALI patients with hepatitis A, acute hepatitis B (HB), HB flare, and autoimmune hepatitis were remarkably decreased from 4.1 to 0, 17.9 to 8.7, 39.1 to 7.1, 30.7 to 2.7, respectively. That in patients with drug-induced hepatic injury was not significantly decreased (from 10.5 to 8.9). GSA scintigraphy, USE and CEUS could all accurately evaluate the severity of liver damage and predict the prognosis of severe ALI patients. In conclusion, the early initiation of preemptive treatment for severe ALI patients based on the accurate prediction of HE-development effectively improved the prognosis of ALI and reduced the cost associated with expensive treatments for ALF, such as artificial liver support and liver transplantation.
ACLF-Korean Experiences

Acute-on-chronic liver failure (ACLF) is a syndrome that rapidly deteriorated in liver function by acute insult and had high short-term mortality. Asian Pacific consortium and European consortium announced different ACLF definitions. Recently, Alcohol-related Problems Study Group of Korea made a nationwide retrospective ACLF cohort in 2015. This ACLF cohort included patients according to both Asian and European definition for investigating discrepancies between two definitions. In this ACLF cohort, discrepant ACLF definitions resulted in differences in mortality and patient characteristics, which arise because underlying chronic liver disease, precipitating factors and organ failures are differently. In addition, the most common acute insult and/or underlying chronic liver disease of ACLF have been changed from HBV to alcohol in Korea. In Korean ACLF cohort, prognostic scoring systems were validated. The scores which developed in the European cohort were useful in patients with ACLF according to European definition, but not the Asian definition. We found that long-term outcomes of patients who survive ACLF were influenced by a prior history of acute decompensation. So, we suggested that the prevention of a first acute decompensation episode may improve the long-term survival of cirrhotic patients. We also reveal that prognostic scores performed well with good predictive ability for short-term mortality in patients with alcoholic hepatitis. Now, we are conducting a prospective multicenter ACLF study and this prospective study is expected to be an important study that can narrow the gap between Asian and European ACLF definitions.
Liver-resident NK Cells Suppress Autoimmune Cholangitis and Limit the Proliferation of CD4⁺ T Cells

Primary biliary cholangitis (PBC) is an autoimmune liver disease characterized by lymphocytic infiltration in portal tracts, destruction of intrahepatic small bile duct epithelial cells, and anti-mitochondrial antibodies (AMAs). Clinical studies suggest that frequency and absolute number of NK cells is increased in both liver and peripheral blood from PBC patients. Here, we extensively investigated the impact of NK cells in the pathogenesis of autoimmune cholangitis utilizing the well-established mouse model.

Firstly, we noticed the progression of disease in PBC mouse model was negatively correlated with the number of liver-resident NK cells. Next, taking advantage of NK cell deficient (Nfil3⁻/⁻) mouse, adoptive transfer and antibody-mediated NK depletion, we demonstrated that loss of NK cells in mouse model resulted in aggravated biliary disease, associated with an increase in T cells, especially CD4⁺ T cells. Furthermore, we found that only DX5⁻ NK cells, but not DX5⁺ NK cells inhibited CD4⁺ T cell proliferation and co-localize with CD4⁺ T cells. Finally, we demonstrated that the suppressive function of DX5⁻ NK cells was enhanced in an inflammatory environment.

Our data demonstrated the immunosuppressive role of liver-resident NK cells in the pathogenesis of biliary disease. Targeting liver-resident NK cells may serve as a tissue specific therapeutic strategy for PBC.
Epigenetic Changes in Autoimmune Diseases

The pathogenesis of autoimmune disorders (AIDs) such as systemic lupus erythematosus (SLE) and psoriasis have been intensively studied but remains far from clear. Although multiple genes take part in determining the predisposition to SLE and Psoriasis, environmental factors have been demonstrated to be crucial for the pathogenesis of SLE and psoriasis. Accumulating evidence has indicated that aberrant DNA methylation, histone modifications and miRNAs in CD4+T cells contributes to the onset and development of SLE and Psoriasis. Apart from those classic methylation-sensitive autoimmunity-related genes in lupus, such as ITGAL, TNFSF7, PRF1 and PP2Acα, the genome-wide methylation pattern has also been explored recently, providing us a more and more full-scale picture of the abnormal status of DNA methylation in SLE. Lupus primarily affects women. The reason is unknown. We demonstrated that regulatory sequences on the inactive X chromosome demethylate in T cells from women with lupus, contributing to CD40LG overexpression uniquely in women. Demethylation of CD40LG and perhaps other genes on the inactive X may contribute to the striking female predilection of this disease. In recent years, certain miRNAs, RFX1, defective ERK pathway signaling, Gadd45α and DNA hydroxymethylation have been proposed as potential mechanisms leading to DNA hypomethylation in lupus. Ten-eleven translocation (TET) family of demethylases catalyzes the conversion of 5-methylcytosine into 5-hydroxymethylcytosine, and regulates DNA methylation dynamically. We have recently demonstrated that TET2 promoters IL-17A expression through demethylation of its promoter and regulating its chromatin accessibility in SLE CD4+ T cells.

In addition to SLE, we demonstrated the specific methylated genes identified from psoriasis patients, which were related to immune response, T cell polarization and signal transduction. We also found that aberrant histone modifications in PBMCs from psoriasis patients. Moreover, we demonstrated that the increased expression of miR-210 induces helper T (TH) 17 and TH1 cell differentiation but inhibits TH2 differentiation by inhibiting its targets STAT6 and LYN, contributing to several aspects of the immune imbalance in psoriasis. Both miR-210 ablation in mice and inhibition of miR-210 by intradermal injection of antagonimr-210 blocked the immune imbalance and the development of psoriasis-like inflammation in imiquimod-induced (IMQ-induced) psoriasis-like mouse model.

Based on the progress made in elucidating molecular mechanisms underlying AIDs, novel biomarkers for prediction, early diagnosis, prognosis and treatment response, and therapeutic strategies are proposed, which represents a promising future in the battle against AIDs. However, challenges remain regarding the clinical application of these potential new tools.
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Autoimmunity against Biliary Epithelial Cells

Biliary epithelial cells (BEC) are main target for primary biliary cirrhosis (PBC). The hallmark of PBC is the presence of auto-reactive T and B cell responses against BEC. We have previously demonstrated that biliary cell cytotoxicity is dependent upon the initiation of innate immune responses followed by chronic adaptive as well as bystander mechanisms. Critical to these mechanisms are the interactions between BEC and natural killer (NK) cells that are activated by toll like receptors ligands (TLRLs) and IFN-a. We have taken advantage of our ability to isolate relatively pure viable preparations of liver-derived NK cells and BEC, and studied the interactions between NK cells and BEC and focused on the mechanisms that activate auto-reactive T cells, their dependence on IFN-g, and the expression of BEC MHC class I and class II molecules. Importantly, we demonstrate that at a high NK/BEC ratio, NK cells are cytotoxic for autologous BEC, and lytic BEC release micro-particles that contain auto-antigen pyruvate dehydrogenase complex E2 component that activate auto-reactive CD4+ T cells in the presence of antigen presenting cells (APC). In contrast, at a low NK/BEC ratio, BEC are not lysed, but IFN-g production is induced, which facilitates expression of MHC class I and class II molecules on BEC and, interestingly, protects them from lysis upon subsequent exposure to auto-reactive NK cells. Furthermore, IFN-g secreted from NK cells after exposure to autologous BEC is essential for this protective function.

Earlier works have demonstrated that patients with PBC have reduced expression of the anion exchanger 2 (AE2) on BEC and deletion of AE2 gene has led to a PBC-like disorder in mice. We showed the hydrophobic bile acids reduced AE2 expression. AE2 reduced BEC up-regulated expression of CD40 and HLA-DR as well as production of chemokines in response to TLRLs, and reduced AE2 expression enhances the migration of autologous lymphocytes towards BEC. TLRLs stimulated IFN-g production from lymphocytes and IFN-g farther reduced AE2 expression from BEC.

In this way, we found IFN-g have a central role in the maintenance of PBC pathology.
Genetics of Autoimmune Liver Diseases

Genome-wide association studies (GWAS) have identified thousands of genetic loci associated with disease-susceptibility in various complex diseases. In addition, the progress of post-GWAS approach is unraveling the causal variants, causal genes and the mechanisms by which disease-susceptibility is conferred. The study of disease-pathways and their upstream and/or downstream regulators based on GWAS is now leading to the identification of the novel molecular targets for treatment. In this lecture, we firstly overview the recent progress of genetics in the pathogenesis of autoimmune liver diseases such as primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and autoimmune hepatitis (AIH) in European descents and East-Asian populations. We will then focus on the genetics of PBC as a model of human autoimmune disease and present the results obtained from integrated analysis of GWAS and transcriptome in the Japanese population. In addition, we will refer to the latest findings of international meta-analysis obtained from the collaboration study among PBC-GWAS consortium in Japan, UK, Italy, Canada, USA, and China.
Female Predominance in Autoimmune Liver Diseases

Most of the autoimmune liver diseases are characterized by a striking female preponderance, in particular in patients with primary biliary cholangitis (PBC). The mechanisms behind this predominance are still to be elucidated, although multiple theories have been postulated and investigated. Among the proposed involved factors, sex hormones have been the first to be studied, but unfortunately data have been inconclusive or conflicting. Similarly, fetal microchimerism has received a huge attention in the past, but data in PBC have been unsatisfactory especially if compared to other autoimmune diseases like systemic lupus erythematosus. Studies focused on genetic factors have generated more intriguing and robust data, reporting a few abnormalities on the X chromosome in PBC patients. However, these data are able to explain only a part of the phenotypic variability attributed to the genetic component, and most importantly, need to be validated in larger series. More recently, a novel mice model of PBC, characterised by a constitutive expression of Interferon-γ (IFN-γ), has been developed and it is notable for being the first one with female predominance. At the same time, there has been a wide interest in the role of microbiome in health and disease, as well as in epigenetics, which have tried to explain differences in biological phenotypes not covered by genetics.
The geoepidemiology of primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH) is a global scale study of distribution and determinants of disease gradients. It leads to delineating disease burden, understanding of genetic and environmental factors related to the etiology and pathogenesis, and ultimately advancing diagnosis and treatment of the autoimmune liver disease (AILD). However, common limitations in geoepidemiology refer to temporal factors (e.g., diagnostic advances, lack of up-to-date data), socioeconomic factors (e.g., access to medical care or diagnostic procedures, medical expertise,) and methodological factors (i.e., community based or hospital based study, small or big data).

Reported PBC prevalences per million people in Eastern countries including South Korea, China, and Japan are 48-78, showing a lower prevalence than those (132-582) in Western countries. Male to female ratio of PBC was 1:4-9, and the overall morality in male was significantly higher than in female patients. The dominant antigen is lipoyl domain of the enzyme family located in the inner membrane of mitochondria including pyruvate dehydrogenase. Genetic factors associated with PBC were HLA class II genes (HLA-DRB1*08) and gene loci in the IL-12 pathway (IL12RB2 and STAT4) and tumor necrosis factor (TNF)-alpha pathway. Environmental factors associated with PBC were infection by *E. coli* or *Novospingobium aromaticivorans*, xenobiotics including 2-octynamide, and dysbiosis of gut microbiota.

Reported AIH prevalences per million people in Eastern countries including South Korea, China, and Singapore are 40-80, showing a lower prevalence than those (150-430) in Western countries. Male to female ratio of AIH was 1:2-7, and there was no gender difference in the overall mortality. Main auto-antigen was not identified in AIH. Genetic factors associated with AIH were HLA class II alleles (HLA-DRB1*04, DRB1*03), and environmental factors associated with AIH were viral infection and drugs including nitrofurantoin, minocycline, and various herbal medications.

In conclusion, conducting multicenter studies in different regions using improved diagnostic criteria may reveal the genetic and environmental factors related to pathogenesis of AILD, which can offer a more selective and effective treatment in the future.
Noninvasive Prediction of Liver Fibrosis in Autoimmune Liver Disease

Autoimmune liver diseases are complex conditions that arise from interactions between genetic susceptibility and environmental triggers. Type 1 autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) are the two most common autoimmune liver diseases in Japan and are characterized by an autoimmune reaction towards hepatocytes and the destruction of intrahepatic bile ducts, respectively. Although liver biopsy is the gold standard to assess the degree of liver fibrosis, it is often limited by invasiveness and pain, sampling error, and interobserver disparity. As severe fibrosis and cirrhosis are generally related to a poor outcome, simple and reliable noninvasive methods are urgently needed to better assess liver fibrosis and predict the progression of the disease. Since several biomarkers such as WFA+-M2BP, autotaxin were developed as noninvasive method of estimating liver fibrosis, I will review the diagnostic performance of those markers for the evaluation of fibrosis and clinical outcome in AIH and PBC.
Autoimmune Hepatitis: 2019 Update

Autoimmune hepatitis (AIH) is a worldwide immunological liver disease which has been considered as a relatively rare disease, especially in Asia-Pacific area. Albeit the diagnosis criteria and immunosuppressive treatment regimens have been established, validated and demonstrated in a series of clinical trials, there are still some challenges. According to the diverse presentations and courses, the individualized managements of these patients, including classical chronic onset, acute or acute severe onset, acute-on-chronic liver failure, cirrhosis, patients with the context of other liver diseases such as non-alcoholic steatohepatitis (NASH) or chronic hepatitis B (CHB) are necessarily descriptive. Each subgroup of patients should receive a personalized analysis of the benefits and side effect risks of drugs.
Autoimmune hepatitis (AIH) is the chronic liver disease which is characterised by circulating auto-antibodies, high IgG levels, absence of viral hepatitis and histological findings showing interface hepatitis. AIH progresses into liver failure and death if remains untreated. Therefore, early diagnosis and effective management are essential to prevent liver related complications and death. Prednisolone alone or in combination with azathioprine, is the current standard therapy of AIH. This therapy improves inflammatory activity, prevents fibrosis progression and prolongs survival in majority of patients. However, up to 20% of AIH patients do not respond or intolerant to standard therapy. Mycophenolate mofetil, tacrolimus and infliximab are rescue therapies for this group of patients.

A smaller group of AIH patients may present or develop PBC features. The term ‘overlap syndrome’ describes this rare clinical condition, although there are no well-established diagnostic criteria. These patients should be treated based on liver histology. Patients who have moderate interface hepatitis can be initially treated by UDCA alone while those with severe hepatitis require additional immunosuppression.
In order to enhance healthcare quality and outcomes of patients with primary biliary cholangitis (PBC) in the Asia-Pacific region, a panel of clinicians with an interest in PBC wrote and discussed this guidance. The major recommendations are drafted as following:

1. The diagnosis of PBC can be established when two of the following three criteria are met:
   (1) Biochemical evidence of cholestasis based mainly on elevation of alkaline phosphatase and GGT with exclusion of extrahepatic biliary obstruction by imaging studies;
   (2) Presence of AMA;
   (3) Histologic evidence of non-suppurative destructive cholangitis and destruction of interlobular bile ducts. (Ⅰ, 1)

2. AMA reactivity alone is not sufficient to diagnose PBC. AMA-positive patients with normal serum liver tests should follow up with annual biochemical reassessment for the presence of liver disease. (Ⅲ, 2)

3. If liver biopsy is not available, a diagnosis of AMA negative PBC can be considered in patients with cholestasis and specific ANA immunofluorescence (nuclear dots or perinuclear rims) or ELISA results (sp100, gp210). (Ⅲ, 1)

4. We recommend that oral UDCA (13–15mg/kg/day) is administrated as the standard therapy for all PBC patients. UDCA treatment should last for life. (Ⅰ, 1)

5. Budesonide might be added to noncirrhotic PBC patients with suboptimal response to UDCA. (Ⅱ, 2)

6. We recommend that bezafibrate (400 mg/d) or fenofibrate (200 mg/d) combined with UDCA for patients with an inadequate response to UDCA. Side effects should be closely monitored specially in cirrhotic patients with PBC. (Ⅱ, 2)

7. We recommend that OCA could be added to UDCA therapy for PBC patients with Child-Pugh-Curcotte A and an inadequate response, or as monotherapy in those intolerant to UDCA. Potential risks and side effects of OCA should be carefully evaluated and appropriately monitored. (Ⅰ, 2)
Global PBC – What Can We Learn from Global Collaboration?

Novel treatments and personalized medicine for liver diseases are fast gaining ground as new insights in immunology and genetics are evolving. In parallel we must gain deeper understanding of these diseases and their interactions with the host to define the needs and possibilities. In the setting of rare diseases or rare events working together across sites and across countries is crucial. At first simply because of numbers, but with engagement of specialists with different expertise new enriched science is thriving.

The Global PBC Study Group was founded in 2011 with the aim to support research on Primary Biliary Cholangitis (PBC). A retrospective database of more than 6000 patients was built in 1.5 year from more than 20 sites worldwide with a mean follow-up of 8 years and detailed visits reports. The first project aimed to find a surrogate endpoint to use for evaluation of new therapies. The big database allowed robust results with an active study group a platform evolved to study multiple questions related to PBC. Now special working groups have materialized focusing on specific topics.

The Global PBC Study Group is one of many new networks on rare diseases or rare outcomes. The formula to a successful network depends on multiple factors melting together at the right time: people, speed, precision, clear message/simplicity, and most of all need. The trajectory of the Global PBC Study Group is highlighted – with examples and lessons learned – and a recent example of one of our findings is presented: The advantage of a big data enabled us to design a prediction score of liver transplantation or death, the GLOBE score, to identify patients in high need of need treatment. The score was validated in a mainly Caucasian population and may need validation and calibration in populations with different ethnic background. To understand PBC worldwide; to study differences in response to therapy and the prognosis of PBC with respect to ethnic background is a next step in collaboration between networks. This creates an even bigger need to extend collaboration between networks.

The anno-2019 way of collaboration asks alongside engagement of scientists also involvement of pharmaceutic industry, regulatory and most important involvement of the patients; bringing new layers and creating forums. For the success to continue avoiding competition and working together across fields will benefit all, but we must remember to keep the need of the patient central.
Whole Genome and Immuno-genome Landscape of Liver Cancer

Cancer is essentially a “disease of the genome” which develops and evolves with the accumulation of a variety of mutations, based on the background of its genomic instability, and some driver mutations were successfully targeted for treatment. Cancer also has been proved to have a feature of “immune reaction” and have been affected by immune editing in carcinogenic steps. Now immune therapies are a real in most types of cancer including liver cancers. To explore whole genomic and immuno-genomic features of cancer, we have been addressing cancer whole genome sequencing (WGS) analysis for liver cancers. As one of the Japanese ICGC projects, we sequenced whole genomes of 300 liver cancer, which were mainly affected by virus infection (Fujimoto et al. Nat Genet 2016). The median number of somatic mutation of liver cancer was approximately 10,000. We identified several mutated driver genes and pathways in liver cancer, including TERT, TP53 and Wnt/CTNNB1 pathway. We found several non-coding mutational clusters, such as TERT promoter and lincRNAs. WGS and RNA-seq detected virus integrations of HBV and AAV in liver cancer genome, and deep sequencing analysis targeting HBV detected 1,684 HBV integration sites in cancer and liver tissues, which preferentially occurred in the open chromatin regions and mitochondria genome in mouse HBV infection model (Furuta et al. Oncotarget 2018). From WGS data, we extracted several mutational signature such as smoking signature (Sig4) and alcohol signature (Sig16), and estimated cell-of-origin through whole genome mutation distribution, which indicated some of ICCs are likely to be originated from hepatocyte, as well as HCC (Wardell, Fujita et al. J Hepatol 2018).

Liver cancer develops in chronic hepatitis where various types of immune cells are activated and suppressed. Although the background liver is highly inflamed, liver cancer is generally considered as immune suppressive. We analyzed RNA and WGS of 234 liver cancers and matched non-tumorous livers with chronic hepatitis, and characterized their immunological feature by comparing the immune profiles in liver cancers and hepatitis livers. Anti-tumor immunity was associated with significantly better prognosis. Tumor had lower expression levels of immune genes than adjacent hepatitis liver, indicating predominant immune suppression in tumor. Gene signature for Treg and CTNNB1 immuno-suppressive signature were overexpressed in tumor.

These approaches combined with mathematical analysis and other -omics analysis can clarify the underlying carcinogenesis and cancer immunology and achieve molecular sub-classification of cancer, which facilitates discovery of genomic biomarkers and personalized cancer medicine.
Adaptive Immunotherapy against HCC Recurrence after Transplantation

Development of an effective adjuvant therapy to prevent HCC recurrence after liver transplantation (LT) is an important medical requirement. Most immunosuppressants reduce the proportion of adaptive components of cellular immunity while maintaining the innate components. Natural killer (NK) cells play a central role in innate immunity against neoplastic cells; therefore, their augmentation is a promising immunotherapeutic approach against HCC recurrence after LT. We propose that adoptive transfer of IL-2-stimulated TNF-related apoptosis inducing ligand (TRAIL)+ NK cells extracted from donor liver graft perfusate can mount an anti-tumor response without causing toxicity to intact recipient tissues. Since 2006, we have successfully performed NK-cell immunotherapy in 30 living donor LT (LDLT) recipients with HCC in Hiroshima. We also applied the proposed approach to the deceased donor LT (DDLT) recipients in collaboration with Miami University group since 2009 (phase I study included 17 subjects). No cases related adverse events were noted in either of the studies. In the series of LDLT with HCC, among the 52 patients who met the Milan criteria (MC) on preoperative imaging (NK group n=25; control group n=27), 18 patients (35%) had HCC exceeding MC on postoperative pathology. Of these 18 patients, the recurrent free survival (RFS) rates were significantly improved in the NK group (n=10) as compared to those in the control group (n=8). Their 5 year-RFS were 79% and 14%, respectively (p=0.004). A sub-analysis showed that the incidence of blood stream infection/bacteremia significantly decreased in the NK group (p=0.012). After infusion of NK cells, the NK cytotoxicity and the proportion of TRAIL+ NK cells in the peripheral blood of patients increased significantly (p<0.05). The inoculated donor NK cells could be confirmed up to 1 month through the analysis of peripheral blood chimerism. In the series of DDLT with HCC, among the 17 patients who met MC on preoperative imaging, 9 patients (53%) had HCC exceeding MC on postoperative pathology. None of the patients have shown any symptom of HCC recurrence. Thus, the administration of IL-2-stimulated NK cells derived from both living and deceased donor liver allografts is a potential novel adjuvant immune treatment for further improving the outcome of LT patients with HCC meeting MC.
Systemic Therapy of Hepatocellular Carcinoma: Korean Experience

In South Korea, the cancer-related mortality rate of primary liver cancer is the second highest across all age groups. Hepatocellular carcinoma (HCC) is most common cancer (approximately 85%) of primary liver cancer. The age-standardized mortality and incidence rates of primary liver cancer appear to be declining; however, this is not because of a reduced burden of liver cancer, but because of the rapid aging of the entire Korean population. Crude rates, incidence rates, and the absolute number of patients associated with primary liver cancer mortality are still increasing. Hepatitis B virus (HBV) is the predominant etiology of HCC in Korea and according to several retrospective studies, HBV accounted for 62~75% of HCC.

A substantial proportion of Korean patients with HC are diagnosed at an advanced stage; more than 35% of patients had Barcelona Clinic Liver Cancer C or D stage tumors at the time of diagnosis. Transarterial chemoembolization (TACE) remains the mainstay of treatment modalities. Sorafenib is the standard of care in the management of advanced HCC. Recently, lenvatinib is available but not covered by national insurance reimbursement program yet in Korea. A retrospective observational study based on national health insurance data (2008-2014) showed that the mean duration of sorafenib administration was 106 days and initial sorafenib dose was 600-800mg in 71% of patients. The most commonly applied treatment after sorafenib failure was TACE but this sequence is changing after availability of regorafenib and nivolumab as a second-line treatment. Recently, a multicenter prospective study showed the median overall survival 10.8 months with sorafenib treatment in patients with far advanced HCC. A multicenter retrospective observational study of regorafenib after progression on sorafenib in Korean patients with HCC reported that the median progression-free survival was 3.7 months and the 1 year OS rate was 54.6%.

2018 Korean Liver Cancer Association-National Cancer Center Korea Practice Guidelines for the Management of Hepatocellular Carcinoma recommended sorafenib and lenvatinib as a first-line systemic treatment, and regorafenib, nivolumab, cabozantinib and ramucirumab as a second-line systemic treatment.
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**Personalised TCR T-cell Immunotherapy for HBV-HCC**

**Background & Aims:** Hepatocellular carcinoma (HCC) is often associated with hepatitis B virus (HBV) infection. Most of the HBV-related HCC contain integrations of HBV-DNA fragments that have been primarily studied for their potentially pro-carcinogenic effects. Here, we aim to determine whether the presence of integrated HBV-DNA fragments can be utilized to select HBV-specific T-cell receptors for personalized anti-HCC T-cell immunotherapy.

**Methods:** We first analysed HBV-serologically negative HCC cells for the presence of HBV-transcripts using real-time PCR, sequencing and Nanostring approaches. The ability of HBV-transcript+ HCC cells to generate functional HBV-specific T-cell epitopes from short integrated HBV-DNA fragments was tested by co-culturing with HBV-specific T-cells. We then utilized the HBV-transcript profiles of primary HCC and metastasis present in two liver transplanted patients with HCC relapses for the selection of HBV-specific T-cell receptors used to engineer T-cells for HCC-specific T-cell immunotherapy.

**Results:** Despite the serological negativity of HBV antigens, HCC cells containing short HBV-transcripts can generate functional T-cell epitopes capable of activating HBV-specific T-cells. In vivo, the volumetric reduction of almost all the lung metastases in one patient after adoptive T-cell transfer suggest that the HBV-transcriptome profiling of HCC cells can indeed guide the selection of HBV-specific TCRs used to personalize T-cell immunotherapy in HBV-HCC patients.

**Conclusion:** A wider population of HCC patients than previously estimated by serological analysis can exploit HBV antigens as tumour-specific targets for T-cell immunotherapy.
In contrast to the curative treatments, such as radiofrequency ablation, surgery, and transarterial chemoembolization, systemic chemotherapy has been a supporting player for the treatment of human hepatocellular carcinoma (HCC). In 2009, sorafenib was approved as the first tyrosine kinase inhibitor (TKI) effective for unresectable HCC patients; it had been the only agents applicable for advanced HCC cases that were unsuitable or had been unsuccessful for locoregional intervention and surgery with enough scientific basis. In 2017, regorafenib, another TKI, was shown to provide survival benefit in HCC patients who progressed after sorafenib. It was also reported that lenvatinib was non-inferior to sorafenib in overall survival in patients with untreated advanced hepatocellular carcinoma.

Currently, both sorafenib and lenvatinib can be used as the first-line systemic chemotherapy for advanced HCC. Although both are multikinase inhibitors that target growth signal and neovascularization, anti-kinase profile is somewhat different among them; lenvatinib inhibits receptor tyrosine kinase of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) more than sorafenib. More importantly, based on the phase III clinical trial, progression-free survival, time-to-progression and objective response rate were better in lenvatinib group than sorafenib, suggesting that anti-tumor effect could be stronger in lenvatinib.

On the other hand, for the patients who progressed HCC after sorafenib, regorafenib was proved to be effective; it could not be applicable for patients who were intolerable for sorafenib because profile of side effects was quite similar but could be more severe in regorafenib than in sorafenib. Another TKI, cabozantinib, and anti-VEGFR-2 antibody, ramucirumab, also showed superiority on overall survival compared to placebo in phase III clinical trials and became available for clinical use as the second-line systemic chemotherapy, although the latter was limited for HCC cases with AFP ≥ 400ng/mL.

In addition, several phase III clinical trials are also ongoing for immune checkpoint inhibitors, such as nivolumab, pembrolizumab, and tislelizumab. Safety and effectiveness of combination therapy of anti-PD-1/PD-L1 antibodies and TKI (lenvatinib), anti-VEGF antibody (bevacizumab), or anti-cytotoxic T-lymphocyte-associated antigen 4 antibody (tremelimumab) are also under investigation; these are showing promising results. Therefore, treating HCC patients using systemic chemotherapy should change drastically in near feature.
Recent Advance of Immune-therapy for Hepatocellular Carcinoma

Recently, some immuno-oncologic (I-O) agents such as ani-PD-1 antibody, anti-PD-L1 antibody and anti-CTLA-4 antibody have begun to attract much attention in attempts at developing effective anticancer agents for advanced hepatocellular carcinoma (HCC). Nivolumab, which is a fully human IgG4 PD-1 immune-checkpoint-inhibitor antibody, demonstrated favorable tumor shrinkage effects and remarkable prolongation of the overall survival with manageable toxicity profile. Based on the results, the Food and Drug Administration (FDA) in United State has approved the use of nivolumab for advanced HCC patients who fail to respond to first-line treatment, although it is not yet covered by the national health insurance in the other countries. Pembrolizumab, which is also anti-PD-1 antibody, has been reported to be similar tumor response and acceptable toxicities to nivolumab in patients treated previously with sorafenib. FDA also approved the use of pembrolizumab for advanced HCC patients who fail to respond to first-line treatment. To explore the potential usefulness of monotherapy with I-O agents, a phase III trial of nivolumab vs. sorafenib as first-line setting and a phase III trial of pembrolizumab as compared to placebo in the second-line setting is under way. Furthermore, various combination regimens of I-O plus I-O agents, such as durvalumab plus tremelimumab, and I-O plus molecular-targeted agents, such as lenvatinib plus pembrolizumab, and bevacizumab plus atezolizumab have also been reported to be favorable efficacy in patients with advanced HCC. These combination regimens have been examined in some phase III trials, such as durvalumab plus tremelimumab vs. durvalumab vs. sorafenib (HIMALAYA), lenvatinib plus pembrolizumab vs. lenvatinib (LEAP-002) and bevacizumab plus atezolizumab vs. sorafenib (IMbrave150).

In the future, the advent of favorable systemic regimens that offer superior survival benefit to loco-regional treatments are expected in patients with advanced HCC.
Liver Function Disorder as irAE by Immune Checkpoint Inhibitor

With the advent of immune checkpoint inhibitor (ICI), the standard therapy for advanced cancer is changing dramatically. The immune checkpoint molecule suppresses the activation of immune cells by transmitting an inhibitory signal, maintains the immune tolerance state to the self, converges the immune response and maintains the co-inhibitory molecule that maintains the homeostatic state of the immune response. Typical immune checkpoint molecules include CTLA-4 (Cytotoxic T lymphocyte-associated antigen 4) and PD-1 (Programmed cell death-1) expressed on T cells. Cancer cells express molecules that contribute to suppression of T cell activation on their cell surface and construct a mechanism to escape from the immune system of the living body by suppressing T cell activation. Agents that inhibit this suppressive regulatory function are collectively referred to as ICI.

Although ICI is expected to have higher efficacy than conventional treatments, it may develop immune related adverse events (irAE) like autoimmune diseases and inflammatory diseases. Therefore, the management that is different from cytotoxic chemotherapy side effects is necessary. Although irAE is expressed relatively more in the skin, gastrointestinal tract, liver and endocrine organs, it is also known to be expressed in kidney, nerve, muscle and eyes. Among hepatic, biliary and pancreatic disorders caused by irAE, the most frequent is hepatic dysfunction, most of which are low grade, but cases of grade 3 or 4 have also been reported.

The point of irAE management is early and appropriate treatment according to early diagnosis and management algorithm. In the treatment of irAE, as with the autoimmune disease according to severity, corticosteroid is used as the first choice, and if the effect of the steroid is insufficient, other immune-suppressants are used.

irAE may be fatal if delayed and become serious, and may lead to irreversible obstacles, but many cases can be managed if it was diagnosed early and appropriate treatments were done. In addition to sharing knowledge and experience in cooperation with experts of each organ, patient education is also necessary for early detection of irAE. In the future, with the expansion of adaptive cancer types, the combination of different ICIs and the introduction of combination therapy with molecular targeted drugs, it is getting important that hepatologists participate in ICI treatment teams and perform an appropriate liver dysfunction management at an early stage of irAE.
Liver Transplantation – Graft Injury and Cancer Recurrence

The post-transplantation tumor recurrence remains the major obstacle worldwide. The intragraft regional specialization of immune system may play an important role in tumor recurrence after liver transplantation. With the application of systems biology approach, we may able to elucidate the mechanism of the intragraft regional specialization of immune system on tumor recurrence and metastasis. Educating the intragraft immune micro-environment will be a prospective way to prevent tumor recurrence after transplantation. It will also feasible to develop a unique computer modeling system essential for both disease prognoses and therapeutic guidance.
Living-donor Liver Transplantation for PBC and PSC in Japan

Cholestatic diseases, primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are two major indications for liver transplantation. Despite the advance of medical treatments for these diseases, not a few patients develop cirrhosis and finally require liver transplantation. With the accumulation of experiences of liver transplantation for PBC and PSC and the improvement in the long-term survival after liver transplantation, the disease recurrence has become a matter of debate. While recurrent PBC is usually slow growing and has minimal impact on patient’s prognosis, recurrent PSC impairs patient and graft survival significantly. In addition, in Japan where living donor liver transplantation (LDLT) is a mainstay for liver transplantation, LDLT-specific factors related with the development and the progression of the recurrent disease have been identified by the recent nation-wide studies. PBC is one of the best indication for liver transplantation in terms of the post-transplant survival, however, the disease recurrence in the long-term is not uncommon and the genetic factors and the sensitization for the donor have been attributed to the recurrent disease. In contrast, the recurrent PSC after liver transplantation is frequent, as high as 50%, with the worse graft and patent survival. In the previous nation-wide survey, the donor-recipient relationship in the first degree was found to be an independent significant predictor for the recurrent disease, indicating the autoimmunity as the cause of disease. In the updated nation-wide survey with the latest data collection, we found that the outcome of LDLT for PSC in Japan is improving along with the era and that the immunosuppressive modulation after LDLT will be of help in decreasing the recurrence rate. In this presentation, we review the outcomes of LDLT for PBC and PSC in Japan, and present some new insights for the recurrent disease of PBC and PSC after liver transplantation.
Long-term Outcomes of Patients with Pre-transplant Portal Vein Thrombosis after Living Donor Liver Transplantation

**Background:** As high as 25% of pre-transplant candidates have portal vein thrombosis (PVT) and its incidence and severity are well-correlated with the degree of hepatic decompensation. Novel techniques for portal reconstruction during living donor liver transplantation (LDLT) gives the transplant surgeon numerous options to curtail challenges in establishing adequate portal inflow especially in patients with high grade PVT, but long-term outcomes of these patients are limited and contrasting.

**Methods:** 1,530 consecutive LDLT’s were performed from June 1994 to June 2018 at Kaohsiung Chang Gung Memorial Hospital. Preoperative PVT was diagnosed using combined CT/MRI and was graded using Yerdel’s classification. A total of 122 (8%) patients with pre-transplant PVT were analyzed and long-term outcomes were compared against patients without PVT.

**Results:** Of the 122 patients with pre-transplant PVT, 97 (80%) were adults while 25 (20%) belonged to the pediatric population. Grade 1 PVT was noted in 64 (52%), grade 2 in 44 (36%), grade 3 in 9 (7%) and grade 4 in 5 (4%) patients. Physiologic or portal to portal inflow reconstruction was achieved in 107 (88%) patients while non-physiologic inflow reconstruction was done in 15 (12%) patients, majority of which utilized an engorged spontaneous portosystemic shunt. Re-thrombosis rate for this study was 5% while the rate of post-transplant portal vein stenosis was 7%. Overall 9-year patient survival was 85% vs. 84% in patients without PVT.

**Conclusion:** LDLT is safe and equally effective in patients with pre-transplant PVT with comparable long-term survival outcomes when compared to those without PVT. In high volume centers, pre-transplant PVT is no longer considered as a contraindication for LDLT except in patients with grade 4 PVT without a sizable collateral vein.
Treatment of Hepatitis C: so far and from now on

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). An estimated 71 million people have chronic HCV infection worldwide. The primary goal of hepatitis C is to cure the infection. Because of the recent progress in HCV treatment, almost all patients with HCV infection are presently curable by interferon-free treatment 30 years after the HCV discovery. In fact, real-world efficacy of glecaprevir/pibrentasvir treatment in patients with HCV genotype 1-6 was reported to be near 100% sustained virological response (SVR) with little virologic failures. In my presentation, I would like to summarize how to treat hepatitis C along the APASL and JSH (Japan Society of Hepatology) guidelines.

Although HCC development reduces by SVR, it never become zero even after SVR. Thus, the prevention of HCC development after viral control is still an important unmet medical need in hepatitis C. I would like to introduce a new option to suppress HCC development applying the activation of innate immune response against HCC cells.

Another unmet need is the treatment of advanced HCC. Recently, systemic therapy against HCC was remarkably advanced and the prognosis of patients with advanced HCC is dramatically improving. Until now, clinical trials with four tyrosine kinase inhibitors, sorafenib, regorafenib, lenvatinib and cabozantinib, and one anti-VEGFR2 antibody, ramucirumab have succeeded. Moreover, clinical trials with immune checkpoint inhibitors and the combination of immune checkpoint inhibitors and molecular targeted drugs are ongoing. In the near future, several treatment options against advanced HCC may become available. Although there is no doubt as to the paradigm shift of HCC treatment, it becomes more complexed and difficult to select the most suitable treatment option for patients with advanced HCC. I would like to also introduce new treatment strategies against HCC, and our future challenges against HCC.
The Real-World Experience of DAA for Hepatitis C: Benefits and Challenges after Virological Cure Revealed by Nation-Wide Cohort

The efficacy of recent DAA regimens was validated through a nation-wide registry cohort of Japanese Red Cross Liver Study Group. More than 5,500 patients were registered. Testing for baseline resistance-associated substitutions lead to optimal selection of regimen and high rate of SVR of >95%. Cumulative incidence of hepatocellular carcinoma (HCC) after SVR was 1.8%/year. Liver-stiffness after SVR and serum M2BPGi predicted future risk of HCC development.
Evening Seminar 1 by Sysmex Corporation

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Impact of Serum M2BPGi Measurement on Clinical Practice of Chronic Liver Disease

M2BPGi is serum marker of liver fibrosis. Serum M2BPGi levels correlate with stage of fibrosis or with the amount of fibers in liver tissue. The optimal cut off value to predict significant fibrosis differs according to the etiology of liver disease. M2BPGi levels are predictive of the presence of esophageal varices or the risk of hepatocellular carcinoma development. This simple marker has significant impact on clinical practice of chronic liver disease.
Clinical Significance of M2BPGi in Patients with Autoimmune Liver Disease

M2BPGi has been reported as a novel marker to assess liver fibrosis in various liver diseases. Since M2BPGi was first found to predict liver fibrosis in chronic hepatitis C patients, later many studies validated serum M2BPGi as useful for assessing the stage of liver fibrosis in patients with chronic hepatitis C and B. In addition, M2BPGi has been found to predict the cumulative incidence of hepatocellular carcinoma and a predictor of HCC development after a sustained virological response in patients with chronic hepatitis C. However, there are a few reports regarding the usefulness of M2BPGi as a liver fibrosis marker or predictor of outcome in patients with autoimmune hepatitis and primary biliary cholangitis. I will introduce the clinical significance of M2BPGi in autoimmune liver disease.
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Survival Benefit of BCAA for Patients with Liver Cirrhosis  
-Prevention of Hepatic Failure and HCC-

Liver cirrhosis patients with decreased hepatic functional reserve are associated with various nutritional/metabolic disorders. Particularly, protein energy malnutrition (PEM) is deeply involved in the prognosis and deterioration of quality of life (QOL) in patients with liver cirrhosis. When liver cirrhosis progresses, the patients are frequently associated with abnormal glucose metabolism such as postprandial hyperglycemia and hyperinsulinemia, both of which are involved in the progression of liver fibrosis and the development of hepatocellular carcinoma (HCC).

With liver cirrhosis, a decrease in branched chain amino acids (BCAA) and an increase in aromatic amino acids are observed. Among BCAA, leucine in particular promotes protein synthesis. BCAA administration for protein malnutrition raises the serum albumin levels and improves QOL and survival of patients with liver cirrhosis. BCAA supplementation is also effective for improving hepatic encephalopathy, which is one of the most important clinical manifestations in decompensated liver cirrhosis. Particularly, BCAA-enriched supplementation given as late-evening snack improves nutritional status and increases body protein content. The results of randomized trial (LOTUS) showed that BCAA granules (LIVACT®) administered for a long period could improve event-free survival, serum albumin level and QOL in patients with cirrhosis.

BCAA plays an important role in maintaining and increasing skeletal muscle mass. Therefore, the decline in BCAA in liver cirrhosis patients is deeply involved in the development of sarcopenia, which is a syndrome characterized by reduced skeletal muscle mass and muscle strength. With liver cirrhosis, progression of PEM, decline in BCAA, the development of sarcopenia and the onset of impaired glucose tolerance are observed as a series of pathological conditions.

In addition to malnutrition, hypernutrition also exacerbates the prognosis of cirrhotic patients. Obesity and diabetes have been reported to increase the risk of HCC and liver cirrhosis with backgrounds of nonalcoholic steatohepatitis related to obesity and lifestyle diseases is also increasing. Importantly, sub-analysis of LOTUS revealed BCAA supplementation inhibits liver carcinogenesis in cirrhotic patients with obesity. BCAA might be a chemopreventive agent for HCC development, especially when the patients have metabolic disorders.

In conclusion, BCAA supplementation improves event-free survival, increases serum albumin levels, improves QOL and suppresses, at least, obesity-related HCC in patients with liver cirrhosis. BCAA is a key agent for the total management of liver cirrhosis.
Reconsideration of Treatment Strategy in the Era of multi-TKIs

Now a day, 5 drugs (sorafenib, regorafenib, lenvatinib, cabozantinib, and ramucirumab) indicated positive results in phase III studies, and those drugs have been approved successively in all over the worlds. In Japanese filed practice, lenvatinib has been used not only front line but also second or later lines after sorafenib or regorafenib since May 2018. Transarterial chemoembolization (TACE) is the most widely used treatment for unresectable hepatocellular carcinoma (HCC) that, if applied correctly, can produce survival benefits and favorable response without adversely affecting hepatic functional reserve. Guidelines of both East and West, not to mention APASL latest guideline (2017), indicate TACE is the initially recommended treatment for patients without both macrovasular invasion (MVI) and extrahepatic metastasis (EHM) (i.e. intermediate stage HCC patients). Since it is well known that repeated TACE is associated with not only decreased expectation rate of response but also increased side effects and liver damage, conversion from TACE to systemic therapy in the appropriate timing is one of the key strategies of HCC in the present era. Hepatic arterial infusion chemotherapy (HAIC) is a traditional treatment for advanced HCC patients in the Asia-Pacific region especially in Japan. So far, HCC patients have pro-longed overall survival due to, early diagnosis by screening programs, early detection of recurrence by high functioning radiological modalities, improvement of several treatments, and maintaining liver function by anti-hepatic viral agents. However, HCC have high frequency rates of recurrence because of its unique malignancy characteristics. Thus, most of patients switch to one treatment to another in their clinical courses. In this session, latest evidences of TKIs, TACE, and HAIC would be summarized and the information would help for treatment selections in the daily practice.
Diversity of Lenvatinib Treatment Strategy and Management of Lenvatinib Treatment for Unresectable Hepatocellular Carcinoma

Sorafenib, a multikinase inhibitor, has been recognized as the only systemic therapeutic agent for advanced hepatocellular carcinoma (HCC) in the past decade after approval of sorafenib. Other systemic drugs have failed to improve outcomes over sorafenib. The use of sorafenib is associated with long-lasting stable disease without objective response; therefore, it is difficult to realize the efficacy of treatment. Recently, a phase III study (REFLECT trial) proved that the multikinase inhibitor lenvatinib was inferior to sorafenib in improving overall survival when used for the treatment of advanced HCC. In this study, the use of lenvatinib showed a high response of 40.6% as per the modified RECIST criteria, and significant prolonged progression-free survival was observed with lenvatinib than with sorafenib. The divergence between outcomes of overall survival and progression-free survival was considered; however, the high objective response by modified RECIST criteria leads to the improvement in overall survival. Lenvatinib with the high objective response may be the first-line therapeutic agent for advanced HCC. High objective response enables both physicians and patients to directly realize the efficacy of treatment and remain motivated. Moreover, the high objective response of lenvatinib in the intermediate stage can advance the timing of the switch from transarterial chemoembolization to systemic therapy. Furthermore, a high objective response leads to curative conversion surgery by the reduction in the size of unresectable tumor. In short, a high objective response may vary the purpose of treatment. Lenvatinib enables not only maintenance chemotherapy to maintain tumor condition but also intensive chemotherapy aimed at conversion surgery. On the other hand, the profile of adverse events between sorafenib and lenvatinib is very different. One of the peculiar adverse events of sorafenib is the hand-foot syndrome reaction; however, those of lenvatinib are hypertension, hypothyroidism, etc. Especially in clinical practice, fatigue, malaise, and anorexia become serious problems causing cessation or discontinuation of treatment. Management of fatigue and malaise is being elucidated. A brief use of steroid or appropriate dose modification may be effective. This report demonstrates how a treatment strategy for advanced HCC changed with the development of lenvatinib and skillful management of lenvatinib, including our clinical experiences.
Hepatitis Action Plan and Changing Trend of Liver Disease in Japan

In Japan, the research on National Database estimated number of chronic HBV infection on treatment was 0.16 million and that of chronic HCV was 0.47 million in 2015, respectively. The mortality of hepatocellular carcinoma (HCC) had been increasing and hit the peak at around 2002, which subsequently started to decrease.

Japan has a national action plan for addressing viral hepatitis called, Basic Act on Hepatitis Measures, established in 2009. Basic Guidelines for Promotion of Control Measures for Hepatitis is issued in 2011 and was updated in 2016, comprising 9 principles in order to promote measures to prevent hepatitis B and C (Oza N, Kanto T, Hepatol Res 2017). According to these guidelines, national and local government share costs for testing HBV and HCV for those who are over 40 years old residents. Real-world clinical data have proven that direct anti-viral agents (DAAs) successfully eradicate HCV from more than 95% of the infected patients. In Japan, most of DAAs have been approved and registered, which enables us to choose multiple treatment options. In addition, for patients on treatment, drug prices of nucleotide analogues, interferon (IFN) or DAAs and examination expenses should be covered by special subsidy program for viral hepatitis. The national and local government cover the amount in excess of 100-200 USD of the cost of treatment.

From December 2018, special coverage program of medical expenses, shared by central and local government, is going to start for patients with HBV- or HCV-induced liver cancer and decompensated cirrhosis. However, in the cascade-of-care of viral hepatitis in Japan, significant gaps still remain in the diagnosis, treatment and their transition of patients in need (Kanto T, Yoshio S. Euroasian J Hepatogastroenterol 2017). Questionnaire analysis for general people in Japan, which was performed in 2011 and 2017 by the government-funded research group including us, revealed that information regarding clinics and hospitals where testing is available for free has not been well disseminated. In addition, lack of correct knowledge on viral hepatitis in ordinarily people sometimes induce stigma and discrimination against patients. Therefore, awareness raising in general people still in great demand for promoting hepatitis policy in Japan.

Several advantages have been prevailed in Japanese health care systems for patients with viral liver disease compared to those in countries in Western Pacific Region. Therefore, Japan should take a lead in helping the implementation of practical hepatitis action plan to each country where in need.
Gut Pathobionts Underlie Intestinal Barrier Dysfunction and Liver Th17 Immune Response in Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is a chronic inflammatory liver disease and its frequent complication with ulcerative colitis (UC) highlights the pathogenic role of epithelial barrier dysfunction, yet its underlying mechanism remains unknown. Here, we aimed to identify the specific microbiota that contribute to the pathogenesis of PSC using humanized gnotobiotic mice. Patients with PSC/UC (n = 18), UC (n = 16), and healthy controls (HCs) were included in this study. 16s rRNA metagenome analysis and quantitative PCR to detect specific microbiota in human fecal samples were performed. We generated gnotobiotic mice by inoculating fecal samples from patients with PSC/UC (PSCUC mice), UC (UC mice), or Healthy controls (HCs) (HC mice). Metagenomic analyses revealed lower diversity and a distinct taxonomic trend with a higher prevalence of Enterobacteriaceae in the microbiota of PSC/UC patients. In contrast to germ-free (GF) or HC gnotobiotic mice, PSCUC mice demonstrated higher Th17 response in the liver and strong susceptibility to the experimental hepatobiliary inflammation by DDC or taurocholic acid, which was reversed by administration with RORgt inverse agonist. We identified three bacterial species, Klebsiella pneumoniae, Proteus mirabilis, and Enterococcus gallinarum from the mesenteric lymph nodes of PSCUC mice by bacterial culture. Importantly, these pathobionts were specifically enriched in the feces of PSC/UC patients both in our cohort (K. pneumoniae; 17/18, P. mirabilis; 5/18, E. gallinarum; 12/18) and European validation cohort. The mono-inoculation of GF mice with PSC mice-derived K. pneumoniae caused mucosal invasion detected by FISH with an EUB338 probe, increased serum endotoxin level, and the induction of RORgt^+IL17^+CD4^+ T cells in the liver, but was insufficient to induce a robust Th17 response observed in mice inoculated with the three mixed strains. To explore the interaction between bacteria and the intestinal epithelium, a monolayered human intestinal organoids were cultured with the specific bacteria in vitro. Using the culture system, we demonstrated that PSC/UC-derived K. pneumoniae unlike commercially available K. pneumoniae strains, directly induced epithelial-pore formation with apoptosis-related genes upregulation. Finally, we confirmed that antibiotic treatment targeting K. pneumoniae and E. gallinarum was effective in the reduction of pathogenic Th17 response in the liver in PSCUC mice. Collectively, our results identified specific pathobionts in PSC/UC patients that collude in intestinal barrier disruption and subsequent Th17-priming in the liver, and provide insights into the implication of the gut microbiota in the pathogenesis of PSC.
Sarcopenia and Hand Grip Strength (HGS) in the Patients with Chronic Liver Disease

Sarcopenia or loss of skeletal muscle mass is the major component of malnutrition and is a frequent complication in cirrhosis that adversely affects clinical outcomes including survival, quality of life, development of other complications and post liver transplantation survival.

In 2015, the Japan Society of Hepatology (JSH) decided to establish its own assessment criteria for sarcopenia in liver disease, because the number of liver disease patients with sarcopenia is expected to increase and there is cumulative evidence to indicate sarcopenic patients have poor clinical outcomes. A working group to create assessment criteria for sarcopenia has thus been established by the JSH. JSH's diagnostic criteria for sarcopenia is that it is evaluated by two factors of grip strength and muscle mass, and practicality is high because it is simplified except evaluating for walking speed.

Hand grip strength (HGS) is used for the diagnosis of sarcopenia and frailty. Several factors have been shown to influence HGS values during measurement. HGS is appealing as a simple, quick, and inexpensive means of stratifying an individual's possibility of development for sarcopenia. There are also reports that examined and examined many papers related to grip strength and obstacles, diseases, and mortality rates. Weakness of grip strength consistently increases the possibility of early death and causes disability, complications, We are concerned with the high risk of prolonging hospitalization after surgery and we should consider taking HGS measurement as a vital sign of middle and high elderly people. Meta analysis on HGS and mortality rate based on recent papers has also been conducted, and relationships between increased grip strength per kg and mortality rate have also been found.

I would like to talk about the significance of sarcopenia and grip strength measurement in liver diseases, including the results of own facility.
Safe Radiofrequency Ablation for Low Platelet Count Patients with Thrombopoietin (TPO) Receptor Agonist (Lusutrombopag)

Bleeding is one of the major complications of radiofrequency ablation for liver tumor. Low platelet count is a risk factor for bleeding. Blood transfusion of platelet was the practical way to increase the number of platelet count before invasive hepatic procedure such as radiofrequency ablation (RFA) for liver cancer. Recently, novel drug which raising platelet count by acting on thrombopoietin receptor has become available.

Lusutrombopag was orally administered 3mg daily for seven days for the patients underwent RFA for liver tumor with low platelet count (mainly less than 50 thousands/µL). Medication was started 5-27 days before procedure. We collected demographic data, liver function, and platelet count of the patients. Lustrombopag was administered for 78 patients between April 2016 and December 2018. Platelet count was elevated from $4.3 \times 10^4 \pm 1.4 \times 10^4$ to $8.3 \times 10^4 \pm 2.6 \times 10^4$. Seventy patients (90%) out of 78 need not platelet blood transfusion by Lustrombopag administration. No patients had bleeding complication after RFA procedure. One had portal vein thrombosis after Lustrombopag use.

We will report an experience of Lustrombopag administration in detail, including overview the prevention of bleeding complications in RFA procedure.
The Role of Lusutrombopag for the Multidisciplinary Treatment of Hepatocellular Carcinoma

It was widely accepted that multidisciplinary treatment could improve the treatment response of hepatocellular carcinoma (HCC). Recently, molecular-targeted agents such as sorafenib, regorafenib and lenvatinib have emerged as promising treatment for advance HCC. However, locoregional therapies such as radiofrequency ablation (RFA), microwave coagulation (MWA) and transcatheter arterial chemoembolization (TACE) still play an important role in management of HCC. Radiofrequency ablation and MWA technique need enough amount of platelet to avoid bleeding. Lusutrombopag is a small molecule thrombopoietin receptor agonist designed to temporarily increase the platelet count in patients with liver cirrhosis. Lusutrombopag could decrease the usage of platelet infusion and increase platelet count appropriately. Multidisciplinary treatment increases the HCC patients who could be treated by RFA and MWA. We frequently used the RFA and MWA after TACE to avoid the locoregional recurrence. Moreover, locoregional treatment will be more important as conversion therapy when the molecular-targeted agents, immune check points inhibitor and hepatic arterial infusion chemotherapy will be widely used for HCC treatment. Therefore, lusutrombopag could be an important drug in the multidisciplinary treatment of HCC. In this lecture, we will present the efficacy and safety of lusutrombopag for HCC. Moreover, the efficacy and safety of repeated use of lusutrombopag will be also shown in this lecture.
APASL Single Topic Conference 2019 in Tokyo

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Abstracts
Oral Free Papers
O-01
Pathway-Analysis Using Datasets of GWAS and mRNA Expression Array Identified IFNG as the Most Significant Upstream-Regulator in Primary Biliary Cholangitis

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Genome-wide association studies (GWAS) in European descents and East Asian populations have identified more than 40 disease-susceptibility genes in primary biliary cholangitis (PBC). However, the disease-pathways and their upstream-regulators remain to be elucidated. The aim of this study is to objectively identify them in PBC by integrated analysis of GWAS and mRNA microarray in the Japanese population. Disease-pathways and their upstream-regulators were analyzed by Ingenuity Pathway Analysis (IPA) using the dataset 1 of GWAS (1920 PBC cases and 1770 controls) which included 261 annotated genes derived from 6760 SNPs (p-value<0.00001), and dataset 2 of mRNA microarray of liver biopsy specimens (15 PBC cases and 5 normal controls) which included 1506 genes with fold expression change >2 as compared to controls (p<0.05). There were 34 genes that were overlapped between the datasets 1 and 2, while there were 17 overlapped pathways and 144 overlapped upstream regulators between the datasets 1 and 2. Among these 144 upstream regulators, 10 showed fold expression change >2 in the microarray. Among these 10 upstream-regulators, IFNG, CD40LG, SPIB, CD2, FASLG and B2M showed the strongest correlation with serum AST, ALT and ALP levels. Furthermore, the meta-analysis of upstream regulators using 2 datasets and public datasets of peripheral blood mononuclear cells revealed that IFNG was the most significant upstream regulator associated with PBC (p=5.02E-49). Our hypothesis-free integrated analysis using GWAS and microarray datasets predicted that IFNG is the most significant upstream-regulator in PBC.

O-02
Altered Expression of Various Inflammation and Immune-related Genes in Senescent Biliary Epithelial Cells Relating to Primary Biliary Cholangitis

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Background: We reported that senescent biliary epithelial cells (BECs) might play various roles in the pathogenesis of primary biliary cholangitis (PBC) and other various cholangiopathies. Senescent BECs are known to produce senescence-associated secretory phenotypes (SASPs) and also considered as a target of senolytic therapy. Taking advantage of microarray analysis, we examined comprehensive profiles of senescent BECs relating to cholangiopathies.

Methods: Cellular senescence was induced in cultured BECs by treatment with serum depletions or glycochenodeoxycholic acid (GCDC, 200 μM)) for 7 days. mRNA was extracted and genome-wide expression profiling was performed using 3D-Gene Oligo chip 24 k (Toray Industries, Inc., 23,522 distinct genes). Cellular senescence was evaluated by senescence-associated β-galactosidase (SA-β-gal) activity.

Results: Cellular senescence was induced in BECs treated with serum depletions or GCDC for 7 days. Functional analysis performed by Gene Ontologies and KEGG pathway analysis showed that genes up-regulated (fold change >2) in senescent BECs induced by serum depletions or GCDC were involved in pathways related to “antigen processing and presentation” such as beta-2-microglobulin, histocompatibility 2 regions, and “systemic lupus erythematosus” such as Fc receptor, IgG, high affinity (FcgR1), complement component and histocompatibility 2, class II antigen A, beta 1 (H2-Ab1). A number of chemokines such as CCL2 and CCL20 and cytokines such as IL3 and IL15 were highly expressed in the senescent BECs.

Conclusion: Senescent BECs showed altered expression of various genes related to immunity and inflammation, which may be closely related to pathophysiology of PBC and other cholangiopathies.
O-03 Validation of Risk Scoring Systems in Ursodeoxycholic Acid Treated Patients with Primary Biliary Cholangitis

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Background: Risk stratification based on biochemical variables is a useful tool for monitoring ursodeoxycholic acid (UDCA) treated patients with primary biliary cholangitis (PBC). Several UDCA-response criteria and scoring systems have been proposed for risk prediction in PBC, but these have not been validated in external large cohorts.

Patients and Methods: We performed a study on data of 1746 UDCA-treated patients with PBC from 25 centres in Europe, USA and Canada. The prognostic performance of the risk scoring systems (GLOBE and UK-PBC) and the UDCA-response criteria (Barcelona, Paris-I, Paris-II, Rotterdam and Toronto) were evaluated. We regarded cirrhosis related complications (ascites, variceal bleeding and/or hepatic encephalopathy) as clinical endpoints.

Results: During a median seven years (range 1–16) follow-up, 171 patients reached a clinical endpoint. The 5-, 10- and 15-year adverse outcome-free survivals were 95%, 85%, and 77%. The GLOBE risk status showed a better discriminative performance than biochemical response criteria including, Barcelona, Paris-I, Rotterdam and Toronto, but was not superior to Paris II (table 1). The GLOBE and UK-PBC scores showed similar and excellent prognostic performance (C-statistic, 0.93; 95% CI, 0.91-0.95 vs.0.94; 95 % CI: 0.89-0.99).

Conclusions: In our international, multi-center PBC cohort, the GLOBE and UK-PBC scoring systems were good predictors of future cirrhosis-related complications.

Key words: Primary biliary cholangitis, UDCA, GLOBE, UK-PBC.

O-04 Access to PBC Foundation Services Improves Patient Self-Management and Mental Well-being

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Introduction: The PBC Foundation provides information and support to over 13,000 patients with primary biliary cholangitis (PBC) in 76 countries. Feedback from service users highlight feeling ‘lost’ and ‘alone’ before accessing PBC Foundation services and of being “more knowledgeable”, and “more empowered” afterwards. International guidelines recommend PBC patients should be signposted to a PSO.

Aim: We aimed to determine if PBC Foundation services support people with PBC.

Materials and Methods: Approved by 40 PBC patients, the 32-question survey captured information on clinical experience and patient support needs. This abstract discusses three key questions with a four point Likert scale (‘strongly agree’, ‘agree’, ‘disagree’ and ‘strongly disagree’).

Results: 727 (n) patients (96% females) were surveyed over 5 months. 609 (83.7%) identified as members of the PBC Foundation with 32% from outside UK. To ‘Accessing PBC Foundation services has enabled you to better manage your symptoms’, 27.2% strongly agreed and 60.4% agreed. Similarly, 34% ‘strongly agreed’ and 54.9% ‘agreed’ to ‘Accessing PBC Foundation services has enabled you to make more informed decisions about your PBC and its treatment’. Responding to ‘Accessing the PBC Foundation services has made you feel less alone’ 49.5% strongly agreed and 43.7% agreed. Overall, the proportion of positive responses to above statements was ~90%, suggesting a significant positive impact.

Conclusion: This survey of PBC patients highlights the importance of PSOs and consequential improvement to quality of life. Large proportions of PBC patients reported improvement in: disease self-management, decision making, and social and mental wellbeing. The study suggests high level of satisfaction with the PBC Foundation.

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O-05
Specific Inhibition of Cancer Cell Glycolysis Enhances Antitumor Immunity in Hepatocellular Carcinoma

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Background and Aim: The immunity checkpoint blockades are expected to be an effective treatment for advanced HCC, but immunosuppressive mechanisms are enhanced in the microenvironment of advanced cancers. Recently, it has been reported that enhanced glycolysis in cancer cells (Warburug effect) suppresses antitumor immunity. The aim of this study was to verify whether specific inhibition of cancer cell glycolysis enhances antitumor immunity in HCC.

Methods: We have developed 2-deoxy-D-glucose (2DG)-encapsulated poly (lactic-co-glycolic acid) (PLGA) nanoparticles (2DG-PLGA) as a cancer cell-specific inhibitor of glycolysis. We investigated in vitro and in vivo whether 2DG-PLGA exerts antitumor effect on HCC through the enhancement of antitumor immunity.

Results: We confirmed specific accumulation of PLGA nanoparticles in HCC xenograft tumor, but not in other tissue, until day 7 of the administration, using PLGA encapsulating the fluorescent dye ICG and in vivo imaging system. 2DG significantly increased chemokine (CXCL10) production in Huh7 cells in the presence of IFN- and TNF, and promoted chemotaxis of T cells toward Huh 7 cells ex vivo. Of note 2DG-PLGA exhibited more vigorous antitumor effect accompanied with lymphocyte infiltration into HCC in immunocompetent mice (STAM™ and DEN-induced HCC mouse models) than in immunosuppressive mice (xenograft HCC in nude mice), suggesting the involvement of enhanced immunity in antitumor effect. Finally, we found that 2DG-PLGA exhibited antitumor effect in C57BL/6 mice transplanted with PD1-resistant melanoma cell line (B16F10).

Conclusion: Specific inhibition of cancer cell glycolysis enhances antitumor immunity in HCC microenvironment, and 2DG-PLGA is expected to become a new anti-HCC drug.

O-06
Structure Guided Design of a Therapeutic Inhibitor of SALL4 Positive Advanced Liver Cancer

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Aggressive cancers and ES cells share similar characteristics. In cancer cell, ES genes are often re-activated and drive the progression of carcinogenesis. SALL4 is an embryonic nuclear factor and a key component of the Oct4/Nanog/SALL4/Sox2 core transcription network that is essential for the maintenance of stem cell pluripotency. It is absent in adult tissues but is re-activated in cancer. SALL4 drives up to 55% of hepatocellular carcinoma (HCC). Specifically, it identifies a subtype of HCC with progenitor-like features associated with poor prognosis. In these cells, SALL4 recruits remodelling complex NuRD to promoters of tumor suppressors, resulting in transcriptional repression. This SALL4–NuRD interaction offers an intriguing therapeutic target for HCC.

Methods: We solved the crystal structure of SALL4-RBBp4 (subunit of NuRD) at 2.7Å. A series of peptides were designed based on the structural information of the binding pocket. These peptides were tested with Flourescence Polarization (FP), Surface Plasmon Resonance (SPR), Isothermal Calorimetry (ITC) and Cell viability assay. A final peptide was determined and treated to mice implanted with SALL4+ HCC xenografts, with or without resistance to Sorafenib.

Results: The elucidation of the SALL4-RBBp4 crystal complex reveal a large binding groove where we subsequently design a therapeutic peptide FFW that blocks the interaction. We demonstrate that FFW has a target affinity of 23nM, exhibits superior anti-proliferative and anti-migratory effect in SALL4+ HCC cells, and exhibits potent anti-tumor activity in HCC xenograft mouse models (tumor growth inhibition 85%). Furthermore, it shows synergistic effect with Sorafenib in a chemo- and radio-resistance xenograft model, supporting FFW as a highly viable drug candidate.

Conclusion: Here, we report the development of a potent anti-tumor therapeutic peptide. This peptide could potentially fulfill the unmet need in liver cancer and other malignancies characterized by SALL4 expression.
O-07

A Hepatocyte Differentiation Model Reveals Two Subtypes of Liver Cancer with Different Oncofetal Properties

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Background: Clinical observation of the association between cancer aggressiveness and embryonic development stage implies the importance of developmental signals in cancer initiation and therapeutic resistance. However, the master oncogenic drivers are still unclear, which impeded the efficient elimination of poor prognostic tumors, including human hepatocellular carcinoma.

Methods: Human embryonic stem cells were induced to differentiate into adult hepatocytes along hepatic lineages in vitro. Combining transcriptomic data from liver cancer patients with the hepatocyte differentiation model, the active genes derived from different hepatic developmental stages and the tumor tissues were selected. Bioinformatic analysis followed by experimental assays were used to validate the tumor subtype-specific oncogenic signatures and potential therapeutic values.

Results: Hierarchical clustering analysis revealed the existence of two subtypes of liver cancer with different oncofetal properties. The gene signatures and their clinical significance were further validated in an independent clinical cohort and the cancer genome atlas database. Upstream activator analysis and functional screening further identified E2F1 and SMAD3 as master transcriptional regulators respectively. Small molecule inhibitors HLM6474 and SIS3 specifically targeting the oncofetal drivers extensively down-regulated subtype-specific developmental signaling and inhibited tumorigenicity. Liver cancer cells with different oncofetal properties also showed selective vulnerability to their specific inhibitors.

Conclusions: Two subtypes of liver cancer with different oncofetal properties and upstream oncogenic drivers were found to exist in clinical patients with poor prognosis. Precise targeting the tumor initiating steps and driving events according to sub-type specific biomarkers might eliminate tumor progression and provide novel therapeutic strategy.

O-08

A Subgroup of Hepatocellular Carcinoma Rich in Druggable Immune Checkpoints

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Background: Immune checkpoint inhibitors poise to revolutionize the therapeutic landscape as key players in cancer immunotherapy. The clinicopathological characteristics and tumor immune microenvironment of hepatocellular carcinoma (HCC) with high expression of druggable immune checkpoint genes have not been fully explored yet.

Methods: Total 55 patients with surgically resected primary HCC were recruited. Total mRNA was isolated from the macrodissected FFPE tumor tissues and analyzed by the nCounter PanCancer Immune Profiling Panel. Unsupervised hierarchical clustering of normalized expression of six druggable immune checkpoint genes (CD274, PDCD1, CTLA4, HAVCR2, IDO1, LAG3) was performed by using complete linkage method with Euclidean distance.

Results: A cluster of HCCs with high expression of druggable immune checkpoint genes were identified (Fig. 1A), and was associated poorer histological differentiation (62.5% vs. 10.6%, P=0.003), lymphoepithelioma-like histological subtype (100% vs. 19.1%, P<0.001), high immunohistochemical expression of stemness markers [EpCAM (50.0% vs. 2.6%, P=0.002) and CK19 (37.5% vs. 0%, P=0.002)] and epithelial-mesenchymal transition markers [E-cadherin (25.0% vs 88.9%, P=0.024) and Vimentin (25.0% vs 0%, P=0.026)]. This group was associated with immunological active tumor microenvironment with higher gene signatures of B-cells, pan T-cells, CD8+ T-cells, T-helper cells, Treg cells, tertiary lymphoid structures, M1 macrophages, MHC types I and II (Fig. 1B).

Conclusions: HCC with high expression of druggable immune checkpoint genes is a subgroup of HCC with distinctive pathological features. Therapeutic blockade of over-expressed immune checkpoints in this subgroup would reactivate the existing immunologically active tumor microenvironment to achieve antitumor effects.
O-09
Ex Vivo Detection and Characterization of Hepatitis B Virus-specific CD8+ T Cells in Patients Considered Immune Tolerant

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In this study, we aimed to detect and characterize the ex vivo virus-specific CD8+ T cells in patients considered immune-tolerant hepatitis B virus (HBV) infection. We investigated a Korean chronic hepatitis B cohort comprising 15 patients in the immune-tolerant phase, 17 in the immune-active phase, and 13 under antiviral treatment. We performed enzyme-linked immunospot (ELISpot) assays ex vivo and intracellular cytokine staining after in vitro culture using a mixture of human leukocyte antigen A2-restricted epitopes of HBV proteins. We also performed ex vivo multimer staining assays and examined the expression of programmed death-1 (PD-1) and CD127 in pentamer-positive cells. Ex vivo ELISpot revealed that HBV-specific T cell function was weaker in immune-tolerant patients than in those under antiviral treatment. Short-term culture of peripheral blood mononuclear cells revealed that some immune-tolerant patients had HBV-specific CD8+ T cells able to produce interferon-γ. We detected HBV-specific CD8+ T cells ex vivo (using the HBV core 18-27 pentamer) in patients from all three groups. The PD-1+ subset of pentamer+ CD8+ T cells was smaller ex vivo in the immune-tolerant phase than in the immune-active phase or under antiviral treatment. Interestingly, the proportion of PD-1+ CD8+ T cells in HBV-specific CD8+ T cells correlated with patient age. Hydrodynamic injection of HBV genotype C-harboring plasmid into CBA/caj mice generated a persistent HBV-carrying mouse model with very low levels of ex vivo interferon-γ-producing HBV-specific T-cells. **Conclusion:** Although their ex vivo functionality is very weak, HBV-specific CD8+ T cells are present in patients considered immune-tolerant. Despite the high viral load, the proportion of PD-1 expression in HBV-specific CD8+ T cells is lower in the immune-tolerant phase than in other phases.

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O-10
mTORC2 - Related Protein Kinase B Phosphorylation is Associated with Nucleotide Analogues Treatment for Chronic Hepatitis B

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**Background:** Nucleos(t)ide analogues (NAs) effectively suppress hepatitis B virus (HBV) replication and correlate with the anti-HBV-specific immune response, such as regulatory T cell (Treg) suppression. Moreover, mTORC2 signaling is emerging as a critical regulator of immune function as well as metabolism and is identified to be a negative regulator of HBV replication. In this study, we assessed mTORC2 mediated expression in NA-treated HBV-replicating cells and clinical liver tissues.

**Methods:** The levels of mTORC mediated factors were measured by qPCR and western blotting after the administration of nucleoside analogues (lamivudine [LAM] and entecavir [ETV]) and nucleotide analogues (adefovir pivoxil [ADV] and tenofovir disoproxil fumarate [TDF]) to HBV-replicating cell lines. Since Notch signaling is reported to regulate the mTORC2 pathway, we assessed also Notch signaling expression. Clinical liver tissues under NA treatment were analyzed using immunohistochemistry and western blotting.

**Results:** While Foxp3 expression was down-regulated in any NA, mTORC2-related genes were upregulated after ADV or TDF administration but were not altered by LAM or ETV. Moreover, mTORC2-related mTOR (Ser2481) and protein kinase B (Akt [Ser473]) phosphorylation was increased after ADV or TDF treatment, whereas mTORC1-related S6K phosphorylation was not changed by any NA. Notch signaling and TGFbeta expression exhibited similar patterns with mTORC2 regulation. Immunohistochemistry and western blotting results indicated higher levels of mTORC2-related proteins in ADV- and TDF-treated patients.

**Conclusion:** Nucleoside and nucleotide analogues display different patterns of signal transduction regarding mTORC2 and Notch signaling activity under HBV infection and these results indicate different HBV infection related immune response.
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The Risk Factor of Developing Hepatocellular Carcinoma in Chronic Hepatitis B Patients with Long-term Administration of Oral Antiviral Therapy

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Background and Aims: Hepatitis B virus (HBV) infection is a major public health threat that increases the risk of developing liver failure and hepatocellular carcinoma (HCC). Nucleos(t)ide analogs (NAs) were seemed to suppress HBV replication and reduce the risk of HCC and HBV-associated mortality. However, hepatic carcinogenesis was not completely suppressed. The aim of this study is to identify carcinogenesis risk of hepatitis B infection patients who was treated with current NAs.

Methods: We included 383 hepatitis B infection patients who received entecavir (ETV)/tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) therapy for more than 1 year. Patients were excluded if they had hepatitis C, HIV, or prior HCC to 1 year of study entry. Cumulative incidence and HCC risk were analyzed using the Kaplan-Meier method and Cox proportional hazard analysis.

Results: The median observation period was 5.2 years. HCC developed in 35 patients. The 3-year and 5-year cumulative HCC rates were 5.4% and 9.2%, respectively. Among HCC cases, median age 59 (28-85) years, majority male (80%), most of cases had been suppressed virus replication and hepatitis activity (ALT was less than 30 IU/ml, median HBV-DNA was less than 1.0 Log IU/L). On the other hand, mean HBV core related antigen (HBCrAg) was 4.3 (3.1-6.3) Log U/ml. According to analysis, age (≧60 years: p=0.014), male (p=0.019), platelets (≤150×10^3/µL; p<0.001), alcohol use (p<0.001), advanced fibrosis and cirrhosis at baseline (p<0.001) were identified as independent factors significantly associated with HCC development. We further investigated with REAL-B risk score which is the carcinogenic prediction score (AASLD. 2017) in NAs administration patients calculated from these factors. REAL-B score classified patients to 137 (0-3 points: low risk)/224 (4-7 points: intermediate risk)/22 (8-13 points: high risk), and 5-year cumulative HCC incidence rates of 2.4% /8.1% /45.3%, respectively (p<0.001).

Conclusion: Carcinogenesis was also observed in patients whose virus was suppressed by long-term administration of NAs. REAL-B risk score was also able to stratify the risk of HCC development. The high-risk patients need to receive cancer surveillance more carefully.

O-12

Undiagnosed Iron Overloaded Liver Disease Cases in Non-Caucasian Group: Iron-Copper Panel Suggested

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Background: HFE C282Y homozygosity for hemochromatosis is seen in 1/250 Caucasians and 80.6% of the patients. In this study, the accountability of HFE and other non-HFE genes was tested in Chinese iron overloaded cases.

Methods: Liver disease patients with persistently elevated serum transferrin saturation (>45%) and ferritin (>500µg/L) from year 2017-2018 were enrolled. Subjects with concurrent infection, anemia of chronic disease, non alcoholic fatty liver disease and alcohol abuse were excluded. Other undiagnosed liver disease patients were taken as disease control. Healthy controls were also included. Exome sequencing and Sanger validation were carried out in cases and controls. Iron-copper genes were analyzed and statistically compared.

Results: Among 352 undiagnosed liver disease patients, 18 cases were included after strict review. along with 48 liver disease controls and 19 healthy controls. Age and sex were comparable among groups. None of the cases had HFE biallelic mutations. Non-HFE gene biallelic mutations occurred in 33.3% of the cases. In one particularly case with known family history, in addition to the iron genetic defects, disease causing mutation was also present in ATP7B, the known copper gene. No iron-copper gene mutation was found in the control.

Conclusions: Despite the low regional HFE mutant carrier rate, iron overloaded cases were not rarely seen in undiagnosed liver cases. Non-HFE genes accounted for most of the patients herein studied. The presence of copper gene defect suggests its influence on the phenotypic expression. We suggest iron-copper panel screening in undiagnosed iron overloaded liver disease cases in non-Caucasian cases.
Gene Mutation PHKA2 Leading to Child Glycogen Storage Disease Type IXa: A Case Report

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Introduction: Glycogen storage disease type IX is the most frequent in all types of GSD. The incidence rate of GSD type a is only about 1/100000 according to current reports over the world and the case report of GSD type IXa is relatively rare in China. Now we present the first case of GSD type IXa in the Northeast China caused by gene mutation PHKA2.

Main Symptoms and Important Clinical Findings: A 11-year-old boy was referred to our hospital with a onset of vomiting, and the further physical examinations indicated the growth delay, relaxation of joint ligament and liver enlargement. Blood tests confirmed a significant increase of aminotransferase level. However, the decrease blood glycogen and high level of triglyceride were noticed.

Diagnosis and Therapeutics Interventions: We made detailed analysis to excluded common factors that might cause liver injury. Eventually we performed ultrasound-guided liver biopsy with the permission of the child’s parents and the histopathological result confirmed our primary diagnosis of GSD. Further gene test shows exact typing of GSD—GSD type IXa, which are caused by the c.133C>T gene mutation of PHAK2. We regulated dietary structure of the child with a high protein and high starch diet and the patient was informed of regular re-examinations.

Conclusion: This is the first reported case of GSD type IXa in the area of Northeast China. We hope the detailed and complete process about this case could provide reference for the diagnosis of unknown liver enlargement in the future clinical practice.

Usefulness of Serum Mac-2 Binding Protein Glycosylation Isomer in Children with Primary Sclerosing Cholangitis

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Background: Mac-2 Binding Protein Glycosylation Isomer (M2BPGi) is a novel serum marker of hepatic fibrosis in adults with chronic hepatitis C. However, it remains unclear whether serum WFA+-M2BP levels are associated with the progression of liver histology in primary sclerosing cholangitis (PSC).

Methods: Twenty-eight children and adolescence with pediatric onset-PSC (M/F = 20/8, median age at diagnosis: 9 years) were enrolled in this study. The relation between serum M2BPGi levels and clinical characteristics was retrospectively evaluated. Moreover, receiver operating characteristic (ROC) analysis was performed to determine whether serum M2BPGi levels could be a reliable marker to identify PSC patients with advanced liver histology.

Results: According to Ludwig classification of liver histological stage, twenty-eight patients were classified into the 4 stages. M2BPGi, and AST to platelet ratio index (APRI) correlated significantly with liver histological stage. Moreover, M2BPGi showed a significant positive correlation (p<0.05) with autoimmune hepatitis overlap, AST, ALT, GGT, total bilirubin, IgG, and APRI. ROC analysis was performed to distinguish the patients with advanced stage (stage 3 and 4) from those with the early stage (stage 0, 1 and 2). M2BPGi yielded the highest area under the ROC curve value (0.898) among 4 surrogate makers (APRI: 0.850, Fib-4 index: 0.806, AST/ALT ratio: 0.802). Moreover, M2BPGi yielded the highest sensitivity, specificity, positive predict value and negative predict value among the four markers.

Conclusion: Serum M2BPGi levels are useful to identify patients with advanced liver histology in pediatric PSC.
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Autoimmune Hepatitis with Acute Presentation; Are There Clinical Characteristics could Contribute its Early Diagnosis?

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Background/Aim: Autoimmune hepatitis with acute presentation (AIH AP) could be difficult to diagnose, because of absence of auto antibodies or normal Immunoglobulin G (IgG) in sera unlike classical AIH. Aim of the current study was to clarify clinical features useful for early diagnosis of the disease.

Methods: Retrospective, cross-sectional and cohort study were performed for patients with AIH AP defined as ALT ≥ 400 U/L or total bilirubin ≥ 5mg/dl on presentation without prior liver disease. Clinical courses were analyzed before corticosteroid. For diseases control, 134 patients with acute hepatitis A, B, or E were recruited.

Results: Fifty-five patients with AIH AP (48 females, 55 ± 14 y-o, 14.9 ± 2.5 in revised AIH score) were enrolled. They were older, and female ratio was higher than those in control (p<0.001, p<0.01), respectively. When comparing with control, the first AST were lower (962 ± 552 vs. 2275 ± 2994 U/L, p<0.001), and, among 32 AP patients followed in early phase, AST at the first visit and its peak level were 841 ± 542, 1186 ± 716 U/L, respectively, and they were lower than those in control (2069 ± 2871, 4112 ± 3701, p<0.001). ALT showed similar changes in the same time points as above. Duration from the first AST/ ALT to their peak point were shorter than those in control.

Conclusions: On early phase in AIH AP, aminotransferases might increase slowly to reach relatively lower peak levels. These observations would help us to make early diagnosis for the disease.

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Clinical Course and Correlation with Ulcerative Colitis in Japanese Patients with Primary Sclerosing Cholangitis

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Background: The comorbidity rate of primary sclerosing cholangitis (PSC) and concurrent ulcerative colitis (UC) is lower in East Asian countries than in Western countries. The number of patients with UC in East Asia has markedly increased. The present study aimed to reveal the clinical course and correlation with UC in Japanese patients with PSC.

Methods: We retrospectively retrieved medical records of patients with PSC (69) and UC (1242) who were diagnosed at Chiba University Hospital between June 1991 and August 2017.

Results: In the present cohort, 37 patients had PSC-UC; the cumulative risks of PSC in patients with UC and of UC in patients with PSC were 3.0% and 53.6%, respectively. The median observation periods in the PSC and UC cohorts were 6.1 and 7.7 years, respectively. In the PSC cohort, younger patients with PSC had a notably high possibility of association with UC. In the UC cohort, the occurrence of the right-sided type was significant higher in patients with PSC-UC than in those with UC (16.2% vs. 4.2%, P = 0.003). From the initial diagnosis of UC, overall survival was significantly lesser in patients with PSC-UC than in those with UC (log-rank; P <0.001). Patients who occurred UC ≤25 years old tended to increase in patients with UC and those with PSC-UC.

Conclusion: In our cohort, the comorbidity rate of PSC-UC was higher than that obtained in previous reports. The incidence of PSC-UC and UC may increase in the future in East Asia, particularly in Japan.
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Characterization and Identification of Differentially Regulated Proteins may Identify Biomarkers for Early Diagnosing and Prognosis Drug-induced Liver Injury (DILI)

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Background and Aims: DILI is a frequent side effect of many drugs, but the diagnosis is mainly by exclusion of other causes as there are no biomarkers to diagnose DILI. Reliable non-invasive methods to identify proteins and determine urinary micro-albumin (µA) are yet not known in DILI.

Methods: - Early morning urine samples from successive patients with biopsy proven DILI (n=35), age, sex matched healthy controls (HC), (n=40) were collected at ILBS from September 2017. Out of the 40, 10 had hepatocellular, 15 cholestatic and 15 mixed types of DILI based on ‘R’ value >5, <2 or between 2-5 respectively at baseline LFT. Urinary micro-albumin, and Label Free Quantification-Mass Spectrometry (LFQ-MS) analyses were done.

Results: - Urinary µA level was significantly higher in DILI [50 mg/dl] compared to HC [0.17mg/dl] (p<0.05) at baseline. On LFQ-MS we identified 1860 proteins in the urine samples. 1292 were common, 235 found only in HC and 333 in DILI. We further analysed Samples Abundance, PCA Plots, Abundance Plot and Biological Process of identified proteins. Used ‘STRING Pathway of differentially expressed proteins to characterize the significant function and clinical role of some expressed proteins in DILI. Which were unique to DILI, like- charged multivesicular body protein-5,Cystatin, Catenin alpha-1, DNA-binding protein, Heparan, Vinculin etc. Functions were assessed in the Uniport data.

Conclusion: - The relevance of its significant abundance in DILI patients needs further assessment. The Urinary µA and these unique proteins can be used as a reliable and exclusive non-invasive diagnostic biomarker for outcome prediction of suspected DILI.

O-18
Cytomegalovirus-Based HBsAg Vaccine Induces Robust T Cell Responses and Results in Viral Clearance in HBV Persistent Mice

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Curative approaches for chronic hepatitis B (CHB) are urgently needed. Cytomegalovirus (CMV) can elicit a remarkable impact on the immune system of their hosts. CMV-based vaccines are currently in the spot-light as they showed superb control of chronic viral infections and tumors growth in animal models. In this study, we examined the potential of using CMV as vectors for generating HBV vaccines. To test the ability of MCMV-based HBV vaccines against HBV, C57BL/6 mice were vaccinated either with MCMV-HBsAg or Δm27 -HBsAg (MCMV replication deficiency strain), and were challenged with HBV through hydrodynamic injection. Compared to untreated or MCMV-vaccinated control mice, mice vaccinated with MCMV-HBsAg or Δm27 -HBsAg showed significantly accelerated HBV clearance in the serum and liver. A rapid development of serum HBsAb after HBV challenge was detected in MCMV-HBsAg vaccinated mice but not control mice. And significantly increased numbers of HBsAg-specific CD8+ T cells in the liver was only observed in MCVM-HBsAg but not MCMV vaccinated mice. A rapid development of robust HBsAg and HBeAg specific CD8+ T cell responses was also observed in the liver of MCMV-HBsAg vaccinated mice compared to control mice. Moreover, we also explored the therapeutic effect of MCMV-HBsAg vaccines in HBV persistent replication mice. Both Δm27- HBsAg and MCMV-HBsAg vaccination resulted in significant HBV suppression. In conclusion, our results demonstrated that MCMV-HBsAg vaccine could elicit robust anti-HBV immune responses and mediate HBV clearance in mice.
The Role of Intrahepatic Type I Interferon Signaling in HBV-Specific T Cell Responses

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Backgrounds: Systemic administration of interferon (IFN)-α to chronic hepatitis B patients rarely terminates hepatitis B virus (HBV) infection partially because it is ineffective to induce HBV-specific T cell responses. In the current study, we examined the impact of intrahepatic activation of type 1 IFN (IFN-I) signaling on HBV-specific CD8 T cell responses and HBV clearance.

Methods: Three groups of mice were treated with NaCl or poly I: C that was either dissolved in NaCl (poly I: C-NaCl) or emulsified in lipid nanoparticle (poly I: C-LNP) and compared IFN-I signaling related genes expression between three groups. In addition, HBV transgenic mice were adoptively transferred with wild-type or IFN-I receptor deficient (IFNαβR-/-) HBV-specific CD8 T cells and then treated with poly I: C-LNP. The number and effector differentiation of intrahepatic HBV-specific T cells after adoptive transfer were analyzed and associated with HBV-mRNA expression.

Results: Poly I: C-LNP induced much stronger and longer expression of IFN-β and interferon stimulated genes (ISGs) in the liver than poly I: C-NaCl. Strikingly, poly I: C-LNP treatment stimulated wild-type HBV-specific CD8 T cells to expand vigorously and differentiate into cytokine-producing, cytotoxic effector cells. Furthermore, poly I: C-LNP treatment strongly suppressed HBV-mRNA expression. In contrast, much fewer IFNαβR-/- T cells expanded and differentiated into effector CD8 T cells in response to poly I:C-LNP treatment, indicating that IFN-I signaling in T cells is indispensable for the induction of functional HBV-specific CD8 T cell responses by poly I:C-LNP administration.

Conclusions: Intrahepatic activation of IFN-I signaling effectively induces functional HBV-specific CD8 T cell responses.

CHI3L1 is a Non-Invasive Surrogate Serum Marker for Effectively Identifying Chronic HBV Patient with Normal ALT Levels but with Advanced Liver Fibrosis for Treatments

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Background: Hidden and undetected ongoing liver fibrosis is a major factor that causes under-treatment of chronic HBV patients, resulting in serious consequences as these patients often manifest as cirrhosis patients when they enrolled in hospitals. CHI3L1 is a new marker that has been recently demonstrated to be an excellent non-invasive staging marker for liver fibrosis caused by HBV and other factors.

Method: We test the applicability of using CHI3L1 for the identification of occult ongoing liver fibrosis for those chronic HBV patients with normal ALT levels. We used the CHI3L1 serum cutoff values of 79 ng/ml for the identification of those chronic HBV patients with liver fibrosis greater than F2. Antiviral treatments (entecavir, ETV) were applied to these patients and their serum CHI3L1 levels were monitored after 12 weeks of antiviral treatments.

Results: We identified 10 naïve chronic patient (no prior antiviral treatments) with CHI3L1 > 79 ng/ml but normal ALT (<40), and subjected them to entecavir treatments. Nine of the 10 patients showed reduced CHI3L1 levels at week 12, and one patient showed increased CHI3L1 level. In addition, 37 chronic HBV patients with ongoing antiviral treatment but with normal ALT levels, 31 of them have reduced CHI3L1 levels with continued 12 weeks of antiviral treatments.

Conclusion: CHI3L1 is a non-invasive surrogate serum marker for effectively identifying chronic HBV patient with normal ALT levels but with occult ongoing liver fibrosis passing stage S2, and for monitoring antiviral (e.g. ETV) treatment responses.
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Analysis of Factors Associated with Reduction of HBs Antigen Levels in Chronic Hepatitis B Patients Treated with TDF + Peg-IFN Combination Therapy

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Aim: Factors associated with reduction of HBsAg level at 48 weeks after starting TDF+Peg-IFN combination therapy were analyzed in chronic hepatitis B patients with high HBsAg levels.

Patients and Methods: A total of 83 patients with CH-B with HBsAg levels higher than 2.9 log IU/ml were enrolled. Of those, 32 patients underwent the combination therapy (20 men, median age 43 years) and 51 underwent TDF monotherapy (32 men, median age 44 years). HBsAg levels were measured at 0, 12, 24, 36, and 48 weeks. Th1/Th2 ratio was measured at baseline.

Results: The median level of HBsAg at the start was 3.8 log IU/ml (3.1 - 4.5) in combination therapy group and was 3.5 log IU/ml (3.0 - 4.2) in monotherapy group. Patients with HBsAg reduction rate over 0.3 log IU/ml/year were significantly (P< 0.001) more common in combination therapy group (15/32, 47%) than in monotherapy group (0/51, 0%). Multivariate analysis showed that the combination therapy (OR 44.6, P <0.001), body weight <60 kg (OR 15.4, p = 0.006), Th2 < 2.6% (OR 13.7, p = 0.015), and ALT  35 U/L (OR 13.0, p = 0.029) at baseline were associated with the reduction rate over 0.3 log IU/ml/year.

Conclusion: Lower body weight, lower Th2 ratio and higher ALT level at baseline were associated with the rapid reduction of HBsAg level in patients with TDF + Peg-IFN combination therapy.

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High Antibody Response to Standard and Double Dose of Hepatitis B Vaccine in Children after Liver Transplantation: A Randomized Controlled Trial

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Background: High prevalence of hepatitis B (HB)-antibody loss after liver transplantation (LT) and de novo HB infection was documented; hence strategy to prevent HB infection by revaccination will be considered. We aim to determine the antibody response to 2 different regimens of HB vaccine.

Methods: Children after LT who had anti-HBs before LT ≥10 IU/L but ≤10 IU/L after LT and clinical stable, were randomized into 2 groups; one received standard (0.5 ml) 3-dose and another received double (1 ml) 3-dose HB vaccine at 0, 1 and 6 month. Anti-HBs was assessed at 0, 1, 6 months after the first injection. DTH skin tests with HB vaccine by Mantoux method in a single blinded fashion was performed at the beginning. Average induration size ≥5 mm at 48 hours defined as positive.

Results: In total, 37 children were recruited. The geometric mean titer of anti-HBs before and after LT were 401.9(270-588) and 0(1.72-3.88) IU/L, respectively. Time of anti-HBs loss (anti-HBs <10 IU/L) after LT was 1.6(0.64-7.9) years. The age of revaccination was 5.21(1.29-14.87) years. There was significant rising of anti-HBs after immunization (P=0.03). However, there was no significant difference of the anti-HBs response in standard (N=19) and double-dose (N=18) group. No serious side effect in all participants was reported. No significant difference of anti-HBs in children who had positive and negative DTH skin test.

Conclusion: Children after LT had a good response to standard and double-dose HB revaccination without any serious side effect. DTH skin test with HB vaccine was useless to predict anti-HBs response.
O-23  
Genetic Polymorphism and Reduced mRNA Expression of HLA Class II DP Genes are Associated with Hepatitis B Virus Reactivation in Japanese Patients Treated with Immunomodulatory Agents

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Hepatitis B virus (HBV) reactivation can be triggered by immunosuppressive chemotherapy. HLA class II molecules may play a role in HBV reactivation. Genetic polymorphism and mRNA expression of HLA class II were examined in Japanese patients with latent HBV infections that were treated with immunosuppressive therapies. Patients with resolved HBV infections who had undergone immunosuppressive chemotherapy treatment were retrospectively enrolled (n = 42) and divided into reactivated (n = 9) and non-reactivated groups (n = 33). Patients were genotyped for 17 single nucleotide polymorphisms (SNPs) within HLA class II DPA1 and DPB1, and mRNA expression levels of HLA class II genes were assessed. The frequency of the AA genotype of rs872956, a SNP in HLA-DPB1, was significantly higher in the reactivated group than in the non-reactivated group (55.6% vs. 12.1%, p < 0.05), and multivariate logistic regression identified the AA genotype of rs872956 as an independent protective factor against HBV reactivation (odds ratio [OR] = 18.1, 95% confidence interval [CI] = 2.6–126.7, p < 0.01). HBV-DNA levels of all nine patients with HBV reactivation fell below the detection sensitivity by administration of nucleic acid analogues. mRNA expression of HLA-DPB1 was lower in the HBV reactivated group than in the non-reactivated group (276.1 ± 165.6/β-actin vs. 371.4 ± 407.5/β-actin [p < 0.05]). These results suggest the involvement of HLA class II molecules in HBV reactivation after treatment with immunomodulatory agents, and rs872956 could be a novel marker for detecting patients at high-risk of HBV reactivation in Japan.

O-24  
Lipoprotein-apolipoprotein Changes in Chronic Hepatitis C Patients Treated with Direct-Acting Antivirals

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Background and Aims: Apolipoproteins binding to lipids to form lipoproteins have been linked to hepatitis C virus infection. This study addressed lipoprotein profile changes in chronic hepatitis C under direct-acting antivirals (DAAs).

Methods: Of 140 chronic hepatitis C patients receiving DAAs, 133 (66 male, median age: 69 years) achieved a sustained virological response (SVR). Thirty-two healthy controls (HC) were also included for lipid profile comparisons at pretreatment (Pre) and SVR12 of apolipoprotein (Apo)-A2, -B, -C2, and -C3 and the Apo-A2 isoforms ApoA2-ATQ/ATQ, -ATQ/AT, and -AT/AT.

Results: Total cholesterol (TC), LDL-C, and HDL-C increased significantly from Pre to SVR12 (all P<0.001) to levels comparable with those for HC. Respective values for Pre, SVR12, and HC were 154, 190, and 175 mg/dL for TC, 52, 98, and 88 mg/dL for LDL-C, and 44, 53, and 59 mg/dL for HDL-C. Apo-B, Apo-C2, and Apo-C3 increased significantly from Pre to SVR12 (all P<0.001) while Apo-A2 decreased (P<0.001); respective values for Pre, SVR12, and HC were 71, 87, and 70 mg/dL for Apo-B, 2.0, 2.3, and 2.7 mg/dL for Apo-C2, 5.6, 7.4, and 7.6 mg/dL for Apo-C3, and 30.9, 27.8, and 29.2 mg/dL for Apo-A2. ApoA2-ATQ/ATQ decreased significantly whereas ApoA2-AT/AT increased from Pre to SVR12 (both P<0.001), whereby respective values for Pre, SVR12, and HC were 87.1, 73.1, and 57.0 µg/mL for ApoA2-ATQ/ATQ and 56.1, 65.8, and 133.4 µg/mL for ApoA2-AT/AT.

Conclusion: Lipoprotein-apolipoprotein profiles change dramatically during DAA treatment. Further studies are needed on the qualitative changes of these lipids in relation to clinical outcome.
O-25

Albumin-bilirubin Score Indicates Liver Fibrosis Staging and Prognosis in Chronic Hepatitis C Patients

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**Background and Aims:** Albumin-bilirubin (ALBI) grade was investigated to predict prognosis of patients with cirrhosis. It was defined using the ALBI score calculated based on serum total bilirubin and albumin, which represent liver functions. Currently, the diagnostic accuracy for liver fibrosis staging in patients with chronic hepatitis using ALBI score has not been investigated well.

This study aimed to evaluate the diagnostic abilities of ALBI score for liver fibrosis staging in chronic hepatitis and cirrhosis in Japanese patients with hepatitis C virus (HCV) infection.

**Methods:** Japanese patients with HCV infection who underwent liver biopsy examinations were enrolled in a retrospective study. Fibrosis staging and activity grading were assessed using a modified META VIR score. ALBI score was calculated according to the following equation: \((\text{Log} 10 \text{ T-Bil (µmol/l)} \times 0.66 + \text{Alb (g/l)} \times (-0.085)).\)

**Results:** A total of 382 patients were enrolled in this study. The ALBI score differentiated fibrosis stage 4 from stage 3 and stage 3 from stage 2 \((p<0.05)\). When an ALBI score of -2.125 was adopted as a cut-off value, the sensitivity and specificity were 73.2% and 87.1%, respectively, with a positive likelihood ratio of 5.67 to differentiate stage 4 from stages 1-3. Kaplan-Meier analysis showed that smaller ALBI score at baseline correlated with better hepatocellular carcinoma (HCC)-free and overall survival \((p<0.05)\).

**Conclusions:** ALBI score indicates liver fibrosis staging in Japanese patients with HCV infection. Furthermore, smaller ALBI score predicts better HCC-free survival and overall survival. ALBI score has the potential to expand its application from cirrhosis to chronic hepatitis.

O-26

High Burden of Hepatitis C Infection and Increase Risk of Liver Fibrosis on Chronic Kidney Disease Underwent Hemodialysis Patients in Hasan Sadikin General Hospital, Bandung, Indonesia: A Preliminary Study Prior of Hepatitis C Eradication Program in Hemodialysis Patients

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**Background:** Direct Acting Antiviral (DAA) with high cure rate contribute to global elimination of Hepatitis C Virus (HCV) infection. Although considered late, several DAA had been accepted to treat HCV patients in Indonesia. Nevertheless, recent DAAs in Indonesia were inapplicable to HCV-infected with CKD underwent hemodialysis patients. The aims of this study are to observe the HCV infection rate and the risk of liver fibrosis in hemodialysis patients, prior approval of Elbasvir – Grazoprevir in one major center in Indonesia.

**Methods:** This was a single center, cross-sectional study comparing liver fibrosis score by APRI and Fib-4 score of HCV-infected to those without HCV-infected on hemodialysis patient admitted in Hasan Sadikin General Hospital, Bandung, Indonesia. Subjects enrolled between November to December 2018 through consecutive sampling. Exclusion criteria were HBV coinfection, acute liver failure, and decompensated cirrhosis state. Frequency, distribution, and results were analyzed with Student t-test, Mann Whitney, or Chi-square where appropriate.

**Results:** One hundred sixty patients registered, 26 patients excluded. Of 134 patients included, HCV infected was 59 (44.0%). Baseline characteristic were normally distributed in both groups. There was significant increase in both APRI and Fib-4 score of HCV-infected group \((p=0.003)\) and \((p=0.003)\).

**Conclusion:** The HCV-infected rate in our dialysis center is noticeably high and liver fibrosis score was increase significantly in this group.
Th17 Cell Activation of T Lymphocytes in Peripheral Blood in Patients with Chronic Hepatitis Delta

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In progression of HDV infection cirrhosis develops 10 years earlier than with monoinfection of HBV (Moatter T, Abbas Z, 2007). The immune response has a significant participation in the ongoing damage, and progression from chronic viral hepatitis to cirrhosis, driving the activation and maintenance of main fibrogenic pathways. The aim of the study to investigate the percentage Th17 cells and the circulating cytokine profile.

Materials and Methods: A total of 105 patients with chronic viral hepatitis D and liver cirrhosis in the outcome of viral hepatitis D were examined. 70 people were included in the healthy group. All subjects underwent the following: general clinical study, blood test, the levels of cytokines (TNF-α, IL-12, IL-17, IL-10, TGFβ1) in the serum by ELISA (IBL (Germany), and Th17 in blood were investigated by flow cytometry (Monoclonal anti-CD3 FITC, anti-CD161-PE, anti-CD4- PerCP and anti-CD196-Alexa Flour ab (BD Biosciences, United States). The fibrosis stage was determined by elastometry (Fibroscan-502).

The results shown that the percentage of the Th17 cells in patients with CHD without cirrhosis significance higher compared with health control group (P = 0.037). We detected no difference regarding the percentage of Th17 cells between the group of different stages of fibrosis

The Results of the study of the level of signaling fibrogenesis molecules showed a significant increase of TNF-α (P = 0.009), IL-10 (P = 0.002), depending on the stage of fibrosis. Levels of IL17, IL12/23, and TGFβ1 also appeared to be elevated in patients with the F3-F4 fibrosis stage compared to patients with fibrous stage F0-F2, however, there is no significant change. The TNFα value has a positive statistically significant correlation with the level of ALT (r = 0.358, P < 0.0001), AST (r = 0.452, P < 0.0001) in the blood and negative with creatinine values (r = -0.396, P = 0.002), urea (r = -0.280, P = 0.019), platelets (r = -0.290, P = 0.005); and leukocytes (r = -0.342, P = 0.001). The activity of IL-10 in the has a positive statistically significant correlation with ALT level (r = 0.256, P = 0.004), AST (r = 0.380, P < 0.0001), bilirubin in blood (r = 0.194, P = 0.037), and negative with albumin values (r = -0.386, P = 0.005), creatinine (r = -0.389, P = 0.003), urea (r = -0.267, P = 0.026), platelets (r = -0.379, P < 0.0001); and leukocytes (r = -0.382, P < 0.0001). The value of IL-17 has a negative statistically significant relationship with the values of ALT and AST (r = -0.209, P = 0.021 and r = -0.249, P = 0.006).

Conclusion: The Th17/IL-17 axis has been found involved in several points of fibrogenesis chain from the activation of stellate cells to induction of imbalance between matrix metalloproteinases and tissue inhibitors of metalloproteinases (F.Ch.Paqquisi, 2017) Significantly higher level of IL17 in patients with CHD in whole group and on the initial stages of fibrosis (F0, F2) explains the more rapid progression of fibrosis and the formation of cirrhosis in patients with chronic HDV infection.

O-28
Soluble Siglec-7 as a Macrophage Activation Marker in Patients with Non-Alcoholic Fatty Liver Disease

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Background and Aims: Macrophage activation plays a key role in the liver disease progression of non-alcoholic fatty liver disease or steatohepatitis (NAFLD/NASH). Siglec (sialic acid-binding immunoglobulin-like lectins) are a family of immune regulatory receptors predominantly found on the immune cells. The impact of Siglecs on the pathogenesis of NAFLD is unknown. We aimed to clarify the significance of Siglec7 in NAFLD patients.

Methods: We enrolled 52 NAFLD patients (33 chronic hepatitis, 19 liver cirrhosis) and 15 healthy donors (HD). We measured sSiglec-1, -7, -9, and macrophage activation markers (sCD163, YKL-40) in the sera. Huh7, HepG2, LX-2 cell lines, primary fibroblasts, and monocyte-derived macrophages (MDMs) and PBMCs were cultured with or without LPS or inflammatory cytokines.

Results: Serum sSiglec-7 levels were significantly higher in NAFLD patients than HD. Serum sSiglec-7 levels were positively correlated with FIB-4, sCD163, YKL-40, but inversely correlated with albumin, PT, ChE. Soluble Siglec-7 levels had no correlation with ALT levels. MDMs produced large amounts of sSiglec-7 with the stimulation of LPS, TNF-α, or IL-1β, which were positively correlated with Siglec-7 expression on MDMs. NK cells and monocytes expressed high levels of Siglec-7 but produced small amounts of sSiglec-7, which was not increased by LPS, TNF-α, or IL-1β. Siglec-7 was not expressed on Huh7, HepG2, LX-2 cells and primary fibroblasts. These results suggest that activated macrophages are main source of sSiglec-7 in NAFLD patients.

Conclusions: Soluble Siglec-7 could serve as a surrogate marker of a macrophage activation in the progression to liver cirrhosis in patients with NAFLD.
Multi-region Whole-genome Sequencing on Hepatocellular Carcinoma with Nodule-in-Nodule Feature Reveals Clonal Evolution of Cancer Cells

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Background: Recent comprehensive genetic analyses have revealed the heterogeneity of genetic aberrations in HCC. However, it is still unclear how genetic alterations contribute to a multistage progression of HCC.

Methods: We examined five cases with hypovascular early HCC with hypervascular foci, so called nodule-in-nodule HCC. Multiregional sampling was performed both from inner hypervascular region (IN) and outer hypovascular region (OUT), together with noncancerous liver tissue and lymphocytes as controls. Comprehensive analysis on somatic mutations and chromosomal structural variations were performed by whole-genome sequencing using HiseqX platform.

Results: On average, 4,329 point mutations, 132 indels and 40.7 structural variants per each tumor region were detected. The number of coding and nonsynonymous mutations did not differ between IN and OUT samples. TP53 nonsense mutations were shared in both IN and OUT samples with relatively high variant allele frequencies. Of note, “chromothripsis”, a phenomenon of extensive genomic rearrangement, was detected as a truncal event in two cases. In HBV-positive case, HBV polymerase gene was integrated into TERT promoter region and TERT mRNA level was significantly elevated in both IN and OUT samples. The integrated site was identical in both samples, suggesting HBV integration and telomerase reactivation were the early event of tumor development. In contrast, PTEN missense mutation was found as the branch mutation, indicating the aberration of mTOR pathway might be related with hypervascular transformation.

Conclusion: Multi-region whole-genome sequencing uncovered the truncal and branch genetic aberrations in the identical HCC, which might be helpful to understand the process of multi-step hepatocarcinogenesis.

Plasma Cell-Free RNA Profiles Distinguish Healthy, Liver Cirrhosis and Cancer Patients

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Liver cancer is the sixth most common cancer and the second leading cause of cancer mortality worldwide with approximately 850,000 new cases and 800,000 deaths per year. It is difficult to treat and lethal if not caught early with the median survival of less than 4 month for stage D patients. This is mainly due to the low sensitivity and inconvenience of current diagnostic methods, ultrasound and AFP blood level with approximately 63% - 73% sensitivity, to predict and monitor the disease and its high-risk population, liver cirrhosis.

Human plasma contains a wide range of cell-free RNA species including small RNA, messenger RNA, long non-coding RNA and circular RNA. The initiation and development of a tumour can alter expression profile at both tumour sites including cancer cells and the microenvironment, as well as distant cells such as cells of the immune system. As a result, the cell-free transcriptome represents both amplified signals from the systemic reaction from the body to a tumour and information about the tissue of tumour origin.

We performed cell-free miRNA and total RNA sequencing to examine the potential of both miRNA and mRNA to distinguish three groups: Liver cancer, Liver cirrhosis and normal controls. Using a pilot patient sample set, we demonstrated that cell-free RNA profiles distinguish three groups. We found a set of markers whose combination yields classification models that differentiate liver cirrhosis from normal control and liver cancer from cirrhosis with high accuracy.
Increased Expression of a Disintegrin and Metalloproteinase 9 (ADAM9) in Advanced Hepatocellular Carcinoma (HCC) and its Role of HCC Immunotherapy

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Background/Aims: MHC class I-related chain A (MICA), a ligand for natural killer (NK) group 2 member D (NKG2D, a stimulatory receptor on NK cell), is expressed in human hepatocellular carcinomas (HCC). ADAMs are membrane anchored proteins. ADAM9 is involved in ectodomain shedding of MICA, and it is an important mediator of invasion and metastasis of HCC. This study aimed to investigate roles of ADAM9 during HCC immunotherapy.

Methods: Quantitative RT-PCR was performed to measure the expression of ADAM9 mRNA using blood samples from 10 HCC patients (mean age 57) and matched healthy controls. The HCC patients comprised 8 chronic hepatitis B (CHB), 1 chronic hepatitis C (CHC) and 1 non-viral patients. Among those, 4 patients (2 CHB, 1 CHC, 1 non-viral HCC) were treated with nivolumab more than 2 cycles, and the changes of ADAM9 mRNA were evaluated before and after the nivolumab treatment. Some of the 4 patients had been administered either sorafenib or regorafenib before nivolumab therapy.

Results: The mean level of ADAM9 mRNA in the 10 HCC patients was significantly higher than that of healthy controls (p < 0.05). Regarding the 4 patients treated with nivolumab, the serum level of ADAM9 mRNA significantly decreased (p < 0.05) in 2 patients (1 CHB and 1 non-viral HCC) who had tumor response, whereas the other 2 patients (1 CHB and 1 CHC HCC) who did not have tumor response showed no significant decrease.

Conclusion: Decreased expression of ADAM9 was significantly associated with clinical response to nivolumab therapy. ADAM9 may serve as a biomarker predicting clinical response and as therapeutic target of HCC immunotherapy.

Milk Fat Globule-EGF Factor 8 (MFG-E8) as an Early Diagnostic and Postoperative Prognostic Biomarker in Patients with Hepatocellular Carcinoma

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Background: Early diagnosis of hepatocellular carcinoma (HCC) results in improved prognoses for HCC patients. We aimed to clarify whether serum MFG-E8 can serve as a diagnostic or prognostic biomarker of HCC.

Methods: Serum MFG-E8 levels of 282 HCC patients, including 185 HCC patients who underwent primary hepatectomy, were examined by ELISA. We also quantified serum MFG-E8 levels in patients with chronic hepatitis (CH), liver cirrhosis (LC), as well as in healthy volunteers (HVs).

Results: Serum MFG-E8 levels were significantly lower in HCC patients than in HVs regardless of the etiology of liver disease (3.6±0.1 vs 5.8±0.2 ng/mL, p<0.0001), and recovered after treatment of HCC. Serum MFG-E8 levels in CH and LC patients were comparable to those of HVs. Serum MFG-E8 was superior to α-fetoprotein or des-γ-carboxy prothrombin (DCP) in discriminating HCC patients from CH and LC patients. Our new HCC prediction model using MFG-E8 and DCP (Logit(p) = 2.061 – 0.7 × serum MFG-E8 – 0.0239 × serum DCP) distinguished HCC patients from CH and LC patients with an area under the ROC curve (AUC) of 0.925, a sensitivity of 78.3%, and a specificity of 92.6%. Furthermore, low preoperative serum MFG-E8 was an independent predictor of poor overall survival. Levels of serum extracellular vesicles (EVs), which can bind MFG-E8 via phosphatidylserine, were significantly decreased in HCC patients. Serum MFG-E8 levels were positively correlated with levels of EVs, which became undetectable in sera depleted of EVs.

Conclusion: Serum MFG-E8 could serve as a feasible diagnostic and prognostic biomarker for HCC.
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SIRveNIB: Selective Internal Radiation Therapy (SIRT) Sorafenib in Mongolian Patients with Hepatocellular Carcinoma

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Aim: To evaluate the efficacy of SIRT using SIR-Spheres yttrium-90 microspheres versus sorafenib in Mongolian patients with locally advanced HCC patients at BCLC stage B and C patients without extra-hepatic metastasis.

Methods: It was a subgroup analysis based on patients enrolled from the National Cancer Center, Mongolia in the SIRveNIB study. SIRveNIB was a multi-center, randomized trial in which eligible patients with locally advanced inoperable HCC was randomized (1:1) to either single injection of SIRT or Sorafenib (oral 400mg BD) and patients were followed up till progressive disease or unacceptable toxicity.

Results: 39 patients (20 SIRT, 19 Sorafenib) were enrolled from Mongolia. BCLC patients without extra-hepatic metastasis comprised 62% of patients, 18% had portal vein thrombosis, 85% were Child-Pugh A, 45% were hepatitis B and 30% were hepatitis C. Altogether 4 of 20 patients (20%) in the SIRT arm failed to receive the study therapy. Intention-to-treat analysis was carried out with the OS in the SIRT and Sorafenib arms being 9.2 and 15.6 months, respectively, (Hazard ratio [HR]0.95, p=0.889). Tumour response rate (TRR) was 10% and 0% (p=0.487) respectively. Time-to-tumor progression (TTP) was 6.2 vs 8.5 months (HR 1.01, p=0.971) and progression-free survival (PFS) 5.9 vs 8.5 months (HR 1.07, p=0.842 for SIRT and Sorafenib, respectively. At least one severe adverse event (≥3 grade) was found in 56% and 47% of patients in the SIRT and Sorafenib arms, respectively.

Conclusions: In this subgroup analysis of a single center which is part of a larger multi-center, randomized controlled trial, 20% of the patients assigned to the SIRT arm failed to receive SIRT. On intention-to-treat analysis, there was no significant difference in OS, TRR, TTP and PFS between the SIRT and sorafenib arms.

O-34

Prospective Validation of New Selection Criteria Considering Pre-transplant Body Compositions in Living Donor Liver Transplantation

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Background: We established new selection criteria for living donor liver transplantation (LDLT) considering pre-transplant body compositions. In the present study, we prospectively validated the usefulness of the criteria.

Methods: We evaluated pre-transplant skeletal muscle mass, muscle quality, and visceral adiposity using skeletal muscle mass index (SMI), intramuscular adipose tissue content (IMAC), and visceral-to-subcutaneous adipose tissue area ratio (VSR) for 277 patients who underwent adult LDLT between January 2008 and July 2016 in our institute. We investigated the impact of these three parameters on survival after LDLT. Based on the findings, we have implemented new selection criteria for LDLT since October 2016 and examined outcomes of 45 consecutive adult LDLT patients between October 2016 and November 2018.

Results: Overall survival rates of patients with low SMI, high IMAC, and high VSR (abnormal factors) were significantly lower than those with high SMI, low IMAC, and low VSR, respectively. Multivariate analysis revealed low SMI, high IMAC, and high VSR were independent risk factors for mortality after LDLT. One-year overall survival rate of patients with no abnormal factor, one factor, two factors, three factors were 98%, 78%, 60%, and 41%, respectively. Based on these findings, we have established new selection criteria for LDLT: 1) to exclude patients with three abnormal factors, 2) to perform perioperative nutrition and rehabilitation therapy. One-year overall survival after LDLT under new criteria was 98%.

Conclusions: We have first established and implemented new selection criteria for LDLT considering pre-transplant body compositions and validated the usefulness of the new criteria.
Serum GPNMB Level Increases in Patients with Acute Liver Failure

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Background and Aim: Glycoprotein Non-metastatic Melanoma protein B (Gpnmb) is expressed by macrophages associated with recovery of acute liver injury. We have shown that Gpnmb-positive macrophages infiltrating during recovery phase have high phagocytic activity and contribute to the balance between fibrosis and fibrolysis. In this study, we measured serum GPNMB levels in patients with acute liver injury.

Methods: Fifty-five patients with acute liver injury were enrolled. GPNMB levels were measured more than three times. The relationship between the maximum peak of GPNMB levels and clinical outcome was analyzed.

Results: The maximum peak of GPNMB levels were associated with the minimum peak of prothrombin activity \( r = -0.6446, p<0.001 \) but not with the maximum peak of alanine aminotransferase \( r=0.02302 \) levels. The maximum peak of GPNMB levels were significantly higher in patients with acute liver failure (diagnosed by prothrombin activity of 40% or less, \( p<0.0001 \)), subsequent development of hepatic coma \( p=0.002 \), and in patients who needed liver transplantation or died \( p=0.005 \).

Conclusion: Serum GPNMB levels increased in patients with acute liver failure and poor prognosis. GPNMB levels seems to reflect the magnitude of liver injury. This indicates that serum GPNMB may be a prognostic marker for acute liver injury.

Hepatic Intracellular Stress Responsible for the Development of HBV-Related Fulminant Hepatitis

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Background: Detailed mechanism of the development of fulminant hepatitis (FH) have not yet been clarified.

Methods: In HBs transgenic mice (HBs-tg) which retains HBs large protein (HBs-L) in the hepatocytes and known to develop FH after HBs antigen-specific CTL transfer, we examined the mechanism of hepatitis exacerbation by applying concanavalin A (Con A)-induced liver injury model to the mice.

Results: Pretreatment analysis for mRNA expression of cytokines/chemokines, coagulation factors, and cellular stress markers in the liver indicated that mRNAs of MCP-1 and CHOP, an endoplasmic reticulum (ER) stress marker, were increased compared with controls (non-transgenic littermates) without differences in those of other cytokines/chemokines and coagulation factors. Twenty-four hr after Con A administration, HBs-tg showed significantly higher ALT elevation, and histologically marked increase of apoptosis-like hepatocyte degeneration in the liver compared with controls. During first 3 hrs after Con A administration when the cytokines and coagulation factors responsible for Con A-induced liver injury, such as IFN-α, TNF-α, IL-6, plasminogen activator inhibitor-1 (PAI-1), tissue factor (TF), are known to be increased, expression of those mRNAs showed significant increase but without differences between HBs-tg and controls. On the other hand, high expression levels of CHOP mRNA in HBs-tg were maintained compared to controls even during this period.

Conclusions: From these results, it is suggested that HBV-infected hepatocytes are exposed to ER stress depending on the amount of intracellular viral antigen, like HBs-L, and that the ER stress induces fragility in the infected cells thereby leading to the development of FH.
Antibiotics-mediated Intestinal Microbiome Perturbation Aggravates Tacrolimus-Induced Glucose Disorders in Mice

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Both immunosuppressors and antibiotics are indispensable for transplant patients. However, the former increases risk of new onset diabetes, whereas the latter impacts intestinal microbiota. It is still unclear whether and how interaction between immunosuppressors and antibiotics alters the intestinal microbiota and thus leads to metabolic disorder of glucose. This study examined alterations of glucose and lipid metabolism and intestinal microbiota in mice exposure to tacrolimus with or without antibiotics. We found that tacrolimus-induced glucose tolerance was further aggravated by antibiotics, although they increased insulin secretion. Combined treatment resulted in exacerbated lipid accumulation in the liver. Tacrolimus-altered microbial community was further amplified by antibiotics administration, which was characterized as the reduction trend of tacrolimus on phylum Firmicutes, and its family Lachnospiraceae, genus Coprococcus. Analyses based on the metagenomics profiles revealed that antibiotics augmented the effect of tacrolimus on microbial metabolic function mostly related to lipid metabolism. The altered components of gut microbiome and predicted microbial functional profiles showed significant correlation with hepatic lipid accumulation and glucose disorders. In conclusion, antibiotics aggravated the effect of tacrolimus on microbiome and its metabolic capacities, which might contribute to hepatic lipid accumulation and glucose disorders. These findings suggest that altered microbiome by antibiotics can amplify the diabetogenic effect of tacrolimus, and could be a novel therapeutic target for patients.

H2-BI/HLA-G as a Regulator of the Inflammatory Process Following Partial Hepatectomy

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Human Leukocyte Antigen G (HLA-G) is a major compatibility complex class I antigen that has a role as an immunotolerance protein. HLA-G expression has been documented in a few tissues in physiological conditions. However, in inflammatory and autoimmune diseases, ectopic HLA-G expression exhibits distinctive effects which depending on the cellular reaction could be dangerous or beneficial for restoring the homeostasis. A previous report has shown through hepatic transcript profiling and a further molecular assessment that H2-BI (mouse homologue of HLA-G) is a likely candidate crucial for survival after partial hepatectomy and that it is induced by the Growth Hormone (GH). Using C57BL/6 mice with partial or total loss of GH activated pathways, a partial hepatectomy was performed to these mice and morphological and histological features of liver injury were assessed. Immune-related factors were also quantified using real-time PCR. Mice were subsequently treated with HLA-G protein through osmotic pump infusion or used mixed background mice to confirm the positive role of HLA-G in mice survival. Finally, RNAi constructs targeting H2-BI were delivered into C57BL/6 mice to test the effect of partial hepatectomy on inflammatory markers including fas, fas ligand, NK1.1, F4/80 and IFNγ. We identified H2-BI as a key immunotolerance protein involved not just in the process of liver regeneration but in the inflammatory reaction driven by immune mediators which are regulated by H2-BI expression. This study uncovers the possibility of using H2-BI/HLA-G as a potential treatment for early stages of liver disease or to avoid rejection following liver transplantation.
Bone Marrow Monocytes Dysfunction in Chronic Liver Disease

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Background and Aims: Monocytes and macrophage are very pleotropic in nature and show pro as well as anti-inflammatory nature in condition dependent manner, in chronic liver disease (CLD) condition monocytes dysfunction occurs and show proinflammatory nature, but status of BM monocytes is not known in CLD, Aim of the study to investigate the distribution and functional of monocytes.

Methods: BM aspirates collated as part of clinical investigation in CLD group (n=15) and as a Non cirrhotic portal hypertension control (NCPF) as Control (n=8). For phenotyping, done by using CD14 CD16 and HLA-DR For M2 macrophage we used marker CD14+/CD163+,CD1206+,MitoSOX are used for mitochondrial reactive oxygen species (ROS). For phagocytosis we used FITC labeled E.coli, LPS stimulation (10 ng/ML) was given 24 hrs. to study functional nature of monocytes. Bactrial killing assay was done with E.coli K12, Stratagene species.

Result: FACS data show total number of CD14+ cells as well as classical non classical and intermediate monocytes all are less in CLD is significantly low (p<0.0001) in CLD Expression of HLA-DR show lower expression of in CLD patient (p<0.01).Phagocytosis of CD14+ monocytes is high in NCPF than CLD patient (P=0.0051).Mitochondrial ROS level is high in NCPF than CLD (P=0.0041),biomassfunctional mitochondrial is high in control in control than CLD,NCPF patient show more scavenger receptors (CD163) (p< 0.0001),And Bacterial killing nature decrease in CLD. Upon LPS stimulation CLD monocytes TNF alpha, IL-8, IL-6, IL1 beta gene level was compromised in than NCPF.

Conclusion: In CLD condition monocytes became priming in niche and they are already lost their most of function.
APASL Single Topic Conference 2019 in Tokyo

“Liver Immunology and Genetics”

Abstracts
Poster Free Papers
P-001
Hepatic Arterial Infusion Chemotherapy with Reservoir for Advanced Hepatocellular Carcinoma, Results of Under Indication Limit

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Background: From 2000 to 2014, 1026 patients with unresectable advanced hepatocellular carcinoma (HCC) who underwent hepatic arterial infusion chemotherapy with reservoir were performed. Good prognosis is expected in Child A, B and no case of intractable ascites. Since 2016, we established a standard for indication of HAIC was not enforced for Child C and refractory ascites, but only one shot infusion chemotherapy.

Purpose: We retrospectively examined the therapeutic effect of HAIC (Low dose FP) satisfying the adaptation criteria.

Method: Between 2011 and 2016, HAIC was performed in 211 cases. Treatment effect was evaluated for each course, and 2 to 4 courses of treatment until for PD were continued. Median survival time (MST) stratified liver function, response rate, survival rate by response (Kaplan - Meier method) were compared. And we compared with the results before the limit of adaptation.

Results: HAIC performed for 211 cases (169 males), average age 66.3 years (30-82), median observation period 11.2 months, Patient background HBV / HCV / nonBnonC is 45/100/68 cases, Child A / B is 125/86 cases, portal vein tumor invasion (VP 0/1/2/3/4) is 47/5/ 39/64/56 cases, extrahepatic metastases in 37 cases. MST was 9.1 months (6.9 months before adaptation restriction), MST in CR was 28.7 months, PR 12.1 months, SD 8.6 months, PD 5.6 months. The response rate was 29.3% (29% before the restriction of adaptation), 31 cases (15%) in CR, 31 cases in PR (15%), 46 cases in SD (22%), 103 cases in PD (49%).

Conclusion: Response rate did not change by restricting therapeutic indications, but MST was prolonged for 2 months and better results were obtained than before.

P-002
The Analysis of Genetic Mutations Related to Expression of Receptor Tyrosine Kinases of Hepatocellular Carcinoma from TCGA Data

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Background: Receptor tyrosine kinases (RTKs) are main targets for the treatment of cancers. Inhibitors of RTKs, including VEGFR, PDGFR, and FGFR, have been developed as the molecular targeted agents for hepatocellular carcinoma (HCC). Recently, exhaustive analysis of genetic mutations in HCC has been carried out due to the development of sequencing technology, and the results are reported. In this study, the relationship between gene expressions of RTKs and genetic mutations was examined using data of TCGA (The Cancer Genome Atlas).

Methods: Pretreated data of genetic mutations and gene expression from 364 HCC cases were downloaded from the GDAC Firehose browser (https://gdac.broadinstitute.org) and analyzed with software R. The mutations of 4,731 genes were observed in more than four cases. Differences in expression of RTKs were analyzed between with and without each mutation of 4,731 genes by t test. The 34 RTK genes included EGFR, ERBB2, ERBB3, INSR, IGF1R, PDGFRA, PDGFRB, CSF1R, KIT, FLT1, KDR, FLT3, FLT4, FGFR1, FGFR2, FGFR3, FGFR4, PTK7, MET, MST1R, NTRK1, NTRK2, NTRK3, AXL, MERTK, TYRO3, TIE1, TEK, ROS1, RET, ALK, ROR1, ROR2.

Results: Among 4,731 mutations, the beta-catenin mutation influenced most significantly on the expression of 13 RTKs, including IGF1R, PDGFRA, PDGFRB, FGFR1, FGFR2, PTK7, MST1R, NTRK2, AXL, MERTK, TYRO3, RET, and ROR2 (p < 10^-4). Especially, the expression of FGFR2 were significantly suppressed in the beta-catenin mutated cases (p < 10^-38). The HNF1A mutation affected the expression of FGFR4 and ROS1 most significantly, and analyzing the relationship between the expression of FGFR4 and HNF1A mutation, p-value was remarkably low (p<10^-24). Between the cases with and without p53 mutation, FLT1, KDR, FLT4, TIE1, and TEK expressed most differentially (p<10^-4). These RTKs expressed in vascular endothelial cells rather than cancer cells. This result suggested that p53 mutation might affect the proportion of the cells consisting the tumor tissue. Conclusion: This study suggested that the gene mutations might affect the RTK expressions of not only cancer cells, but also normal cells in tumor microenvironment of HCC.
P-003

Study on Validity of Biomarkers DKK1 and HBx-LINE1 in Diagnosis and Posttreatment Monitoring of Hepatocellular Carcinoma

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Objective: Evaluate validity of DKK1 and HBx-LINE1 in diagnosis and post-treatment of HCC/HBV and analyse relationship with clinical and paraclinical some characteristics.

Subject and methods: Study on 114 HCC patients at 108 Military Central Hospital, 103 Military Hospital and 175 Military Hospital from 1/2016 to 3/2018 with DKK1 and HBx-LINE1, re-examination after surgery.

Results: With DKK1 ≥2.15 ng/mL, the positive rates of serum protein DKK1 were significant increased when compared with those of AFP (97.37% so với 62.92%). The mean of serum protein DKK1 of HCC was significant higher than it in liver cirrhosis patients with p<0.05. Combination between AFP and DKK1 expression will improved positive rates and help more diagnosis in 12.3% of HCC cases. Using combination of AFP, serum protein DKK1 and DKK1 expression may help diagnosis for negative AFP of HCC cases. Logistic regression analysis showed the risk of HCC will increased about 18.5 times when DKK1 ≥2.15 ng/mL. The level of DKK1 expression reduced in patients with ≥2 tumors when compare with one tumor and in cases with size ≥5 cm when compare with <5 cm. This correlation was the same with reducing of AFP related with quantity and size of tumor. The level of DKK1 expressions reduced in posthepatectomy when compare with those before surgery and in time of more than one year when compare with those from 1 to 12 months. We did not identify HBx-LINE1 fusion transcript in all 114 (100%) HBV-related HCC patients.

Conclusion: Biomarkers serum protein DKK1 and DKK1 expression have validity in diagnosis and post-treatment of HCC, especially for AFP-negative patient. HBx-LINE1 fusion transcript was not identified in our study.

Keywords: Hepatitis B virus, hepatocellular carcinoma, biomarker DKK1, biomarker HBx-LINE1.

P-004

Spiral CT in the Clinical Significance of Portal Cavernous Change in Hepatocellular Carcinoma

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Background: Cavernous degeneration of portal vein (cavernous transformation of portal vein,CTPV) refers to the formation of a large number of collateral branches or recanalization after complete or partial obstruction of the main portal vein or its branches. It is a compensatory disease to ensure the liver blood flow and liver function.

Methods and Results: 88 cases of hepatocellular carcinoma with portal cavernous change were enrolled. The abdominal CT data were analyzed, including blood vessel cross-sectional features, hepatic parenchyma perfusion, CT findings and features of portal hypertension.

The main manifestations of CT were several small blood vessels around the portal vein in the occlusion position, the heavy ones were beaded, and the hepatic parenchyma was transient perfusion abnormality in the arterial phase, showing a zonal high-density shadow around the liver, the main findings were as follows: At the arterial stage, the hepatic parenchyma was abnormal. In the portal phase, the whole liver showed uniform density. In cirrhotic patients with portal hypertension, collateral circulation vessels (27/88), ascites (18/88), splenomegaly (45/88) were seen around the hilum of the spleen.

Conclusions:Spiral CT dual phase scanning is an effective method for diagnosis of portal cavernous degeneration of liver cancer, which can provide necessary basis for clinical treatment and prognosis.
P-005

Association Between Type-2 Diabetes Mellitus and Platelet Distribution Width in Patients with Primary Liver Cancer

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Background: To determine the association between type-2 diabetes mellitus (T2DM) and platelet distribution width (PDW) in patients with primary liver cancers (PLC).

Methods and Results: We included 576 patients with a hospital discharge diagnosis of PLC treated in the Guangdong General Hospital from January 2000 to January 2017 in this investigation. Demographic and clinical characteristics data were analyzed. The multivariate logistic regression model was used to determine the association between T2DM and PDW in PLC patients. Of the total 576 patients, 103 (17.9%) cases were diagnosed with T2DM, the mean hemoglobin was (122.7±18.8)g/L, the median/interquartile range of PDW was 15.2%(11.1%-17.3%). A statistical difference was found by univariate analysis for the PDW [11.0%(8.6%-12.4%) vs. 16.7%(15.4-18.0%), P=0.037]. The two groups, with statistical difference (OR=0.437, 95%CI: 0.236-0.638, P=0.04) was found by the multivariate analysis, after controlling the age, HBV infection, hemoglobin.

Conclusions: PLC patients with T2DM have obviously decreased PDW, compared with those without T2DM, which is modified by other factors.

P-006

The Role of Interferon-Induced Transmembrane Protein-3 in Progression of Hepatitis HBV Related HCC in China

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Background and Aims: Interferon-induced transmembrane protein 3 (IFITM3) has been recognized as a key signal molecule regulating cell growth in some tumors such as gastric carcinoma, colon cancer and lung cancer, and it plays different role in different tumors. However, the function of IFITM3 in the hepatocellular carcinoma (HCC) related with hepatitis HBV remains unknown. Our study investigated the role of IFITM3 in HCC progression in China.

Methods: Forty-eight liver specimens including 15-pair hepatoma tissues and self paired normal liver tissues were surgically obtained between June 2016 and May 2017 at the Beijing You’an Hospital, Capital Medical University (Beijing, China). IFITM3 expression in hepatic tissues of HCC patients were performed by immunohistochemically staining technique and Quantigen PLEX 2.0 assay. To investigate the function of IFITM3 in HCC cell lines, LV-IFITM3 vector lentiviral constructs were modified to knockdown IFITM3 in PLC/PRF/5 cell line. Using Cell Counting Kit (CCK)-8 and wound-healing test assessed the effect of IFITM3 of tumor cell lines proliferation and migration ability. RNA-seq technology was used to identify the IFITM3-related signaling pathways which was further verified by Western blot analysis.

Results: 1. IFITM3 gene regulates HCC cell proliferation and migration
The IFITM3 gene knockdown in PLC/PRF/5 cell line transfected LV-IFITM3 vector lentiviral constructs was confirmed by western blot and RT-qPCR analysis. The function of the proliferation and migration was decreased in IFITM3 knockdown PLC/PRF/5 cells. Vimentin level which is a marker of mesenchymal was decreased in IFITM3 knockdown PLC/PRF/5 cells. That suggested IFITM3 may regulate the migration of the hepatic cell lines by vimentin.

2. RNA sequencing and the verification of RT-PCR
DEGs between the LV-NC and the LV-IFITM3 were screened by using NOIseq. A total of 891 significantly upregulated genes and 3998 downregulated genes compared with the LV-NC were screened under the criteria of a minimum 2-fold-change and a P value < 0.001. By KEGG enrichment, we found out that PI3K-AKT signaling pathway which played an essential role in regulating EMT of HCC progressing had larger number of DEGs (9 genes). The expression of PI3K and mTOR was downregulated (P<0.01) after IFITM3 knockdown. The relationship between IFITM3 and PI3K/AKT/mTOR signal pathway as figure2C.

3. IFITM3 expression in hepatic tissues of HCC patients
we firstly examined the expression of IFITM3 protein in 15 pairs of hepatic tissues compared with adjacent normal hepatic tissues. The date showed that IFITM3 was strong positive staining in the HCC tissues, whereas mildly weak staining in the adjacent normal tissue. The IFITM3 IOD value was higher in hepatoma tissues than adjacent liver tissues (P<0.05).

4. IFITM3 rs12252-CC genotype was associated with HCC progression by increasing IFITM3 level
A significant upregulation of IFITM3 expression was observed in liver tissues with IFITM3-CC genotype compared with the IFITM3-TT. The expression level of IFITM3 RNA was higher in low pathological differentiation of HCC and tumor metastasis. The proportion of IFITM3 rs12252 CC genotype increased gradually from high to low differentiation of hepatocellular carcinoma (10.3% - 30.9% - 38.8%). The proportion of TT genotype decreased with the differentiation of HCC (28.2% - 17.6% - 10.2%). The relative risk of poorly differentiated HCC in patients with this genotype is 5.542.

Conclusion: IFITM3 plays a vital role in the progress of HCC through regulating HCC cell proliferation and migration by PI3K/AKT/mTOR signaling pathway. The HCC patients with IFITM3 rs12252 CC genotype has a risk on poor differentiation tumor and trend of metastasis.

Key Words: HCC, IFITM3, PI3K, AKT, mTOR, tissue differentiation
P-007
The Characterization of Genetic Alterations Using Whole-Genome Sequencing Data in HBV-Related Hepatoma Cell Line

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Background/Aim: The chronic infection with hepatitis B virus (HBV) is one of the biggest primary risk factors for the development of hepatocellular carcinoma (HCC). Recent advances in next generation sequencing (NGS) technologies have enabled complete analyses for the genetic alterations at single nucleotide resolution. Moreover, the emergence of databases such as OncoKB (JCO PO 2017), in which the curated information about the effects of specific cancer-related gene alterations is collected, make it easier to understand the significance of genetic alteration in cancer genomes. In this study, we tried to characterize the genetic alterations in HBV-related hepatoma cell line using NGS data and OncoKB.

Methods: 1) We performed the whole-genome sequencing (WGS) on Hep3B cells, a hepatoma cell line established from HBV-related HCC, and investigated the genetic alterations such as single nucleotide variant (SNV), insertion/deletion (Indel), and copy number variation (CNV).
2) We explored the significant alterations which led to gain- or loss-of-function of cancer-related genes according to OncoKB classification.

Results: 1) The genetically homozygous alterations occurred at approximately 3,700,000 SNVs and 890,000 Indels through the whole genome. Regarding CNV, the high-grade gain regions were found in 11q13.
2) According to OncoKB classification, 30 cancer-related gene alterations were found. Among these alterations, 23 genes were led to gain-of-function, and 7 genes were led to loss-of-function.

Conclusion: Using WGS data on HBV-related hepatoma cell line, we identified 30 cancer-related gene alteration. The identified cancer-related genes in this study might be attractive targets in the treatment of HBV-related HCC.

P-008
Sorafenib-Regorafenib Sequential Therapy in Japanese Patients with Hepatocellular Carcinoma in Real World Practice Including the Early Experiences of Lenvatinib as a 3rd-Line Agent

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Background and Aim: In Japan, regorafenib has been approved since June 2017 and lenvatinib has been used as a new molecular-targeted agent since March 2018. We investigated the clinical outcome of regorafenib therapy including the early experiences of lenvatinib as a 3rd-line agent.

Methods: This study contained 30 patients treated with regorafenib at our institution. Overall survival (OS), progression free survival (PFS), and radiological response evaluated by mRECIST criteria were analyzed.

Results: The median age was 74 years old, 28 patients were Child-Pugh A, and 17 patients were BCLC stage C. Median observation time was 8.1 months and 13 patients died from HCC progression. Median OS and PFS were 12.6 months and 5.1 months. Median OS from the beginning of sorafenib was 38 months. Tumor response was evaluated in 29 of 30 patients. CR in 0, PR in 5, SD in 17, and PD in 7 patients. ORR and DCR were 17.2% and 75.9%. There was no significant difference in OS and PFS between the initial dose-modified group (n=24) and non-modified group (160mg/day, n=6). In 16 of 20 patients who stopped regorafenib, post-regorafenib anticancer therapies were performed and 9 patients received lenvatinib. Based on mRECIST, CR was shown in 1 ,PR in 3 , SD in 3 ,and PD in 1 patient. The ORR and DCR were 44% and 78%.

Conclusions: The clinical outcome of regorafenib in real world practice was similar to the RESORCE trial. Eighty percent of the patients could receive anticancer therapies after regorafenib. In lenvatinib treatment as 3rd-line, the radiological responses including ORR and DCR were equivalent to the REFLECT trial.
**P-009**

**Immunomodulation After Radiofrequency Ablation of Hepatocellular Carcinoma**

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**Objective:** Most patients with hepatocellular carcinoma (HCC) are not eligible for radical surgery due to poor liver function or late disease status. Radiofrequency ablation (RFA) has been demonstrated to be feasible and safe with a positive impact on survival. The aim was to investigate whether an immune reaction is activated after ablation.

**Methods:** Peripheral Blood samples of HCC-bearing rats were obtained before RFA and on post-operative days 1, 3 and 28. Evaluated parameters were: cells CD4+, CD8+ and activated subsets, T-Reg, Monocytes, myeloid Dendritic cells (DC) and cytokines [Interleukin (IL)-6, IL-1b, Tumor-Necrosis Factor (TNF)-alpha, Interferon (IFN)-gamma, Vascular Endothelial Growth Factor (VEGF), chemokine (C-C motif) ligand 5 (CCL-5), Transforming-Growth Factor (TGF)-beta].

**Results:** CD4+, CD8+ and CD3+ increased from day 3 suggesting the activation of the adaptive response. Immunosuppressive T-Reg cells were decreased despite the possibility that heating might favor their expansion until day 28. Myeloid DCs, that present tumor-associated antigens, increased on day 28. RFA dramatically increased circulating IL-6 and IFN-gamma on day 1 and 3 but this decreased to baseline by day 28, consistent with the supposed anti-tumor effect. RFA did not significantly modulate essential chemokines, such as CCL-5, VEGF, TGF-beta and TNF-alpha.

**Conclusions:** This study provides the evidence of RFA-based immunomodulation in HCC. We observed a general activation of adaptive response along with a decrease of immunosuppression. Furthermore, most cells showed prolonged activation some weeks after the procedure, suggesting true immunomodulation rather than a normal inflammatory response.

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**P-010**

**Sensitive Detection of Circulating Tumor Cells in Patients with Chronic Liver Disease and Hepatocellular Carcinoma Using a Microcavity Array**

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**Background:** As hematogenous spread is the major route of metastasis and recurrence in patients with hepatocellular carcinoma (HCC), detection of circulating tumor cells (CTCs) has important clinical significance. The aim of this study was to establish a novel CTC isolation strategy using a microcavity array (MCA) system and evaluate the clinical implication of CTCs in patients with chronic liver disease and HCC.

**Methods:** To enrich CTCs from whole blood, a fabricated filter with a MCA was integrated with a miniaturized device. The shape and porosity of the MCA were optimized to efficiently capture tumor cells on the microcavities under low flow resistance condition, while allowing other blood cells to effectively pass through. We defined cells characterized by immunofluorescent intensities that were positive for cytokeratin and DAPI and negative for CD45 as CTCs. To validate the performance of MCA system, we examined the recovery rate of the tumor cells and mRNA expression of the genes in the cells captured on the filter by qPCR after preamplification of the cDNA using HCC cell lines (HepG2, HuH7 and PLC/PRF/5). The examined genes were AFP, glypican 3 (GPC3), EpCAM, albumin (ALB) and GAPDH.

Peripheral blood samples were processed by MCA assay from 7 healthy donors, 14 patients with liver cirrhosis (LC) without any cancers and 31 patients with HCC. In 11 patients with HCC, we investigated mRNA expression in the same way by qPCR.

**Results:** The average recovery rates of HepG2, HuH7 and PLC/PRF/5 cells using the MCA system were 65%, 77% and 99%. Furthermore, mRNA expression of five genes was detectable by qPCR from very few HCC cells (ten HCC cells) spiked into 3mL whole blood in almost all HCC cell lines. In the samples of HCC patients, positivity rate was 90.3%(28/31) and the mean number of detected CTCs was 47.6±114.1. The number of CTCs were significantly increased in patients with metastatic HCC (102.2±160.6) compared to localized HCC (8.2±7.7) (P<0.05). The cumulative survival for ≥10 CTC patients was significantly shorter compared to <10 CTC patients (P<0.05). In 13 HCC patients, mRNA detection rate of AFP, GPC3, EpCAM and ALB was 15.4%(2/13), 7.7%(1/13), 15.4%(2/13) and 46.2%(6/13) respectively. In each group including LC, Localized and Metastatic patients, mRNA detection rate of ALB was 20%(1/5), 14.3%(1/7) and 83%(5/6).

**Conclusion:** The MCA system has a new potential to isolate CTCs at high sensitivity and analyze mRNA expression from CTCs in HCC patients. The results suggest that the MCA system may provide a predictive tool for poor prognosis in HCC patients and a new strategy to analyze mRNA expression for precision medicine.
A man in his seventies with hepatitis C virus (HCV) infection underwent anti-viral drug treatment from February to May 201X. HCV infection recurred after a month. In November 201X, a known benign tumor in segment (S) 5 had increased in size and hepatocellular carcinoma (HCC) was suspected. The man was referred to our hospital. Imaging studies showed 35, 27, 18 and 16 mm tumors in S5, S7, S8 and S4, respectively. The S5 and S8 tumors were considered typical HCC. The S7 tumor differed from typical HCC because of unusually poor uptake in the hepatobiliary phase. The S4 tumor was considered a vascular anomaly without a typical HCC wash-out pattern. AFP and PIVKAII were negative. Liver function tests conducted were Child–Pugh A and Liver Damage B. In February 201X+1, a left lobectomy and partial resection of S5 and S7 were performed. The patient was discharged from hospital on postoperative day 27. No recurrence was diagnosed for 10 months. Histopathological examination revealed the S7 tumor was a poorly differentiated adenocarcinoma with sarcomatous change. The S4 tumor was a scirrhous HCC with a cholangiole component. The S5 tumor was an early HCC. The S8 tumor was a high-grade dysplastic nodule. Liver fibrosis was severe (Inuyama, F4). The relationship of tumors with anti-viral therapy needs further investigation. We report this case as an interesting native model of a degenerated and precancerous state of cirrhotic liver suggestive of multicentric tumorigenesis and multipotent differentiation patterns.

Melatonin, a hormone secreted by the human pineal gland, plays a key role in regulating the sleep-wake cycle. Recent studies have proven that melatonin has anti-cancer activity against liver cancer and many other types of cancer; however, the detailed regulatory mechanisms remain unclear. The present study investigated the regulatory role of noncoding RNAs in the anti-liver cancer mechanism of melatonin. A liver cancer cell line was treated with melatonin, and functional analysis was then performed to determine its influence. To investigate the noncoding RNAs that may participate in the anti-liver cancer mechanism of melatonin, whole-transcriptome sequencing analysis was performed; reverse transcription-PCR (RT-PCR) and western blotting were utilized to explore the regulatory role of noncoding RNAs. The results indicate that melatonin could significantly inhibit cell growth, migration, and invasive properties. It also reduced the expression of long noncoding RNA CCAT1, which functions as a sponge for the miRNA let-7i-3p, thereby increasing the let-7i-3p level and consequently inhibiting RAF-1 protein translation, and activation of the downstream MAPK signaling pathway. Inhibition of tumor growth by melatonin through regulation of these molecules was also proven in an animal model. Thus, the present study is the first to report that melatonin can achieve anti-liver cancer effects by inhibiting the expression of long noncoding RNA CCAT1, and proves the potential of melatonin application in liver cancer treatment.
**P-013**

**Investigation of ARID Family Gene Mutation in Hepatocellular Carcinoma**

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**Background:** Frequent mutation in ARID family including ARID1A, ARID1B and ARID2 has been reported in a wide range of cancers. In this study, we assessed the biological significance of ARID mutation in hepatocellular carcinoma (HCC).

**Methods:** We analyzed genome data and clinical information of 361 TCGA liver cancer dataset using UCSC Xena Browser. Number of somatic mutations (short nucleotide variations and short Indels with amino acid substitution) per 1Mb was defined as tumor mutation burden (TMB). Expression levels of mismatch repair (MMR) genes (MSH2, MSH6, MLH1, and PMS2), TMB, and overall survival (OS) were compared between wild type (WT) and mutated type (MT) of each ARID gene. Furthermore, the relationship between TMB and OS was also analyzed.

**Results:** Mutations in ARID1A, ARID1B and ARID2 were found in 7.76%, 3.88%, 5.54% cases, respectively. The expression levels of MMR genes except for MSH6 were significantly lower in ARID1B MT compared with WT, but there was no significant difference for ARID1A and ARID2. Longer OS and higher TMB was observed in WT than in MT for any ARID. In patients with TMB≥4 (13.6%), OS was significantly shorter than patients with TMB<4 (median survival 601 vs 2110 days).

**Conclusion:** ARID mutation is closely associated with survival and TMB in HCC. Considering that immune checkpoint inhibitors (ICIs) exhibits favorable treatment effect in patients with high TMB tumors, ARID-mutated HCC might be a good indication for ICIs.

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**P-014**

**Autophagy of Hepatic Stellate Cells Promotes HCC Progression**

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**Background:** Autophagy in hepatoma cells promotes tumor growth. On the other hand, the effect of autophagy in hepatic stellate cells (HSCs) on hepatocellular carcinoma (HCC) growth has not been fully clarified.

**Methods:** HepG2 cells were used as human hepatoma cells and LX2 cells as human HSCs. HSC-specific Atg7 knockout (HSC-Atg7 KO) mice were generated by crossing GFAP-Cre mice and Atg7 fl/fl mice.

**Results:** After co-culturing LX2 cells with HepG2 cells, autophagy in LX2 cells was promoted with increase of IL-6 expression and cell viability of HepG2 cells was increased. siRNA-mediated knockdown of Atg7 in LX2 cells decreased IL-6 expressions. Co-culture with LX2 cells increased cell viabilities with STAT3 activation in HepG2 cells, which was attenuated by siRNA-mediated Atg7 knockdown in LX2 cells.

HepG2 cells, but not LX2 cells, were successfully engrafted into NOG mice. The growth of xenograft tumor of HepG2 cells in NOG mice was accelerated by co-transplantation with LX2 cells. The acceleration was suppressed by Atg7 knockout in LX2 cells.

Wild-type (WT) mice or HSC-Atg7 KO mice were administrated with streptozotocin at the age of 2 days followed by a high-fat diet feeding. At the age of 20 weeks, 100% of WT mice and 83% of HSC-Atg7 KO mice developed HCCs. Both the maximum size and the number of liver tumors were significantly suppressed in HSC-Atg7 KO mice with decrease in pSTAT3 expression levels of tumor lesions.

**Conclusion:** HSC autophagy, which induced by co-culturing with HCCs, increases IL-6 expression levels and promotes HCCs.
P-015
EpCAM-high Liver Cancer Stem Cells Show Resistance to Natural Killer Cell-Mediated Cytotoxicity Via CEACAM1 in Both in vitro and in vivo Models of Hepatocellular Carcinoma

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Introduction: Natural killer (NK) cells are the prototype innate lymphoid cells endowed with potent cytolytic function that provide host defense against microbial infection and tumors. However, there are conflicting results on the cytotoxicity of NK cells against cancer stem cells. In this study, we investigated whether NK cells exhibit enhanced or decreased cytotoxicity against Epithelial Cell Adhesion Molecule (EpCAM)-expressing liver cancer stem cells (CSCs) and the underlying mechanism of the phenomenon.

Methods: EpCAMhigh and EpCAMlow Huh-7 cells were sorted by flow cytometry. Human NK cells were isolated by magnetic sorting from peripheral blood mononuclear cells of healthy donors. NK cell and hepatoma cells were co-cultured and cytotoxicity assay were performed. Immunohistochemistry and western blot was performed using human HCC tissues obtained from surgical specimens. In vivo experiments were performed using Hepa1-6 mouse hepatoma cells and C57/BL6 mice.

Results: Patients with positive EpCAM expression in their tumors showed higher serum α-fetoprotein levels and histological Ki-67 expression. The frequency of early and massive recurrence was higher in patients with positive EpCAM expression in their tumors. Co-culture experiment demonstrated that EpCAMhigh Huh-7 cells resisted NK cell-mediated cytotoxicity. We have identified that carcinoembryonic antigen cell adhesion molecule 1 (CEACAM1) was upregulated on the surface of EpCAMhigh Huh-7 cells. Silencing of CEACAM1 restored cytotoxicity of NK cells against EpCAMhigh Huh-7 cells. Moreover, neutralizing CEACAM1 on NK cell surface also enhanced killing of Huh-7 cells, suggesting that homophilic interaction of CEACAM1 is responsible of attenuated NK cell-mediated killing of CEACAM1high cells. In vivo mice experiments with Hepa1-6 cells demonstrated that EpCAMhigh Hepa1-6 cells form larger tumors and show higher CEACAM1 expression after NK cell depletion. Neutralizing CEACAM1 in mice HCC model showed shrinkage of tumor. Tumor-infiltrating NK cells showed more activated phenotype after CEACAM1 blocking. Finally, we found that CEACAM1 expression positively correlated with EpCAM expression in human liver tissues at both mRNA and protein levels.

Conclusion: Overall, our data clearly demonstrate that EpCAMhigh liver CSCs resist NK cell-mediated cytotoxicity by upregulation of cell surface CEACAM1 expression.

P-016
Massive Hemothorax Caused by Diaphragmatic Vessel Injury after Radiofrequency Ablation for Hepatocellular Carcinoma

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Radiofrequency ablation (RFA) has been widely used as an alternative treatment to surgical resection for small hepatic tumors. Goto et al. reported that hemothorax was complicated in 0.3% percutaneous RFA treatments. The cause of hemothorax was mostly derived from the injury of intercostal arteries because of the upward tilt of puncture needle through intercostal route for ablation of right liver dome tumors. The hemothorax was usually self-limited and rarely required tube drainage or even thoracostomy. In the patient demonstrated below, massive hemothorax was related to the injury of diaphragmatic vessel during the procedure of RFA.

A 63-year-old woman was admitted due to her recurrent hepatocellular carcinoma with Barcelona Clinic Liver Cancer stage of A4. She had been treated on 6 previous courses with hepatectomy and radiofrequency ablation (RFA). The further application of percutaneous RFA via the intercostal approach for her recurrent tumor in segment VII of the liver was performed this time (Figure 1a). Several hours after the procedure, she experienced shortness of breath, chest tightness and low blood pressure. The plain film of chest demonstrated fluid accumulation in the right thorax (Figure 1b). Urgent chest tube insertion for thoracic drainage and blood transfusion was performed. Due to her blood loss from the chest tube more than 3000cc, she received further Video-Assisted Thoracic Surgery. One active bleeder over the diaphragm was found (Figure 2a, white arrow). Evacuation of blood clots and suture of the bleeder was done successfully (Figure 2b). The puncture hole of visceral pleura was also shown (Figure 2b, white arrow). Her lung was fully expanded later and she was discharged under stable conditions after the removal of chest tube.
P-017  
Effects of Regorafenib on the Toll-like Receptor Signaling Pathways in HCC

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**Background and Aims:** Oral kinase-inhibitor sorafenib is a first-line treatment for advanced hepatocellular carcinoma. Sorafenib-resistance is often seen, and innate immunity is involved in its mechanism. Regorafenib, an oral kinase-inhibitor, is also available for patients with sorafenib-resistance. We examined the effects of regorafenib on the innate immunity, including toll-like receptor (TLR) signaling pathway, in human hepatoma cell line.

**Materials and Methods:** (1) HepG2 cells were treated with or without regorafenib for 24 hours. (2) Cell viability was evaluated by MTS assay. (3) After cellular RNA was extracted, real-time RT-PCR-based arrays were performed to examine the effects of regorafenib on 84 TLR-associated signaling molecules. (4) Results with p < 0.05 were considered statistically significant using F-test and T-test.

**Results:**  
(1) Viability of HepG2 cells treated with or without 2μM for 24 h are 93.4% and 100%, respectively.  
(2) BTK, CXCL10, TLR2, TLR7, TLR8, LY86, IL2, IL10, IFNG, CSF2, CLECE4, and IFNB1 were equal to or more than 2-fold upregulated in HepG2 treated with regorafenib (n=3, p<0.05).  
(3) TNF was 4.4-fold down-regulated, and  
(4) IL6, FOS, PTGS2, TICAM2, TRAF6, NR2C2, MYD88, UBE2N, MAP2K4, CHUK, MAP3K7, and MAP4K4 were significantly down-regulated in HepG2 treated with regorafenib (n=3, p<0.05).

**Conclusion:** We previously reported that activation of AP-1 is involved in sorafenib-resistance. Of interest, regorafenib-treatment could lead to downregulation of FOS. It is possible that regorafenib may partly inhibit TLR signaling pathway and cytokine production. Further studies are needed regarding the association between molecular targeting drugs-resistance and innate immunity.

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P-018  
Alpha-Interferon Increases Methylation of Hepatitis B Virus DNA in Human Hepatoma Cells

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**Background:** Alpha-interferon (IFN-α) has been used for the treatment of chronic hepatitis B virus (HBV) infection, but the exact mechanisms of antiviral effect have not been elucidated at molecular levels. HBV covalently closed circular DNA (cccDNA) can be methylated in liver tissues of chronic hepatitis B patients and cccDNA methylation is associated with suppression of viral transcriptional activity. We tried to test the hypothesis that INF-α may induce methylation of HBV cccDNA, which in turn affects transcriptional activity of HBV cccDNA in a cell-based model.

**Methods:** HepG2 human hepatoma cells were infected with HBV viral particle and were treated with INF-α for 2 days. Methylation of HBV covalently closed circular DNA was assessed by methylation-specific PCR, bisulfite sequencing and dot blot. HBV relaxed circular DNA was quantified by real-time qPCR and Southern blotting. The association of Argonaute with HBV cccDNA was assessed by chromatin immunoprecipitation (ChIP).

**Results:** INF-α increased degree of methylation of HBV genome. Methylation of HBV genome was associated with down-regulation of HBV expression. We found that AGO2 was present in the nucleus and AGO2 was associated with HBV cccDNA.

**Conclusion:** IFN-α induces methylation of hepatitis B virus DNA in human hepatoma cells leading to suppression of HBV replication.
P-019
Quantitative Analysis of TERT Promoter Mutation with Blood during HCC Treatment

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Background: The clinical significance of circulating tumor DNA (ctDNA) using liquid biopsy in HCC has not been established. Since TERT promoter mutation was high frequently observed in HCC, the kinetics of TERT promoter mutation in the blood may reflect the change of whole tumor.

Methods: Method 1: The prevalence of TERT promoter mutation and variant allele frequency (VF) were analyzed with DNAs collected from tumor and non-tumor area from FFPE specimens of 109 surgically resected HCCs. Method 2: TERT promoter mutant copy numbers in 1ml of plasma were analyzed with DNAs collected from plasma before TACE or multikinase inhibitor (TACE n=12, lenvatinib n=4, sorafenib n=1). In the cases treated by TACE, we examined the change in the number of mutated copies before and after treatment.

Results: Result 1: The prevalence of TERT promoter mutation in tissues was 62.4%. VFs of each pathological grade were 28.8%/25.4%/34.1%/29.7% (early/well/moderately/poorly). Result 2: The average mutant copy numbers in 1ml of plasma were 0/1.0/4.4/121.6 (stage I / II / III / IV) and more detected in stage III/IV (P = 0.039). Mutant copy numbers detected in plasma before and after TACE were 3.6/94.6/22.4 (before TACE/after 1day/after 3 or 4days), reflecting the release of ctDNA into the blood by treatment.

Conclusion: The TERT promoter mutation in HCC is constant at any progressive stage and it may reflect the total tumor volume. Quantitative examinations of TERT promoter mutations in the blood may be applicable to therapeutic effect monitoring.

P-020
The Association between HMG-CoA Inhibitors and Cholangiocarcinoma

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Introduction: As HMG-CoA inhibitors, statins have been studied widely for cardiovascular disease risk modification. However, statins inhibit cholesterol metabolism which has the potential to impact cancer behavior. This study aims to study the association between statins and risk of developing cholangiocarcinoma.

Methods: We searched PubMed, EMBASE, and Cochrane electronic libraries systematically using the relevant keywords. After identifying the studies eligible, data from the studies were pooled, and a meta-analysis was conducted to estimate the Odds Ratio (OR) of developing cholangiocarcinoma in statin users. Mantel Hazard method was used, and both fixed-effects and random-effects models were conducted. Statistical analyses were performed with use of RStudio® software package (meta) (RStudio, Boston, MA).

Results: 27,729 patients from five studies were included in the final analysis. A high degree of heterogeneity was identified between the included studies, tau^2 = 0.2558; H = 5.75 [4.47; 7.40]; I^2 = 97.0% [95.0%; 98.2%]. Both the fixed-effects and the random-effects models showed a significant decrease in the risk of cholangiocarcinoma in statin users, (OR: 0.81, 95% CI [0.76-0.86], p-value < 0.0001), and OR: 0.55, 95% CI [0.34-0.87], p-value = 0.0112), respectively.

Conclusions: Cholangiocarcinoma is a very aggressive malignancy that needs more studies to understand the molecular mechanisms behind tumor aggression. From the current study, it appears that inhibition of lipid metabolism has a significant favorable impact on cancer behavior. Further studies are needed to evaluate the effects of statins on the survival of cholangiocarcinoma as well as the possible synergistic effect if combined with immunological modulators.

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**P-021**

**MicroRNA-30b-5p Promotes Hepatitis B Virus-Induced Hepatocarcinogenesis through Modulation of Proliferation and Metastasis**

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**Background:** Hepatocellular carcinoma (HCC) is one of the most common malignant cancers, and thousands of genes have been well demonstrated to play important roles in HCC progression. In this study, we explored the promotion mechanism of microRNAs (miRNAs) for tumorigenesis via hepatocyte metabolic pathways.

**Methods:** The differential expression profiles of miRNA between HBV-induced hepatocarcinogenesis and carcinogen-induced hepatocellular carcinoma were obtained by high-throughput sequencing. Bioinformatics, multi-omics analysis were combined with experiments to find miRNA and mRNA interaction targets that regulate occurrence, development, invasion, and metastasis of tumors. Results of cell lines demonstrated the modulating mechanism of miRNA in the biological behaviors of tumor cells.

**Results:** The sequencing results showed 110 and 24 differentially expressed mRNAs and miRNAs, respectively, in which we found the up-regulated expression of hsa-miR-30b-5p was negatively correlated with the down-regulated expression of MINPP1. Luciferase reporter assays was used to validate the target of miR-30b-5p. Real-time PCR confirmed that hsa-miR-30b-5p had higher expression in Hep3B cell line, which was consistent with high-throughput sequencing results. Functional experiments indicated that down-regulated expression of hsa-miR-30b-5p inhibited the proliferation, cell migration and invasion of tumor cells.

**Conclusion:** These findings suggest a tumor promotion role of miR-30b-5p in HBV-induced hepatocarcinogenesis. MiR-30b-5p could serve as a potential biological targets for clinical diagnosis and treatment of HBV-induced hepatocarcinogenesis.

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**P-022**

**Macrophage Colony-stimulating Factor (M-CSF) Receptor Antagonist Inhibits Progression of Hepatocellular Carcinoma in vivo**

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**Aim:** The purpose of this study was to investigate effects of M-CSF receptor antagonist on HCC.

**Materials and Methods:** C57/BL6 mice were treated with diethyl nitrosamine (DEN) intraperitoneally. For treatment group, M-CSF receptor antagonist (GW2580) was treated every day. Incidence of tumors was assessed 38 weeks after treatment. Furthermore, angiogenesis and distribution of CD163-positive M2 Mφs were assessed. Mouse HCC cells (MH134) were implanted to same strain C3H mice by subcutaneous injection (1 × 10^5/animal). Tumor progression was assessed after 3 weeks. In the nude mouse, human HCC cells (HuH7) were implanted by intra-splenic injection (1 × 10^6/animal). Tumor progression in the spleen was assessed after 3 weeks. MH134 or HuH7 (1 × 10^4/well) was cultured with media containing M-CSF (100ng/ml) in the presence or absence of GW2580 (1ng/ml) for 7 days in vitro, and cell proliferation was assessed.

**Results:** Hepatic tumors diagnosed as hepatocellular adenoma or HCC were observed in animals treated with DEN. In contrast, tumor incidence was significantly reduced in DEN-treated animals with GW2580. Enhanced angiogenesis M2Mφ population observed in the DEN-treated animals was significantly blunted by treatment of GW2580. Growth of implanted both of HCC was significantly inhibited by GW2580 in vivo. Proliferations both of HCC were not significant difference between in cells cultured with M-CSF and cells cultured without M-CSF. Furthermore, GW2580 did not inhibit proliferation of these cells in vitro.

**Conclusions:** M-CSF and/or its receptor could be a new therapeutic target for HCC.
P-023
The Real World Practice of Lenvatinib in Patients with Advanced Hepatocellular Carcinoma in Japan

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Background: Lenvatinib, the third tyrosine kinase inhibitor (TKI) after sorafenib and regorafenib, has been used in the treatment of advanced hepatocellular carcinoma (HCC) in Japan since March 2018. We collected clinical data of cases administered with lenvatinib for advanced hepatocellular carcinoma in our hospital, and analyzed the initial data on safety and efficacy in real world.

Methods: Between March 26, 2018, and the end of October 2018, we retrospectively collected data on advanced HCC patients who were administrated lenvatinib from our institution. Radiological assessments were evaluated according to both the Response Evaluation Criteria in Solid Tumors (RECIST) and the modified Response Evaluation Criteria in Solid Tumors (mRECIST).

Results: A total of 56 patients received lenvatinib. The overall response rate (ORR) according to mRECIST was 41% and disease control rate (DCR) was 59%. The most common any-grade adverse events were fatigue [24(43%)], hypertension [13(23%)], and anorexia [13(23%)]. Among 23 patients with portal systemic shunt diagnosed before treatment started, hepatic encephalopathy was observed in 6 patients. Adverse events led to drug interruption or dose reduction in 40 patients(71%), and the main causes were anesthesia, fatigue, and hepatic encephalopathy.

Conclusions: In real world, lenvatinib had high ORR and DCR as well as REFLECT study. However, unlike the REFLECT study, fatigue and anorexia often required dose adjustment. All patients who developed hepatic encephalopathy were cases with portal systemic shunt. But even in cases of hepatic encephalopathy, treatment can be continued by prophylactic treatment and dose reduction of lenvatinib, and antitumor effect can be expected.

P-024
Prognosis of Primary Sclerosing Cholangitis (PSC) That Merges with Ulcerative Colitis (UC) May Depend on Inflammatory Control of UC

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Primary sclerosing cholangitis (PSC) is a disease characterized by intrahepatic and/or extrahepatic bile duct inflammation and fibrosis, leading to cirrhosis or liver failure at high frequency. Fifty to 75% of patients with PSC are merging with inflammatory bowel disease (IBD). About 5% of patients with ulcerative colitis (UC) will have PSC. Both IBD and PSC are positive for MPO-ANCA. It is obvious in the genome-wide association analysis that the immune-genetic background of PSC partially overlaps that of UC. But they are different things. Patients with PSC who have symptoms develop cirrhosis and liver failure in 5 to 10 years and ultimately require transplantation. The correlation between inflammatory control of UC and progression of PSC is unclear. We experienced patients suffering from UC with PSC who dropped out from outpatient clinic. A case is a 43-year-old man. The patient had UC with PSC at age 31. The patient dropped out from outpatient clinic at the age of 34. When patient reached an outpatient visit again at the age of 41, the patient had deteriorated to 11 points of Child-Pugh Score from 5. Currently, the patient is preparing for living liver transplantation. We will report on the correlation between inflammatory control of UC and the progression of PSC including literature consideration.
POGLUT1, The Effector Gene Driven by rs2293370 in Primary Biliary Cholangitis (PBC) Susceptibility Locus Chromosome 3q13.33 in The Japanese Population

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Primary biliary cholangitis (PBC) is a chronic and cholestatic autoimmune liver disease caused by the destruction of intrahepatic small bile ducts. Our previous genome-wide association study (GWAS) identified HLA, TNFSF15, and four other loci as susceptibility loci for PBC in the Japanese population. Here, in order to further elucidate the genetic architecture of PBC in the Japanese population, a GWAS was performed on an additional independent sample set, then a genome-wide meta-analysis with our previous GWAS was performed based on a whole-genome single nucleotide polymorphism (SNP) imputation analysis of a total of 4,045 Japanese individuals (2,060 PBC cases and 1,985 healthy controls). A susceptibility locus on chromosome 3q13.33 (including ARHGAP31, TMEM39A, POGLUT1, TIMMDC1, and CD80) was identified in the Japanese population (odds ratio [OR] = 0.7241, P = 3.5 × 10^{-9}). Subsequent in silico and in vitro functional analyses identified rs2293370 as the primary functional SNP. When the rs2293370 was conditioned on, significant associations of other SNPs in chromosome 3q13.33 totally disappeared by the conditional logistic regression analysis. Moreover, eQTL analysis in various organs indicated that the effector gene of rs2293370 was Protein O-Glucosyltransferase 1 (POGLUT1) (P < 0.0005). This is the first study to demonstrate that POGLUT1, and not CD80 which encodes a well-known co-stimulatory signaling molecule necessary for antigen presentation from HLA class II to T cell receptor (TCR), is the effector gene regulated by the primary functional SNP rs2293370, and that increased expression of POGLUT1 might be involved in the pathogenesis of PBC.

P-026

Hypercholesterolemia is Not a Risk Factor for Cardiovascular Disease–Related Deaths among Primary Biliary Cholangitis Patients in Japan

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Background: Hypercholesterolemia is a common feature of primary biliary cholangitis (PBC). In general, hypercholesterolemia is associated with an increased risk of cardiovascular disease (CVD)–related deaths. We have investigated whether hypercholesterolemia is a risk factor for especially CVD-related deaths among PBC patients in Japan.

Methods: 8514 PBC patients from 520 hospitals or clinics were enrolled and analyzed. The average prevalence of hypercholesterolemia is defined as a total cholesterol (T–Cho) ≥ 240 mg/dl (6.2 mmol/l).

Results: The mean serum T–Cho levels were 215.12 ± 78.08 mg/dl in 7576 PBC patients (data deficient; 938). The hypercholesterolemia ratio in PBC patients was 24.4% (1854/7576). In s2PBC group (serum bilirubin level equal or over 2.0 mg/dl), the mean T–Cho levels were 255.53 ± 163.34 mg/dl (n=557), higher significantly compared to that of aPBC and s1PBC group (211.91 ± 65.77 mg/dl, n=6559 ; p<0.001). There was a significant positive correlation between serum ALP level and T–Cho level (r=0.3, p<0.001, n=6444). In the last determination of T–Cho and ALP levels were significantly decreased compared to the both levels at the initial determination in PBC. In PBC patients, total mortality rate was 6.48% (317/4890). The most prevalent cause of death was hepatic failure (2.25%). There were only eleven deaths (0.2%) from CVD–related causes. No relationship emerged between CVD related–deaths and T-Cho levels in a multiple logistic regression analysis.

Conclusions: This large study brought us that hypercholesterolemia was not a risk factor for CVD–related deaths among PBC patients.

Acknowledgement: This nationwide survey was conducted by the Primary biliary cholangitis Group, a subgroup of the Intractable Hepato-Biliary Disease Study Group supported by the Ministry of Health, Labour and Welfare of Japan.
Validation of The Usefulness of The UDCA Response Score in Japanese Patients with Primary Biliary Cholangitis

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Background: A model for pretreatment prediction of response to ursodeoxycholic acid (UDCA) in patients with primary biliary cirrhosis (PBC), named the UDCA Response Score (URS), was derived from the Italian PBC Study Group and the UK-PBC Consortium. However, the usefulness of the URS in Japanese patients with PBC has been enigmatic.

Methods: We retrospectively validated the usefulness of the URS in 190 Japanese patients with PBC. The probability of response (POR) based on URS and laboratory data after 1 year of UDCA treatment was analyzed. Cut-off values were determined using receiver operating characteristic curve analysis.

Results: There were significant correlations between URS and alkaline phosphatase (ALP) or γ-glutamyl transpeptidase (GGT) after 1 year of UDCA treatment, (ALP, r=-0.59, p<0.001; GGT, r=-0.48, p<0.001). POR was significantly higher in patients with normalized aminotransferase, ALP, and GGT after 1 year of UDCA treatment than in patients without normalized values (p<0.001). While significantly higher values of POR were found in responders defined by the Barcelona criteria or Paris II criteria (Barcelona: p<0.05, Paris II: p<0.001), there was no difference between responders and non-responders defined by the Paris criteria. The POR cut-off values for prediction of responders defined by Barcelona criteria or Paris II criteria were 0.949, with sensitivity of 0.51 and specificity of 0.75; and 0.812, with sensitivity of 0.85 and specificity of 0.55, respectively.

Conclusion: URS is useful for pretreatment prediction of response to UDCA in Japanese patients with PBC.

The Prognostic Factors for Primary Sclerosing Cholangitis(PSC) Including Oxidative Stress Markers

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Background: Primary sclerosing cholangitis (PSC) is a rare chronic liver disease, with 0.95 cases reported per 100,000 people in Japan. Because of its rarity in Japan, the clinical characteristics are still not clear. Recent investigations have shown that general conditions, such as oxidative stress, affect the course of chronic diseases. We investigated the clinical course and oxidative stress-related condition of PSC to determine the prognostic factors of PSC.

Methods: We analyzed 41 PSC patients who attended our department from June 2008 to June 2018. The clinical characteristics, including concomitant inflammatory bowel disease (IBD) or bile duct carcinoma and liver transplantation experience, were investigated in order to define the prognostic factors. The oxidative stress status was evaluated by two types of markers: serum d-ROM (oxidative stress marker) and OXY (antioxidant marker).

Results: The median age was 36.4 years (14-74 years), and male sex was predominant (27:14). The average observation period was 1085 days, and 9 patients died. The Mayo risk score and the Child-Pugh score were able to significantly discriminate the bad overall survival (p<0.01). Significant poor prognostic factors were higher age, higher Mayo risk score, higher FIB-4 index, and positive anti-nuclear antibody according to the log rank test. A lower anti-oxidant marker OXY was also included as a poor prognosis-related factor, while d-ROM was not. In a multivariate analysis, the FIB-4 index was the only marker proven to be significant.

Conclusion: The FIB-4 index was shown to be a prognostic factor for PSC in our study. Maintaining antioxidant stress will lead to a good prognosis and suggests the efficacy of antioxidant treatment.
Investigation of Liver Immune Responses of Males and Females in the Initiation of Primary Biliary Cholangitis

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Primary biliary cholangitis (PBC) is a chronic autoimmune liver disease which caused by inflammation targeting intrahepatic small bile ducts. A sex disparity happens in PBC with a female-to-male ratio of 10:1. However, the reason why females outnumber males remains unclear. In this study, we investigated whether different liver immune responses exist in the initiation of PBC by using xenobiotic (2-OA-OVA)-induced PBC murine model. The female PBC mice expressed more exacerbating liver inflammation and cell infiltration reacted to 2-OA-OVA immunization than the male group. In liver, the CD8⁺ T/ Foxp3⁺ Treg ratio in the female group was higher than the male group, which was caused by the percentage of CD8⁺ T cells increased in female PBC mice while Foxp3⁺ Treg cells were comparable. The two groups exhibited parallel functions of CD8⁺ T cells such as expression of cell activation, development, and cytokine secretion, suggesting that gender difference did not affect the CD8⁺ T cells function but imbalanced the Teff/ Treg ratio of the model. The mRNA levels of chemokine such as CXCL9 and CXCL10 were both significantly higher in liver homogenate of the female group. These results indicated the chemokines affected by gender might play an important role in regulating the PBC severity. In summary, our data indicated that the female mice exhibited more effector immune response may result from imbalance of Teff/ Treg and different chemokine expression, nevertheless the exact mechanism deserves further exploration.

Risk Factors for Primary Biliary Cholangitis in South Korea: A Case-Control Study

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Background/Aim: The risk factors for development of primary biliary cholangitis (PBC) is unclear. This study aimed to investigate the risk factors associated with PBC in South Korea through a questionnaire survey.

Methods: Consecutively enrolled 103 PBC patients from six referral hospitals and 100 age and sex-matched community controls participated in this study. Standardized questionnaire survey including demographics, lifestyle, individual and familial medical history and reproductive history were prospectively collected and analyzed.

Results: Demographics were similar between PBC and control groups (mean age 58.3 years vs 56.8 years, female 86.4% vs 85%). Smoking including both firsthand and secondhand (odds ratio (OR) 1.95, 95% confidence interval (CI) 1.21–3.83), history of autoimmune diseases (OR 2.56, 95% CI 2.51–6.33) and family history of PBC (OR 17.32, 95% CI 1.73–2351.95) were significantly associated with PBC, whereas alcohol intake was negatively associated with PBC. Among reproductive factors, nulliparity and artificial abortion were significantly associated with PBC.

Conclusion: Family history of PBC, accompanying autoimmune diseases, smoking, artificial abortion and nulliparity were significant risk factors for PBC, while alcohol intake was negatively associated with PBC. Further studies to validate the results of this study and search for clues to the pathogenesis of PBC are warranted.
P-031

Immunoglobulin G4-Related Liver Disease Overlapping with Non-Alcoholic Steatohepatitis That was Diagnosed Simultaneously with Autoimmune Pancreatitis: A Case Report

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A 70-year-old female visited a nearby clinic due to dry eyes, a dry mouth, and epigastric pain. She was referred to our gastrointestinal medicine outpatient facility, and distal pancreatic swelling, liver edge dullness, and liver surface irregularities were found on abdominal computed tomography. She was positive for serum anti-nuclear antibodies and had elevated immunoglobulin (Ig) G (1837 mg/dL) and IgG4 (558 mg/dL) levels. A diagnosis of autoimmune pancreatitis (AIP) was made based on images obtained using endoscopic retrograde cholangiopancreatography and endoscopic ultrasound and a histopathological examination conducted using endoscopic ultrasound-guided fine-needle aspiration. Her AIP improved after prednisolone treatment was started. A histopathological examination of the liver showed interface hepatitis together with lymphoplasmacyte and IgG4-positive plasma cell infiltration into the portal region. Non-alcoholic steatohepatitis (NASH) was diagnosed based on the detection of parenchymal steatosis, ballooning hepatocytes, and pericellular fibrosis. We experienced a case of unique liver disease, which was considered to have involved IgG4-related liver disease overlapping with NASH.

P-032

Association between Serum Leucine-Rich α2-glycoprotein Predict and the Prognosis in Primary Biliary Cholangitis

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Background: A noninvasive biomarker to predict prognosis in patients with primary biliary cholangitis (PBC) is needed. Leucine-rich alpha 2 glycoprotein (LRG) has been reported as a biomarker of autoimmune disease. We evaluated the association between LRG and the development of cirrhosis-related conditions in PBC.

Methods: We retrospectively reviewed the clinical data of 129 individuals with biopsy-proven PBC. All patients were treated with ursodeoxycholic acid. We analyzed serum LRG in patients with PBC at the biopsy (N = 129) and patients with after treatment (N = 80) by enzyme-linked immunosorbent assays using stored sera. Serum of healthy subject was used as control (N = 15).

Results: LRG in the patients with PBC at biopsy was significantly higher than that of the healthy subjects (58.0 μg/mL vs 25.5 μg/mL, P < 0.001). LRG was significantly decreased after treatment (55.8 μg/mL vs 39.7 μg/mL, P < 0.001). Neither LRG nor change of LRG was associated with transaminase or histological findings. In multivariate analysis including histological stage, increased LRG after treatment was independently associated with the high rate of development of cirrhosis-related conditions (hazard ratio 5.46, 95% confidence interval 1.50–19.9, P = 0.010). Patients with increased LRG after treatment had a significantly increased rate of cirrhosis-related conditions (log-rank, P = 0.009).

Conclusion: Increased LRG after treatment predicted PBC prognosis but were not associated with transaminase and histology. Change of LRG after treatment might be useful to evaluate response to treatment in PBC patients.
P-033
Efficacy and Safety of Long-Term Denosumab Therapy for Osteoporosis in Patients with Autoimmune Liver Diseases

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**Background:** Osteoporosis is a major complication in patients with primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH). Denosumab is a fully human monoclonal antibody against the receptor activator of nuclear factor-κB ligand (RANKL), inhibiting the development and activity of osteoclasts, decreasing bone resorption, and increasing bone density. In this study, we aimed to clarify the efficacy and safety of long-term denosumab therapy for osteoporosis in patients with PBC or AIH.

**Methods:** We enrolled 10 consecutive PBC or AIH patients with osteoporosis who received subcutaneous denosumab treatment at a dose of 60 mg every 6 months for 36 months or more between November 2014 and September 2018, whose bone mineral density (BMD) was less than -2.5 of T-score (corresponding to Young Adult Mean [YAM] in Japan: 70%) at the lumbar spine by dual-energy X-ray absorptiometry. All patients received supplementation with oral calcium (305 mg/day) and vitamin D (200 IU/day). Changes in BMD, serum TRACP-5b (bone resorption marker), ALP, ALP 3 (bone-related isozyme), calcium levels were evaluated using Wilcoxon signed-rank test.

**Results:** All patients were postmenopausal women (6 PBC and 4 AIH) with median (range) age of 68.5 (59-79) years. Baseline values (median [range]) of T-score, serum TRACP-5b, ALP, ALP 3, calcium levels were -2.95 (-3.50 to -2.52), 593 (304-1480) mU/dL, 404 (192-896) IU/L, 153 (83.6-627.2) IU/L, 9.25 (8.8-9.7) ml/dL, respectively. BMD gradually and significantly improved with denosumab treatment: increase rates of percentage to YAM were 3.1% (P = 0.007), 9.9% (P = 0.007), 11.5% (P = 0.008), and 15.3% (P = 0.005) in 6, 12, 24, and 36 months, respectively. Serum TRACP-5b, ALP and ALP3 levels significantly reduced in 6, 12, 24, and 36 months. In this study period, fresh vertebral fractures and denosumab-related adverse events including hypocalcemia were not observed.

**Conclusions:** These results suggest that long-term denosumab therapy may be safe and effective for osteoporosis in patients with autoimmune liver diseases and warrant a large-scale prospective study.

P-034
Correlation between the Oral and Gut Microbiota in Patients with Autoimmune Liver Disease

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**Background and Aim:** Recently, it is known that gut microbiota is involved in the pathogenesis of autoimmune liver disease (AILD), mainly primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH). This study aimed to analyze and compare the composition of the oral microbiota of 56 patients with AILD and 15 healthy controls (HCs) and to evaluate its association with gut microbiota.

**Methods:** Subjects were 39 PBC and 17 AIH patients diagnosed at our hospital. The control population included 15 matched HCs. Salivary samples were collected, and microorganisms were analyzed by the bacterial 16S rRNA gene. AILD patients had the following characteristics: mean age, 63 years; male/female ratio, 5:34; laboratory parameters at sample collection: ALT 27±16 U/L, ALP 321±111 U/L, γGTP 59±44 U/L, 26 patients with PBC underwent ursodeoxycholic acid (UDCA) treatment and 11 underwent UDCA and bezafibrate treatment. AIH patients had the following characteristics: mean age, 60 years; male/female ratio, 2:15; laboratory parameters at sample collection: ALT 19±10 U/L, IgG 1473±821 mg/dl, 11 patients with AIH underwent prednisolone treatment and 4 underwent prednisolone and azathioprine treatment. The results showed that the genera Veillonella was significantly increased with a concurrent decrease in the genera Streptococcus in the salivary microbiota of AIH patients compared with HCs. The genera Eubacterium, Veillonella were significantly increased and the genera Fusobacterium was significantly decreased in salivary microbiota of PBC patients compared with HCs. The genera Eubacterium, Veillonella were significantly increased in the genera Streptococcus in the salivary microbiota of AIH patients compared with HCs. The genera Eubacterium, Veillonella were significantly increased and the genera Fusobacterium was significantly decreased in salivary microbiota of PBC patients compared with HCs. Interestingly, our study suggested that while the relative abundance of Lactobacillales in feces positively correlated with the relative abundance of Veillonella in saliva from patients with AIH, the relative abundance of Bifidobacterium in feces negatively correlated with the relative abundance of Veillonella in saliva from patients with PBC. Moreover, the relative abundance of Clostridium subcluster XIVa in feces positively correlated with the relative abundance of Neisseria and negatively correlated with the relative abundance of Eubacterium in saliva from patients with AIH. Dysbiosis of the oral microbiota reflects changes in the gut microbiota in patients with AILD.

**Conclusions:** The dysbiosis may play an important role in the pathogenesis of AILD.
A Case of Drug-Induced Autoimmune Hepatitis which can be Caused by Clarithromycin and Fexofenadine

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A 58-year-old Japanese woman was referred to our hospital because of acute liver dysfunction. Blood examination revealed her elevated values of T-Bil (3.2 mg/dL), AST (582 IU/L), ALT (592 IU/L) and IgG (3547 mg/dL). And her prothrombin time was prolonged (69%). She had no history of alcohol abuse. Every serological finding for viral hepatitis A, B, C, E, Epstein-Barr virus and cytomegalovirus was negative. ANA, SMA and anti-LKM-1 were also negative. She exhibited negative for HLA DR3 and DR4. She had been treated for sinusitis and food allergy with clarithromycin and fexofenadine before the development of liver injury. The drug-induced lymphocyte stimulation test showed positive for both drugs. Ultrasonography and computed tomography showed no significant findings. Histologic features were characterized by interface hepatitis and portal fibrous expansion with lymphocytes, plasma cells and some eosinophils infiltration. Intralobular necrosis was also noted. Since diagnostic score for AIH was 12 points based on the criteria of the International Autoimmune Hepatitis Group, she was considered compatible with probable autoimmune hepatitis (AIH). In addition, because score applying diagnostic criteria from the Digestive Disease Week-Japan 2004 workshop was 4 points, she was also considered to exhibit possible drug-induced liver injury. Consequently, she was diagnosed as drug-induced AIH. Liver function tests were improved rapidly with discontinuation of clarithromycin and fexofenadine followed by oral administration of prednisolone (40mg/day=1mg/kg/day). Prednisolone was gradually reduced to a maintenance dose of 5 mg/day without relapse of hepatitis.

In conclusion, we report a rare case of drug-induced AIH which might be caused by clarithromycin and/or fexofenadine.

Histopathological Analysis and Validation of Different Scoring Systems for Autoimmune Hepatitis: Experience of Two Institutes

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Background: The currently used criteria for autoimmune hepatitis (AIH) include the revised original (1999) and simplified (2008) scoring systems proposed by the international autoimmune hepatitis group (IAHG). Recently, new histologic criteria have been proposed (UCSF criteria). Here, we sought to evaluate the histological features of AIH according to the three proposed criteria.

Materials and Methods: Clinical data and liver biopsies were reviewed for 49 patients with AIH diagnosed at Seoul National University Hospital and Seoul National University Bundang Hospital between 2009 and 2014.

Results: Interface hepatitis (≥ Ishak Gr3), plasmacytic infiltrates, rosettes and emperipolesis were seen in 61.2%, 100%, 69.4% and 75.5%, respectively. Patients presented with cirrhosis in 22.4%, acute hepatitis pattern was seen in 38.8%, and co-existing steatosis was present in 34.7%. Except one patient with HBV hepatitis, the rest of patients met the 1999 IAHG criteria (65% “definite”, 33% “probable”). Only 67.3% of those meeting the 1999 criteria also met the 2008 criteria (46.9% “definite”, 20.4% “probable”). By the UCSF criteria, definite/probable AIH increased from 67.3% to 83.7% (57.1% “definite”, 26.5% “probable”).

Conclusion: The concordance rate of 1999 IAHG and 2008 IAHG were 69%. The recently proposed UCSF criteria increased the diagnosis of AIH by 16.4%.

Reference:
Impact of Apoptosis Inhibitor of Macrophage on the Disease Progression in Patients with Primary Biliary Cholangitis

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Background: Apoptosis inhibitor of macrophage (AIM) plays pivotal roles in obesity-associated inflammatory disease. Several autoimmune diseases are associated with obesity and/or insulin resistance. However, the correlation between AIM and obesity/insulin resistance remains unclear in patients with primary biliary cholangitis (PBC).

Methods: Obesity and insulin resistance were defined as a body mass index (BMI) exceeding 25 and a HOMA-IR value higher than 2.0, respectively. Hepatic steatosis was estimated on the basis of the classification proposed by Brunt and colleagues. The histological stage was determined using Scheuer’s classification.

Results: Twelve (25%) and seven (15%) of 48 patients with PBC had concurrent obesity and type 2 diabetes mellitus (T2DM), respectively. PBC patients with obesity had significantly lower serum ALP levels (383 ± 126 vs. 650 ± 383 IU/L, p=0.0172) and higher frequency of hepatic steatosis grade 1(40% vs. 3%, p=0.0062) than those without obesity. Serum ALT levels were significantly higher (68 ± 24 vs. 47 ± 27 IU/L, p=0.0486) and the histological stages were more advanced (2.8 ± 1.0 vs. 1.8 ± 0.9, p=0.0313) in PBC patients concomitant with T2DM than in those without T2DM. PBC patients had significantly higher serum AIM levels than normal healthy controls (33.1 ± 28.9 vs. 3.0 ± 5.4 ng/ml, p=0.0116). Serum AIM levels were not associated with BMIs and HOMA-IR values, but with serum IgG (r=0.5329, p=0.0012), albumin (r=-0.4962, p=0.0028), TNF-α (r=0.4224, p=0.013) levels and the histological stages (r=0.4114, p=0.0165).

Conclusion: These data imply that AIM may be involved in the disease progression among patients with PBC.

Case of Immune-Mediated Sclerosing Cholangitis Induced by Atezolizumab

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Background: As immune checkpoint inhibitors have become established treatment agents for metastatic cancers, reports of immune-related adverse events (irAE), including drug-induced liver injury (DILI), have increased. Clinical trials show that atezolizumab, a monoclonal antibody against the programmed death-ligand 1 protein, can cause grade 3 or higher DILI in 3.0% of patients. Here, we report a unique case of atezolizumab-induced sclerosing cholangitis (SSC).

Case Presentation: An 86-year-old woman was hospitalized owing to epigastralgia and anorexia three weeks after receiving atezolizumab treatment. She had been diagnosed with lung adenocarcinoma a year prior and underwent first-line chemotherapy. However, owing to disease progression, atezolizumab was administered. Liver function had been normal until atezolizumab was administered. At admission, computed tomography showed wall thickening in intrahepatic bile ducts (IHBD); laboratory tests revealed the following: ALT, 67 U/L; AST, 72 U/L; ALP, 976 U/L; GGT, 295 U/L; CRP, 7.06 mg/dL; and bilirubin, 0.37 mg/dL. Cholangitis was suspected; hence, antibiotic administration was started. However, liver function exacerbated to grade 3. We suspected irAE and started prednisolone treatment. Magnetic resonance cholangiopancreatography (MRCP) revealed mild narrowing and dilatation of IHBD, and liver biopsy showed mild non-specific periportal inflammation. Although liver function improved after steroid therapy, liver enzyme levels increased again after prednisolone was tapered. MRCP was repeated, revealing apparent deformities and IHBD with a beaded appearance, which was compatible with SSC. She died 5 months after admission due to the exacerbation of lung cancer.

Conclusions: To our knowledge, this is the first reported case of atezolizumab-induced SSC.
P-039
Post-liver Transplantation Survival-Related Factors and Regulatory T Cell Frequency in Primary Biliary Cholangitis

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Background and Aims: Primary biliary cholangitis (PBC) is a leading cause of orthotopic liver transplantation (OLT) in Japan. The one- to three-year survival rates after OLT are usually reasonably good; however, the long-term survival-related factors, including immune-related factors, are not clearly defined. Regulatory T cells (Treg) are known to be decreased in PBC, but their status post-OLT is poorly understood. In the present study, we investigated the survival-related clinical factors and conducted a Treg population analysis in PBC patients after OLT.

Methods: The patient background before OLT was investigated in order to predict the post-OLT PBC recurrence and survival. We investigated 26 patients, 5 of whom died and 7 who had histologically confirmed PBC recurrence. We determined Treg frequencies after OLT in 4 patients with PBC, 8 with hepatitis B and 23 with hepatitis C.

Results: There were no PBC recurrence-predicting factors, nor were there any significant factors predicting the survival, but the history of acute cellular rejection and HLA mismatch number (≥3) was relatively frequent in the dead group (p=0.05 and 0.09, respectively). The Treg frequency was significantly lower in PBC than in healthy volunteers and hepatitis C virus-related OLT patients.

Conclusion: As the immune background was correlated with the post-OLT PBC survival and the Treg frequency was lower in post-OLT PBC than in hepatitis C, immune regulation should be strictly managed in post-OLT PBC patients.

P-040
Identification of Risk Factors for Histological Progression in Patients with Primary Biliary Cholangitis

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Aim: To identify laboratory predictors of histological progression (HP) in primary biliary cholangitis (PBC)

Methods: Sequential biopsies were performed for 35 of 308 PBC patients treated with ursodeoxycholic acid (UDCA); patients were divided into UDCA responders (R: n = 14) and non-responders (NR: n = 21) based on decreased γ-glutamyl transpeptidase levels 1 year post-UDCA initiation (Nara definition). Patients were categorized as demonstrating HP (progressive group, PG) or lacking HP (nonprogressive group, NPG) according to the Scheuer and Nakanuma classifications; the latter grades liver fibrosis (fibrosis score, F) and bile duct loss (BDDL; BDL score, B).

Results: In the R group, proportions of patients with improved, stable, or worsened stage were 0%, 78.6%, and 21.4%, respectively, according to the Scheuer classification and 14.3%, 64.3%, and 21.4%, respectively, according to the Nakanuma classification. Proportions of patients with improved, stable, or worsened F were 0%, 64.3%, and 35.7%, respectively, while these proportions were 14.3%, 71.4%, and 14.3%, respectively, for B. In the NR group, proportions of patients with improved, stable, or worsened stage were 4.8%, 57.1%, and 38.1%, respectively, for B. In the NR group, proportions of patients with improved, stable, or worsened stage were 4.8%, 57.1%, and 38.1%, respectively, for F and 9.5%, 61.9%, and 28.6%, respectively, for B. The rate of γ-GTP level change 1 year post-UDCA initiation was an independent risk factor for increased Nakanuma stage progression.

Conclusions: Biochemical response to UDCA according to the Nara definition may help predict HP in PBC.
P-041
Clinicopathological Study of Acute Presentation of Autoimmune Hepatitis: Focus on Differentiation from Drug-Induced Liver Injury

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Background: Acute presentation of autoimmune hepatitis (AIH) is often difficult to differentiate from drug-induced liver injury (DILI). This study aimed to address the issue by characterizing the clinical and histological features for comparison of the acute presentation of AIH vs. DILI.

Subjects and Methods: In 35 patients who underwent liver biopsy and were clinically well-characterized with acute presentations of AIH (24 cases) and DILI (11 cases), we compared the clinical, biochemical and histological characteristics of AIH vs. DILI. The biopsy slides were evaluated by 3 experienced pathologists, in which each histological feature was predefined by consensus based on the diagnostic criteria. The evaluation of diagnostic performance was based on the receiver operating characteristic (ROC) curve.

Results: The IgG level and positive rate of anti-nuclear antibody (ANA) were greater in AIH cases than in DILI cases on clinical evaluation. The scores for pathological findings, such as cobblestone appearance of hepatocytes, lobular necrosis/inflammation, centrilobular fibrosis and bile duct injury in addition to the classic AIH features, such as interface hepatitis, lymphocytic/lymphoplasmacytic infiltrates, and hepatocyte rosette formation were higher in the AIH group than in the DILI group. We found no single feature indicative of AIH or DILI, but the combination of distinct findings, such as cobblestone appearance of hepatocytes, interface hepatitis and portal inflammation was helpful in differentiating DILI vs. AIH.

Conclusion: In the future, we aim to explore the most useful combination of findings for distinguishing AIH vs. DILI.

P-042
Efficacy of Humanized Anti-Human CD20 on Early Stage Autoimmune Cholangitis is Impaired by Anti-Drug Antibodies in Human CD20 and FcγR Expressing dnTGFβRII Mice

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Background: Clinical trials in human primary biliary cholangitis (PBC) using a chimeric antibody against human CD20 (hCD20) has had limited clinical efficacy. Two potential explanations for this disappointing data are the appearance of anti-drug antibody (ADA) and the presence of anti-drug antibodies limits the effectiveness of B cell-targeted therapies and might be overcome by using fully humanized antibodies.

Methods: We developed hCD20 and human Fcγ receptors (hFcγRs) expressing dnTGF-βRII mice. Female mice were treated beginning at 4 to 6 weeks of age, with either a humanized anti-hCD20 antibody coined TKM-011 (n=16) or a control vehicle (n=20).

Results: After 16 weeks treatment, we observed a significant reduction in portal inflammation, concurrent with a decrease in liver infiltrating mononuclear cells and liver CD8+ T cells. Importantly, a positive correlation between the numbers of liver non-B cells and B cells, as well as liver memory CD8+ T cells and B cells, was apparent (r=0.7426, p=0.0006; r=0.6423, p=0.0054). Accompanying these changes was a dramatic reduction in anti-mitochondrial antibodies (AMA), Interleukin (IL)-12p40, and IL-5, as well as an elevation of the anti-inflammatory chemokine CXCL1/KC. Of note, in mice that developed mouse anti-humanized antibodies (MAHA), these clinical improvements were less prominent.

Conclusion: Sustainable B cell targeting therapies may provide a broader mechanism of effector pathway reductions in early PBC, However, the presence of anti-drug antibodies limits the effectiveness of B cell-targeted therapies and might be overcome by using fully humanized antibodies.
P-043
The Availability of the GLOBE Score/UK-PBC Risk Scores to Predict Fibrosis in Japanese with Primary Biliary Cholangitis

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Background: Ursodeoxycholic acid (UDCA) is the first choice for treatment of PBC; however, some cases are not adequately responsive and progress to cirrhosis, so it is important to accurately predict long-term clinical course. Therefore, we verified the interaction between a progression of PBC and the GLOBE score/UK-PBC risk scores.

Methods: The total of 56 patients with PBC were diagnosed by liver biopsy from 2006 to 2018 and treated with UDCA over 1 year. Histological stage was evaluated according to the Scheuer classification. The GLOBE score and the UK-PBC risk scores were calculated based on the response to UDCA after 1 year of the initial treatment. The availability of scores as predictor factors for FIB4-index>1.3 or APRI>0.5 at 3 years after the initial UDCA was evaluated.

Results: The GLOBE score of late-stage PBC (stage 3-4) was 0.592±0.213, which was significantly higher than that of early-stage PBC (stage 1-2) (0.002±0.127, P=0.012). In contrast, there was no significant correlation between the PBC stage and the UK-PBC risk scores. The GLOBE score was significantly higher in patients with FIB4-index>1.3 in 3 years after UDCA, the AUC being 0.93, and cut-off value was -0.19. GLOBE score was also significantly higher in patients with APRI>0.5 in 3 years. The UK-PBC risk scores showed significant correlation with APRI>0.5 but not with FIB4-index>1.3 in 3 years after UDCA.

Conclusions: Our findings showed the GLOBE score is well correlated with pathological stage and a promising predictive factor of advanced fibrosis in UDCA-treated PBC patients.

P-044
Efficacy and Safety of Steroid Treatment in Elderly Patients with Autoimmune Hepatitis

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Background & Aim: In autoimmune hepatitis (AIH), few studies focused on the clinical features according to the age at disease onset. The aim of this study was to provide insight regarding the clinical features in elderly patients.

Methods: Eighty-nine patients who were newly histologically diagnosed as AIH in our hospital were retrospectively collected (January 2007 to March 2018). Clinical characteristics, histological findings, AIH score, and therapeutic response and relapse rates were evaluated.

Results: The proportion of patients ≥75 years significantly increased in the latter period (2013-2018) (16/51, 31%) compared with that in the first period (2007-2012) (2/38, 5.3%) (p=0.01). The older group had a significantly lower albumin level and platelet. Severer fibrosis stage of F3/4 at diagnosis was found in older group than in younger group (25%, 5.3%, p=0.02). The older and younger groups received UDCA (46%, 40.4%), prednisolone (PSL) (50%, 51%), and PSL pulse therapy (3.6%, 8.7%). In PSL-treated patients, the older group showed comparable remission rates (ALT ≤ 40U/L) to the younger group with the similar initial dose. None of 14 elderly patients have relapsed so far during or after PSL treatment although 18% of younger patients (6/29) relapsed. Few adverse effects were found even in older group treated with steroid, but one elderly woman died from severe pneumonia during steroid therapy.

Conclusion: Elderly AIH patients were suggested to become more frequently and have more advanced liver fibrosis. If used carefully and properly, steroid therapy in older patients is as effective and safe as in younger patients.
Autoimmune hepatitis (AIH) with acute onset has emerged as a novel disease setting in acute liver injuries. Non-alcoholic steatohepatitis (NASH) has been regarded as increasing cause in chronic liver diseases in Asia-Pacific area. We recently experienced a case of acute onset AIH on NASH.

The patient is a 64-year-old female, who had no abnormal liver dysfunction until 2018, when AST/ALT levels increased to 191/219 IU/L at annual health checkup. Data rose to 562/616 IU/l in three months’ time. She had taken fenofibrate for hyperlipidemia for the last two years. She gained weight over the last 4 years from 60kg to 64kg (BMI 26). Family history was positive for autoimmune disease, as her son had ulcerative colitis. Antinuclear antibody was positive at 1:160; IgG was 1737mg/dl. Viral serological markers were all negative. Abdominal ultrasound showed bright liver. Liver biopsy revealed NAS 3 (A2F2) with Brunt stage 3. Typical finding of AIH including portal inflammation, interface activity, predominance of plasma cells, bridging fibrosis and hepatocellular rosette formation were compatible with AIH (A2F2). Central perivenulitis with plasma cells and fibrosis were also present.

From the clinical aspects, NASH is a reasonable diagnosis. AIH scoring system of 12 points is consistent with AIH. Taking into consideration, we tentatively diagnose acute-onset AIH on NASH for her acute hepatitis.

To our best knowledge, there are very few reports for concomitant AIH with acute presentation on NASH.

Necessity of Steroid Therapy for Patients with Hepatitic Form of Primary Biliary Cholangitis

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Background: About 8–10% of patients with primary biliary cholangitis (PBC) show concomitant features of autoimmune hepatitis (AIH), referred to as hepatitic form of PBC. The disease has more aggressive course than classical PBC, and immunosuppressive therapy have been reported to be effective. However, the optimal treatment strategy remains unclear.

Methods: We retrospectively reviewed medical records of 97 patients with histologically proven PBC at Kurashiki Central Hospital between April 2006 and June 2018. Hepatitic form of PBC was diagnosed if the patients met the criteria of probable/definite AIH in the simplified AIH scoring system, and we examined their clinical course. Biochemical response was defined when laboratory data improved after treatment as following; ALP and AST ≤ 1.5 × ULN spell out, and bilirubin ≤ ULN spell out, according to Paris II criteria.

Results: Hepatitic form of PBC was diagnosed in 21 patients. Ursodeoxycholic acid (UDCA) of 300–900 mg was administered to all patients, and the biochemical response was achieved in 12 patients (57.1%) with UDCA alone. Among the remaining 9 non-responders, 8 patients received prednisolone (0.3–0.5 mg/kg as a starting dose), all the patients achieved biochemical response. At the end of follow-up periods (median, 5.5 years), all of these patients were receiving maintenance prednisolone therapy (2–5 mg). No relapse was observed while prednisolone was reduced.

Conclusions: Hepatitic form of PBC may be treated with UDCA monotherapy, but half of patients will need additional steroid therapy.
Case Report; A Case of Acute Severe Autoimmune Hepatitis with Extensive Zonal Necrosis

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Background/ Aim: There were merely few case reports showed detailed clinical features of autoimmune hepatitis with acute presentation (a-AIH) so far. We, herein, report clinical course of a case showed non-A, -B, -C, -E acute liver injury with massive necrosis in liver histology.

Case:
A 74-year-old female visited primary care doctor with fatigue and anorexia sustaining over 2 weeks. Laboratory data were serious; aspartate transferase 1133 IU/L, alanine transferase 598 IU/L, γ-glutamyl transpeptidase 682 IU/L, and total bilirubin 12.9 mg/dL, and she referred to our liver unit to care liver injury. Her sister had hyperthyroidism and her brother showed rheumatoid arthritis. She had neither dietary supplement nor alcohol abuse.

On admission, her consciousness was lucid but she had jaundice and leg edema. Antinuclear antibody was 1: 80, and antimitochondrial antibodies was negative. Serum immunoglobulin G was 1840mg/dL. Serologies for hepatitis A, B, C, and E were negative. She underwent liver biopsy 1 month after onset, and histology indicated massive necrosis with numerous plasma cell infiltration, piecemeal and bridging necrosis without portal fibrosis, and zone 3 necrosis. The highest value of PT-INR was 1.31. These findings suggested a-AIH could develop acute liver failure. Thus, prednisolone therapy was initiated at a dose of 0.8mg/kg/day and, thereafter, aminotransferase levels returned to reference range immediately. Based on the criteria by International AIH Group, diagnosis was made as “probable” on both pre- and post- treatment periods.

Conclusion: We experienced a case of serious a-AIH with whose clinicopathological feature were compatible with a-AIH.

Clinicopathological Study of Patients with Co-Existing Primary Biliary Cholangitis and Nonalcoholic Fatty Liver Disease

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Aims: Female nonalcoholic fatty liver disease (NAFLD) patients is commonly diagnosed in postmenopausal women, and primary biliary cholangitis (PBC), which is a similar susceptible age. Therefore, we clinicopathologically investigated cases with co-existing PBC and NAFLD (PBC+NAFLD).

Patients: The diagnostic criteria of both diseases were as follows: PBC cases were diagnosed by the Japanese Guideline for PBC, while NAFLD cases were defined as those with histological confirmation after excluding complicated cases of other liver diseases except PBC.

Results: Of 651 PBC cases and 891 NAFLD cases, 24 patients with PBC+NAFLD were diagnosed (4 men: 48, 67, 70, and 78 years, observation periods of 5, 14, 1, and 23 years; 20 women: median age 61 [48–71] years with 5 [1–9] years). Seventy-five percent of male patients were diagnosed with PBC before 5, 10, and 22 years and gained 15, 8, 14 kg. All female patients were diagnosed with PBC/NAFLD at one time. The prevalence and data by gender were as follows: obesity, 50% in men and 50% in women; diabetes, 50/40%; hypertension, 75/40%; dyslipidemia, 50/50%; median total bilirubin level of 0.6/0.8 mg/dL, albumin 4.1/4.3 g/dL, AST 39/41U/L, ALT 47/63U/L, ALP 488/414 U/L, gGTP 165/123 U/L, T-chol 201/219 mg/dL, TG152/132 mg/dL, HbA1c5.9%/5.1%, platelets 24/20 ×10,000/μL, and positivity of anti-M2 antibody of 100/70%. Histological diagnoses were as follows: PBC stage 1–2 in 75/90% and stage 3–4 in 25/10%. All were treated with ursodeoxycholic acid (UDCA), and only 5 of 12 obese patients exhibited deteriorated liver disease severity.

Conclusions: The clinical features of PBC+NAFLD indicated gender differences. In obese patients, the important therapeutic approaches include both the administration of UDCA and a reduction in body weight.
P-049
Therapeutic Effect of Azathioprine in Combination with Prednisolone in Patients with Autoimmune Hepatitis

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Background and Aim: Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease which is relatively rare and heterogeneous. Corticosteroid alone and it in conjunction with or followed by the addition of azathioprine (AZA) are recommended for treatment for AIH in Western countries. A lower dose of prednisolone in combination with AZA is effective for control of active AIH. The combination regimen of prednisolone and AZA is associated with a lower occurrence of corticosteroid-related side effects than the higher dose prednisolone regimen. In Japan, AZA is covered by health insurance as a therapeutic agent for AIH in 2018. We evaluated the clinical course of patients with AIH who were treated with AZA in addition to prednisolone in our hospital.

Subjects and Results: Six patients were collected and reviewed efficacy and safety of the combination therapy retrospectively. We defined continuation of ALT value of ≤40U/L as remission. All but one patients were female. They had median age of 59 year-old at the start of the prednisolone monotherapy and 60 year-old at the combination therapy, and had median observation period of 41 months from the initial therapy. All the patients were treated with ursodeoxycholic acid in conjunction with prednisolone prior to administration of AZA. The median initial dose of prednisolone, ursodeoxycholic acid and AZA were 20mg/day, 600mg/day and 50 mg/day, respectively. AZA was added because of flare or insufficient reduction of ALT during prednisolone mono-therapy. ALT has been decreased in all 6 patients and kept the level of remission in 4 patients after additional therapy of AZA. Besides, all patients have been reduced the dose of corticosteroids. One patient experienced mild anemia after addition of AZA.

Conclusions: Additional therapy of AZA has provided fine courses in our patients who experienced insufficient effect with prednisolone mono-therapy.

P-050
Predictors of Clinical Outcome for Patients with Severe Acute Exacerbation of Chronic Hepatitis B

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Objective: Severe acute exacerbation of chronic hepatitis B sometimes occurs and can lead to liver failure and death. The aim of this study was to identify predictors of the progression of acute-on-chronic liver failure (ACLF) during severe acute exacerbation of chronic hepatitis B without preexisting cirrhosis.

Methods: This retrospective cohort study included 69 consecutive chronic hepatitis B patients for severe acute exacerbation without cirrhosis, which was defined as an abrupt elevation of serum alanine aminotransferase (ALT) ≥ 600 IU/L, a total bilirubin (TB) ≥ 3.0 mg/dl, and plasma prothrombin activity (PTA) ≤ 50%. Clinical outcome and influential factors were analyzed.

Results: Of the 69 patients, 33 (47.8%) severe acute exacerbation cases progressed to liver failure. TB (TB>256umol/dl, P = 0.008) and PTA (PTA<40%, P < 0.001) were identified as significant determinants of liver failure, and PTA<20% showed a significant relationship with liver-related death, according to the multivariate analysis. HBeAg negativity (P = 0.065) and precore (PC) mutation (nt1896) (P = 0.090) were also associated with the progression to hepatic decompensation.

Conclusions: Serum total bilirubin, prothrombin activity, HBeAg status, and PC mutation were predictors of progression to ACLF in severe acute exacerbation patients without preexisting cirrhosis.

Key Words: Chronic hepatitis B; Severe acute exacerbation; Acute-on-chronic liver failure
P-051
Genomic Comparison between Two Liver Tumors from a Patient with HBV-Related Cirrhosis
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Introduction: Thanks to advances in genomic technology, numerous genomic variations involved in carcinogenesis have been found. We tried to elicit critical genomic variance in different liver cancers.

Methods: We sequenced short-insert genomic libraries of two primary multi-centric liver tumors, combined hepatocellular cholangiocarcinoma (CHCCC) and well differentiated hepatocellular carcinoma (HCC), and one non-cancerous liver (NCL) tissue surgically resected from a male with chronic hepatitis B using the HiSeq X-Ten sequencer. After alignment to human reference genome and removal of duplications, three genomes were compared with each other.

Results: We obtained nucleotide sequences covering 106.0Gb of CHCCC genome (37.1 x coverage), 102.6Gb of HCC genome (35.9 x coverage), and 106.5Gb (37.3 x coverage) of NCL genome. The sequenced reads covered 99.5% on all three genomes. Comparison of the CHCCC and NCL genomes showed 13,544 somatic single nucleotide variants (SNV), 3,789 small insertions and deletions, and 57 structural variants in CHCCC genome. Distinct SNVs were composed of 2.2% on exon, 37.3% on intron, and 60.5% on intergenic regions. Comparison of the HCC and NCL genomes showed 3,675 somatic single nucleotide variants (SNV), 3,491 small insertions and deletions, and 18 structural variants in HCC genome. Distinct SNVs in HCC were composed of 2.6% on exon, 39.9% on intron, and 57.5% on intergenic regions.

Conclusion: The prevalence of somatic SNVs in CHCCC is much more than HCC when compared with NCL. And that indicates more complex process were involved in CHCCC. Following study is warranted for finding significant changes and process, and validation.

P-052
Projection of Long-Term Bone and Renal Outcomes Using Tenofovir Alafenamide (TAF) for the Management of Chronic Hepatitis B (CHB) in China
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Background: Chronic hepatitis B infection burden in China is high, with ~96M patients infected. Treatments goals are to suppress viral replication and achieve normalization of alanine aminotransferase (ALT) levels to avoid liver damage and related liver complications; however, CHB treatments can increase risk of bone and renal adverse events. In this study, we simulated the long-term bone and renal consequences of nucleos(t)ide analog therapies on 100,000 Chinese CHB patients comparing TAF to tenofovir disoproxil fumarate (TDF) and entecavir (ETV).

Methods: The lifetime health outcomes model used an individual patient simulation framework. Risk of Stage 3 chronic kidney disease (CKDIII) and end-stage renal disease (ESRD) were based on treatment-specific changes on estimated glomerular filtration rates (eGFR). Risk of non-traumatic fracture was based on applying treatment-specific fracture risks (TAF hazard ratio vs. TDF: 0.956 from trials; ETV equivalent to TAF) to 10-year fracture rates from the Fracture Risk Assessment Tool algorithm. Life expectancy was influenced by treatment efficacy-related impacts on liver disease progression. Model inputs were sourced from randomized controlled trials and peer-reviewed Chinese literature and validated by Chinese hepatologists.

Results: Over a lifetime, TAF-treated patients were expected to have fewer events of CKDIII and ESRD (Table 1). Specifically, compared to TDF/ETV, TAF resulted in reductions of 51%/25% on CKDIII events, respectively. TAF patients had a lower lifetime rate of non-traumatic fractures, but experienced more events due to increased life expectancy.

Conclusions: Driven by its improved efficacy & safety profile, TAF is projected to reduce bone and renal complications compared to TDF and ETV.

Table 1: Number of events over lifetime horizon (based on 100,000 simulated CHB patients)
<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Number of Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKDIII</td>
<td>4864</td>
</tr>
<tr>
<td>ESRD</td>
<td>276</td>
</tr>
<tr>
<td>TDF/ETV</td>
<td>51%/25%</td>
</tr>
</tbody>
</table>

*an average starting age of 50 years old based on age distributions from clinical trial
P-053
IFNα Directly Induces Apoptosis in Hepatocytes That Accumulate Hepatitis B Large S Proteins

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Background: Chronic hepatitis B patients often experience hepatic flares during IFNα treatment, but the underlying molecular mechanisms are currently unknown. The aim of this study is to determine the impact of IFNα on hepatocytes that produce the hepatitis B large S envelope proteins (LHBs).

Methods: LHBs-transgenic (LHBs-Tg) and normal mice were injected with IFNα, and then sacrificed at various time points to analyze serum ALT, interferon-stimulated genes (ISGs), caspase-3 cleavage and molecules associated with unfolded protein responses (UPR), such as CHOP, GRP78, phospho-PERK and spliced-XBP1. To dissect how IFNα induces cell death, hepatocytes isolated from LHBs-Tg mice were cultured with or without IFNα and LDH in the supernatant was monitored. In addition, several UPR-related molecules were downregulated by siRNA before IFNα treatment.

Results: ALT increased after IFNα treatment in the LHBs-Tg mice, but not normal mice, despite similar ISG induction. The liver disease was associated with CHOP and GRP78 upregulation. Transient suppression of phospho-PERK and spliced-XBP1 occurred during ISG induction, suggesting that IFNα modulates UPR to induce apoptosis. In vitro, LDH and cleaved-caspase 3 significantly increased in IFNα-treated LHBs-Tg hepatocytes, indicating that IFNα directly induces apoptosis. Interestingly, suppression of GRP78 but not CHOP or XBP1 reduced LDH in association with upregulation of phospho-PERK and s-XBP1, suggesting that UPR upregulation rescues LHBs accumulating cells from the cytolytic effect of IFNα.

Conclusions: IFNα directly induces LHBs accumulating hepatocyte apoptosis. UPR activation by GRP78 suppression ameliorates IFNα-induced cell death.

P-054
Factors Associated with Clearance of Hepatitis B Virus Surface Antigen in Patients Infected with Human Immunodeficiency Virus

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Background: HBV co-infection is frequently observed in HIV-infected patients. Compared to only HBV-infected patients, patients with HIV/HBV co-infection tend to develop persistent infection. However, factors influencing HBsAg clearance in HIV-infected patients have not been clearly demonstrated. Therefore, we sought to determine factors associated with HBsAg clearance.

Methods: HIV-infected patients who visited our hospital from 1994 to 2017 were retrospectively examined. Among patients whose HBsAg had been positive, patients whose HBsAg disappeared afterward were determined as “cured”, while patients who remained HBsAg positive until 2017 were “persistent”. SNP analysis was performed by Taqman SNP Genotyping Assays. Fisher’s exact test and Mann-Whitney U-test were used for statistical analysis.

Results: Among 734 HIV-infected patients whose HBV-related tests were available, 28 patients were determined as cured while 21 were persistent. Three patients treated with anti-HIV drugs before the first HBsAg detection and a sole female patient were excluded. Compared to cured patients, persistent patients were older and had lower CD4 and platelet count. Higher levels of hepatic transaminases were observed in cured patients. By multivariate analysis, only an age was an independent factor for the clearance of HBsAg. SNPs in HLA-DPA1 and HLA-DPB1 were not associated in this cohort as far as we examined.

Conclusion: Considering CD4 and platelet counts were higher in cured patients, host immune status and degree of liver fibrosis are associated with HBsAg clearance. Higher transaminases may reflect host immune responses leading to HBsAg eradication. Younger patients tend to have higher CD4 and transaminases levels, which may explain the age as an independent factor.
**P-055**

**Serum IFNL3 Quantification in the Chronic Hepatitis B Patients Receiving Antiviral Therapy**


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**Background and Aim:** IFNL3 have been associated with anti-HBV activity. Nucleotide, but not nucleoside analogues have been reported to induce IFNL3 production. We measured serum IFNL3 levels in the chronic hepatitis B patients during antiviral therapy.

**Methods:** Two cases of asymptomatic carriers, 12 cases who switched to tenofovir disoproxil fumarate (TDF) from entecavir (ETV), and 22 cases who switched to tenofovir alafenamide (TAF) from TDF were included. Serum samples were collected before and after treatment switch and tested for IFNL3 quantification by ELISA. Positive control was prepared using culture media of HepG2 expressing IFNL3 (IFNL3-HepG2). According to the standard curve assay, lower limit of quantification (LOQ) was set at 50pg/ml. Mean blank value +2σ was considered as lower limit of detection.

**Results:** IFNL3 was not detectable in the culture media of HepG2, while >1000pg/ml of IFNL3 was detected in the culture media of IFNL3-HepG2. Serum samples were evaluated for the presence of IFNL3, and only 6 of 70 samples detected IFNL3 positive with <LOQ values (0 of 2 samples from asymptomatic carriers, 2 of 12 samples collected during ETV treatment, 1 of 22 samples collected during TAF treatment, and 3 of 34 samples collected during TDF treatment.) In the cases switched to TDF from ETV, 1 case showed increase of IFNL3, while 11 cases remained IFNL3 negative.

**Conclusions:** Our results indicated that serum IFNL3 levels were low in the chronic hepatitis B patients even in the treatment of nucleic acid analogs. Higher sensitive assay with larger sample size will be needed in the future studies.

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**P-056**

**Pruritus in Hepatitis B Virus Infection**

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**Background and Aim:** Pruritus is common pathogenesis in liver diseases including chronic viral hepatitis B but its prevalence and effect of hepatitis B virus (HBV) infection phase on pruritus remains unclear.

**Methods:** Of the 1,631 patients who attended our joint research facilities and were interviewed regarding their pruritus, 196 patients with HBV infection were enrolled. Prevalence of pruritus was analyzed according to HBV infection phase. One-to-one propensity score-matching using 13 variables was performed between participants in the hepatitis B e antigen (HBe-Ag)-positive/negative immune-active phase group and the inactive chronic hepatitis B phase group and multivariate analysis was performed whether these HBV infection phase associates with pruritus.

**Results:** The prevalence of pruritus in the inactive chronic hepatitis B phase was significantly lower than in the HBe-Ag-positive/negative immune-active phase (22.2% vs. 40.6%, p=0.014). Being in the inactive chronic hepatitis B phase was an independent risk factor for pruritus (odds ratio, 0.32; 95% confidence interval, 0.13–0.82; p=0.017).

**Conclusions:** In conclusion, pruritus tends to be suppressed during the inactive chronic hepatitis B phase in patients with HBV infection.
P-057
Neurometabolites Alterations Judged by Magnetic Resonance Spectroscopy may Underlie Chronic Hepatitis C Associated Fatigue


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Objectives: To evaluate the relationship between chronic hepatitis C (CHC) and severity of fatigue and to determine neurochemicals alterations using magnetic resonance spectroscopy (MRS) Patients & Methods: 100 CHC were categorized using modified Child-Pugh classification and underwent evaluation of quality of life using CLDQ questionnaire, sense of fatigue using FSS, global fatigue using the 11-point visual analogue fatigue scale (VAFS) and to determine the impact of fatigue on their daily life using MFIS. All patients underwent MRI and MRS examinations. Results: Mean total CLDQ score and VAFS mean score as well frequency of lower MFIS of Class A patients was significantly higher than Class B and C and in Class B than Class C. Mean FSS of Class A patients was significantly lower compared to other patients. Mean total MFIS score was significantly lower in Class A than in Class C patients. Neurochemicals concentration was significantly decreased in all patients than controls with significantly lower NAA concentration in Class C than in Class A patients. Conclusion: Fatigue is a prominent complaint of CHC patients and is magnified with the more liver derangement and mostly associated with altered concentrations of neurochemicals judged by MRS.

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P-058
Clinical Outcome of 5-Year Consolidation Therapy Following Virological Response in HBeAg-Negative Chronic Hepatitis B Patients Treated with Nucleos(t)ide Analogues

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Background/Aim: The durability of response after discontinuing nucleos(t)ide analogue (NA) in chronic hepatitis B (CHB) patients remains unknown. The consolidation therapy before the discontinuation of NA is suggested to be at least one year although the duration of consolidation is yet to be validated. We studied the long term outcome of HBeAg negative CHB patients who discontinued NA after 5 years of consolidation therapy.

Method: We retrospectively studied the outcomes of 88 HBeAg negative CHB patients who discontinued NA after 5-year consolidation therapy with virologic response. Consolidation therapy was defined as NA treatment which was sustained after the first undetectable serum HBV DNA before NA discontinuation. Relapse was defined as HBV DNA > 2000 IU/mL measured twice at 6 months apart within one year, or retreatment after the initial HBV DNA elevation.

Results: NAs used at discontinuation were entecavir 0.5mg (51%), lamivudine (25%), lamivudine with adefovir (12%), adefovir (6%), clevudine (3%), or telbivudine (3%). Median follow-up from the initial therapy and from the discontinuation after 5-year consolidation therapy was 85 (range 62-107) and 27 (range 7-44) months. The relapse was noted in 38 (43%) of 88 patients after discontinuing NA even with 5-year consolidation therapy. After relapse, retreatment was started in out 36 of 38 patients (95%). There was no significant factor predicting relapse after discontinuation.

Conclusion: After 5-year consolidation therapy in HBeAg negative patients, 43% of patients experienced a relapse after discontinuation of NA. This study suggests that CHB patients who discontinue NA therapy require close monitoring and proper treatment.
P-059
Differential Genetic and Immunological Response to an Acute Hepatitis B Virus F1b Infection could explain the High Frequency of Occult B Infection in the Native-American Population

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Background: Hepatocellular carcinoma in Caucasian patients infected with hepatitis B virus (HBV) F1b and occult B infection in Native populations is common in Latin America. These features may be related to differences in the immunological response due to the ethnic/genetic background.

Objective: This study aimed to describe the virological and immunological profile of an acute HBV infection with subgenotype F1b in a Mexican subject.

Methods: A 30-year old man with clinical hepatitis was evaluated at baseline and during a 9-month follow-up without antiviral therapy. HBV serology, HBV-DNA viral load, HBV genotype were assessed by standard procedures, whereas 17 serum cytokines were measured using the MAGPIX® system and MILLIPLEX® Analysis software 5.1.

Results: An incubation period of 7.5 weeks was estimated. Rapid normalization of liver enzymes and bilirubin were detected between week 15-20 post-infection. The percentage of lymphocytes increased while the viral load decreased. At week 10 post-infection, viral load was undetectable, and the level of lymphocytes remained high. A stage of occult hepatitis B was detected at week 20. The main elevated cytokines were SFas, Perforin, Granzyme, and sFasL when compared to healthy controls. The total duration was 6.9 months (27.6 weeks).

Conclusions: During the acute HBV sub-genotype F1b infection, the dynamics of liver enzymes and lymphocytes differed from the established clinical model. The levels of Perforin, Granzyme A, SFas and SFasL could play a key role in the control of acute HBV infection. HBV sub-genotype F1b may cause different clinical outcomes depending on the ethnicity of the host population.

P-060
Seroconversion of Hepatitis B Surface Antigen after Allogenic Bone Marrow Transplantation From Hepatitis B Virus-naïve Donor; A Case Report

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Rationale: It is known that allogenic bone marrow transplantation (BMT) changes immune reaction including antibody production for hepatitis virus B (HBV) infection. We report a case with hepatitis B surface antigen (HBsAg) seroconversion after BMT.

Summary: A 63-year-old female patient had BMT in 2014 against acute myelogenous leukemia. HBsAg, hepatitis B surface antibody (HBsAb) and hepatitis B core antibody (HBcAb) were negative in the donor. In the recipient, positive HBsAg, positive HBV-DNA (3.5 log10IU/ml) and negative HBsAb was observed and administration of entecavir (ETV) was maintained before and after BMT. After BMT, tacrolimus and prednisolone were used for prevention and treatment of graft-versus-host disease (GVHD). Negative and positive HBV-DNA levels (< 2.1 log10IU/ml) were observed under ETV administration. Immunosuppressant was gradually decreased according to improvement of GVHD. Two years after BMT, acute hepatic injury was suddenly observed and HBsAg seroconversion was identified. Positive HBsAb, negative HBV DNA and negative hepatitis B core-related antigen remained for 2 years after seroconversion and ETV was discontinued. Liver function test has been within normal range and HBsAb has remained positive.

Assessment: Previous reports show that HBsAg seroconversion occurs in BMT with HBsAb positive donor, which is thought to be an antigen recognition by lymphocytes from donor; however, seroconversion after BMT with HBV-naïve donor has been never reported. Immunosuppressant for several GVHDs and lowered virus level by ETV might affect the delayed activation of killer T cells from donor.

Conclusion: BMT with HBV-naïve donors could induce HBsAg seroconversion in recipients.
P-061

Serum HBV-RNA is a Potential Surrogate Marker of cccDNA

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Background: It has been reported that HBV-RNA is detectable in serum of hepatitis B patients, but its clinical significance has not been fully elucidated. We compared serum HBV-RNA with other serum markers to estimate its clinical significance.

Methods: We analyzed the serum of 178 cases of hepatitis B patients (4 asymptomatic carriers, 118 cases of active hepatitis, 56 cases of inactive carriers). We compared the detection of HBV-RNA and other HBV serological markers.

Results: HBV-RNA was detected in 46 cases (26%). The detection rates by stage were asymptomatic carriers 4 (100%), active hepatitis 39 (33%), inactive carriers 3 (5%). Among active hepatitis patients, factors related to RNA detections are HB core related antigens (HR 2.43, p = 0.0109) and serum HBV-DNA (HR 1.53, p = 0.0351) in multivariate analysis. Among 63 patients treated with nucleot(s)ide analogue, the relation between HBV-RNA with HBV-DNA was not significant. HBV-RNA detection was significantly related to HB core-associated antigen.

Conclusions: Serum HBV-RNA is related to HBV-DNA and HB core-related antigens. Even in cases treated with nucleot(s)ide analogue, the HB core-related antigen also associated with HBV-RNA detection. Since RNA transcription from cccDNA is theoretically not suppressed by nucleic acid analogs, serum HBV-RNA would reflect the transcriptional activity of cccDNA as well as core-associated antigen. Conclusion: Serum HBV-RNA is a surrogate of transcriptional activity of cccDNA.

P-062

Comprehensive Analysis of the mRNA-lncRNA Co-expression Profile and ceRNA Networks Patterns in Chronic Hepatitis B

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Background: Long non-coding RNAs (lncRNAs) are emerging as important regulators in the modulation of virus infection by targeting mRNA transcription. However, their roles in chronic hepatitis B (CHB) remain to be elucidated. To explore the lncRNAs and mRNA expression profiles in CHB and asymptomatic HBsAg carriers (ASC) and construct mRNA-lncRNA co-expression profile and ceRNA networks to identify the potential targets of diagnosis and treatment in CHB.

Methods: We determined the expression profiles of lncRNAs and mRNAs in CHB and ASC using microarray analysis. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to explore their function. We also constructed co-expression, cis-regulatory, and competing endogenous RNA (ceRNA) networks with bioinformatics methods.

Results: We identified 1634 mRNAs and 5550 lncRNAs that were differentially expressed between CHB and ASC. Significantly enriched GO terms and pathways were identified, many of which were linked to immune processes and inflammatory responses. Co-expression analysis showed 1196 relationships between the top 20 up/downregulated lncRNAs and mRNA, especially 213 lncRNAs interacted with ZFP57. The ZFP57-specific ceRNA network covered 3 lncRNAs, 5 miRNAs, and 17 edges. Cis-correlation analysis showed that IncRNA T039096 was paired with the most differentially expressed gene, ZFP57. Moreover, by expanding clinical samples size, the qRT-PCR results showed that the expression of ZFP57 and T039096 increased in CHB compared to ASC.

Conclusion: Our study provides insights into the roles of mRNA and lncRNA networks in CHB, highlighting potential applications of IncRNA-T039096 and mRNA-ZFP57 for diagnosis and treatment.
P-064
Prevalence of Hepatitis C Infection among Individuals in Central Taiwan

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Background: Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV). The global HCV prevalence is about 2.5%. The average 3.87% of HCV infection in Taiwan is determined by Taiwan CDC in 2011. Opening government data organized by Ministry of Health and Welfare revealed that the ratio of liver cancer-associated death in Yunlin located in central Taiwan is highest (25%). We would like to determine the prevalence and distribution of hepatitis C infection among populations from Yunlin.

Methods: The 8,049 blood samples from Mailiao located in Yunlin County in 2017 were collected and examined by ISO15189-certified medical laboratories. The quantitative electrochemiluminescence method was used for the detection of the anti-HCV Ab. HCV genotyping is identified by reverse transcription real-time PCR. The clinical and laboratory raw data were analyzed by SAS (Statistical Analysis System).

Results: The total of 1017 (12.6%) out of 8049 residents showed positive for anti-HCV Ab screening, 10.7% in men and 14.3% in women. HCV genotype 1b is the most epidemic subtype isolates (49.8%), followed by genotype 2 (43.9%), mixed genotype infections (2.4%), 1a (1.4%) and 6 (1.4%). The high prevalence (43.2%) in age from 70 to 80 was observed among all age groups.

Conclusion: In conclusion, the prevalence of HCV in central Taiwan is higher (12.6%) than previously reported average 3.87%. In order to reduce the risk of further complications such as liver cancers caused by HCV, the improving screening coverage could promptly find and treat the potential patients with HCV.

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P-065
Clinical Significance of the Serum Leptin Levels of Hepatitis C Patients and the Blood Lipid Levels Detection

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Background: To investigate the correlation between the serum leptin levels and blood lipids levels detection in patients with hepatitis C.

Methods and Results: 94 patients with hepatitis C in our hospital were randomly selected as the experimental group, while 88 cases of the healthy check-up as the control group. The serum leptin levels and blood lipids levels of all the subjects were detected and their results were statistically analyzed.

The average results of all the levels in the experimental groups were higher than that those of the control group. Serum leptin was increased significantly in patients with hepatitis C compared with the healthy controls (23.17±6.46) ng/ml vs (5.47±2.71) ng/ml, P<0.01). TC in the hepatitis C group increased significantly compared with that in the control group, which was of significant difference (P<0.01); HDL-C decreased more obviously in the hepatitis C group, which was of statistical difference (P<0.01).

Conclusions: Through the comprehensive indexes of the serum leptin levels and the combined detection of blood lipid in patients with Hepatitis C liver disease, it can accurately reflect the severity of the hepatitis C liver disease, and it is of guidance significance in clinical diagnosis and treatment.
P-066

Combination Therapy with Glecaprevir and Pibretasvir for Dialysis Patients Infected with Hepatitis C Virus

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Hepatitis C virus (HCV) infection is more prevalent and is associated with higher mortality in patients receiving dialysis than in the general population. Glecaprevir/pibretasvir (GLE/PIB) therapy and elbasvir+grazoprevir (EBR+GZR) therapy are 1st choice for dialysis patients with HCV according to the most recent version of Japan Society of Hepatology guideline. However, the indication of EBR+GZR therapy is limited to genotype 1 and the evidence of the efficacy and safety of GLE/PIB therapy for dialysis patients is insufficient. We evaluated the effects of GLE/PIB therapy in 6 dialysis patients with genotype 1 or 2. The treatment period was 8 or 12 weeks according to the package insert. All patients were chronic hepatitis as disease severity except 1 cirrhotic patient. The each number of patients with HCV genotype 1, 2a, or 2b was all 2. All patients were direct acting antivirals-naïve except 1 patient with the past history of viral breakthrough due to daclatasvir/asunaprevir therapy. All 6 patients achieved a rapid viral response and a sustained virological response at 12 weeks after treatment. Five patients complained of pruritus and the numbers of its grade 3, 2, or 1 according to CTCAE ver. 5.0 were 3, 1, or 1, respectively. Nalfurafine hydrochloride, antihistamine agent, and steroid ointment therapy managed to complete the scheduled treatment period in these patients. Although the efficacy of GLE/PIB therapy for dialysis patients was promising regardless of genotypes, the development of severe pruritus was a critical issue and appropriate medical care should be needed to maintain medication adherence.

P-067

Assessment of Predictors of Response to Generic Sofosbuvir-containing Regimens in the Treatment of Chronic Hepatitis C Egyptian Patients

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Background: Despite aggressive programs toward education, care, and treatment over the last 10 years, Egypt faces the largest burden of HCV infection in the world, predominantly genotype 4.

Methods: 324 patients diagnosed with chronic HCV underwent sofosbuvir-containing regimen in the treatment and followed up during and after the end of the therapy for 3-6 months. Patients were studied as regard, stage of liver fibrosis and/or presence of cirrhosis, naive versus experienced patients regarding previous treatment of chronic hepatitis c, interferon (IFN) free versus IFN containing regimen, side effects of the treatment.

Results: As regard treatment regimens, all patients received the following regimens, IFN-based therapy, (sofosbuvir + Simeprevir) and (sofosbuvir + daclatasvir) showed 100 % response while only 83.87 % of patients received (sofosbuvir + ribavirin) achieved SVR 12 and 92.31 % of patients received (sofosbuvir + daclatasvir + ribavirin) achieved SVR 12, and these results were statistically highly significant.

It was found that all patients with liver stiffness stages F0, F1, F2, and F3 achieved 100 % response (i.e.: SVR 12), Among cirrhotic patient, our result of SVR 12 was (87.5%), So, among patients who were non responders i.e. "non-SVR 12.

Conclusion: Generic sofosbuvir containing regimens are highly effective in the treatment of HCV Egyptian patients. Predictors of response concluded from our study, were female patient, aged less than 65 years old, treatment-naive, with lower liver stiffness values, higher serum albumin and a lower total bilirubin level while baseline viral load had no impact on the response to the therapy.
Successful Treatment with Glecaprevir and Pibrentasvir for Hepatitis C Complicated by Overlap Syndrome of Primary Biliary Cholangitis and Autoimmune Hepatitis

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Case report: Seventy-four years old female was referred to our hospital for elevated serum transaminase levels. She had been diagnosed as chronic hepatitis C (CHC) for 10 years. Laboratory data showed elevated IgG (2883 mg/dL), antinuclear-antibody (80x), anti-mitochondrial M2 antibody (12.8 IU/mL), and HCV-RNA (6.5 log IU/mL). Neither liver tumor nor portal hypertension were observed by contrast-enhanced computed tomography. Liver biopsy revealed significant infiltration of lymphocytes and plasma cells in portal triad, moderate interface hepatitis with mild bridging fibrosis and chronic non-suppurative destructive cholangitis. By the scoring system of International Autoimmune Hepatitis Group for the diagnosis of autoimmune hepatitis (AIH), she was diagnosed as probable AIH (score 11) and pathological findings were compatible with AIH/PBC overlap syndrome rather than CHC. The combination therapy with glecaprevir/pibrentasvir (GLE/PIB) rapidly improved serum transaminase and HCV-RNA. She achieved sustained viral response 24 weeks after GLE/PIB. No adverse events were observed, and IgG levels were normalized (1573 mg/dL) 196 days after GLE/PIB. The second liver biopsy performed 301 days after GLE/PIB demonstrated the remarkable improvement of hepatitis activity. Discussion: CHC complicated by AIH or PBC has been reported to deteriorate by the interferon-based therapy. Recent advance of CHC treatment using direct acting antivirals has been thought to have less immune-modulating effect and safer for CHC with AIH or PBC. This case is the first report of CHC with AIH/PBC overlap syndrome safely and effectively treated by DAA. Conclusions: GLE/PIB could be the effective and tolerated choice for the treatment in cases of HCV-AIH/PBC overlap syndrome.

Highly Effective Treatment Response and Well Tolerability by All Oral Direct Acting Antivirals for Chronic Hepatitis C Patients Post Organ Transplantation

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Background: For CHC patients received organ transplantation (Tx), an accelerated course of disease progression was documented due to immunosuppressants use. SVR rates for these patients treated by interferon (IFN) are poor. Besides, IFN was contradictory for patients post renal Tx due to high risks of graft rejection. Aims: To investigate the efficacy and safety of all oral direct acting antivirals (DAAs) for CHC patients after organ Tx. Patients and Methods: 32 consecutive post organ Tx (liver: 17, kidney: 13, kidney then liver: 1, heart: 1) CHC patients who treated with all oral DAAs (paritaprevir/ritonavir, ombitasvir and dasabuvir: 11, daclatasvir and asunaprevir: 4, sofosbuvir-based: 17) were enrolled. Selection of DAAs was based by genotype, drug to drug interaction profiles and Health-Insurance reimbursement criteria.

Results: Mean age of patients was 61.4±1.7 years, 50.0% of them was male and 15.6% have cirrhosis. Fourteen (43.7%) of them were failed to previous IFN. Genotype distribution was as follows: 1a: 6, 1b: 17, 2: 7, 3: 1 and 6: 1. Mean time between Tx and DAAs therapy was 77.3±11.0 months. Baseline HCV RNA was 6.20±0.19 log10 IU/mL. After DAAs, undetectable HCV RNA (< 15 IU/mL) rates at week 2, week 4 and end-of-treatment were 53.1%, 93.8% and 100%, respectively. Subjective adverse events during therapy were generally mild and no patients early terminated therapy. After post-treatment follow up, all 32 patients (100%) achieved SVR12.

Conclusions: For difficult-to-treat subpopulation like CHC post organ Tx, highly effective treatment response and well tolerability were achieved by all oral DAAs.
P-070
Treatment Efficacy and Safety of Sofosbuvir Plus Daclatasvir in Genotype 2 and 3 Chronic Hepatitis C Patients with or without Liver Cirrhosis

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Background: Based on previously published results, 12 weeks of sofosbuvir 400mg/day plus ribavirin, the current DAA regimen reimbursed by Bureau-of National-Health-Insurance (BNHI) of Taiwan for genotype-2 CHC is suboptimal in efficacy, especially for difficult-to-treat subpopulations such as liver cirrhosis, previous interferon treatment failure and high viral-load. This study aimed to evaluate the efficacy and safety of sofosbuvir (SOF) plus daclatasvir (DCV) for Taiwanese genotype-2 or -3 CHC patients.

Methods: Between March 2017 to May 2018, 50 consecutive genotype-2 or -3 CHC patients who received 12 weeks combination of SOF (400mg/day) plus DCV (60mg/day) with or without ribavirin by investigators were enrolled for analyses. When ribavirin (RBV) was added, weight-based(800-1200 mg/day) approach was applied. Sustained virological response (SVR12) was defined by undetectable HCV RNA (< 15 IU/mL) at the end and 12 weeks after the completion of therapy.

Results: The mean age was 61.4±12.2 years, 15 (30.0%) of them was male and 19 (38.0%) diagnosed to have liver cirrhosis. The mean baseline HCV RNA level was 6.16±0.89 log10 IU/mL and 96.0% (48/50) were infected by HCV genotype-2. The rates of undetectable HCV RNA (< 15 IU/mL) at week-2, week-4 and end-of-treatment were 40%, 94% and 100% respectively. Grade-2 (1.5-3.0 x ULN) hyperbilirubinemia was found in 16.0% (8/50) patients during study period and all belonged to unconjugated hyperbilirubinemia. After post-treatment follow-up, all 50 patients (100%) achieved SVR12.

Conclusion: Our real-world data in Taiwan revealed combination therapy with SOF/DCV, a more potent DAA regimen recommended by foreign professional organizations, is well-tolerated and highly effective for genotype-2 or -3 CHC patients.

P-071
The Efficacy of Glecaprevir and Pibrentasvir Therapy for Patients Who Fail Direct-acting Antiviral Therapy

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Background and Aims: Glecaprevir and Pibrentasvir (GLE/PIB) therapy was approved for chronic hepatitis type C patients who have failed direct-acting antiviral (DAA) therapy. However, NS5A resistance-associated substitutions (RASs), such as NS5A-P32del, have shown extreme resistance to NS5A inhibitors. We therefore examined the retreatment efficacy with GLE/PIB therapy and factors associated with virologic failure.

Methods: We investigated 26 patients with HCV infection who had a history of all-oral DAA therapy and had been treated with GLE/PIB. RASs at baseline and failure were evaluated by direct sequencing.

Results: Of the 26 patients, 18 had been treated with Daclatasvir and Asunaprevir (DCV/ASV) therapy, 3 had been treated with Sofosbuvir and Ledipasvir (SOF/LDV) therapy, 1 each had been treated with Ombitasvir and Paritaprevir or Elbasvir and Grazoprevir therapy, and 3 had been treated with DCV/ASV and SOF/LDV combination. The sustained virologic response (SVR) rate of GLE/PIB retreatment patients was 85.0%. All patients who had been treated with DCV/ASV and SOF/LDV achieved SVR12. Three patients showed virologic failure; two had NS5A Q24, L28, R30 and A92K RASs at baseline, and one had NS5A L31I and P32del RAS. All failure patients had Interleukin (IL) 28b singlenucleotide polymorphism (SNP) minor allele. A univariate analysis showed IL28b minor allele and NS5A A92K RAS to be associated with virologic failure (p=0.035 and 0.046, respectively)

Conclusion: On retreatment with GLE/PIB, viral factors, such as NS5A P32del and A92K RAS, and host factors, such as IL28b minor allele, might affect virologic failure.
SVR in HCV GT1b-patient with Alanine Aminotransferase Elevation Who Discontinued Grazoprevir/Elbasvir Combination Treatment at Week 8

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Hepatitis C virus (HCV) infection could lead to acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). For the prevention of disease progression in HCV-infected patients, it is important to eradicate this virus and achieve a sustained virologic response (SVR) by anti-viral therapy. We describe a Japanese female who experienced alanine aminotransferase (ALT) elevation of more than 500 IU/L during combination therapy with grazoprevir/elbasvir against HCV infection; she achieved SVR after stopping therapy at week 8. In the present report, we also focused on ALT elevation and SVR during and after antiviral therapies. Close monitoring of liver function tests is required during direct-acting antiviral treatment against HCV infection because it is now possible to treat patients with other complications, patients with polypharmacy, patients with chronic kidney disease, patients with compensated cirrhosis or aged patients. ALT elevation should be closely monitored during the use of combination therapy with grazoprevir/elbasvir.

Effectiveness of Glecaprevir/Pibrentasvir for Hepatitis C: Real-world Experience and Clinical Features of Retreatment Cases

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Background/Aims: Glecaprevir/Pibrentasvir (GLE/PIB) are direct-acting antiviral (DAA) agents that achieve a high sustained virological response (SVR) rate for hepatitis C virus (HCV) infection. We investigated GLE/PIB effectiveness for HCV patients based on real-world experience and the clinical features of retreatment cases.

Methods: HCV patients (n=130) were compared for clinical features and outcomes between first treatment (n=111) and retreatment (n=19) GLE/PIB groups.

Results: Fifty-five (42.3%) patients were male, median age was 68 years, chronic hepatitis/cirrhosis numbered 110/20 cases, and 78/47/1/4 cases had genotype 1/2/1+2/3. Excluding one case of treatment cessation (diarrhea), GLE/PIB SVR rate was 99%. There were no remarkable differences between the first treatment and retreatment groups for male (42.3 vs. 42.1%), median age (67 vs. 69 years), cirrhosis (16.5 vs. 10.5%), prior hepatocellular carcinoma (10.5 vs. 5.4%), or the fibrosis markers APRI (0.53 vs. 0.46) or FIB-4 index (2.2 vs. 2.1). The retreatment group had a significantly more frequent history of interferon treatment (16.5 vs. 47.4%, p<0.01) and Y93H mutation (26.3 vs. 63.4%, p=0.02). Pretreatment in the retreatment group was triple therapy (simeprevir or vaniprevir), DCV/ASV, LDV/SOF, EBR+GZR, SOF+RBV, and DCV/ASV+LDV/SOF. DCV/ASV (6 cases) and LDV/SOF (2 cases) pre-treated by DAAs had a Y93H mutation but no P32 deletion, while EBR+GZR (2 cases) had both Y93H and L31M.

Conclusions: GLE/PIB was effective for both HCV first treatment and retreatment despite the retreatment group having specific resistance mutations for other prior DAAs. As GLE/PIB treatment failure has been reported for P32 deletions, clinicians should consider resistance mutations during DAA selection.
P-074
The Prevalence and Risk of Hepatocellular Carcinoma after Direct-acting Antiviral Drug Treatment in Patients with Hepatitis C Virus

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Background: The direct-acting antivirals (DAAs) treatment for hepatitis C virus (HCV) have been showing high efficacy, however, their effects on hepatocarcinogenesis were not clear. Here, we examined the prevalence and risk of hepatocellular carcinoma (HCC).

Methods: This study enrolled 349 patients with HCV who underwent DAA treatment at our hospital between 2014 and 2018. Their median age was 65 years and 184 were male; 30 1 cases were of HCV serotype 1 and 48 were serotype 2. The DAA treatment was daclatasvir/asunaprevir in 107 cases, sofosbuvir (SOF)/ledipasvir in 147 cases, ritonavir-boosted ombitasvir/paritaprevir in 28 cases, elbasvir/grazoprevir in 19 cases, and SOF/ribavirin in 48 cases. HCC history in 45 cases, liver transplant (LT) in 10 cases, and kidney transplant (KT) in 17 cases were included.

Results: Of 349 patients, 335 cases (96%) were obtained sustained virologic responses. DAA was initiated with no complication of HCC, however, 15 cases (33%) had recurrence of HCC after a median of 11.6 months and 3 cases (1%) developed de novo HCC. No HCC was observed after DAA in LT and KT cases. The incidence of HCC was significantly higher in the patients with the HCC history in the Cox hazard model (hazard ratio 1.664, 95% confidence interval 1.134–2.441, p<0.01).

Conclusions: DAA did not increase the rate of HCC, even in immunosuppressed patients. In the patients with HCC history, the risk of HCC was increased even after DAA treatment. Therefore, careful follow-up for HCC is required in previously treated cases.

P-075
Association of Viral and Immunogenetic Factors with Sustained Viral Response among HCV Infected Patients Treated with Direct Acting Antivirals

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Background: In April 2015, Georgia launched national HCV elimination program in collaboration with CDC, Gilead and other international partners. Free of charge treatment with DAAs is available for all HCV infected persons. The purpose of this study was to evaluate the predictive role of viral and immunogenetic factors (HLA/KIR polymorphisms) for sustained viral response (SVR) among patients treated with direct acting antivirals (DAA) within HCV elimination program in Georgia.

Methods: Data from the HCV treatment program database of clinic Neolab, one of the major clinical sites providing HCV treatment, were analyzed. Association of baseline viral load with SVR was assessed and adjusted with other potential predictive factors of SVR, including age, viral genotype, HLA/KIR types and degree of liver fibrosis (advanced liver fibrosis if >=F3 by liver elastography or >3.25 by FIB4 score). HCV viral load, HCV genotype and HLA/KIR profiles were defined by PCR-based molecular methods. Multivariate analysis using logistic regression was conducted.

Results: SVR result was available for 2294 patients by the time of data analysis. HLA/KIR types were defined for randomly selected 460 patients from this cohort. By bivariate analysis, patients with higher baseline VL had significantly higher chance of achieving SVR, but after adjustment, fibrosis stage, virus genotype and specific HLA/KIR profile (homozygous KIR2DL3-HLA-C1 genotype) were found to be independent predictors, while VL was not significantly associated with SVR.

Conclusions: Our study showed that several viral (HCV genotype) and host (liver fibrosis stage and HLA/KIR polymorphism type) are independent predictors of SVR after antiviral treatment.
P-076
Direct Acting Anti-viral Agents in Hepatitis C Patients with Chronic Kidney Disease-Outcome Analysis

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Backgrounds: Chronic hepatitis C (CHC) is not uncommon among patients with chronic kidney disease (CKD). Treatment of CHC of the CKD patients has advanced dramatically after the introduction of direct acting anti-viral agent (DAA).

Aims: Evaluate outcomes of CHC patients with CKD treated by DAA in a medical center.

Patients and methods: Between January 2017 and Oct 2018, a total of 249 CHC patients received DAA treatment in our hospital. We defined two groups for subsequent comparisons: CKD group (CKD-G): individuals with eGFR < 60 ml/min/1.73m2 and non-CKD group (NCKD-G): individuals with eGFR >= 60 ml/min/1.73m2. Baseline characteristics and clinical outcomes were compared between two groups. A $p$ value <0.05 is considered as significant.

Results: A total of 37 and 212 patients were enrolled as CKD-G and NCKD-G respectively. The proportion of gender, baseline HCV viral loads and distribution of viral genotypes revealed no significant difference between two groups. CKD-G appear older, tend to have higher FIB-4 value and have more co-morbid conditions ($p<0.05$). Sustained virologic response at 12 weeks (SVR12) of CKD-G vs. NCKD-G revealed: 24/25 (96%) vs. 172/176 (97.7%) ($p=0.61$). After a median follow-up of 12 months, incident hepatocellular carcinoma (HCC) in CKD-G and NCKD-G were 0/37 (0%) and 8/191 (4.2%) respectively ($p=0.36$).

Conclusions: CHC patients with CKD can reach a high SVR12 by DAA treatment. CKD-G carried more co-morbid conditions, highlights the importance of monitoring drug-drug interactions. Incident HCCs were detected only in NCKD-G, though not statistically significant, a longer observation is mandatory to clarify the unexpected trend.

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P-077
Rifaximin Alleviates Endotoxemia and Intestinal Hyperpermeability with Partially Modified Fecal Microbiota in Cirrhotic Patients

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Background: Rifaximin is a minimally absorbed antibiotic effective for hepatic encephalopathy (HE). However, the mechanism of how rifaximin affects HE remains unclear. In this study, we assessed the mechanistic effect of rifaximin on intestinal permeability and gut microbiota in patients with decompensated cirrhosis.

Methods: Thirty patients with decompensated cirrhosis (Child–Pugh score >7) were assessed by cognitive neuropsychological testing, endotoxin activity (EA), and serum proinflammatory cytokines at baseline and after 4 weeks of rifaximin treatment (400 mg thrice a day). Intestinal permeability was assessed by serum levels of soluble CD163 (sCD163), mannose receptor (sMR), and zonulin. Fecal microbiome was analyzed by 16S ribosomal RNA (rRNA) gene sequencing.

Results: Treatment with rifaximin improved hyperammonemia and cognitive impairment, and decreased EA. Rifaximin also lowered serum levels of sCD163 and sMR, but not zonulin. Decreases in sCD163 and sMR were positively correlated with EA decrease ($ΔsCD163: R = 0.680, p = 0.023; ΔsMR: R = 0.613, p = 0.014, \text{vs} \Delta EA$). Rifaximin did not change the diversity or major components of the gut microbiota, although the relative abundance of Veillonella was reduced. Serum levels of proinflammatory cytokines were also unchanged.

Conclusions: Rifaximin alleviated HE and endotoxemia with improvement of intestinal hyperpermeability in patients with decompensated cirrhosis. This effect is partially associated with a change in bacterial composition, although further investigation is required to clarify other key mechanisms to preserve intestinal barrier function.
Efficacy and Safety of Administration of Zinc Acetate Hydrate (N0BELZIN) to Patients with Refractory Hepatic Encephalopathy

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Purpose: Inosine acetate hydrate (NOBELZIN) can be used as a hypozincemia ameliorating agent and can be used for patients who cannot expect sufficient effect by ingesting zinc by meals or supplement. Patients with decompensated cirrhosis developed hypozincemia and may lead to intractable hepatic encephalopathy. In this study we investigated efficacy and safety of NOBELZIN administration to patients with refractory hepatic encephalopathy and hypozincemia.

Material and Methods: Subjects were sixteen patients (10 males, 6 females, average age 69 years, C type cirrhosis 8 cases, NASH 4 cases, NBNC cirrhosis 4 cases) diagnosed as intractable hepatic encephalopathy that cannot be controlled even when using two or more hepatic encephalopathy medicines at our hospital. Blood tests before administration are Hgb 12.3, platelets 9.54 million T, bil 1.91, albumin 3.05. In addition, patients were treatment before zinc administration, non-absorbable disaccharide 10 cases, non-absorbable antibiotic 12 cases, BCAA preparation 11 cases and carnitine preparations 5 cases. NOBELZIN administration was started at 100 mg and we examined the transition of blood test (ammonia, zinc, copper et al).

Results: Ammonia decreased from 119 to 114 before or one month after administration, zinc increased from 52.2 to 99.6, and copper decreased from 87 to 76. As a side effect, it stopped with itching in one case, hypocopperemia occurred in the case of decreased renal function, stopped NOBELZIN by anemia progression.

Conclusion: In patients with refractory hepatic encephalopathy, we frequently saw hypozincemia and serum zinc was found to increase by administration of zinc acetate hydrate (NOBELZIN). Although there are many cases in which blood ammonia concentration decreases, there are few cases in which it does not decrease, so more examination is necessary. In the case of impaired renal function, serum copper value decreases, so it is necessary to check serum copper levels.

Evaluating the Coagulation Abnormalities in Liver Cirrhosis

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Background: Liver cirrhosis patients of the blood coagulation basic parameters 80-85% changed (thrombocite, quick value, fibrinogen, antithrombin III, blood flow time). S.I.Rapaport determined alcohol inhibit the growth of platelet and Buevich E.I study in XIIa, prekallikrein failure found 57% of the 93 cases.

Results: Virus and Alcoholic Liver Cirrhosis Comparative Analysis Parameters

Prothrombin time of viral liver cirrhosis group 19.29 ± 0.46, alcoholic liver cirrhosis group 19.91 ± 0.81, thrombin time of viral liver cirrhosis group 14.18 ± 0.29, alcoholic liver cirrhosis group 14.5 ± 0.45, fibrinogen viral liver cirrhosis group1.69 ± 0.12, alcoholic liver cirrhosis group 1.41 ± 0.12. There was statistically significant (p> 0.05). APTT was viral liver cirrhosis group 38.14 ± 1.21, alcoholic liver cirrhosis group 41.76 ± 1.3 (p<0.01). When alcohol causes liver cirrhosis more determined VIII, IX, XI, XII, prekallikrein, high molecular kininogen deficiency.

Virus and Co-induced Liver Cirrhosis Comparative Analysis Parameters

Blood coagulation test in prolonged prothrombin time 1.04 times, thrombin time 1.05 time, APTT 1.02 times. There wasn’t statistically significant (p>0.05). Fibrinogen decreased in viral liver cirrhosis group 1.64±0.14, co-induced liver cirrhosis 1.31±0.05 (p<0.01).

Alcohol and Co-induced Liver Cirrhosis Comparative Analysis Parameters

Alcohol liver cirrhosis blood coagulation test of APTT 40.44±1.37, co-induced cirrhosis 38.83±1.24 (p<0.01). Alcoholic cirrhosis more determined VIII, IX, XI, XII and prekallikrein, high molecular kininogen deficiencies. Prothrombin time in alcoholic liver cirrhosis 19.36 ± 0.82, co-induced liver cirrhosis group 18.95 ± 0.51, thrombin time alcoholic liver cirrhosis group 14.08 ± 0.41, co-induced liver cirrhosis group 14.01 ± 0.29, fibrinogen alcoholic liver cirrhosis group 1.48 ± 0.15, co-induced liver cirrhosis group decreased by 1.32 ± 0.08. There wasn’t statistically significant (p> 0.05).

Conclusion:

1. When alcoholic liver cirrhosis of blood coagulation determined VIII, IX, XI, XII, prekallikrein, high molecular kininogen deficiencies.
2. When virus induced liver cirrhosis decreased fibrinogen, determined disfibrinogenemia.
Identification of a Novel Barbituric Acid Derivative to Inhibit Hepatic Stellate Cells Activation and Liver Fibrosis Via Blocking NF-κB and TGF-β Signaling Pathways

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Background: Liver fibrosis is the first step toward the progression of liver cirrhosis and liver cancer. Hepatic stellate cells (HSCs) are the major cells which promote the pathogenesis of liver fibrosis. Lipopolysaccharide (LPS) and transforming growth factor-β1 (TGF-β1) signaling plays a critical role to induce inflammation and HSCs activation. Until now, there is no drug treatment for the liver fibrosis. The barbituric acid derivatives have been used as anti-asthmatic drugs in the clinical; however, the effect of treating liver fibrosis remains unknown.

Methods: The inhibition effect of HSCs activation by different barbituric acid derivatives were examined by western blot. The BAMBI expression, NF-κB signaling pathway and p65 nucleus translocation were evaluated. The secretions of inflammatory cytokines were analyzed by QPCR and ELISA. Carbon tetrachloride (CCl4) was used to induce liver fibrosis in mice. Liver injury was measured by H&E stain and macrophage marker F4/80.

Results: We identified a barbituric acid derivative (compound 1D) has the best ability to inhibit TGF-β1 induced α-SMA and collagen1A2 expression. Furthermore, the compound 1D can alleviate LPS-induced BAMBI protein reduction. The LPS-induced phosphorylation of IKKα/β, IκBα and NF-κB were decreased by pre-treating with the 1D. The p65 nucleus translocation and MCP-1 protein secretion were also inhibited. In the animal model, the administration of compound 1D reduced CCl4-induced liver injury and F4/80 expression.

Conclusion: Our study showed that the compound 1D can inhibit HSCs activation through blocking LPS-induced NF-κB and TGF-β signaling and further alleviate the liver fibrosis and macrophage recruitment in the CCl4 mouse model.

Significance of Serum Myostatin in Patients with Chronic Liver Disease

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Background: Myostatin belongs to transforming growth factor β family which has been reported as a key mediator of fibrosis in several organs, and negatively works for differentiation of skeletal muscle cells. Recent study showed higher myostatin level in liver cirrhosis associated with unfavorable outcome, however, the precise mechanism between myostatin level and poor prognosis is still unknown. The aim of our study is to elucidate the correlation between serum myostatin level and clinical parameters.

Method: 162 patients with chronic liver disease and 20 healthy control were enrolled, and serum myostatin level was calculated by ELISA method. We measured handgrip strength which has been reported to correlate with skeletal muscle function.

Results: 53 patients were diagnosed liver cirrhosis. The median serum myostatin level was 4780 pg/ml in patient group, 4329 pg/ml in healthy control group, respectively (P=0.298). Child grade B or C patients (n=145) showed significantly higher myostatin level than Child grade A patients (n=17) (P=0.004). Serum myostatin level significantly correlated with platelets (r=-0.346, P<0.001), Child Pugh score (r=0.546, P<0.001), and M2BPGi (r=0.615, P<0.001). Multiple regression analysis in patients revealed handgrip strength (P=0.018), Child Pugh score (P=0.028), and M2BPGi (P=0.001) were independent factors associated with serum myostatin level.

Conclusion: Increased serum myostatin level reflected deteriorated liver function, and we presented M2BPGi and handgrip strength significantly correlated with myostatin level. These results imply high myostatin level in liver disease mirror sarcopenia, severe liver fibrosis and potential of hepatocarcinogenesis.
P-082
Serum Myostatin is Associated with the Survival Rate and the Risk of Developing Hepatocellular Carcinoma in Patients with Liver Cirrhosis

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Background: Myostatin is a negative regulator of skeletal muscle growth and has been reported to be associated with sarcopenia that causes poor prognosis in patients with cirrhosis (LC). We evaluated the relationship between survival and hepatocellular carcinoma (HCC) incidence rate and serum myostatin concentration in LC patients.

Methods: A total of 213 consecutive patients with LC who visited our hospital during 2009-2010, were enrolled. We prospectively evaluated association between serum myostatin concentration and clinicolaboratory variables. In addition, we evaluated cumulative survival and HCC incidence rate in high and low myostatin groups.

Results: Our study cohort included 52.1% male with a median age of 63.1 years. The median followup period was 4.4 years. The median concentration of serum myostatin for the entire cohort was 5885pg/ml and for Child-Pugh A (n=172), B (n=33) and C (n=8) were 6258, 7973 and 10984pg/ml, respectively. 28 patients (13.1%) died during the follow-up period. The 4-year cumulative survival rate was 68.4% in the high-myostatin group \( \geq 7800 \text{pg/ml} \) (n=50), 89.3% in the low-myostatin group \(<7800 \text{pg/ml} \) (n=163) (P < 0.001). After excluding HCC patients (at baseline) from our analysis (n=166), the 4-year cumulative rate of HCC was 40.4% in the high-myostatin group (n=33), 20.4% in the low-myostatin group (n=133) (P =0.017).

Conclusions: Our study revealed that LC patients with high serum myostatin concentrations have low cumulative survival rate and high risk of developing HCC.

P-083
Comparison of Real-Time Shear Wave Elastography with APRI and FIB-4 to Identify Liver Fibrosis in Chronic Liver Disease

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Background: Shear wave elastography (SWE) was shown to be a non-invasive tool for quantification of liver fibrosis and had limited comparisons with other available fibrosis biomarkers. The aim of this study was to assess the performances of SWE for the diagnosis of liver fibrosis.

Methods: We prospectively enrolled 124 patients who underwent liver biopsy from January 2016 to December 2017. All participants received measurement of Toshiba shear imaging (Aplio 500), fibrosis-4 (FIB-4) score and aspartate transaminase to platelet ratio index (APRI). Areas under the receiver operating curves (AUROCs) were performed and compared for various degree of liver fibrosis.

Results: Liver fibrosis was METAVIR F1 in 17 (13.7%), F2 in 44 (35.5%), F3 in 50 (40.3%), and F4 in 13 (10.5%) patients. SWE and FIB-4 correlated significantly with histological fibrosis score \((r=0.33, p=0.0002; r=0.28, p=0.02,\) respectively) rather than APRI \((r=0.14, p=0.13).\) The optimal cut-off values of SWE for significant fibrosis \((\geq F2),\) severe fibrosis \((\geq F3)\) and cirrhosis \((F4)\) were 10.8, 13.1 and 14.6 kPa, respectively. AUROCs of SWE, FIB-4 and APRI were 0.69, 0.72 and 0.68 for the diagnosis of significant fibrosis \((\geq F2),\) 0.75, 0.71 and 0.70 for the diagnosis of severe fibrosis \((\geq F3).\) However, no statistically significant difference was observed for the diagnosis of significant and severe fibrosis between SWE and biomarkers.

Conclusion: The performance of SWE for the diagnosis of liver fibrosis was similar to those of biomarkers. The SWE showed a better accuracy for the diagnosis of severe fibrosis \((\geq F3)\) but more patients are needed to confirm the preference.
Thrombin Cleavage of Osteopontin Promotes Activation of Hepatic Stellate Cells

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**Backgrounds:** Studies indicated that osteopontin (OPN) plays a pivotal role in a variety of liver diseases. However, the molecular mechanism underlying the role of OPN in liver fibrosis is unclear. In the present study, we examined the role of thrombin-cleaved OPN (Thr-OPN) in the pathogenesis of liver fibrosis.

**Methods:** We investigated the correlation between Thr-OPN level and the severity of liver fibrosis. We further analyzed the involvement of Thr-OPN in liver fibrosis in both mouse models and LX-2 cells.

**Results:** Thr-OPN level was significantly higher in liver cirrhosis (LC) patients than that in chronic hepatitis B patients. Importantly, Thr-OPN level was positively correlated to LC degree. The correlation between hepatic Thr-OPN levels and LC degree in mouse models was consistent with the above results. Thr-OPN neutralization alleviated LC in wild-type mice, whereas Thr-OPN peptides exacerbated liver fibrosis in OPN-deficient mice. Compared with full-length OPN (FL-OPN), Thr-OPN exhibited greater ability to promote hepatic stellate cell (HSC) activation, proliferation and migration via MAP kinase and NF-κB signaling pathways.

**Conclusions:** Thr-OPN, not FL-OPN, was critically involved in the pathogenesis of liver fibrosis via MAP kinase and NF-κB signaling pathways, thus representing a novel diagnosis biomarker and treatment target for liver cirrhosis.

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microRNA Profile Analysis in Liver Fibrotic Tissue and Hepatic Differentiated Human Bone Marrow-derived Mesenchymal Stem Cells

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**Background:** Although the therapeutic mechanisms of Bone Marrow-derived Mesenchymal Stem Cells (BM-MSC) are still unclear, BM-MSCs play key therapeutic roles in liver fibrosis. Furthermore, microRNAs (miRNAs) are regulators in hepatic differentiation and liver fibrosis. The purpose of this study is to identify opposite miRNAs during hepatic trans-differentiation and stage of liver fibrosis.

**Methods:** To detect the miRNAs of hBM-MSC into functional hepatocytes and liver fibrotic tissue, the next-generation sequencing (NGS) was performed in hBM-MSCs before and after differentiation, hepatocyte, normal liver tissue, portal fibrotic tissue, and septal fibrotic tissue. Three miRNAs (miR-26a-5p, miR-26b-5p, and miR-101-3p) were selected to assess their potential to improve fibrosis.

**Results:** As a result of NGS analysis, three miRNAs showed a significantly higher expression level in differentiated hepatocyte-like cells and hepatocyte than hBM-MSC but lower expression level in liver fibrosis than normal liver. At the validation phase, Quantitative real-time PCR (qRT-PCR) results showed that the level of three miRNAs was down-regulated in TGF-β1 activated human HSC line (LX-2) and bile duct ligated (BDL) rats. In contrast, three miRNAs expression was up-regulated during hepatic differentiation of hBM-MSCs. Also, that was increased in BDL rats one week after transplanting BM-derived hepatocyte-like cells. As well as, three miRNAs mimic inhibited activation of LX-2 by TGF-β1.

**Conclusions:** In this study, we identified novel miRNAs (miR-26a-5p, miR-26b-5p, and miR-101-3p) that can regulate the hepatic fibrosis. Our results demonstrate that three miRNAs may be a biomarker, monitoring the response to therapeutic effect by BM-MSC in liver fibrosis.

**Keywords:** Human bone marrow-derived mesenchymal stem cells (hBM-MSCs), Hepatic differentiation, Next-generation sequencing (NGS), microRNAs (miRNAs), Fibrosis
Association between Polymorphisms in TGFBR1 and TGFBR2 Genes with Cirrhosis – Cytokine Gene Polymorphisms in Ethnic Indonesians with Hepatitis B and C Virus Infection

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Background: Components of the transforming growth factor-β (TGF-β) family are potent regulatory cytokines that affect multiple immune system cell types mediating pro-inflammatory or anti-inflammatory responses and are essential for tissue and organ homeostasis. TGF-β signaling participates in different stages of disease progression, from initial liver injury toward fibrosis, cirrhosis and cancer. This study aimed to investigate the association between SNPs in the TGF-β Receptor genes and their susceptibility to Cirrhosis in ethnic Indonesians.

Methods: This study consisted of cohort comprising of 113 controls, 131 Cirrhosis (HBV) and 69 Cirrhosis (HCV). 101 SNPs were identified in the promoter and exon 1-9 regions of TGBR1 and 119 SNPs were identified in the promoter and exon 1-8 regions of TGFBR2. SNP genotyping was conducted using PCR-based DNA direct sequencing. Fifteen SNPs with MAF > 0.05 were included in the association study. Logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) with adjustment to gender and age.

Results: Genotype frequencies of rs10819635, rs10760667, rs1888223, rs334354, rs334349, rs3739798 and rs1590 in TGFBR1 and genotype frequencies of rs1155705, rs764522 and rs2228048 in TGFBR2 were statistically different in Cirrhosis and control subjects. Association analysis suggested that genetic variants were statistically associated with the risk of Cirrhosis in both HBV and HCV.

Conclusions: Genetic polymorphisms in promoter region, intron 7 and 3'UTR of the TGFBR1 gene, and in promoter region, intron 3 and exon 5 of the TGFBR2 gene are associated with increased risk of Cirrhosis in our study population.

Phosphorylation of Smad3 Linker Lesion Promotes Fibro-Carcinogenesis in Nonalcoholic Steatohepatitis of Hepatocellular Carcinoma

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Background: Recently, a growing number of case reports demonstrate that hepatocellular carcinoma (HCC) sometimes develop in non-cirrhotic liver, although cirrhosis is a major risk factor for HCC development. Useful biomarkers to predict HCC development in patients with non-alcoholic steatohepatitis (NASH) are needed. Transforming growth factor (TGF)-β type I receptor (TβRI) and c-Jun N-terminal kinases (JNK) phosphorylate Smad3 differentially to create 2 isoforms phosphorylated (p) at the COOH-terminus (C) or at the linker region (L) and regulate hepatocytic fibro-carcinogenesis. This study aimed to elucidate how phospho-Smad signaling affected hepatic fibro-carcinogenesis in NASH.

Methods: Thirty patients who were histologically diagnosed as NASH and were followed more than 10 years or until onset of liver carcinogenesis. We divided patients into two groups: 17 patients not developing HCC after a diagnosis of NASH (non-HCC group; stage I: 8 cases, II: 4 cases, III: 4 cases, IV: 1 cases) and 13 patients who developed HCC concurrently or after diagnosis of NASH (HCC group; stage I: 0 cases, II: 2 cases, III: 4 case, IV: 7 cases). To investigate the phosphorylation of Smad3 in the hepatocytes, we performed immunohistochemistry and compared the staining properties in each group.

Results: Hepatocytic tumor-suppressive pSmad3C signaling shifted to fibro-carcinogenic pSmad3L signaling as liver diseases progressed. Compared with phosphorylation states of Smad3 in hepatocytic nuclei in non-HCC group, hepatocytes in HCC group showed high phosphorylation of Smad3L.

Conclusions: Phospho-Smad3 profiles are useful predictive biomarkers to predict risk of HCC development in NASH patients.
Combining Probiotics and an Angiotensin-II Receptor Blocker has Beneficial Effects on Hepatic Fibrogenesis in a Rat Model of Nonalcoholic Steatohepatitis

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Background: Intestinal endotoxin is important for the progression of nonalcoholic steatohepatitis (NASH). By improving the gut microbiota and restoring intestinal barrier functions, probiotics are effective for NASH. Angiotensin-II receptor blocker (ARB) can attenuate hepatic stellate cells and lead to suppress liver fibrosis. We evaluated the effect of combining probiotics and ARB on liver fibrosis using a rat NASH model.

Methods: Fisher 344 rats were fed a choline-deficient/L-amino acid-defined (CDAA) diet for 8 weeks to generate the NASH model. MIYAIRI 588 were used for probiotics and losartan was used for ARB. Animals were divided into probiotics, ARB, and combination groups. We analyzed the liver fibrosis, intestinal barrier function, and gut microbiome (by analyzing next-generation sequencing) of the rats.

Results: Liver fibrosis was suppressed in both probiotics and ARB group, compared to that in the CDAA group. A more potent inhibitory effect on liver fibrosis was observed in the combination group, compared to that with either drug alone. Hepatic TGF-β and TLR4 expression were decreased in both probiotic and ARB groups, compared to that in the CDAA group. In the probiotics group, LBP mRNA decreased compared to that in the CDAA group. As well as hepatic LBP, probiotics, not ARB, reduced intestinal permeability by rescuing ZO-1 disruption induced by the CDAA diet. CDAA group showed different cluster compared with control group. In addition, probiotics administration improved the microbiome disrupted by the CDAA diet.

Conclusions: The combination of probiotics and ARB are effective in suppressing liver fibrosis via different mechanisms.

Non-Alcoholic Fatty Liver Disease in Patients with Autoimmune Hepatitis

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Background and Aim: The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing all over the world. NAFLD develops in patients with liver disease, including patients with autoimmune hepatitis (AIH). NAFLD and AIH have some similar laboratory and histological findings. The aim of this study was to elucidate the characteristics of AIH patients with NAFLD.

Methods: We re-evaluated the nationwide survey performed in Japan in 2015 of AIH patients diagnosed between 2009 and 2013.

Results: A total of 1151 subjects (144 men and 1007 women) were enrolled in the present study. The overall prevalence of NAFLD was 17.0%. Compared to AIH without NAFLD, AIH patients with NAFLD had the following characteristics: (i) low female-to-male ratio, (ii) older age, (iii) mild elevation in hepatobiliary enzymes, (iv) histologically progressive fibrosis and mild plasma cell infiltration or mild lobular hepatitis, (v) lower prevalence of prednisolone administration and higher prevalence of ursodeoxycholic acid administration, (vi) higher levels of hepatic enzymes and immunoglobulin G after treatment, and (vii) similar prevalence of autoimmune and malignant complications.

Conclusion: AIH patients with NAFLD have many features that are different from AIH patients without NAFLD. Understanding these differences is essential for the proper diagnosis and treatment of AIH patients with NAFLD.
Identification of Novel Factors for Diet Induced NASH Susceptibility

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is an alarmingly rising metabolic disorder with a high worldwide prevalence. Nonalcoholic steatohepatitis (NASH) is the progressive liver damage from NAFLD with inflammation, which progress in to liver fibrosis and carcinogenesis. Exact underlying factors for NASH pathophysiology is unclear. Interestingly, we observed that high cholesterol diet (HCD) fed gene X point mutated mice (B6 X) are highly susceptible for NASH compared to Wild type mice (WT), a phenotype not reported before.

Objectives: To understand the underline mechanism of elevated NASH susceptibility of B6 X mice. Based on this understanding, develop a simple and fast diet induced NASH mouse model using B6 X mice.

Method and Results: B6 X mice carries a point mutation in gene X and this is the only genetic difference compared to WT mice. B6 X mice and WT were fed with HCD for 10 weeks. Normal diet fed mice used as controls. Body weights, Blood indices and NASH related serum parameters were monitored. Liver samples were histologically analyzed. Surprisingly, liver injury was observed in B6 X mice from post day 1 HCD feeding, with elevated serum ALT and AST levels. 2 weeks of HCD induced NASH in B6 X mice, but no symptom was observed in WT mice even after 10 weeks of diet. HCD fed B6 X mice showed significantly high mortality rate. Histological analysis of liver revealed significant inflammatory cell and lipid infiltration, and severe fibrosis. Serum cholesterol species analysis showed significantly high chylomicron and VLDL levels in B6 X mice.

Conclusion: We believe that our work will advantage the identification of susceptible genetical factors for NASH development and to expand the understanding on NASH pathophysiology.

Effects of Knockdown of ARRDC3 on Inflammasome-Associated Pathways in Human Hepatocytes

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Background and Aims: Prevalence of NAFLD and NASH is increasing worldwide. We previously observed that arrestin domain-containing protein 3 (ARRDC3) mRNA was significantly higher expressed in the liver of NASH model rat at week 4 after feeding a normal diet compared with those of the stroke-prone spontaneously hypertensive rat. We investigated the role of ARRDC3, which is linked to obesity in men and regulates body mass, adiposity and energy expenditure, in progression of NAFLD and NASH.

Methods: Inflammasomes and cytokines are major players in induction of hepatocyte apoptosis in NAFLD and NASH. To further explore mechanism, we have examined inflammasome-related gene expression profiles using real-time PCR-based focused microarrays to compare between HepG2 cells transfected with si-ARRDC3 and those with siRNA. Inflammasome-associated gene expression between HepG2 cells transfected with si-ARRDC3 and si-control were compared using inflammasomes-associated signaling target PCR array.

Results: (1) Five genes (CCL5, CASP5, IL6, IFNB1 and CXCL2) were downregulated 3-fold or more. HSP90AA1 was the only gene that was significantly upregulated. (2) Most of inflammasome-associated genes were downregulated in HepG2 cells transfected with si-ARRDC3, compared with the si-control. Among negative regulation molecules of inflammasomes, HSP90AA1 was significantly upregulated and BCL2L1, cathepsin B CTSB, HSP90AB tended to be upregulated. (3) Among NOD-like receptor-related molecules, NLRC4 and NLRP9 tended to be downregulated, and NLRX1 and NOD1 tended to be upregulated.

Conclusions: We identified ARRDC3 as an important positive regulator in NAFLD and NASH. Targeting ARRDC3 may be a good strategy to develop a novel therapeutic method against NAFLD and NASH.
P-092
B Cell Activating Factor Exacerbates Unhealthy Adipose Tissue Expansion and Liver Steatosis During Obesity

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Background: Obesity-induced adipose-tissue dysfunction is a critical contributor to the pathogenesis of nonalcoholic fatty liver disease (NAFLD). B cell-activating factor (BAFF) is an adipokine related to impaired insulin sensitivity, and the serum BAFF concentration is associated with NAFLD severity. In this study, we aimed to determine the direct in vivo role of BAFF in the development of insulin resistance, adipocyte dysfunction, and hepatic steatosis using BAFF−/− mice fed a high-fat diet (HFD).

Methods: Metabolic, histological, and biochemical analyses were performed using wild-type (WT) and BAFF−/− mice fed a high-fat diet (HFD) for 24 weeks.

Results: HFD-fed BAFF−/− mice exhibited significantly improved insulin sensitivity despite their increased weight gain and adiposity relative to HFD-fed WT mice. Moreover, inflammation, especially accumulation of CD11c+ adipose-tissue macrophages, and fibrosis of epididymal adipose tissue were reduced, which contributed to healthy adipose-tissue expansion in HFD-fed BAFF−/− mice. In line with metabolically healthy obesity, hepatic steatosis was decreased in HFD-fed BAFF−/− mice compared to HFD-fed WT mice.

Conclusions: Our data revealed that BAFF serves as a potential stimulator of unhealthy adipose-tissue expansion by triggering inflammation and fibrosis and leading to enhanced insulin resistance and NAFLD. These results suggest BAFF as a promising target for diabetes and NAFLD treatment.

P-093
Roles of CC Chemokine Receptor 9 in the Progression and Carcinogenesis of Non-Alcoholic Steatohepatitis

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Background: The number of non-alcoholic steatohepatitis (NASH) patients are increasing. This research aimed to examine role of CCR9/CCL25 axis against NASH.

Methods: Serum CCL25 level was measured by ELISA from 50 NAFLD patients. Male C57BL/6 (WT) and CCR9 deficient (CCR9−/−) mice were fed 60% high-fat and 1% high-cholesterol diet (HFHC) for 24 weeks. Bone marrow transplantation (BMT) was performed among Ly5.1 and Ly5.2 CCR9−/− mice, and they were fed with HFHC diet. WT mice fed with HFHC diet were subsequently administrated CCR9 antagonist for 6 weeks. Furthermore, WT and CCR9−/− mice administrated diethylnitrosamine and fed 60% HF diet (HF+DEN) for 45 weeks for analysis of carcinogenesis.

Results: We found serum CCL25 level was significantly higher in NAFLD patients than that of healthy volunteer. Next, we analyzed WT and CCR9−/− mice fed with HFHC diet and found CCR9−/− mice fed with HFHC ameliorated the NASH progression both compared with WT mice. Also, we found the increased TNF-α-producing CCR9+CD11b+ macrophages in WT mice fed with HFHC. Besides, we found CCR9 expression was significantly higher on hepatic stellate cell (HSC) in WT fed with HFHC by immunofluorescence analysis. BMT showed CCR9+ HSC played important role in NASH. Reduction of liver fibrosis in mice administered with CCR9 antagonist further support the pathogenic role of CCR9. Finally, we found the number and diameter of tumor were suppressed in CCR9−/− mice in carcinogenesis model.

Conclusion: CCR9/CCL25 axis mainly in HSCs plays a pathogenic role both in NASH progression and carcinogenesis, suggesting a clinical therapeutic target against NASH.

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P-094
The Plasma and Tissue MicroRNA-122 Levels of Non-alcoholic Steatohepatitis (NASH) Patients Regarding its Role in the NASH Associated Hepatocarcinogenesis
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Background: Non-alcoholic fatty liver disease (NAFLD), which can progress to non-alcoholic steatohepatitis (NASH), cirrhosis and eventually hepatocellular carcinoma (HCC), is an emerging medical concern in many countries. Circulating microRNA (miR)-122 has been regarded as a predictive biomarker of NAFLD progression to NASH, because it is a liver-specific miR that mediates fat deposition into liver. The expression level of miR-122 is expected to decrease during progression of NASH to cirrhosis and HCC due to the replacement of hepatocytes with collagen and tumor tissue.

Aims: This study aimed to investigate the significance of miR-122 blood and tissue levels during NAFLD progression to NASH and its association with NASH related HCC.

Methods: We recruited 11 simple steatosis (SS) [NAFLD fibrosis score (NFS) < 0.3] and 7 NASH fibrosis (NFS > 0.3) subjects and collected plasma samples to measure miR-122 level. We also recruited eight NASH associated HCC subjects and collected both tumor and adjacent non-tumor liver tissue after surgery of HCC. MiR-122 levels were measured with qRT-PCR and normalized by snRNA. For tissue miR-122 analysis of 8 NASH associated HCC patients, extracted RNA was analyzed by the same qRT-PCR method.

Results: Plasma miR-122 levels of NASH fibrosis were much lower than that of SS patients (p=0.05). Moreover, miR-122 levels of NASH HCC tumors were significantly lower compared to that of non-tumorous liver tissues from the same subjects (p=0.016).

Conclusion: Circulating and tissue miR-122 in NASH fibrosis and NASH HCC patients can suggest that miR-122 would be a potential biomarker for NASH associated hepatocarcinogenesis.

P-095
Assessment of the Progression of Liver Fibrosis by Body Comparison Analysis by Bioelectrical Impedance Analysis
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Background: Obesity and impaired glucose tolerance (IGT) are major risk factors for the onset of non-alcoholic fatty liver disease (NAFLD) and the development of its liver fibrosis. Body mass index (BMI) is a measure of obesity, and is greatly involved in the onset of IGT and NAFLD. However, in clinical practice, there are so-called “hidden obesity type” which has high body fat percentage even though BMI is normal, and cases with low muscle mass and liver disorder even if the body fat percentage is normal. Therefore, it is considered important to measure body composition such as body fat and muscle mass in medical treatment of NAFLD. The present study aims to investigate the relationship between various parameters obtained by body composition measurement and the progression of liver fibrosis in NAFLD.

Methods: We measured the body composition such as body fat percentage, muscle mass per body weight (muscle percentage), BMI, etc. of NAFLD patients by the bioelectrical impedance analysis (BIA). Fibrosis-4 (Fib-4) index was also measured in all cases and correlations with various parameters obtained by BIA were examined.

Results: BMI, body fat percentage, and muscle mass had no significant correlation with Fib-4 Index on their own. However, classifying the body type into nine groups with a combination of the degree of body fat and muscle percentage (high, medium, low) shows that the Fib-4 indexes in the groups with high-fat/medium-muscle and high-fat/high-muscle were significantly higher than that of the standard group (medium-fat/medium-muscle). Particularly in the high-fat/high-muscle group, the Fib-4 index tended to increase markedly with age.

Conclusions: Body composition measurements and body type determination by BIA method are considered to be useful for predicting the progress of liver fibrosis in NAFLD patients.
A Case Report: Sepsis after Liver Transplantation

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**Background:** Sepsis caused by infection is more common in patients after liver transplantation for the extensive use of high efficiency immunosuppressants. This paper reports a case of Acinetobacter baumannii infection and sepsis after liver transplantation.

**Methods and Results:** The clinical features and treatment methods of 1 patient developing sepsis after liver transplantation, who was admitted and treated in the ICU of the Guangdong general Hospital in December 2014, were retrospectively studied. One male patient at the age of 52 years old developed high fever and decrease of blood pressure at the first day after liver transplantation, and was diagnosed as septic shock. The symptoms were relieved after the appropriate treatment like goal directed fluid resuscitation, anti-infection and blood purification, etc. And the patient was discharged in stable conditions.

**Conclusions:** Liver transplantation is the most effective method for the treatment of end-stage hepatopathy. However, postoperative infection is one of the most important factors affecting the prognosis and the leading cause of death after kidney transplantation. Risk factors for Acinetobacter baumannii infection include prolonged hospitalization, intensive care, mechanical ventilation, invasive operations, exposure to antimicrobial agents, and severe underlying diseases. To sum up, patients after liver transplantation are prone to infection. Therefore, once sepsis occurs, clinicians should actively follow relevant guidelines for early goal-oriented treatment and bundle therapy. According to the results of pathogen culture, the selection of sensitive drugs can reduce the fatality rate.

Prevalence of Hepatitis E Infection in Liver Transplanted Children: A Single Centre Experience

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**Objectives and Study:** Hepatitis E virus (HEV) infection is an acute and self-limiting hepatitis but can be chronic after organ transplantation. This study aims to determine the prevalence of HEV infection in children after liver transplantation (LT).

**Methods:** Children after LT from 2003 to 2017 were retrospectively reviewed. The demographic data, HEV serology and other investigations were collated. HEV RNA were tested if they had positive anti-HEV IgM.

**Results:** Thirty one patients investigated for HEV serology because of the persistent elevated transaminases. Fourteen (45.2%) and 17 (54.8%) patients were seropositive and negative, respectively. Four patients (13%) had seropositive for anti-HEV IgM and only one (patient No1) had been detected HEV RNA in stool. Interestingly and anti-HEV serology can persist more than 3 years after transaminases resolved. For patient No2, she had persistent elevated transaminases and positive anti-HEV IgM, however, treatment as acute rejection was initially started before the result of liver pathology and serology for hepatitis profiles came back. Two weeks later, she had EBV viraemia and developed posttransplant lymphoproliferative disease (PTLD). Other 2 patients (No3 and 4) had elevated transaminases off and on but the aetiology was not clearly identified. Theses 2 patients also had persistent positive anti-HEV IgM and IgG.

**Conclusion:** The prevalence of HEV infection in children after LT is 45.2%. Chronic HEV infection were evidenced in 2 and was suspected in 2 patients. Investigation for HEV infection in children after LT with persistent elevated transaminases should be considered as the result can guide the proper management.
A Case of Immune-Mediated Drug-Induced Liver Injury Caused by Neuraminidase Inhibitor

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Laninamivir octanoate hydrate is one of treatments for influenza. It is used as a single inhalation, which is convenient and improves compliance. Commonly reported adverse drug reactions are psychiatric disorders, such as abnormal behavior, and most adverse drug reactions emerge within 3 days of inhalation. But drug-induced liver injury by laninamivir has been few reported. We report a case of drug-induced liver injury after administration of laninamivir. A 15-year-old woman was diagnosed with influenza. She was prescribed 40 mg laninamivir and inhaled the drug. Six weeks after administration of laninamivir, she was admitted to our hospital because of jaundice and fatigue. Laboratory examinations showed elevated levels of hepatobiliary enzymes, and acute liver injury was suspected. A drug-induced lymphocyte stimulating test for laninamivir was positive. Liver histological findings revealed characteristic of autoimmune hepatitis. The association between laninamivir and liver injury was deemed “probable” using the criteria of the Roussel Uclaf Causality Assessment Method scale (score of 5). In addition, the score of the revised international diagnostic scoring system of AIH was 12 points “probable”. So steroid treatment was started, but it was ineffective, and azathioprine was added to the treatment. Liver function tests normalized 2 months after azathioprine initiation. Twenty-two months after onset, second biopsy revealed absence of inflammatory infiltrations. With this result, steroid and azathioprine were withdrawn. To our knowledge, this is the first case of a patient with immune-mediated drug-induced liver injury caused by laninamivir.

Th1/Th2 Balance Changed in Idiosyncratic Drug-Induced Liver Injury: 5 Cases with Chronological Evaluation of Cytokines

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Background: Idiosyncratic drug-induced liver injury (DILI) causes acute liver injury. We previously reported DILI-induced acute liver failure (ALF) shows a poor response to treatment, leading to unfavorable outcomes. This finding indicated that progression to ALF had already determined after DILI induction. Pathophysiology underlying in the early phase of DILI needs to be understood to identify the therapeutic target. Thus, to elucidate the pathophysiology of DILI-related acute liver injury, several chemokines/cytokines were evaluated before/after DILI onset.

Methods: After identifying DILI patients, we confirmed whether patients’ serum samples obtained before DILI development were stocked. Patients whose pre- and post-DILI samples were available were enrolled. Several chemokines/cytokines were evaluated by a multiplex assay.

Results: Five DILI patients were included. DILI was first detected on day 11 [5-14] (median [range]) after the first administration of the causal agent. Transaminase levels peaked on day 14 (5-21) after the first administration. One and 4 cases were classified into the mixed and hepatocellular type, respectively. Alanine aminotransferase (ALT) level was 23 U/L (17-34) on the day of the causal agent administration, and the maximum ALT level was 213 U/L (125-1037). All patients recovered after the discontinuation of causal agents. IL-1β and IFNγ/IL-4 levels significantly increased and RANTES significantly decreased after DILI development.

Conclusion: Th1/Th2 balance changed in the early phase of DILI development. Correction of the Th1/Th2 balance can be a therapeutic strategy for prevention of progression to ALF in the patients with DILI.
P-101
Drug Induced Liver Injury (DILI) a Potential Killer in Hospitalized Patients: A Study from a Tertiary Care Hospital in Pakistan

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Background and Aim: In light of the paucity of data on DILI especially from South East Asia, this study aims to evaluate the clinical spectrum and predictors of mortality and morbidity of hospitalized patients with suspected DILI.

Methods: 462 cases were identified and categorized on basis of COIMS/RUCAM score1 and the exclusion of other liver diseases. Ratio (R value) between ALT (Alanine transerase) and AP (alkaline phosphatase) expressed as R>5; hepatocellular, R<2;cholestatic, and R >2 <5;mixed. Clinical and laboratory parameters were analyzed to identify the predictors of hospital mortality and morbidity in terms of prolonged hospital stay (>5 days).

Results: Out of 462 patients, there were 264 (57.6%) males with the mean age being 50.83 years (range, 20-94 years). DILI was classified as definite or highly probable in 31.1%, probable in 62.5%, and possible in 7.4% of cases. Pattern of liver injury was hepatocellular in 25.1%, cholestatic in 56.17% and mixed in 18.72% of patients. Mean total bilirubin levels, ALT and AP levels were 5.37mg/dl, 358.65 IU/L and 168.68 IU/L respectively.

Antituberculous (ATT) drugs were found to be the most common cause (63.9%) followed by homeopathic or herbal meds (9.5%), antiarrhythmic drugs (8.7%), statins (6.1%), antifungals (5.6%), chemotherapeutic agents (4.4%) and antiepileptics (1.7%). Altered mental status was present in 98 (21.6%) of patients while rest presented with abdominal pain, vomiting, jaundice & pruritus; 57.1%, 57.1%, 54.1%, and 42.3% respectively.

In-hospital mortality was 26.5% and prolonged hospital stay was observed in 35.93% of patients. On multivariate analysis mortality was significantly greater in patients with altered mental status, male gender, hepatocellular pattern of DILI, increased INR (>1.5) and use of ventilator support. Likewise, prolonged hospital stay was associated with female gender, increased ALT, AST aspartate aminotransferase levels, use of ventilator support  and mixed pattern of DILI. 25% patients received N-Acetylcystiene (NAC); a subgroup analysis was conducted to compare with patients who received supportive care. These patients had high INR values (p<0.001), significantly higher number had altered mental status (34.8 vs 17.9; p<0.001) and required ventilator support (29.4 vs 10.9; p<.001). A higher mortality rate was observed in NAC group (41.3%) with prolonged hospital stay in 50 (54.3%).

Conclusion: The most frequent cause of DILI was ATT in hospitalized patients in the present study. The common pattern of DILI was found to be cholestatic. More than a quarter of patients died during hospital stay. Hence, awareness among physicians is required while prescribing potentially hepatotoxic agents.

P-102
A Case of Hepatic Amyloidosis Presenting with Liver Cirrhosis of Uncertain Etiology

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Background: Primary amyloidosis (AL amyloidosis) frequently involves the liver, but rarely causes clinically apparent liver disease. Hepatomegaly and mild elevation of alkaline phosphatase are the most common clinical and biochemical findings. Here we report a case of primary amyloidosis presenting with liver cirrhosis of unknown etiology diagnosed by liver biopsy.

Case: A 54-year-old woman visited hepatology department for jaundice. She had no medical past history. There was no history of alcohol or drug use. Complete blood count showed WBC 6,500/mm3, Hb 12 g/dL and platelet 100,000/mm3. Biochemical tests showed serum AST 238 IU/L, ALT 109 IU/L, ALP 975 IU/L, albumin 2.4 g/dL, total bilirubin 7.2 mg/dL. PT(INR) was 1.5. Abdominal CT scan showed surface nodularities of liver, hepatosplenomegaly and ascites. Esophagogastroduodenoscopy revealed esophageal varices. The Child-Pugh classification was B. Viral hepatitis, metabolic causes, hepatic vascular disorders were excluded. Anti mitochondrial, anti-nuclear, anti-smooth muscle antibodies were negative. A ultrasound-guided liver biopsy was done. Liver biopsy showed deposition of amorphous materials in the peri-sinusoidal spaces. Staining with Congo red showed the classical apple-green birefringence under polarized light microscopy, diagnostic for amyloidosis. Bone marrow biopsy showed 30% k-clonal plasma cells and positive amyloid confirming the diagnosis of multiple myeloma and AL amyloidosis. She was died after 1 month of hospitalization due to multiple organ failure despite systemic chemotherapy.

Conclusions: Since hepatic amyloidosis usually does not present specific laboratory or imaging hallmarks, diagnosis is difficult. In cases of liver cirrhosis of uncertain etiology, it is very important to perform a liver biopsy and histologic examination.
P-103

Inhibitory Effects of HAV Infection on HBV Replication in Hepatocytes and Japanese Rice-koji Miso has Inhibitory Effects on HAV Replication

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Background and Aims: Hepatitis A virus (HAV) infection causes acute hepatitis including acute liver failure and occasionally results in death. Prevalence of hepatitis B infection (HBV) infection is higher in Asian-Pacific area. It was reported that patients with chronic HBV infection may have a more severe clinical course during HAV infection. We previously demonstrated that Japanese rice-koji miso extract has inhibitory effects on HAV replication. We investigated the replication of HAV and HBV in a hepatocyte coinfection models and whether Japanese rice-koji miso extract inhibits the replication of both viruses.

Methods: Human hepatoma HepG2, HepG2.2.15 cells and human hepatocyte PXB cells are used. Japanese rice-koji miso, Kurasaigetsususuijomiso, which is made from rice, soy, and salt with special Yurara yeast, is purchased from Ando Brewery, Kakunodate, Japan. HAV HA11-1299 genotype IIIA strain is used at a multiplicity of infection (MOI) of 0.01 for HAV infection. Cellular HAV RNA and HBV DNA were evaluated by real-time RT-PCR and PCR, respectively.

Results: (1) HBV replication is inhibited in HepG2.2.15 cells infected with HAV, compared to HepG2.2.15 cells without HAV infection. (2) Both HAV and HBV replications are inhibited in PXB cells coinfected with HAV and HBV, compared to those with HAV or HBV mono-infection. (3) Japanese rice-koji miso extract has inhibitory effects on HAV replication in HepG2.2.15 infected with HAV.

Conclusions: HAV suppressed HBV replication in coinfected hepatocytes. Japanese rice-koji miso extract has inhibitory effects on HAV replication and may be useful for the control of HAV infection.

P-104

Zinc Sulfate Inhibits HAV Replication

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Zinc and metallothionein play roles in antiviral activities during viral infection. We previously reported that Japanese rice-koji miso has an antiviral activity against HAV infection in hepatocytes. In this study, we observed zinc homeostasis pathway as a key pathway of antiviral activity of Japanese rice-koji miso against HAV infection in hepatocytes using RNA-Seq and transcriptome analysis. Further, zinc sulfate inhibits HAV replicon replication and enhances GRP78 expression in dose-dependent manner. Metallothionein of condition medium are enhanced by zinc sulfate in dose-independent manner. We also found an antiviral activity of zinc sulfate against HAV HA11-1299 genotype IIIA strain replication. These data provide a new approach to prevent severe hepatitis A by the use of zinc.
P-105  
Liver Dysfunction in Patients with Chronic Constipation  
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**Background:** Constipation is one of common symptoms frequently seen in Japanese clinical settings due to increasing elderly population, which has been focused on by physicians since new several medicines have been developed recently. Because one of them includes bile acid transporter inhibitor, possible relation between liver function and constipation is considered. However, there is little information regarding the status of liver function in patients with constipation.

**Methods:** From patients’ list of Teikyo University Hospital (July-October, 2018), 24 patients suffered from chronic constipation who did not have organic liver and biliary diseases evaluated by computed tomography or ultrasound, were selected as the subjects. Habitual alcohol drinkers were excluded from the subjects. The result of liver function test including serum total bilirubin, AST, ALT, ALP, γ-GTP, and total bile acid were reviewed from the medical records. Additionally, biographic data, underlying diseases, comorbidities, concomitant medicines, and constipation symptoms of the subjects were also examined.

**Results:** Of all the subjects, 7 patients (29%) showed at least one abnormal value in liver tests; AST: 5 (21%), ALT: 6 (25%), ALP: 3 (13%), γ-GTP: 2 (8%), bile acid: 1 (4%). Patients taking anthraquinone laxatives tended to show high ALT value, and those taking any psychoactive drug had tendency to show higher bile acid concentration.

**Conclusions:** Liver test abnormality seems frequent in patients with constipation. Concomitant medications might be the cause of such abnormalities.

P-106  
Epigenetic Drugs Suppress Cholangiocarcinoma and Pancreatic Cancer Organoids by Inducing an Anti-tumor Immune Response through Demethylation the Promoter Region of ERVs  
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DNA methyltransferase inhibitors (DNMTis) were initially thought to reactivate silenced tumor suppressor genes in cancer cells. However, recent studies demonstrated that DNMTis activated endogenous retroviruses (ERVs) and induced Interferon (IFN)-responsive genes. Thus, DNMTis induce the innate immune response in tumors. A newly developed 3D culture system allows long-term expansion of LGR5-positive stem cells with properties resembling those of the original tissues. We established organoids from human intrahepatic cholangiocarcinomas and also pancreatic cancers. The aim of this study is to investigate the anti-tumor effect of epigenetic drugs including DNMTis and histone deacetylase inhibitors (HDACi) on organoids derived from human cholangiocarcinoma and pancreatic cancer.

5-aza-2’-deoxycytidine (5-Aza-CdR), one of DNMTis treatment significantly reduced cell proliferation of cancer organoids and activated expression levels of ERVs and IFN-responsive genes. Bisulfite sequencing of syncytin-1, one of ERVs, revealed the increase in the number of unmethylated region after exposing 5-Aza-CdR. In addition, suberoylanilide hydroxamic acid (SAHA), one of HDACi treatment also reduced cell proliferation of cancer organoids and activated expression levels of ERVs and IFN-responsive genes. Bisulfite sequencing of syncytin-1 showed that total number of methylated regions was decreased by exposing SAHA. These results indicate that epigenetic drugs, such as 5-Aza-CdR and SAHA could exert the anti-tumor effect on cholangiocarcinoma and pancreatic cancer organoids by activating ERVs through demethylation the promoter region of ERVs and IFN-responsive genes. In addition, these results indicated that the effects of 5-Aza-CdR and SAHA are homologous at least the phenotypic alteration, ERV reactivation and demethylation of CpG island of syncytin-1 gene.
P-107
Investigating Immunomodulatory Roles of CX3CR1 Related with Differentiation and Functional Polarization of Kupffer Cells in Alcoholic Liver Disease

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Background/Aims: Even though there were many studies to identify the origin of Kupffer cells (KCs), resident macrophage in the liver, it has not been elucidated clearly. CX3CR1 which is a kind of chemokine receptor is expressed on various monocyte-macrophage lineage cells and is known to relate with liver diseases. In the present study, we investigated the role of CX3CR1 for development of KC.

Methods: For differentiation of macrophages from monocytes, sorted monocytes from mice liver and human PBMCs were co-cultured with liver sinusoidal endothelial cells (LSECs) and human umbilical venous endothelial cells (HUVECs). Alcoholic liver injury was achieved by feeding with 5% liquid ethanol diet for 8 weeks to CX3CR1+/GFP and CX3CR1GFP/GFP mice.

Results: Isolated monocytes from liver and spleen were changed their morphology and cell surface marker with support of LSECs. During recovery period after clodronate treatment, the population of GFP+ macrophage were significantly decreased in CX3CR1 deficient mice compared to control mice. Interestingly, pro-inflammatory molecules such as IL-6, MCP-1 and TNF-α were highly expressed in F4/80 high cells from CX3CR1+/GFP mice, while F4/80 high cells which were originated from CX3CR1GFP/GFP mice exhibited abundant expression of anti-inflammatory molecule like arginase-1, IL-10 and TGF-β. Moreover, CX3CR1 deficiency reduced serum level of AST/ALT and reduced fat accumulation compared to control in alcohol-induced liver injury.

Conclusions: CX3CR1 is important factor in development and anti-inflammatory functional differentiation of KCs. Therefore, CX3CR1 could be not only novel therapeutic target for various kind of liver disease but also key molecule for studying of macrophage origin.

P-108
Estradiol Enhances Hepatic Innate Immune Responses against Double-Stranded RNA through the RIG-I Signaling Pathway in Mice

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Female predominance in the development of primary biliary cholangitis (PBC) suggests certain gender difference in hepatic innate and autoimmune responses. Estrogens are a family of female sexual hormones with an exceptionally wide spectrum of effects. It has been reported that repeated injections of polyinosinic-polycytidylic acid (poly I:C), a synthetic analog of double-stranded (ds) RNA induces autoimmune-like cholangitis in mice. Therefore, our AIM in this study was to evaluate the role of estrogens on hepatic innate immune responses against dsRNA by utilizing a murine ovariectomy (OVx) model.

Method: Male and female 8-week-old C57Bl/6 mice were given repeated injections of poly I:C twice a week for up to 24 weeks. Serum ALP levels were measured, and liver histology was assessed. Serum anti-AMA was detected by ELISA. In acute model, mice were administrated a single injection of poly I:C. Some female mice were ovariectomized followed by subcutaneous administration of estradiol slow-releasing pellets (OVx-e) or placebo (OVx-p) in 4 weeks prior to single injection of poly I:C. Hepatic mRNA levels were measured by real-time RT-PCR.

Results: In chronic model, mice remarkably developed lymphocyte-dominant inflammatory infiltration surrounding bile ducts, and ALP and AMA levels were increased only in females, but not in male mice. In acute model, hepatic expression of TNFα and IFNβ mRNA levels significantly increased in females compared to male in 1h after single injection of poly I:C. Interestingly, hepatic mRNA levels for RIG-I were significantly higher in female mice as compared to male mice before injection of poly I:C whereas no significant differences in TLR3 or MDA5 mRNA levels between females and males. Moreover, TNFα were higher in OVx-e than in OVx-p. RIG-I were higher in OVx-e compared to those in OVx-p before injection of poly I:C whereas no significant differences in TLR3 and MDA5 mRNA.

Conclusions: These findings clearly demonstrated that female mice are more susceptible to autoimmune-like cholangitis caused by repeated injections of poly I:C. Our data hypothesized that estrogen plays a pivotal role in innate immune responses in cholangitis after poly I:C through RIG-I signaling pathway.