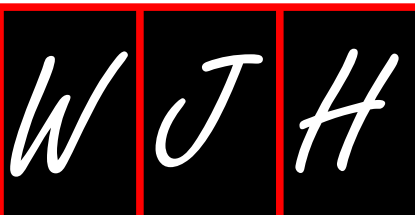


World Journal of *Hepatology*

World J Hepatol 2017 August 18; 9(23): 979-1012





Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 474 members, representing a team of worldwide experts in hepatology. They are from 52 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (2), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (97), Czech Republic (1), Denmark (2), Egypt (12), France (6), Germany (20), Greece (11), Hungary (5), India (15), Indonesia (3), Iran (4), Israel (1), Italy (54), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (12), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (18), and United States (55).

EDITORS-IN-CHIEF

Clara Balsano, *Rome*
Wan-Long Chuang, *Kaohsiung*

ASSOCIATE EDITOR

Thomas Bock, *Berlin*
Silvia Fargion, *Milan*
Ze-Guang Han, *Shanghai*
Lionel Hebbard, *Westmead*
Pietro Invernizzi, *Rozzano*
Valerio Nobili, *Rome*
Alessandro Vitale, *Padova*

GUEST EDITORIAL BOARD MEMBERS

King-Wah Chiu, *Kaohsiung*
Tai-An Chiang, *Tainan*
Chi-Tan Hu, *Hualien*
Sen-Yung Hsieh, *Taoyuan*
Wenya Huang, *Tainan*
Liang-Yi Hung, *Tainan*
Jih RU Hwu, *Hsinchu*
Jing-Yi Lee, *Taipei*
Mei-Hsuan Lee, *Taipei*
Chih-Wen Lin, *Kaohsiung*
Chun-Che Lin, *Taichung*
Wan-Yu Lin, *Taichung*
Tai-Long Pan, *Tao-Yuan*
Suh-Ching Yang, *Taipei*
Chun-Yan Yeung, *Taipei*

MEMBERS OF THE EDITORIAL BOARD



Algeria

Samir Rouabhia, *Batna*



Argentina

Fernando O Bessone, *Rosario*
Maria C Carrillo, *Rosario*
Melisa M Dirchwolf, *Buenos Aires*
Bernardo Frider, *Buenos Aires*
Jorge Quarleri, *Buenos Aires*
Adriana M Torres, *Rosario*



Armenia

Narina Sargsyants, *Yerevan*



Australia

Mark D Gorrell, *Sydney*



Austria

Harald Hofer, *Vienna*
Gustav Paumgartner, *Vienna*
Matthias Pinter, *Vienna*
Thomas Reiberger, *Vienna*



Bangladesh

Shahinul Alam, *Dhaka*
Mamun Al Mahtab, *Dhaka*



Belgium

Nicolas Lanthier, *Brussels*

Philip Meuleman, *Ghent*
Luisa Vonghia, *Antwerp*



Botswana

Francesca Cainelli, *Gaborone*
Sandro Vento, *Gaborone*



Brazil

Edson Abdala, *Sao Paulo*
Ilka FSF Boin, *Campinas*
Niels OS Camara, *Sao Paulo*
Ana Carolina FN Cardoso, *Rio de Janeiro*
Roberto J Carvalho-Filho, *Sao Paulo*
Julio CU Coelho, *Curitiba*
Flavio Henrique Ferreira Galvao, *Sao Paulo*
Janaina L Narciso-Schiavon, *Florianopolis*
Sílvia HC Sales-Peres, *Bauru*
Leonardo L Schiavon, *Florianópolis*
Luciana D Silva, *Belo Horizonte*
Vanessa Souza-Mello, *Rio de Janeiro*
Jaques Waisberg, *Santo André*



Bulgaria

Mariana P Penkova-Radicheva, *Stara Zagora*
Marieta Simonova, *Sofia*



Canada

Runjan Chetty, *Toronto*
Michele Molinari, *Halifax*
Giada Sebastiani, *Montreal*

**Chile**

Luis A Videla, *Santiago*

**China**

Guang-Wen Cao, *Shanghai*
 En-Qiang Chen, *Chengdu*
 Gong-Ying Chen, *Hangzhou*
 Jin-lian Chen, *Shanghai*
 Jun Chen, *Changsha*
 Alfred Cheng, *Hong Kong*
 Chun-Ping Cui, *Beijing*
 Shuang-Suo Dang, *Xi'an*
 Ming-Xing Ding, *Jinhua*
 Zhi-Jun Duang, *Dalian*
 He-Bin Fan, *Wuhan*
 Xiao-Ming Fan, *Shanghai*
 James Yan Yue Fung, *Hong Kong*
 Yi Gao, *Guangzhou*
 Zuo-Jiong Gong, *Wuhan*
 Zhi-Yong Guo, *Guangzhou*
 Shao-Liang Han, *Wenzhou*
 Tao Han, *Tianjin*
 Jin-Yang He, *Guangzhou*
 Ming-Liang He, *Hong Kong*
 Can-Hua Huang, *Chengdu*
 Bo Jin, *Beijing*
 Shan Jin, *Hohhot*
 Hui-Qing Jiang, *Shijiazhuang*
 Wan-Yee Joseph Lau, *Hong Kong*
 Guo-Lin Li, *Changsha*
 Jin-Jun Li, *Shanghai*
 Qiang Li, *Jinan*
 Sheng Li, *Jinan*
 Zong-Fang Li, *Xi'an*
 Xu Li, *Guangzhou*
 Xue-Song Liang, *Shanghai*
 En-Qi Liu, *Xi'an*
 Pei Liu, *Shenyang*
 Zhong-Hui Liu, *Changchun*
 Guang-Hua Luo, *Changzhou*
 Yi Lv, *Xi'an*
 Guang-Dong Pan, *Liuzhou*
 Wen-Sheng Pan, *Hangzhou*
 Jian-Min Qin, *Shanghai*
 Wai-Kay Seto, *Hong Kong*
 Hong Shen, *Changsha*
 Xiao Su, *Shanghai*
 Li-Ping Sun, *Beijing*
 Wei-Hao Sun, *Nanjing*
 Xue-Ying Sun, *Harbin*
 Hua Tang, *Tianjin*
 Ling Tian, *Shanghai*
 Eric Tse, *Hong Kong*
 Guo-Ying Wang, *Changzhou*
 Yue Wang, *Beijing*
 Shu-Qiang Wang, *Chengdu*
 Mary MY Wayne, *Hong Kong*
 Hong-Shan Wei, *Beijing*
 Danny Ka-Ho Wong, *Hong Kong*
 Grace Lai-Hung Wong, *Hong Kong*
 Bang-Fu Wu, *Dongguan*
 Xiong-Zhi Wu, *Tianjin*
 Chun-Fang Xu, *Suzhou*
 Rui-An Xu, *Quanzhou*
 Rui-Yun Xu, *Guangzhou*

Wei-Li Xu, *Shijiazhuang*
 Shi-Ying Xuan, *Qingdao*
 Ming-Xian Yan, *Jinan*
 Lv-Nan Yan, *Chengdu*
 Jin Yang, *Hangzhou*
 Ji-Hong Yao, *Dalian*
 Winnie Yeo, *Hong Kong*
 Zheng Zeng, *Beijing*
 Qi Zhang, *Hangzhou*
 Shi-Jun Zhang, *Guangzhou*
 Xiao-Lan Zhang, *Shijiazhuang*
 Xiao-Yong Zhang, *Guangzhou*
 Yong Zhang, *Xi'an*
 Hong-Chuan Zhao, *Hefei*
 Ming-Hua Zheng, *Wenzhou*
 Yu-Bao Zheng, *Guangzhou*
 Ren-Qian Zhong, *Shanghai*
 Fan Zhu, *Wuhan*
 Xiao Zhu, *Dongguan*

**Czech Republic**

Kamil Vysloulzil, *Olomouc*

**Denmark**

Henning Gronbaek, *Aarhus*
 Christian Mortensen, *Hvidovre*

**Egypt**

Ihab T Abdel-Raheem, *Damanhour*
 NGB G Bader EL Din, *Cairo*
 Hatem Elalfy, *Mansoura*
 Mahmoud M El-Bendary, *Mansoura*
 Mona El SH El-Raziky, *Cairo*
 Mohammad El-Sayed, *Cairo*
 Yasser M Fouad, *Minia*
 Mohamed AA Metwally, *Benha*
 Hany Shehab, *Cairo*
 Mostafa M Sira, *Shebin El-koom*
 Ashraf Taye, *Minia*
 MA Ali Wahab, *Mansoura*

**France**

Laurent Alric, *Toulouse*
 Sophie Conchon, *Nantes*
 Daniel J Felmlee, *Strasbourg*
 Herve Lerat, *Creteil*
 Dominique Salmon, *Paris*
 Jean-Pierre Vartanian, *Paris*

**Germany**

Laura E Buitrago-Molina, *Hannover*
 Enrico N De Toni, *Munich*
 Oliver Ebert, *Muenchen*
 Rolf Gebhardt, *Leipzig*
 Janine V Hartl, *Regensburg*
 Sebastian Hinz, *Kiel*
 Benjamin Juntermanns, *Essen*
 Roland Kaufmann, *Jena*
 Viola Knop, *Frankfurt*

Veronika Lukacs-Kornek, *Homburg*
 Benjamin Maasoumy, *Hannover*
 Jochen Mattner, *Erlangen*
 Nadja M Meindl-Beinker, *Mannheim*
 Ulf P Neumann, *Aachen*
 Margarete Odenthal, *Cologne*
 Yoshiaki Sunami, *Munich*
 Christoph Roderburg, *Aachen*
 Frank Tacke, *Aachen*
 Yuchen Xia, *Munich*

**Greece**

Alex P Betrosian, *Athens*
 George N Dalekos, *Larissa*
 Ioanna K Delladetsima, *Athens*
 Nikolaos K Gatselis, *Larissa*
 Stavros Gourgiotis, *Athens*
 Christos G Savopoulos, *Thessaloniki*
 Tania Siahaidou, *Athens*
 Emmanouil Sinakos, *Thessaloniki*
 Nikolaos G Symeonidi, *Thessaloniki*
 Konstantinos C Thomopoulos, *Larissa*
 Konstantinos Tziomalos, *Thessaloniki*

**Hungary**

Gabor Banhegyi, *Budapest*
 Peter L Lakatos, *Budapest*
 Maria Papp, *Debrecen*
 Ferenc Sipos, *Budapest*
 Zsolt J Tulassay, *Budapest*

**India**

Deepak N Amarapurkar, *Mumbai*
 Girish M Bhopale, *Pune*
 Sibnarayan Datta, *Tezpur*
 Nutan D Desai, *Mumbai*
 Sorabh Kapoor, *Mumbai*
 Jaswinder S Maras, *New Delhi*
 Nabeen C Nayak, *New Delhi*
 C Ganesh Pai, *Manipal*
 Amit Pal, *Chandigarh*
 K Rajeshwari, *New Delhi*
 Anup Ramachandran, *Vellore*
 D Nageshwar Reddy, *Hyderabad*
 Shivaram P Singh, *Cuttack*
 Ajith TA, *Thrissur*
 Balasubramaniyan Vairappan, *Pondicherry*

**Indonesia**

Pratika Yuhyi Hernanda, *Surabaya*
 Cosmas RA Lesmana, *Jakarta*
 Neneng Ratnasari, *Yogyakarta*

**Iran**

Seyed M Jazayeri, *Tehran*
 Sedigheh Kafi-Abad, *Tehran*
 Iradj Maleki, *Sari*
 Fakhraddin Naghibalhossaini, *Shiraz*

**Israel**

Stephen DH Malnick, *Rehovot*

**Italy**

Francesco Angelico, *Rome*
 Alfonso W Avolio, *Rome*
 Francesco Bellanti, *Foggia*
 Marcello Bianchini, *Modena*
 Guglielmo Borgia, *Naples*
 Mauro Borzio, *Milano*
 Enrico Brunetti, *Pavia*
 Valeria Cento, *Roma*
 Beatrice Conti, *Rome*
 Francesco D'Amico, *Padova*
 Samuele De Minicis, *Fermo*
 Fabrizio De Ponti, *Bologna*
 Giovan Giuseppe Di Costanzo, *Napoli*
 Luca Fabris, *Padova*
 Giovanna Ferraioli, *Pavia*
 Matteo Garcovich, *Rome*
 Edoardo G Giannini, *Genova*
 Rossano Girometti, *Udine*
 Alessandro Granito, *Bologna*
 Alberto Grassi, *Rimini*
 Alessandro Grasso, *Savona*
 Francesca Guerrieri, *Rome*
 Quirino Lai, *Aquila*
 Andrea Lisotti, *Bologna*
 Marcello F Maida, *Palermo*
 Lucia Malaguarnera, *Catania*
 Andrea Mancuso, *Palermo*
 Luca Maroni, *Ancona*
 Francesco Marotta, *Milano*
 Pierluigi Marzuillo, *Naples*
 Sara Montagnese, *Padova*
 Giuseppe Nigri, *Rome*
 Claudia Piccoli, *Foggia*
 Camillo Porta, *Pavia*
 Chiara Raggi, *Rozzano (MI)*
 Maria Rendina, *Bari*
 Maria Ripoli, *San Giovanni Rotondo*
 Kryssia I Rodriguez-Castro, *Padua*
 Raffaella Romeo, *Milan*
 Amedeo Sciarra, *Milano*
 Antonio Solinas, *Sassari*
 Aurelio Sonzogni, *Bergamo*
 Giovanni Squadrito, *Messina*
 Salvatore Sutti, *Novara*
 Valentina Svicher, *Rome*
 Luca Toti, *Rome*
 Elvira Verduci, *Milan*
 Umberto Vespasiani-Gentilucci, *Rome*
 Maria A Zocco, *Rome*

**Japan**

Yasuhiro Asahina, *Tokyo*
 Nabil AS Eid, *Takatsuki*
 Kenichi Ikejima, *Tokyo*
 Shoji Ikuo, *Kobe*
 Yoshihiro Ikura, *Takatsuki*
 Shinichi Ikuta, *Nishinomiya*
 Kazuaki Inoue, *Yokohama*

Toshiya Kamiyama, *Sapporo*
 Takanobu Kato, *Tokyo*
 Saiho Ko, *Nara*
 Haruki Komatsu, *Sakura*
 Masanori Matsuda, *Chuo-city*
 Yasunobu Matsuda, *Niigata*
 Yoshifumi Nakayama, *Kitakyushu*
 Taichiro Nishikawa, *Kyoto*
 Satoshi Oeda, *Saga*
 Kenji Okumura, *Urayasu*
 Michitaka Ozaki, *Sapporo*
 Takahiro Sato, *Sapporo*
 Junichi Shindoh, *Tokyo*
 Ryo Sudo, *Yokohama*
 Atsushi Suetsugu, *Gifu*
 Haruhiko Sugimura, *Hamamatsu*
 Reiji Sugita, *Sendai*
 Koichi Takaguchi, *Takamatsu*
 Shinji Takai, *Takatsuki*
 Akinobu Takaki, *Okayama*
 Yasuhiro Tanaka, *Nagoya*
 Takuji Tanaka, *Gifu City*
 Atsunori Tsuchiya, *Niigata*
 Koichi Watashi, *Tokyo*
 Hiroshi Yagi, *Tokyo*
 Taro Yamashita, *Kanazawa*
 Shuhei Yoshida, *Chiba*
 Hitoshi Yoshiji, *Kashihara*

**Jordan**

Kamal E Bani-Hani, *Zarqa*

**Malaysia**

Peng Soon Koh, *Kuala Lumpur*
 Yeong Yeh Lee, *Kota Bahru*

**Mexico**

Francisco J Bosques-Padilla, *Monterrey*
 María de F Higuera-de la Tijera, *Mexico City*
 José A Morales-Gonzalez, *México City*

**Moldova**

Angela Peltec, *Chishinev*

**Netherlands**

Wybrich R Cnossen, *Nijmegen*
 Frank G Schaap, *Maastricht*
 Fareeba Sheedfar, *Groningen*

**Nigeria**

CA Asabamaka Onyekwere, *Lagos*

**Pakistan**

Bikha Ram Devrajani, *Jamshoro*

**Philippines**

Janus P Ong, *Pasig*
 JD Decena Sollano, *Manila*

**Poland**

Jacek Zielinski, *Gdansk*

**Portugal**

Rui T Marinho, *Lisboa*
 Joao B Soares, *Braga*

**Qatar**

Reem Al Olaby, *Doha*

**Romania**

Bogdan Dorobantu, *Bucharest*
 Liana Gheorghe, *Bucharest*
 George S Gherlan, *Bucharest*
 Romeo G Mihaila, *Sibiu*
 Bogdan Procopet, *Cluj-Napoca*
 Streba T Streba, *Craiova*

**Russia**

Anisa Gumerova, *Kazan*
 Pavel G Tarazov, *St.Petersburg*

**Saudi Arabia**

Abdulrahman A Aljumah, *Riyadh*
 Ihab MH Mahmoud, *Riyadh*
 Ibrahim Masoodi, *Riyadh*
 Mhoammad K Parvez, *Riyadh*

**Singapore**

Ser Yee Lee, *Singapore*

**South Korea**

Young-Hwa Chung, *Seoul*
 Jeong Heo, *Busan*
 Dae-Won Jun, *Seoul*
 Bum-Joon Kim, *Seoul*
 Do Young Kim, *Seoul*
 Ji Won Kim, *Seoul*
 Moon Young Kim, *Wonu*
 Mi-Kyung Lee, *Suncheon*
 Kwan-Kyu Park, *Daegu*
 Young Nyun Park, *Seoul*
 Jae-Hong Ryoo, *Seoul*
 Jong Won Yun, *Kyungsan*

**Spain**

Ivan G Marina, *Madrid*

Juan G Acevedo, *Barcelona*
 Javier Ampuero, *Sevilla*
 Jaime Arias, *Madrid*
 Andres Cardenas, *Barcelona*
 Agustin Castiella, *Mendaro*
 Israel Fernandez-Pineda, *Sevilla*
 Rocio Gallego-Duran, *Sevilla*
 Rita Garcia-Martinez, *Barcelona*
 José M González-Navajas, *Alicante*
 Juan C Laguna, *Barcelona*
 Elba Llop, *Madrid*
 Laura Ochoa-Callejero, *La Rioja*
 Albert Pares, *Barcelona*
 Sonia Ramos, *Madrid*
 Francisco Rodriguez-Frias, *Córdoba*
 Manuel L Rodriguez-Peralvarez, *Córdoba*
 Marta R Romero, *Salamanca*
 Carlos J Romero, *Madrid*
 Maria Trapero-Marugan, *Madrid*



Sri Lanka

Niranga M Devanarayana, *Ragama*



Sudan

Hatim MY Mudawi, *Khartoum*



Sweden

Evangelos Kalaitzakis, *Lund*



Switzerland

Christoph A Maurer, *Liestal*



Thailand

Taned Chitapanarux, *Chiang mai*
 Temduang Limpai boon, *Khon Kaen*
 Sith Phongkitkarun, *Bangkok*
 Yong Poovorawan, *Bangkok*



Turkey

Osman Abbasoglu, *Ankara*
 Mesut Akarsu, *Izmir*
 Umit Akyuz, *Istanbul*

Hakan Alagozlu, *Sivas*
 Yasemin H Balaban, *Istanbul*
 Bulent Baran, *Van*
 Mehmet Celikbilek, *Yozgat*
 Levent Doganay, *Istanbul*
 Fatih Eren, *Istanbul*
 Abdurrahman Kadayifci, *Gaziantep*
 Ahmet Karaman, *Kayseri*
 Muhsin Kaya, *Diyarbakir*
 Ozgur Kemik, *Van*
 Serdar Moralioglu, *Uskudar*
 A Melih Ozel, *Gebze - Kocaeli*
 Seren Ozenirler, *Ankara*
 Ali Sazci, *Kocaeli*
 Goktug Sirin, *Kocaeli*
 Mustafa Sunbul, *Samsun*
 Nazan Tuna, *Sakarya*
 Ozlem Yonem, *Sivas*



Ukraine

Rostyslav V Bubnov, *Kyiv*
 Nazarii K Kobylak, *Kyiv*
 Igor N Skrypnyk, *Poltava*



United Kingdom

Safa Al-Shamma, *Bournemouth*
 Jayantha Arnold, *Southall*
 Marco Carbone, *Cambridge*
 Rajeev Desai, *Birmingham*
 Ashwin Dhanda, *Bristol*
 Matthew Hoare, *Cambridge*
 Stefan G Hubscher, *Birmingham*
 Nikolaos Karidis, *London*
 Lemonica J Koumbi, *London*
 Patricia Lalor, *Birmingham*
 Ji-Liang Li, *Oxford*
 Evaggelia Liaskou, *Birmingham*
 Rodrigo Liberal, *London*
 Wei-Yu Lu, *Edinburgh*
 Richie G Madden, *Truro*
 Christian P Selinger, *Leeds*
 Esther Una Cidon, *Bournemouth*
 Feng Wu, *Oxford*



United States

Naim Alkhouri, *Cleveland*

Robert A Anders, *Baltimore*
 Mohammed Sawkat Anwer, *North Grafton*
 Kalyan Ram Bhamidimarri, *Miami*
 Brian B Borg, *Jackson*
 Ronald W Busuttil, *Los Angeles*
 Andres F Carrion, *Miami*
 Saurabh Chatterjee, *Columbia*
 Disaya Chavalitdhamrong, *Gainesville*
 Mark J Czaja, *Bronx*
 Jonathan M Fenkel, *Philadelphia*
 Catherine Frenette, *La Jolla*
 Lorenzo Gallon, *Chicago*
 Kalpana Ghoshal, *Columbus*
 Hie-Won L Hann, *Philadelphia*
 Shuang-Teng He, *Kansas City*
 Wendong Huang, *Duarte*
 Rachel Hudacko, *Suffern*
 Lu-Yu Hwang, *Houston*
 Ijaz S Jamall, *Sacramento*
 Neil L Julie, *Bethesda*
 Hetal Karsan, *Atlanta*
 Ahmed O Kaseb, *Houston*
 Zeid Kayali, *Pasadena*
 Timothy R Koch, *Washington*
 Gursimran S Kochhar, *Cleveland*
 Steven J Kovacs, *East Hanover*
 Mary C Kuhns, *Abbott Park*
 Jiang Liu, *Silver Spring*
 Li Ma, *Stanford*
 Francisco Igor Macedo, *Southfield*
 Sandeep Mukherjee, *Omaha*
 Natalia A Osna, *Omaha*
 Jen-Jung Pan, *Houston*
 Christine Pocha, *Minneapolis*
 Yury Popov, *Boston*
 Davide Povero, *La Jolla*
 Phillip Ruiz, *Miami*
 Takao Sakai, *Cleveland*
 Nicola Santoro, *New Haven*
 Eva Schmelzer, *Pittsburgh*
 Zhongjie Shi, *Philadelphia*
 Nathan J Shores, *New Orleans*
 Siddharth Singh, *Rochester*
 Shailendra Singh, *Pittsburgh*
 Veysel Tahan, *Columbia*
 Mehlika Toy, *Boston*
 Hani M Wadei, *Jacksonville*
 Gulam Waris, *North Chicago*
 Ruliang Xu, *New York*
 Jun Xu, *Los Angeles*
 Matthew M Yeh, *Seattle*
 Xuchen Zhang, *West Haven*
 Lixin Zhu, *Buffalo*
 Sasa Zivkovic, *Pittsburgh*



Contents

Three issues per month Volume 9 Number 23 August 18, 2017

REVIEW

- 979 Innate lymphoid cells in tissue homeostasis and diseases

Ignacio A, Breda CNS, Camara NOS

MINIREVIEWS

- 990 Use of everolimus in liver transplantation

Yee ML, Tan HH

ORIGINAL ARTICLE

Prospective Study

- 1001 MicroRNAs and clinical implications in hepatocellular carcinoma

Mohamed AA, Ali-Eldin ZA, Elbedewy TA, El-Serafy M, Ali-Eldin FA, AbdelAziz H

CASE REPORT

- 1008 Association of autoimmune hepatitis type 1 in a child with Evans syndrome

Jarasvaraparn C, Imran H, Siddiqui A, Wilson F, Gremse DA

Contents

World Journal of Hepatology
Volume 9 Number 23 August 18, 2017

ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Dr. Nicolas Lanthier, MD, PhD, Gastroenterology and Hepatology Unit, Cliniques Universitaires Saint-Luc, 1200 Brussels, Belgium

AIM AND SCOPE

World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/ABSTRACTING

World Journal of Hepatology is now indexed in Emerging Sources Citation Index (Web of Science), PubMed, PubMed Central, and Scopus.

FLYLEAF

I-IV Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Dan Li*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fung-Fung Ji*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
36 Issues/Year (8th, 18th, and 28th of each month)

EDITORS-IN-CHIEF
Clara Balsano, PhD, Professor, Departement of Biomedicine, Institute of Molecular Biology and Pathology, Rome 00161, Italy

Wan-Long Chuang, MD, PhD, Doctor, Professor, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung 807, Taiwan

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjgnet.com>

www.wjgnet.com/1948-5182/editorialboard.htm

EDITORIAL OFFICE
Xiu-Xia Song, Director
World Journal of Hepatology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238243
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLICATION DATE
August 18, 2017

COPYRIGHT
© 2017 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
<http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.f6publishing.com>

Innate lymphoid cells in tissue homeostasis and diseases

Aline Ignacio, Cristiane Naffah Souza Breda, Niels Olsen Saraiva Camara

Aline Ignacio, Cristiane Naffah Souza Breda, Niels Olsen Saraiva Camara, Laboratory of Transplantation Immunobiology, Institute of Biomedical Sciences, Department of Immunology, University of São Paulo, São Paulo, SP 05508-900, Brazil

Author contributions: Breda CNS and Ignacio A contributed equally to this work, generated the figure and table, and wrote the manuscript; Camara NOS designed the aim of the editorial and wrote the manuscript.

Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), No. 2012/02270-2.

Conflict-of-interest statement: The authors declare that there is no conflict of interest regarding the publication of this paper.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Niels Olsen Saraiva Camara, MD, PhD, Professor, Laboratory of Transplantation Immunobiology, Institute of Biomedical Sciences, Department of Immunology, University of São Paulo, Av. Prof. Lineu Prestes, 1730, Cidade Universitária, São Paulo, SP 05508-900, Brazil. niels@icb.usp.br
Telephone: +55-11-30917388
Fax: +55-11-30917224

Received: March 1, 2017

Peer-review started: March 2, 2017

First decision: March 28, 2017

Revised: May 22, 2017

Accepted: June 19, 2017

Article in press: June 20, 2017

Published online: August 18, 2017

Abstract

Innate lymphoid cells (ILCs) are the most recently

discovered family of innate immune cells. They are a part of the innate immune system, but develop from the lymphoid lineage. They lack pattern-recognition receptors and rearranged receptors, and therefore cannot directly mediate antigen specific responses. The progenitors specifically associated with the ILCs lineage have been uncovered, enabling the distinction between ILCs and natural killer cells. Based on the requirement of specific transcription factors and their patterns of cytokine production, ILCs are categorized into three subsets (ILC1, ILC2 and ILC3). First observed in mucosal surfaces, these cell populations interact with hematopoietic and non-hematopoietic cells throughout the body during homeostasis and diseases, promoting immunity, commensal microbiota tolerance, tissue repair and inflammation. Over the last 8 years, ILCs came into the spotlight as an essential cell type able to integrate diverse host immune responses. Recently, it became known that ILC subsets play a key role in immune responses at barrier surfaces, interacting with the microbiota, nutrients and metabolites. Since the liver receives the venous blood directly from the intestinal vein, the intestine and liver are essential to maintain tolerance and can rapidly respond to infections or tissue damage. Therefore, in this review, we discuss recent findings regarding ILC functions in homeostasis and disease, with a focus on the intestine and liver.

Key words: Innate lymphoid cells; Intestine; Liver; Homeostasis; Inflammatory diseases

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Receiving approximately 70% of blood through the portal vein, the liver represents one of the most important sites of defense against invading pathogens. In addition, the liver and the intestine are important immune organs, as they are often in contact with antigens and endotoxins produced by the gut microbiota. These organs are densely populated by innate immune cells such as natural killer cells, dendritic cells, macrophages, natural killer T cells and innate lymphoid cells (ILCs), which are rapidly activated by commensal and pathogenic antigens,

growth factors, cytokines and host metabolites. Recent studies have been focused on discovering the role of ILCs and how these cell populations can regulate the immune response. Our goal is to discuss innovative literature highlighting the importance of ILCs in the context of infectious disease, tissue repair, tolerance of gut microbiota and inflammatory diseases that affect the liver and intestine homeostasis.

Ignacio A, Breda CNS, Camara NOS. Innate lymphoid cells in tissue homeostasis and diseases. *World J Hepatol* 2017; 9(23): 979-989 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v9/i23/979.htm> DOI: <http://dx.doi.org/10.4254/wjh.v9.i23.979>

INTRODUCTION

Innate lymphoid cells (ILCs) are the most recent family of innate immune cells discovered among the myriad of factors that make up the immune system. They belong to innate immune system but develop from the lymphoid lineage. However, contrary to T and B lymphocytes, ILCs do not have RAG-mediated recombined antigen receptors^[1,2]. Their distribution is ubiquitous, being found throughout the body and enriched in mucosal surfaces^[3,4]. These cells are able to communicate with different cell types to orchestrate the immune system during homeostasis and inflammation^[5-7].

The non-cytotoxic ILCs consist of three different groups: ILC1, ILC2 and ILC3^[5,7-10]. The ILC3s also include the lymphoid tissue inducer (LTi) cells. These cells were uncovered in 1997 and are involved in the formation of secondary lymphoid tissues^[4].

Mirroring the Th subsets, the non-cytotoxic ILCs are separated based on cytokine expression, transcription factors during development, surface markers and distinct effector functions^[5,6]. Although parallels between ILCs and Th subsets have been observed, ILCs lack pattern-recognition receptors and therefore cannot directly mediate antigen specific responses^[3,11]. In fact, given that these cells are not directly activated by pathogen-associated molecular patterns it was unclear how ILCs discern infection, tissue injury or disruption of homeostasis. It is now known that ILCs present within adult tissues constitutively express cytokines, alarmins and growth factor receptors making them more sensitive to these mediators in their environment, enabling immediate ILC activation^[3,12]. Despite being present in very low numbers, the wide distribution of ILCs in lymphoid and non-lymphoid tissues across species was seen as an indicator of the fundamental role of these innate cells in regulating multiple physiological processes throughout the body^[7].

Several studies have shown that ILCs are easily recovered in areas susceptible to microbial colonization or invasion by pathogens, such as barrier surfaces. Recently, it became known that ILC subsets play a key

role in host immune responses to bacteria, fungi, viruses and extracellular parasites at these sites^[6,13,14]. In addition, their interaction with the microbiota, nutrients and metabolites^[6,13] highlighted important functions for ILCs in triggering tissue repair and inflammation which, if unregulated, can result in exacerbated immune responses.

Based on the emerging roles of ILCs in controlling tissue homeostasis, this review will highlight the advances in understanding how ILCs can participate in host defense in the context of immunity, microbiota, autoimmunity and tissue remodeling, focusing on the intestinal and liver pathophysiology.

ILCs DEVELOPMENT: AN OVERVIEW

Until the discovery of ILCs, conventional natural killer cells (cNKs) were the only innate cells able to respond to cytokines released by antigen presenting cells (APCs). Therefore, NK cells represent the prototypical member of the ILC family^[1,15-18]. However, NKs have additional roles that set them apart from other ILCs, such as cytotoxicity and the ability to initiate immune responses against virus and tumor cells^[18]. Besides, recent analysis of the progenitor cells and surface markers of the ILC family members indicate that NK cells and non-cytotoxic ILCs group do not come from the same lineage^[7-10].

The identification of the ILC precursors and the key factors required for development of the different ILC subsets is quite recent. It was found that the ILCs arise from a common lymphoid progenitor (CLP). Downstream, the precursors can develop into different ILC subsets and NK cells expressing the integrin $\alpha 4\beta 7$ and the transcription factors Nfil3 (nuclear factor- interleukin 3 regulated) and TOX^[7,9,19,20]. First, the common helper-like ILC progenitor (CHILP), that expresses the transcriptional regulator inhibitor of DNA binding 2 (Id2), gives rise only to ILC1, ILC2, ILC3 and LTi cells^[9,10,19]. Downstream to CHILP, another ILC precursor is able to give rise all ILCs subsets, but not LTi or cNK cells^[10]. This precursor can express the transcription factor promyelocytic leukemia zinc finger (PLZF)^[21,22].

ILC SUBSETS

As mentioned before, ILC populations differ based on their transcription factors and production of signature cytokines, similar to Th cells. However, while ILC2s and ILC3s are well characterized, ILC1s are more complex to identify due to many shared characteristics with NK cells^[23]. Both are responsive to inflammatory cytokines, such as interleukin (IL)-15 and IL-12, and produce interferon (IFN)- γ and tumor necrosis factor (TNF) after activation^[24,25]. ILC1s are enriched in the liver, skin, salivary glands, uterus, thymus and the gut^[23,26]. Regarding transcription factors, T-bet is the most important and regulates the ILC phenotype and functions, such as the production of IFN- γ . NK cells can

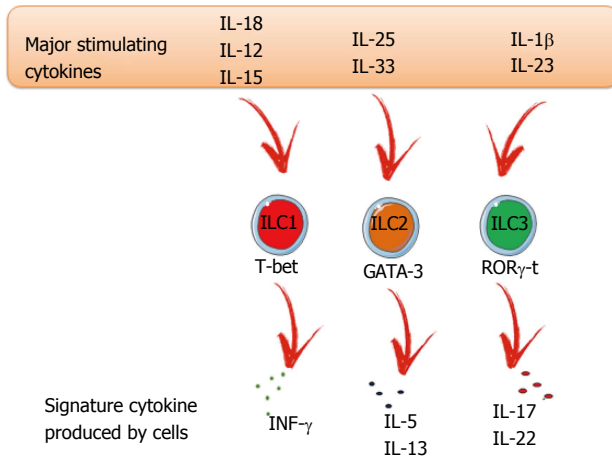


Figure 1 Innate lymphoid cell family. Each individual ILC subset is characterized by differential expression of transcription factors and patterns of expression of cytokines. ILCs can be activated by a diverse array of cytokines and can contribute to immunity, inflammation and maintenance of tissue homeostasis. IL: Interleukin; IFN- γ : Interferon γ ; ILC: Innate lymphoid cell.

also express T-bet (Figure 1). However, in NK cells, this transcription factor is not expressed in the same level, nor is it important to their development, unlike ILC1s. The T-box transcription factor eomesodermin (Eomes) is the marker of NK cells, but in some organs ILC1s can also express this transcription factor^[24,25,27,28]. The general ILC1 surface markers include CD69, NK1.1 and Nkp46 but, depending on the environment, we can find distinct ILC1 phenotypes and, consequently, surface markers. ILC1s are important to promote immunity against intracellular bacteria and parasites^[9,29-31].

ILC2s are known to produce Th2 signature cytokines, IL-5 and IL-13^[32-35] and can also release IL-9, IL-5 and IL-6^[36]. They are characterized by their responsiveness to IL-33, IL-25 and thymic stromal lymphopoietin (TSLP)^[37-40]. Their main function is to promote type 2 inflammation, which is important during allergies, helminth infection, and tissue repair^[32-34,41-43]. ILC2s are found in different tissues including lung and adipose tissue, as well as in the gut, liver and skin^[39,44-46]. Their main surface markers are CD127, c-kit, Sca1 and ST2 (the receptor for IL-33)^[11]. Regarding transcription factors, experiments with GATA-binding protein 3 (GATA-3) knockout mice have shown that this factor is essential for ILC2 differentiation and maturation (Figure 1)^[47,48].

ILC3s are a heterogeneous group found mainly in mucosal tissues^[49]. However, a small number can be found in the spleen^[50], lung^[50] and liver^[51]. Two different ILC3 subsets can be distinguished based on the expression of T-bet and CCR6. Both express RAR-related orphan receptor gamma (ROR γ t), but only LTi cells express CCR6⁺. The LTi population can be further divided based on the expression of CD4 (LTi CD4⁺ or LTi CD4⁻). The CCR6⁻ ILC3 population can express T-bet and consists of two subpopulations that are distinguished based on the expression of the natural cytotoxicity receptor (NCR) Nkp46^[52-54]. The ILC3 subset signature cytokines are IL-22 and IL-17 (Figure

1). The IL-22 acts selectively on stromal and epithelial cells leading to a rapid production of the antimicrobial peptides alpha and beta defensins. ILC3-derived IL-22 is crucial in preventing dissemination of commensal bacteria^[52,55,56].

ROLES OF ILCs SUBSETS: FROM HOMEOSTASIS TO DISEASE

The strategic location of ILCs at the mucosal surfaces ensures the induction of an immune response and shapes the adaptive immune response against invading pathogens. In addition, the presence of these cells in non-lymphoid tissues suggests that, besides regulating the proinflammatory responses, ILCs can also play a role in tissue development and homeostasis (Table 1).

ILCs induce protective immunity in response to infections

Following pathogen invasion and tissue damage, epithelial cells and innate immune cells produce cytokines and alarmins which cooperatively mobilize and activate ILCs subsets^[6]. Studies have shown that ILC-derived cytokines have an important protective function against *S. Typhimurium*^[57], *C. rodentium*^[54,58], *N. brasiliensis*^[32,48], and *C. albicans*^[59] infections.

IFN γ -producing ILC1s contribute to protection against *Salmonella enterica* subsp. *enterica* serovar Typhimurium infection in the colon. In addition, ILC3-derived IL-22 is required for the fucosylation of the intestinal epithelium which helps to protect against *S. Typhimurium* infection. Once bound to the receptor, IL-22 triggers a signaling cascade which induces fucosylation of epithelial cells, activation of the transcription factor STAT3 and consequently secretion of antimicrobial peptides^[57,60]. Murine ILC1s are also important to immune function in response to *T. gondii*^[9]. An increase of human ILC1s was shown in patients with chronic hepatitis B infection^[61], indicating that this population can contribute to immunity in response to specific pathogens in both mice and humans.

Before the onset of adaptive immune responses, the innate immune response to the enteric pathogen *Citrobacter rodentium* is critically dependent on ILC3-derived IL-22. *C. rodentium* is a gram-negative bacterium which causes acute colitis in mice. As mentioned above, the expression of antimicrobial peptides, dependent on the STAT-3 pathway, is induced by IL-22 and contributes to maintenance of the epithelial barrier surface. In addition, mice deficient in IL-22 rapidly succumb to infection due to exacerbated intestinal inflammation, bacterial invasion and proliferation throughout the tissues^[60]. IL-23 production by DCs or CX3CR1⁺ mononuclear phagocytes is necessary for ILC3 activation and it has been shown that ILC3s are the predominant source of IL-22 in the first week of *C. rodentium* infection^[60]. Satpathy *et al.*^[62] showed that *il23a*^{-/-} mice are more susceptible to infection with high

Table 1 Innate lymphoid cell functions across the intestine during homeostasis and inflammatory diseases

ILC subtype	Function	Model	Evidence	Ref.
ILC1	<i>T. gondii</i> infection	Oral infection C57BL/6 mice	Immunity to <i>T. gondii</i> infection is IFN γ -dependent; mice lacking T-bet expression had virtually no IFN-g production in response to <i>T. gondii</i> infection and failed to control parasite replication	[9]
ILC2	<i>N. brasiliensis</i> infection	Balb/c subcutaneous infection	Combined absence of IL-25 and IL-33 signaling led to a defect in worm expulsion, that was rescued by ILC2-adoptive transfer	[34]
ILC3	<i>S. Typhimurium</i> infection	Fut2-deficient C57BL/6 mice	Fucosylation of intestinal epithelial cells is catalyzed by Fut2; IL-22-derived ILC3s induce the expression of Fut2. Disruption of intestinal fucosylation led to increased susceptibility to infection by <i>S. Typhimurium</i>	[57]
ILC3	<i>C. rodentium</i> infection	Oral infection C57BL/6 mice	Mice lacking IL-22-producing ILC3 cells showed heightened susceptibility to the pathogen	[54,62]
ILC3	<i>C. albicans</i> infection	C57BL/6 and BALB/c mice	IL-22 mediates protection in IL-17RA-deficient mice; an early IL-22-dominated response is then followed by Th1/Treg reactivity	[65]
ILC2	Epithelium repair after intestinal inflammation	C57BL/6 DSS- induced colitis	Number of AREG-expressing ILC2s increases following intestinal inflammation. Disruption of the AREG-EGFR pathway exacerbated disease	[74]
ILC3	Repair of lymphoid tissue	C57BL/6 mice	LCMV infection induces the destruction of secondary lymphoid organs ROR γ -deficient WT chimeras had impaired rebuilding of stromal cell compartment after LCMV infection	[70]
ILC3	Regeneration of intestinal epithelium	C57BL/6 mice	Intestinal microbiota represses the ILC3-producing IL-22 through the induction of IL-25 by IECs. RAG-2-deficient mice treated with IL-25 showed significant weight loss in response to DSS treatment	[68]
ILC3	Containment of the gut microbiota	C57BL/6 mice	Depletion of IL22-producing ILC3s resulted in peripheral dissemination of commensal bacteria and systemic inflammation, which was prevented by administration of IL-22	[80]
			Ablation of LT α in ROR γ t + cells abrogated IgA production in the gut and altered microbiota composition	[81]
ILC1	Crohn's disease	Human	ILC1 population is increased in the inflamed intestine of people with Crohn's disease	[29,30]
ILC1	Ulcerative colitis	Anti-CD40 colitis model	IELs from the small intestine of mice treated with anti-CD40 revealed a robust production of IFN- γ by ILC1s. Anti-Nk1.1 treatment reduced inflammatory infiltration and epithelial damage, suggesting that ILC1 can contribute to colitis through IFN- γ secretion	[85]
ILC3	Ulcerative colitis	Anti-CD40 colitis model	ILC3s secrete higher amounts of GM-CSF which in turn recruits pathogenic Ly6C ⁺ inflammatory monocytes, increasing inflammation and tissue damage	[86]
ILC3	Crohn's disease	Human	Inflamed tissue from patients with CD showed accumulation of IL-23-responsive ILCs and increase expression of IL-17	[91]
ILC3	Colorectal cancer	C57BL/6 mice	Absence of IL-23 promotes tumor development accompanied by increased innate immune cell infiltration; tumorigenesis induced by IL23 could not be initiated in RAG2 ^{-/-} IL-2R ^{-/-} double knockout mice; IL-23R expression was identified in gut associated lymphoid tissue	[49] [93]

IFN: Interferon; Fut2: Fucosyltransferase 2; AREG: Amphiregulin; LT α : Lymphotoxin α ; LCMV: Lymphocytic choriomeningitis virus; GM-CSF: Granulocyte-macrophage colony-stimulating factor; EGFR: Epidermal growth factor receptor; IL: Interleukin; ILC: Innate lymphoid cell.

concentrations of this bacterium than wild type mice. As IL-23 was found to be crucial for IL-22 production by ILC3s, but not by Th17 cells, this model suggests that ILC3s are essential for resistance to *C. rodentium* infection. To clarify this phenomenon, experiments with *Rag*^{-/-}*il2rg*^{-/-} mice, which lack T cells and ILCs, showed that ILC-deficient mice are more susceptible to infection when compared to *Rag*^{-/-} mice^[54,58]. However, at a later infection stage, it was observed that T cell-derived IL-22 contributes substantially to *C. rodentium* clearance and tissue repair^[63]. Therefore, whether ILC3s and T cells can perform redundant functions cannot be ruled out. In addition to IL-22 and IL-33, IL-17 was also described to play an important role during viral hepatitis. The intrahepatic subpopulation of ILC3s can induce IL-17 signaling to induce T cell responses in viral hepatitis, improving the clearance of the virus^[64].

In a similar fashion, in the response against ba-

cterial pathogens, ILC3s seem to be the primary source of IL-22 during *Candida albicans* infection. *C. albicans* is a commensal fungus found on mucosal and skin surfaces, but can also cause infection in children younger than 1 mo, elderly and in immuno compromised individuals. A recent study showed that IL-22 acts as the first-line of defense during candidiasis by controlling fungal overgrowth and epithelial integrity. In the second stage, the Th1 response is crucial to prevent fungal dissemination^[65].

Interactions between the epithelium and ILC2s mediate immunity to helminth parasites. The type 2 immune response is characterized by the production of IL-4, IL-5, IL-9 and IL-13 cytokines. The immunity to the mouse nematode *Nippostrongylus brasiliensis* is IL-13-dependent, as this cytokine upregulates macrophage activation, development of goblet cells and smooth muscle contraction that together will induce

parasite expulsion^[7]. Even though CD4⁺ T cells are central in a type 2 immune response, it was shown that T cells are not the major producing cells of IL-13 for the expulsion of *N. brasiliensis*. CD4⁺ T cells from wild type mice were unable to induce worm expulsion when transferred into IL-4- and IL-13-deficient *Rag2*^{-/-} mice^[66]. The secretion of IL-13 by ILC2s was demonstrated by adoptive transfer of IL-13^{-/-} ILC2s, which were not able to promote worm elimination. Moreover, transferring of wild-type ILC2s into mice deficient in IL-13 supported the data whereby IL-13 secretion from ILC2s is enough for worm expulsion^[33].

Although the trigger of the signals is not well understood, these recent studies suggest a crosstalk between epithelial cells and ILCs driving appropriate ILC response. In attempting to explain the mechanisms by which ILCs interact with the non-hematopoietic and other hematopoietic cells, the employment of genetic and imaging tools are necessary to clarify it.

ILCs maintain tissue integrity

ILCs also promote the maintenance of tissue integrity by contributing to tissue remodeling and healing of tissue injury. During embryonic development, a subset of ILC3s known as LTi, promote the formation of secondary lymphoid organs such as Payer's Patches in the gut. LTi cells induce the production of chemokines CXCL13, CCL21 and CCL19 by stromal cells and the upregulation of adhesion molecules (VCAM1, MadCam1 and ICAM1) that attract and bind leukocytes to constitute lymphoid structures^[67]. ILC3s have also been implicated in repair of lymphoid tissue after damage as a result of graft-vs-host disease, acute viral infection^[7], irradiation or treatment with methotrexate. The intestinal epithelial cell regeneration can also be launched through ILC3-derived IL-22 which mediates the regeneration of the cells by acting on intestinal stem cells that express the IL-22 receptor^[60]. In addition, IL-22 can be produced in the liver, acting on hepatocytes and hepatic stellate cells (HSCs) exhibiting hepatoprotective properties by diminishing liver fibrosis and improving acute liver injury^[68-71]. Kong *et al.*^[72] identified high levels of IL-22R1 expression on HSCs. This cell type is the most involved during liver fibrogenesis. They demonstrated that IL-22, *via* STAT3, SOCS3 and p53 activation, has an antifibrotic effect by inducing the senescence of HSCs, ameliorating liver fibrosis^[72]. Matsumoto *et al.*^[73] reported that the IL-22-producing ILC3s play a protective role in a murine acute hepatitis model, by potentially blocking the hepatocyte cell death.

Corroborating the IL-22 protective role, Kudira *et al.*^[51] recently demonstrated that IL-22 contributes to liver regeneration in a partial hepatectomy (PH) model. However, they demonstrated that the source of IL-22, in this model, is ILC1s and cNK. They showed that IL-22 is essential for liver regeneration, and its production depends on the extracellular adenosine triphosphate (ATP) *via* P2X1 receptor^[51].

The fact that dying and damaged epithelial cells discharge alarmins which can be sensed by ILC2s suggests a close interaction between these two cells types. In fact, ILC2s expressing amphiregulin can regulate cell differentiation and proliferation by binding to the epidermal growth factor receptor (EGFR). IL-33-stimulated ILC2s can induce the repair of intestinal epithelial lesions after DSS-induced colitis by amphiregulin secretion^[74]. This cytokine, IL-33, as well as ILC2s, have been in the spotlight due to their contributions to the improvement of obesity-induced insulin resistance. IL-33 can bind the ST2 receptor and induce the production of large amounts of anti-inflammatory cytokines by ILC2s in adipose tissue. These cytokines lead to polarization of the adipose tissue macrophages to an M2 phenotype^[75,76]. In the liver, unlike in DSS-induced colitis and adipose tissue, IL-33 was identified as a key mediator of hepatic fibrosis. It is released in response to chronic hepatocellular stress and, after binding to ST2, culminates in ILC2s activation, as mentioned above. These cells produce anti-inflammatory and tissue remodeling cytokines, such as IL-13 and IL-4. In turn, IL-13 can activate HSCs in an IL-4Ra- and STAT6 transcription-factor-dependent fashion, a pro-fibrotic cascade. Accordingly, IL-33 plays a role in a profibrotic cascade as the apex of the signaling pathway^[77]. Another study showed that HBV infected patients have higher concentrations of IL-33 in serum compared to healthy controls. In addition, that concentration decreases following 12 wk of treatment^[78]. These findings indicate that, in certain conditions, ILC2s can be manipulated, avoiding excessive tissue remodeling, when IL-33-stimulated ILC2s secrete IL-13 and IL-4, inducing fibrosis mediated by liver stellate cells (Figure 2)^[79].

Besides their tissue repair properties, ILC2s play a role in limiting exacerbation of inflammatory responses. This can occur through the production of type 2 cytokines, that can suppress type 1 and type 17 inflammation^[3], showing the diverse roles that ILCs can play.

ILCs and the crosstalk with the intestinal microbiota

Complementary to their role in promoting immunity against pathogens, ILCs are also evolved with tolerance mechanisms regarding interactions between the host and the commensal microbiota. Recent studies have begun to disclose how ILC3s interact with gut bacteria, diet-derived factors and various cell types to maintain intestinal homeostasis.

Although the organization of ILC subsets in the gut-associated lymphoid tissues and murine intestinal tissues occur independently of microbiota colonization, the anatomical retention of lymphoid tissue resident bacteria seems to be related with ILC3s function^[80]. For example, B cells can be activated by ILC3s through lymphotoxin $\alpha 1\beta 2$ which induces the proliferation and the production of immunoglobulin A (IgA), that

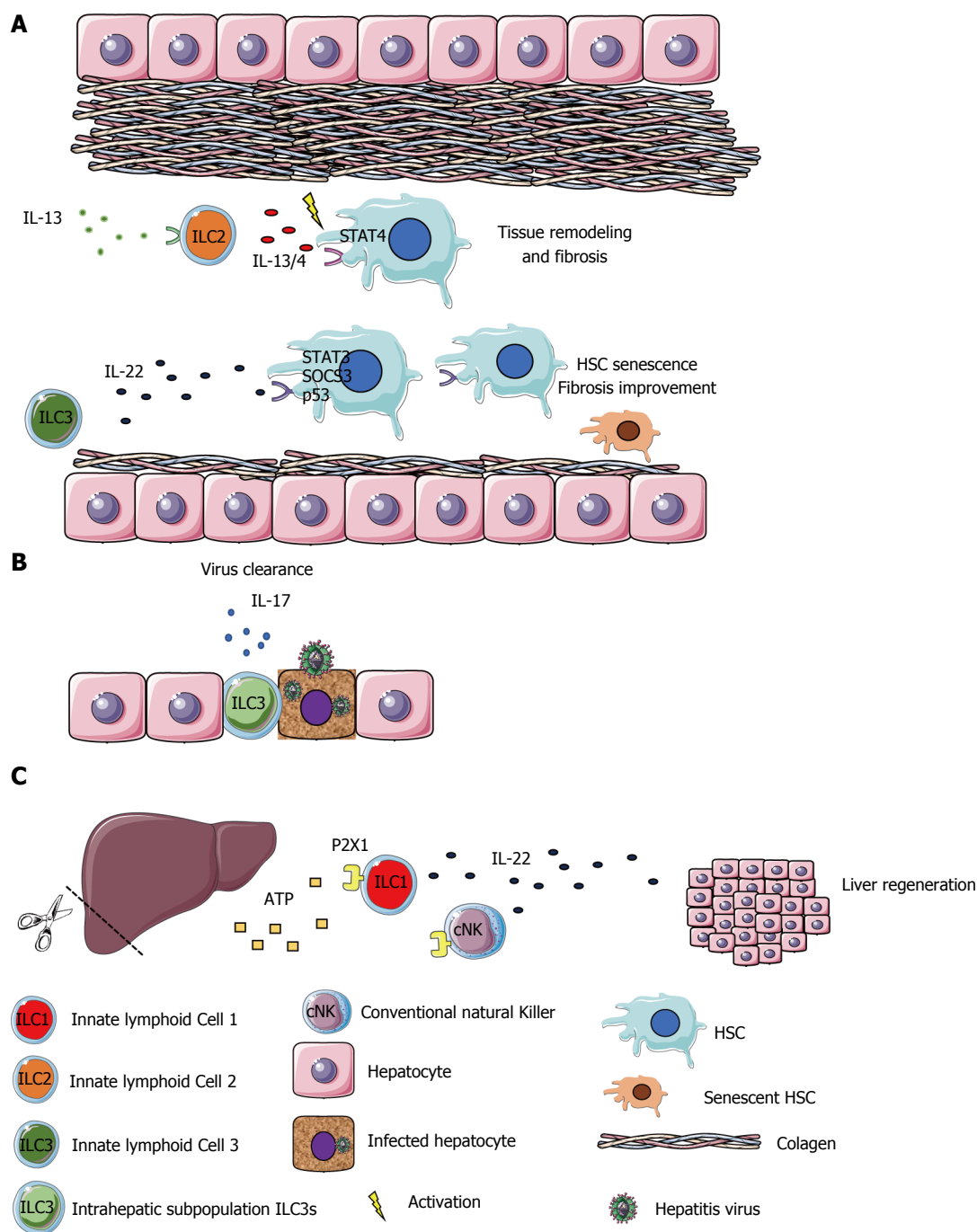


Figure 2 Innate lymphoid cell family plays different roles in the liver. ILCs can develop different functions depending on the organ and environment in which they are found. In the liver (A) IL-33, produced by hepatic cells, can act on ILC2s, promoting the release of anti-inflammatory cytokines, such as IL-13 and IL-4. These cytokines can activate HSCs, via STAT4, promoting tissue remodeling and fibrosis. On the other hand, IL-22, produced by ILC3s, acts on HSCs, via STAT3, SOCS3 and p53, promoting their senescence and ameliorating liver fibrosis; B: IL-17 can be released by the intrahepatic subpopulation ILC3s, during the virus infection promoting the clearance of the virus; C: IL-22 can be produced by ILC1s and cNK cells in the liver, contributing to liver regeneration via ATP-P2X1. Therefore, different cytokines can be manipulated, as therapeutic targets, in benefit of hepatic inflammation, fibrosis and tissue regeneration. IL: Interleukin; IFN- γ : Interferon γ ; ILC: Innate lymphoid cell; HSCs: Hepatic stellate cells.

subsequently contributes to neutralization of commensal bacteria in the lumen and prevents an inappropriate immune response^[81]. Furthermore, it was shown that ILC3s express MHC class II and although they do not express co-stimulatory molecules necessary to activate T-cells, depletion of ILC3s or selective deletion of MHC class II in these cells is associated with exacerbated bacteria-specific Th17 cell response and intestinal

inflammation^[82]. These data suggest that ILC3s can drive a host immune tolerogenic state in the intestine by controlling the functions of other immune cell types.

Controversial results have been found regarding the influence of microbiota on ILC3 function. The production of protective levels of IL-17A, IL-17F and IL-22 and the responsiveness to IL-23 suggest that both human and mice ILC3s contribute to intestinal homeostasis^[14].

Despite some studies that have shown that murine ILC IL-22 production is not affected after alteration of bacterial communities^[80], other works show that microbial products influence the level of IL-22 secretion in mice and humans^[7,14], and that germ-free mice have decreased IL-22-expressing ILC3s^[54]. In addition, epithelial cells stimulated by commensal microbiota release IL-25 which acts on CD11c⁺ cells to limit IL-22 secretion derived from ILC3s^[83]. Future work will be needed to elucidate the mechanisms by which this interaction occurs and how this process is regulated. In attempt to explain how ILC3s communicate with environmental factors in the intestine, recent studies have focused on whether dietary substances can be sensed by ILC3s. Fucose can be used as a carbohydrate source by commensal bacteria. ILC3s facilitate the transfer of fucose to the surface of intestinal epithelial cells which is critical for resistance to infection with *Salmonella Typhimurium* and to maintain the appropriate number of bacteria in the lumen^[57]. Another example is the relationship between the level of vitamin A and ILC3 functions, whereby vitamin A deficiency was related with impaired ILC3 responses^[84], suggesting that these cells sense signals from host-derived nutrients and directly from the microbiota.

ILCs promote chronic inflammatory diseases

Besides their function in promoting tissue homeostasis, the chronic activation of ILCs can also induce inflammation at mucosal surfaces. IL-23 is a powerful activator of ILC3s and this axis is intimately linked to inflammatory bowel disease (IBD). Infection-induced and sterile inflammation models of colitis such as *S. typhimurium*, *Helicobacter hepaticus*, *Helicobacter typhlonius* infectious models or anti-CD40 models have been used to better understand the ILC3 functions, which are thought to be related to stimulation by IL-23 or IL-12 and consequently release of IL-17, GM-CSF and IFN- γ ^[85-87]. IL-17-producing ILC3s have been shown to play a key role in T-cell independent mouse models and, in this context, CD127 blockade seems to reduce ILC3 numbers and ameliorates disease. It is believed that activation of dendritic cells leads to TNF and IL-23 release which in turn results in an expansion of IL-17 producing ILCs^[88]. In contrast, ILC3-derived IL-22 protects mice from intestinal inflammation trigger by *C. rodentium* infections, DSS-induced colitis and the transfer of T cells^[55,86,89]. In some murine colitis models, the blockade of intra-epithelial ILC1s and IFN- γ -producing ILC3 ameliorates the inflammation on the mucosal layer^[6,90]. Conversely, although a consistent role for ILCs in human IBDs continues to be discussed, several studies have reported varying numbers of these cells in intestinal samples. Patients with Crohn's disease presented an increase in ILC1 populations accompanied by decreased levels of IL-22-producing ILC3s in inflamed intestinal tissues^[29,30]. In addition, in pediatric patients with Crohn's disease, a lower expression of MHC class II on ILC3s was observed than

in control subjects without IBD; a reduction of MHC class II was correlated with increased numbers of Th17 cells^[91]. Together, these data suggest that ILC1s and ILC3s might participate in the establishment and the development of inflammation, and ILC3s might reduce pathogenic T cells through MHC class II interactions.

Evidence regarding the function of ILCs in tumorigenesis are emerging from studies investigating the pro-carcinogenic role of cytokines and chronic inflammation. Human colorectal cancer (CRC) samples showed increased expression of IL-23 receptor, and the induced expression of IL-23 in mice led to the development of adenomatous tumors originating in the duodenum. Although the contribution of adaptive cells remains unclear, this model would indicate a potential role for ILC3s^[92,93]. Moreover, IL-22-producing ILC3s might also be related in human CRC because uncontrolled IL-22 production facilitates tumor-infiltrating lymphocytes and IL-22 levels in the tumor were significantly higher than in non-tumor sections from the same patients^[94].

Future perspectives

The liver and intestine are complex organs that have multiple interactions with the microbiota, nutrients, metabolites and diverse types of cell to maintain the host homeostasis. The importance of the ILC family in the immunity panel is growing fast. Many studies have been done to elucidate the specific ILCs function in different sites triggering immunity, tissue repair and inflammation. However, the molecular mechanism by which ILC subsets play specific roles and their consequences for the host homeostasis remain unclear. Future studies focusing on how ILC responses are regulated and how they integrate the immune cells in different organs might provide therapeutic potential in the treatment of diverse diseases.

ACKNOWLEDGMENTS

We gratefully acknowledge MSc Christina Adams, from Division of Translational Medicine, "Hospital For Sick Children - Peter Gilgan Centre for Research and Learning", Toronto, ON, Canada and Tatiana Takiishi, from Immunology Department of Institute of Biomedical Science, University of São Paulo, Brazil for the manuscript editing.

REFERENCES

- 1 Cella M, Miller H, Song C. Beyond NK cells: the expanding universe of innate lymphoid cells. *Front Immunol* 2014; **5**: 282 [PMID: 24982658 DOI: 10.3389/fimmu.2014.00282]
- 2 Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. *Annu Rev Immunol* 2012; **30**: 647-675 [PMID: 22224763 DOI: 10.1146/annurev-immunol-020711-075053]
- 3 Tait Wojno ED, Artis D. Emerging concepts and future challenges in innate lymphoid cell biology. *J Exp Med* 2016; **213**: 2229-2248 [PMID: 27811053 DOI: 10.1084/jem.20160525]
- 4 Mebius RE. Organogenesis of lymphoid tissues. *Nat Rev Immunol* 2003; **3**: 292-303 [PMID: 12669020 DOI: 10.1038/nri1054]

- 5 **Spits H**, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie AN, Mebius RE, Powrie F, Vivier E. Innate lymphoid cells--a proposal for uniform nomenclature. *Nat Rev Immunol* 2013; **13**: 145-149 [PMID: 23348417 DOI: 10.1038/nri3365]
- 6 **Spits H**, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol* 2011; **12**: 21-27 [PMID: 21113163 DOI: 10.1038/ni.1962]
- 7 **Artis D**, Spits H. The biology of innate lymphoid cells. *Nature* 2015; **517**: 293-301 [PMID: 25592534 DOI: 10.1038/nature14189]
- 8 **Yagi R**, Zhong C, Northrup DL, Yu F, Bouladoux N, Spencer S, Hu G, Barron L, Sharma S, Nakayama T, Belkaid Y, Zhao K, Zhu J. The transcription factor GATA3 is critical for the development of all IL-7Ra-expressing innate lymphoid cells. *Immunity* 2014; **40**: 378-388 [PMID: 24631153 DOI: 10.1016/j.immuni.2014.01.012]
- 9 **Klose CS**, Flach M, Möhle L, Rogell L, Hoyler T, Ebert K, Fabiunke C, Pfeifer D, Sexl V, Fonseca-Pereira D, Domingues RG, Veiga-Fernandes H, Arnold SJ, Busslinger M, Dunay IR, Tanriver Y, Diefenbach A. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. *Cell* 2014; **157**: 340-356 [PMID: 24725403 DOI: 10.1016/j.cell.2014.03.030]
- 10 **Constantinides MG**, McDonald BD, Verhoef PA, Bendelac A. A committed precursor to innate lymphoid cells. *Nature* 2014; **508**: 397-401 [PMID: 24509713 DOI: 10.1038/nature13047]
- 11 **Ignacio A**, Morales CI, Câmara NO, Almeida RR. Innate Sensing of the Gut Microbiota: Modulation of Inflammatory and Autoimmune Diseases. *Front Immunol* 2016; **7**: 54 [PMID: 26925061 DOI: 10.3389/fimmu.2016.00054]
- 12 **Simoni Y**, Fehlings M, Kløverpris HN, McGovern N, Koo SL, Loh CY, Lim S, Kurioka A, Fergusson JR, Tang CL, Kam MH, Dennis K, Lim TK, Fui AC, Hoong CW, Chan JK, Curotto de Lafaille M, Narayanan S, Baig S, Shabeer M, Toh SE, Tan HK, Anicete R, Tan EH, Takano A, Klenerman P, Leslie A, Tan DS, Tan IB, Ginhoux F, Newell EW. Human Innate Lymphoid Cell Subsets Possess Tissue-Type Based Heterogeneity in Phenotype and Frequency. *Immunity* 2017; **46**: 148-161 [PMID: 27986455 DOI: 10.1016/j.immuni.2016.11.005]
- 13 **Gury-BenAri M**, Thaïs CA, Serafini N, Winter DR, Giladi A, Lara-Astiaso D, Levy M, Salame TM, Weiner A, David E, Shapiro H, Dori-Bachash M, Pevsner-Fischer M, Lorenzo-Vivas E, Keren-Shaul H, Paul F, Harmelin A, Eberl G, Itzkovitz S, Tanay A, Di Santo JP, Elinav E, Amit I. The Spectrum and Regulatory Landscape of Intestinal Innate Lymphoid Cells Are Shaped by the Microbiome. *Cell* 2016; **166**: 1231-1246.e13 [PMID: 27545347 DOI: 10.1016/j.cell.2016.07.043]
- 14 **Eberl G**. Development and evolution of ROR γ ⁺ cells in a microbe's world. *Immunol Rev* 2012; **245**: 177-188 [PMID: 22168420 DOI: 10.1111/j.1600-065X.2011.01071.x]
- 15 **Yokoyama WM**. Natural killer cell immune responses. *Immunol Res* 2005; **32**: 317-325 [PMID: 16106081 DOI: 10.1385/IR.32:1-3:317]
- 16 **Lanier LL**. Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol* 2008; **9**: 495-502 [PMID: 18425106 DOI: 10.1038/ni1581]
- 17 **Chijioko O**, Münz C. Dendritic cell derived cytokines in human natural killer cell differentiation and activation. *Front Immunol* 2013; **4**: 365 [PMID: 24273539 DOI: 10.3389/fimmu.2013.00365]
- 18 **Vosshenrich CA**, Di Santo JP. Developmental programming of natural killer and innate lymphoid cells. *Curr Opin Immunol* 2013; **25**: 130-138 [PMID: 23490162 DOI: 10.1016/j.coi.2013.02.002]
- 19 **Cherrier M**, Sawa S, Eberl G. Notch, Id2, and ROR γ t sequentially orchestrate the fetal development of lymphoid tissue inducer cells. *J Exp Med* 2012; **209**: 729-740 [PMID: 22430492 DOI: 10.1084/jem.20111594]
- 20 **Possot C**, Schmutz S, Chea S, Boucontet L, Louise A, Cumano A, Golub R. Notch signaling is necessary for adult, but not fetal, development of ROR γ t(+) innate lymphoid cells. *Nat Immunol* 2011; **12**: 949-958 [PMID: 21909092 DOI: 10.1038/ni.2105]
- 21 **Kovalovsky D**, Uche OU, Eladad S, Hobbs RM, Yi W, Alonzo E, Chua K, Eidson M, Kim HJ, Im JS, Pandolfi PP, Sant'Angelo DB. The BTB-zinc finger transcriptional regulator PLZF controls the development of invariant natural killer T cell effector functions. *Nat Immunol* 2008; **9**: 1055-1064 [PMID: 18660811 DOI: 10.1038/ni.1641]
- 22 **Savage AK**, Constantinides MG, Han J, Picard D, Martin E, Li B, Lantz O, Bendelac A. The transcription factor PLZF directs the effector program of the NKT cell lineage. *Immunity* 2008; **29**: 391-403 [PMID: 18703361 DOI: 10.1016/j.immuni.2008.07.011]
- 23 **Jiao Y**, Huntington ND, Belz GT, Seillet C. Type 1 Innate Lymphoid Cell Biology: Lessons Learnt from Natural Killer Cells. *Front Immunol* 2016; **7**: 426 [PMID: 27785129 DOI: 10.3389/fimmu.2016.00426]
- 24 **Daussy C**, Faure F, Mayol K, Viel S, Gasteiger G, Charrier E, Bienvenu J, Henry T, Debien E, Hasan UA, Marvel J, Yoh K, Takahashi S, Prinz I, de Bernard S, Buffat L, Walzer T. T-bet and Eomes instruct the development of two distinct natural killer cell lineages in the liver and in the bone marrow. *J Exp Med* 2014; **211**: 563-577 [PMID: 24516120 DOI: 10.1084/jem.20131560]
- 25 **Zook EC**, Kee BL. Development of innate lymphoid cells. *Nat Immunol* 2016; **17**: 775-782 [PMID: 27328007 DOI: 10.1038/ni.3481]
- 26 **Sojka DK**, Plougastel-Douglas B, Yang L, Pak-Wittel MA, Artyomov MN, Ivanova Y, Zhong C, Chase JM, Rothman PB, Yu J, Riley JK, Zhu J, Tian Z, Yokoyama WM. Tissue-resident natural killer (NK) cells are cell lineages distinct from thymic and conventional splenic NK cells. *Elife* 2014; **3**: e01659 [PMID: 24714492 DOI: 10.7554/eLife.01659]
- 27 **Robinette ML**, Fuchs A, Cortez VS, Lee JS, Wang Y, Durum SK, Gilfillan S, Colonna M. Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets. *Nat Immunol* 2015; **16**: 306-317 [PMID: 25621825 DOI: 10.1038/ni.3094]
- 28 **Gordon SM**, Chaix J, Rupp LJ, Wu J, Madera S, Sun JC, Lindsten T, Reiner SL. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. *Immunity* 2012; **36**: 55-67 [PMID: 22261438 DOI: 10.1016/j.immuni.2011.11.016]
- 29 **Bernink JH**, Peters CP, Munneke M, te Velde AA, Meijer SL, Weijer K, Hreggvidsdottir HS, Heinsbroek SE, Legrand N, Buskens CJ, Bemelman WA, Mjösberg JM, Spits H. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat Immunol* 2013; **14**: 221-229 [PMID: 23334791 DOI: 10.1038/ni.2534]
- 30 **Fuchs A**, Vermi W, Lee JS, Lonardi S, Gilfillan S, Newberry RD, Cella M, Colonna M. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN- γ -producing cells. *Immunity* 2013; **38**: 769-781 [PMID: 23453631 DOI: 10.1016/j.immuni.2013.02.010]
- 31 **Vonarbourg C**, Mortha A, Bui VL, Hernandez PP, Kiss EA, Hoyler T, Flach M, Bengsch B, Thimme R, Hölscher C, Hönig M, Pannicke U, Schwarz K, Ware CF, Finke D, Diefenbach A. Regulated expression of nuclear receptor ROR γ t confers distinct functional fates to NK cell receptor-expressing ROR γ t(+) innate lymphocytes. *Immunity* 2010; **33**: 736-751 [PMID: 21093318 DOI: 10.1016/j.immuni.2010.10.017]
- 32 **Moro K**, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, Furusawa J, Ohtani M, Fujii H, Koyasu S. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. *Nature* 2010; **463**: 540-544 [PMID: 20023630 DOI: 10.1038/nature08636]
- 33 **Neill DR**, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, Bucks C, Kane CM, Fallon PG, Pannell R, Jolin HE, McKenzie AN. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010; **464**: 1367-1370 [PMID: 20200518 DOI: 10.1038/nature08900]
- 34 **Price AE**, Liang HE, Sullivan BM, Reinhardt RL, Easley CJ, Erle DJ, Locksley RM. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci USA* 2010; **107**: 11489-11494 [PMID: 20534524 DOI: 10.1073/pnas.1003988107]

- 35 **Saenz SA**, Siracusa MC, Monticelli LA, Ziegler CG, Kim BS, Brestoff JR, Peterson LW, Wherry EJ, Goldrath AW, Bhandoola A, Artis D. IL-25 simultaneously elicits distinct populations of innate lymphoid cells and multipotent progenitor type 2 (MPPtype2) cells. *J Exp Med* 2013; **210**: 1823-1837 [PMID: 23960191 DOI: 10.1084/jem.20122332]
- 36 **Turner JE**, Morrison PJ, Wilhelm C, Wilson M, Ahlfors H, Renaud JC, Panzer U, Helmby H, Stockinger B. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. *J Exp Med* 2013; **210**: 2951-2965 [PMID: 24249111 DOI: 10.1084/jem.20130071]
- 37 **Zhou B**, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, Gyarmati D, Aye T, Campbell DJ, Ziegler SF. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005; **6**: 1047-1053 [PMID: 16142237 DOI: 10.1038/ni1247]
- 38 **Kim BS**, Siracusa MC, Saenz SA, Noti M, Monticelli LA, Sonnenberg GF, Hepworth MR, Van Voorhees AS, Comeau MR, Artis D. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med* 2013; **5**: 170ra16 [PMID: 23363980 DOI: 10.1126/scitranslmed.3005374]
- 39 **Salimi M**, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, Wang X, Huang LC, Johnson D, Scanlon ST, McKenzie AN, Fallon PG, Ogg GS. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med* 2013; **210**: 2939-2950 [PMID: 24323357 DOI: 10.1084/jem.20130351]
- 40 **Barlow JL**, McKenzie AN. Type-2 innate lymphoid cells in human allergic disease. *Curr Opin Allergy Clin Immunol* 2014; **14**: 397-403 [PMID: 25115682 DOI: 10.1097/ACI.0000000000000090]
- 41 **Mjösberg JM**, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, Fokkens WJ, Cupedo T, Spits H. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol* 2011; **12**: 1055-1062 [PMID: 21909091 DOI: 10.1038/ni.2104]
- 42 **Monticelli LA**, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CG, Doering TA, Angelosanto JM, Laidlaw BJ, Yang CY, Sathaliyawala T, Kubota M, Turner D, Diamond JM, Goldrath AW, Farber DL, Collman RG, Wherry EJ, Artis D. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol* 2011; **12**: 1045-1054 [PMID: 21946417 DOI: 10.1038/ni.2131]
- 43 **Wilhelm C**, Hirota K, Stieglitz B, Van Snick J, Tolaini M, Lahl K, Sparwasser T, Helmby H, Stockinger B. An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. *Nat Immunol* 2011; **12**: 1071-1077 [PMID: 21983833 DOI: 10.1038/ni.2133]
- 44 **Halim TY**, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* 2012; **36**: 451-463 [PMID: 22425247 DOI: 10.1016/j.immuni.2011.12.020]
- 45 **Imai Y**, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, Yoshimoto T, Nakanishi K, Yamanishi K. Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. *Proc Natl Acad Sci USA* 2013; **110**: 13921-13926 [PMID: 23918359 DOI: 10.1073/pnas.1307321110]
- 46 **Roediger B**, Kyle R, Yip KH, Sumaria N, Guy TV, Kim BS, Mitchell AJ, Tay SS, Jain R, Forbes-Blom E, Chen X, Tong PL, Bolton HA, Artis D, Paul WE, Fazekas de St Groth B, Grimbaldston MA, Le Gros G, Weninger W. Cutaneous immunosurveillance and regulation of inflammation by group 2 innate lymphoid cells. *Nat Immunol* 2013; **14**: 564-573 [PMID: 23603794 DOI: 10.1038/ni.2584]
- 47 **Liang HE**, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat Immunol* 2011; **13**: 58-66 [PMID: 22138715 DOI: 10.1038/ni.2182]
- 48 **Hoyler T**, Klose CS, Souabni A, Turqueti-Neves A, Pfeifer D, Rawlins EL, Voehringer D, Busslinger M, Diefenbach A. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity* 2012; **37**: 634-648 [PMID: 23063333 DOI: 10.1016/j.immuni.2012.06.020]
- 49 **Montaldo E**, Juelke K, Romagnani C. Group 3 innate lymphoid cells (ILC3s): Origin, differentiation, and plasticity in humans and mice. *Eur J Immunol* 2015; **45**: 2171-2182 [PMID: 26031799 DOI: 10.1002/eji.201545598]
- 50 **Takatori H**, Kanno Y, Watford WT, Tato CM, Weiss G, Ivanov II, Littman DR, O'Shea JJ. Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. *J Exp Med* 2009; **206**: 35-41 [PMID: 19114665 DOI: 10.1084/jem.20072713]
- 51 **Kudira R**, Malinka T, Kohler A, Dosch M, de Agüero MG, Melin N, Haegeler S, Starlinger P, Maharjan N, Saxena S, Keogh A, Stroka D, Candinas D, Beldi G. P2X1-regulated IL-22 secretion by innate lymphoid cells is required for efficient liver regeneration. *Hepatology* 2016; **63**: 2004-2017 [PMID: 26853442 DOI: 10.1002/hep.28492]
- 52 **Cella M**, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, Doherty JM, Mills JC, Colonna M. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature* 2009; **457**: 722-725 [PMID: 18978771 DOI: 10.1038/nature07537]
- 53 **Cupedo T**, Crellin NK, Papazian N, Rombouts EJ, Weijer K, Grogan JL, Fibbe WE, Cornelissen JJ, Spits H. Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC+ CD127+ natural killer-like cells. *Nat Immunol* 2009; **10**: 66-74 [PMID: 19029905 DOI: 10.1038/ni.1668]
- 54 **Satoh-Takayama N**, Vosschenrich CA, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, Mention JJ, Thiam K, Cerf-Bennsusan N, Mandelboim O, Eberl G, Di Santo JP. Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. *Immunity* 2008; **29**: 958-970 [PMID: 19084435 DOI: 10.1016/j.immuni.2008.11.001]
- 55 **Zenewicz LA**, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 2008; **29**: 947-957 [PMID: 19100701 DOI: 10.1016/j.immuni.2008.11.003]
- 56 **Molofsky AB**, Nussbaum JC, Liang HE, Van Dyken SJ, Cheng LE, Mohapatra A, Chawla A, Locksley RM. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med* 2013; **210**: 535-549 [PMID: 23420878 DOI: 10.1084/jem.20121964]
- 57 **Goto Y**, Obata T, Kunisawa J, Sato S, Ivanov II, Lamichhane A, Takeyama N, Kamioka M, Sakamoto M, Matsuki T, Setoyama H, Imaoka A, Uematsu S, Akira S, Domino SE, Kulig P, Becher B, Renaud JC, Sasakawa C, Umesaki Y, Benno Y, Kiyono H. Innate lymphoid cells regulate intestinal epithelial cell glycosylation. *Science* 2014; **345**: 1254009 [PMID: 25214634 DOI: 10.1126/science.1254009]
- 58 **Sonnenberg GF**, Monticelli LA, Elloso MM, Fouser LA, Artis D. CD4(+) lymphoid tissue-inducer cells promote innate immunity in the gut. *Immunity* 2011; **34**: 122-134 [PMID: 21194981 DOI: 10.1016/j.immuni.2010.12.009]
- 59 **Gladiator A**, Wangler N, Trautwein-Weidner K, Leibundgut-Landmann S. Cutting edge: IL-17-secreting innate lymphoid cells are essential for host defense against fungal infection. *J Immunol* 2013; **190**: 521-525 [PMID: 23255360 DOI: 10.4049/jimmunol.1202924]
- 60 **Wolk K**, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. *Immunity* 2004; **21**: 241-254 [PMID: 15308104 DOI: 10.1016/j.immuni.2004.07.007]
- 61 **Yang Z**, Tang T, Wei X, Yang S, Tian Z. Type 1 innate lymphoid cells contribute to the pathogenesis of chronic hepatitis B. *Innate Immun* 2015; **21**: 665-673 [PMID: 25977358 DOI: 10.1177/1753425915586074]
- 62 **Satpathy AT**, Briseño CG, Lee JS, Ng D, Manieri NA, Kc W, Wu X, Thomas SR, Lee WL, Turkoz M, McDonald KG, Meredith MM, Song C, Guidos CJ, Newberry RD, Ouyang W, Murphy TL, Stappenbeck TS, Gommerman JL, Nussenzweig MC, Colonna M, Kopan R, Murphy KM. Notch2-dependent classical dendritic cells orchestrate intestinal immunity to attaching-and-effacing bacterial pathogens. *Nat Immunol* 2013; **14**: 937-948 [PMID: 23913046]

- DOI: 10.1038/ni.2679]
- 63 **Basu R**, O'Quinn DB, Silberger DJ, Schoeb TR, Fouser L, Ouyang W, Hatton RD, Weaver CT. Th22 cells are an important source of IL-22 for host protection against enteropathogenic bacteria. *Immunity* 2012; **37**: 1061-1075 [PMID: 23200827 DOI: 10.1016/j.immuni.2012.08.024]
- 64 **Jie Z**, Liang Y, Hou L, Dong C, Iwakura Y, Soong L, Cong Y, Sun J. Intrahepatic innate lymphoid cells secrete IL-17A and IL-17F that are crucial for T cell priming in viral infection. *J Immunol* 2014; **192**: 3289-3300 [PMID: 24600029 DOI: 10.4049/jimmunol.1303281]
- 65 **De Luca A**, Zelante T, D'Angelo C, Zagarella S, Fallarino F, Spreca A, Iannitti RG, Bonifazi P, Renauld JC, Bistoni F, Puccetti P, Romani L. IL-22 defines a novel immune pathway of antifungal resistance. *Mucosal Immunol* 2010; **3**: 361-373 [PMID: 20445503 DOI: 10.1038/mi.2010.22]
- 66 **Voehringer D**, Reese TA, Huang X, Shinkai K, Locksley RM. Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *J Exp Med* 2006; **203**: 1435-1446 [PMID: 16702603 DOI: 10.1084/jem.20052448]
- 67 **van de Pavert SA**, Olivier BJ, Goverse G, Vondenhoff MF, Greuter M, Beke P, Kusser K, Höpken UE, Lipp M, Niederreither K, Blomhoff R, Sitnik K, Agace WW, Randall TD, de Jonge WJ, Mebius RE. Chemokine CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation. *Nat Immunol* 2009; **10**: 1193-1199 [PMID: 19783990 DOI: 10.1038/ni.1789]
- 68 **Sonnenberg GF**, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat Immunol* 2011; **12**: 383-390 [PMID: 21502992 DOI: 10.1038/ni.2025]
- 69 **Wolk K**, Witte E, Witte K, Warszawska K, Sabat R. Biology of interleukin-22. *Semin Immunopathol* 2010; **32**: 17-31 [PMID: 20127093 DOI: 10.1007/s00281-009-0188-x]
- 70 **Radaeva S**, Sun R, Pan HN, Hong F, Gao B. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. *Hepatology* 2004; **39**: 1332-1342 [PMID: 15122762 DOI: 10.1002/hep.20184]
- 71 **Zenewicz LA**, Yancopoulos GD, Valenzuela DM, Murphy AJ, Karow M, Flavell RA. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. *Immunity* 2007; **27**: 647-659 [PMID: 17919941 DOI: 10.1016/j.immuni.2007.07.023]
- 72 **Kong X**, Feng D, Wang H, Hong F, Bertola A, Wang FS, Gao B. Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis in mice. *Hepatology* 2012; **56**: 1150-1159 [PMID: 22473749 DOI: 10.1002/hep.25744]
- 73 **Matsumoto A**, Kanai T, Mikami Y, Chu PS, Nakamoto N, Ebinuma H, Saito H, Sato T, Yagita H, Hibi T. IL-22-producing ROR γ T-dependent innate lymphoid cells play a novel protective role in murine acute hepatitis. *PLoS One* 2013; **8**: e62853 [PMID: 23626860 DOI: 10.1371/journal.pone.0062853]
- 74 **Monticelli LA**, Osborne LC, Noti M, Tran SV, Zaiss DM, Artis D. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc Natl Acad Sci USA* 2015; **112**: 10762-10767 [PMID: 26243875 DOI: 10.1073/pnas.1509070112]
- 75 **Han JM**, Wu D, Denroche HC, Yao Y, Verchere CB, Levings MK. IL-33 Reverses an Obesity-Induced Deficit in Visceral Adipose Tissue ST2+ T Regulatory Cells and Ameliorates Adipose Tissue Inflammation and Insulin Resistance. *J Immunol* 2015; **194**: 4777-4783 [PMID: 25870243 DOI: 10.4049/jimmunol.1500020]
- 76 **Castoldi A**, Naffah de Souza C, Câmara NO, Moraes-Vieira PM. The Macrophage Switch in Obesity Development. *Front Immunol* 2015; **6**: 637 [PMID: 26779183]
- 77 **McHedlidze T**, Waldner M, Zopf S, Walker J, Rankin AL, Schuchmann M, Voehringer D, McKenzie AN, Neurath MF, Pflanz S, Wirtz S. Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. *Immunity* 2013; **39**: 357-371 [PMID: 23954132 DOI: 10.1016/j.immuni.2013.07.018]
- 78 **Wang J**, Cai Y, Ji H, Feng J, Ayana DA, Niu J, Jiang Y. Serum IL-33 levels are associated with liver damage in patients with chronic hepatitis B. *J Interferon Cytokine Res* 2012; **32**: 248-253 [PMID: 22304300 DOI: 10.1089/jir.2011.0109]
- 79 **Klose CS**, Artis D. Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis. *Nat Immunol* 2016; **17**: 765-774 [PMID: 27328006 DOI: 10.1038/ni.3489]
- 80 **Sonnenberg GF**, Monticelli LA, Alenghat T, Fung TC, Hutnick NA, Kunisawa J, Shibata N, Grunberg S, Sinha R, Zahm AM, Tardif MR, Sathaliyawala T, Kubota M, Farber DL, Collman RG, Shaked A, Fouser LA, Weiner DB, Tessier PA, Friedman JR, Kiyono H, Bushman FD, Chang KM, Artis D. Innate lymphoid cells promote anatomical containment of lymphoid-resident commensal bacteria. *Science* 2012; **336**: 1321-1325 [PMID: 22674331 DOI: 10.1126/science.1222551]
- 81 **Kruglov AA**, Grivennikov SI, Kuprash DV, Winsauer C, Prepens S, Selezniev GM, Eberl G, Littman DR, Heikenwalder M, Tumanov AV, Nedospasov SA. Nonredundant function of soluble LT α 3 produced by innate lymphoid cells in intestinal homeostasis. *Science* 2013; **342**: 1243-1246 [PMID: 24311691 DOI: 10.1126/science.1243364]
- 82 **Hepworth MR**, Monticelli LA, Fung TC, Ziegler CG, Grunberg S, Sinha R, Mantegazza AR, Ma HL, Crawford A, Angelosanto JM, Wherry EJ, Koni PA, Bushman FD, Elson CO, Eberl G, Artis D, Sonnenberg GF. Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. *Nature* 2013; **498**: 113-117 [PMID: 23698371 DOI: 10.1038/nature12240]
- 83 **Sawa S**, Lochner M, Satoh-Takayama N, Dulauroy S, Bérard M, Kleinschek M, Cua D, Di Santo JP, Eberl G. ROR γ T+ innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nat Immunol* 2011; **12**: 320-326 [PMID: 21336274 DOI: 10.1038/ni.2002]
- 84 **Spencer SP**, Wilhelm C, Yang Q, Hall JA, Bouladoux N, Boyd A, Nutman TB, Urban JF, Wang J, Ramalingam TR, Bhandoola A, Wynn TA, Belkaid Y. Adaptation of innate lymphoid cells to a micronutrient deficiency promotes type 2 barrier immunity. *Science* 2014; **343**: 432-437 [PMID: 24458645 DOI: 10.1126/science.1247606]
- 85 **Buonocore S**, Ahern PP, Uhlig HH, Ivanov II, Littman DR, Maloy KJ, Powrie F. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* 2010; **464**: 1371-1375 [PMID: 20393462 DOI: 10.1038/nature08949]
- 86 **Song C**, Lee JS, Gilfillan S, Robinette ML, Newberry RD, Stappenbeck TS, Mack M, Cella M, Colonna M. Unique and redundant functions of NKp46+ ILC3s in models of intestinal inflammation. *J Exp Med* 2015; **212**: 1869-1882 [PMID: 26458769 DOI: 10.1084/jem.20151403]
- 87 **Klose CS**, Kiss EA, Schwierzeck V, Ebert K, Hoyler T, d'Hargues Y, Göppert N, Croxford AL, Waisman A, Tanriver Y, Dieffenbach A. A T-bet gradient controls the fate and function of CCR6-ROR γ T+ innate lymphoid cells. *Nature* 2013; **494**: 261-265 [PMID: 23334414 DOI: 10.1038/nature11813]
- 88 **Powell N**, Walker AW, Stolarczyk E, Canavan JB, Gökmen MR, Marks E, Jackson I, Hashim A, Curtis MA, Jenner RG, Howard JK, Parkhill J, MacDonald TT, Lord GM. The transcription factor T-bet regulates intestinal inflammation mediated by interleukin-7 receptor+ innate lymphoid cells. *Immunity* 2012; **37**: 674-684 [PMID: 23063332 DOI: 10.1016/j.immuni.2012.09.008]
- 89 **Longman RS**, Diehl GE, Victorio DA, Huh JR, Galan C, Miraldi ER, Swaminath A, Bonneau R, Scherl EJ, Littman DR. CX3CR1+ mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22. *J Exp Med* 2014; **211**: 1571-1583 [PMID: 25024136 DOI: 10.1084/jem.20140678]
- 90 **Hazenber MD**, Spits H. Human innate lymphoid cells. *Blood* 2014; **124**: 700-709 [PMID: 24778151 DOI: 10.1182/blood-2013-11-427781]
- 91 **Geremia A**, Arancibia-Cárcamo CV, Fleming MP, Rust N, Singh B, Mortensen NJ, Travis SP, Powrie F. IL-23-responsive innate lymphoid cells are increased in inflammatory bowel disease. *J Exp Med* 2011; **208**: 1127-1133 [PMID: 21576383 DOI: 10.1084/jem.20101712]
- 92 **Langowski JL**, Zhang X, Wu L, Mattson JD, Chen T, Smith K,

- Basham B, McClanahan T, Kastelein RA, Olt M. IL-23 promotes tumour incidence and growth. *Nature* 2006; **442**: 461-465 [PMID: 16688182 DOI: 10.1038/nature04808]
- 93 **Chan IH**, Jain R, Tessmer MS, Gorman D, Mangadu R, Sathe M, Vives F, Moon C, Penaflor E, Turner S, Ayanoglu G, Chang C, Basham B, Mumm JB, Pierce RH, Yearley JH, McClanahan TK, Phillips JH, Cua DJ, Bowman EP, Kastelein RA, LaFace D. Interleukin-23 is sufficient to induce rapid de novo gut tumorigenesis, independent of carcinogens, through activation of innate lymphoid cells. *Mucosal Immunol* 2014; **7**: 842-856 [PMID: 24280935 DOI: 10.1038/mi.2013.101]
- 94 **Kirchberger S**, Royston DJ, Boulard O, Thornton E, Franchini F, Szabady RL, Harrison O, Powrie F. Innate lymphoid cells sustain colon cancer through production of interleukin-22 in a mouse model. *J Exp Med* 2013; **210**: 917-931 [PMID: 23589566 DOI: 10.1084/jem.20122308]

P- Reviewer: De Ponti F, Shen T **S- Editor:** Ji FF **L- Editor:** A
E- Editor: Lu YJ



Use of everolimus in liver transplantation

Mei-Ling Yee, Hui-Hui Tan

Mei-Ling Yee, Department of Pharmacy, Singapore General Hospital, Singapore 169608, Singapore

Hui-Hui Tan, Department of Gastroenterology and Hepatology, Singapore General Hospital, Singapore 169608, Singapore

Author contributions: Both authors contributed to the writing and review of the manuscript.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Mei-Ling Yee, Senior Principal Clinical Pharmacist, Department of Pharmacy, Singapore General Hospital, Outram Road, Singapore 169608, Singapore. yee.mei.ling@sgh.com.sg
Telephone: +65-63213797
Fax: +65-62274330

Received: January 16, 2017

Peer-review started: January 18, 2017

First decision: May 9, 2017

Revised: May 20, 2017

Accepted: June 12, 2017

Article in press: June 13, 2017

Published online: August 18, 2017

everolimus (EVR) in de novo LT is established and a reasonable time to initiate EVR is 30 d from LT surgery. Initiating EVR early post-LT allows for calcineurin inhibitor (CNI) reduction, thus reducing nephrotoxicity in LT recipients. However, data is inadequate on the appropriate timing for conversion from CNI to EVR maintenance in order to achieve optimal renoprotective effect without compromising drug efficacy. Adverse effects of proteinuria, hypercholesterolemia and hyperlipidemia are significantly higher as compared to standard CNI and long-term implications on graft and patient survival in LT is still unclear. Future research to explore strategies to minimise EVR adverse effects will be crucial for the success of EVR as an important alternative or adjunct immunosuppressive therapy in LT.

Key words: Everolimus; Mammalian target of rapamycin inhibitor; Immunosuppression; Liver transplantation; Nephrotoxicity

© **The Author(s) 2017.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Everolimus is the most recently approved immunosuppressant for use in liver transplantation (LT). Its renoprotective effect is an attractive option for LT recipients who have calcineurin inhibitor-induced nephrotoxicity. This review examines through data published, discovers gaps of evidences and discusses the place in therapy for everolimus (EVR) in LT. At the end of review, it summarises how EVR can benefit LT recipients as well as the caveat in using EVR.

Yee ML, Tan HH. Use of everolimus in liver transplantation. *World J Hepatol* 2017; 9(23): 990-1000 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v9/i23/990.htm> DOI: <http://dx.doi.org/10.4254/wjh.v9.i23.990>

Abstract

In recent years, the use of mammalian target of rapamycin inhibitors has gained traction in their use as alternative or adjunct immunosuppressants in the post-liver transplantation (LT) setting. The efficacy of

INTRODUCTION

Since the first liver transplantation (LT) surgery in 1963,

surgical techniques and immunosuppression therapy have evolved much and improved patient outcomes. Based on Organ Procurement Transplantation Network/Scientific Registry of Transplant Recipients (OPTN/SRTR) data in 2013, the 5-year graft survival rate in LT is as high as 76%^[1]. In most transplant centers, LT immunosuppressive regimes include calcineurin inhibitors (CNI), antimetabolites, steroid with or without induction therapy^[2]. For the past few decades, CNIs have been the cornerstone of immunosuppressant regimens for LT recipients. The overall patient survival at 1-, 5- and 10-years for LT with tacrolimus (FK) were in range of 81%-84%, 70%-72% and 57%-68% respectively^[3,4]. Nonetheless, CNIs, both FK and cyclosporine (CsA), increase the risk of nephrotoxicity, diabetes, hypertension and neurotoxicity^[2]. Ojo *et al*^[5] reported as high as 18% of LT recipients developed renal impairment within 5 years post-LT. Therefore much research has been focused on finding strategies or alternatives to avoid or minimize nephrotoxicity in the past 10 years and one of the more recent drug classes to be used are the mammalian target of rapamycin (mTOR) inhibitors [sirolimus, everolimus (EVR)].

EVR was approved for the prevention of graft rejection in LT when used in combination with both FK and steroid in Europe (October 2012) and in the United States (February 2013).

PHARMACOLOGICAL PROPERTIES OF EVR

EVR is an mTOR inhibitor and has antiproliferative properties. It reduces protein synthesis and cell proliferation by binding to FK binding protein-12 to form a complex that inhibits activation of the mTOR serine threonine kinase activity (Figure 1). It also has antiangiogenic effects by inhibiting expression of hypoxia inducible factor and vascular endothelial growth factor. In addition, mTOR may have additional importance in neuroendocrine cells and EVR has been shown to block the action of IGF-1 in neuroendocrine cells^[6].

EVR is a derivative of sirolimus, differing by one extra hydroxyethyl group at position 40 (Figure 2). Based on pharmacokinetics data, its absorption is rapid and bioavailability is variable, about 16%-20% (higher than sirolimus' 10%-14%)^[7,8]. EVR requires twice daily dosing as its elimination half-life is 32 h, which is shorter than sirolimus' half-life of 62 h. Therefore, no loading dose is required for EVR and steady state can be achieved faster, in 4 d, vs 6 d for sirolimus. EVR is extensively metabolised in the liver *via* cytochrome P450-3A4 (CYP3A4) and has 6 wk metabolites. Similar to sirolimus, it is a substrate of p-glycoprotein (PgP) and CYP3A4 pathways. It interacts with strong and moderate inhibitors, inducers and substrates of CYP3A4 and PgP at different intensities^[9,10]. CsA increases the maximum concentration of EVR by 82%, EVR however

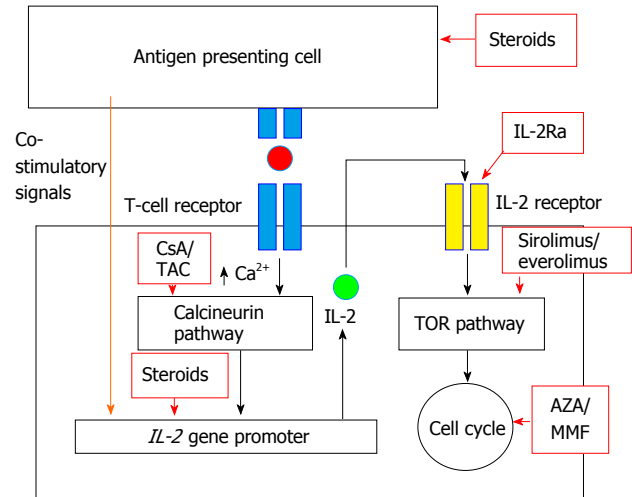


Figure 1 Mechanism of action of efficacy of everolimus and other immunosuppressants in solid organ transplantation (permission from Moini *et al*^[2], *World J Hepatol* 2015). AZA: Azathioprine; CsA: Cyclosporine; IL-2: Interleukin-2; IL-2Ra: Interleukin-2 receptor antagonist; MMF: Mycophenolate mofetil; TAC: Tacrolimus; TOR: Target of rapamycin.

does not influence trough level nor drug exposure (area under the curve, AUC) of CsA^[8]. EVR is excreted mainly (80%) *via* feces and only 5% in urine^[7,8]. There is no dose adjustment required in renal impairment but dose reduction is recommended for moderate and severe liver impairment. As EVR has a narrow therapeutic index and immunogenicity varies post LT, therapeutic monitoring is essential for dose titration and monitoring. The EVR trough level (C_0) correlates well (correlation coefficient of 0.86-0.94) with drug exposure, *i.e.*, AUC, and has been recommended as the standard for EVR monitoring^[7,11].

Key studies on the use of EVR in LT (Tables 1 and 2)

Several studies, both prospective and retrospective, on EVR in LT have been reported. In a phase II study, Levy *et al*^[12] compared different dosing regimen of EVR (0.5 mg BD, 1 mg BD and 2 mg BD) to placebo. The study concluded that EVR in combination with CsA could be a safe and tolerable alternative in LT, despite the increased incidence of adverse effects. There are 3 main phase III studies in the use of EVR in LT, *i.e.*, PROTECT, H2304 and RESCUE studies (Table 1). PROTECT, an open-label multi-center prospective randomised controlled trial (RCT) recruited 203 patients randomised to EVR plus withdrawal of CNI by month 4 post-LT vs continued standard CNI till month 11^[13]. Steroid was optional in either group. The study concluded significant improvement in renal function (estimated glomerular filtration rate, *i.e.*, eGFR improved by 7.8 mL/min) in the group with EVR, despite similar mortality rates, biopsy-proven rejection (BPAR) rates and efficacy failure rates between both groups. However, it also reported a significantly higher incidence of adverse effects mainly oral herpes, leukopenia, hypercholesterolemia, hyperlipidemia and proteinuria in the EVR-treated

Table 1 Outcomes of everolimus-based immunosuppressant for *de-novo* liver transplantation recipients in prospective randomised controlled trial

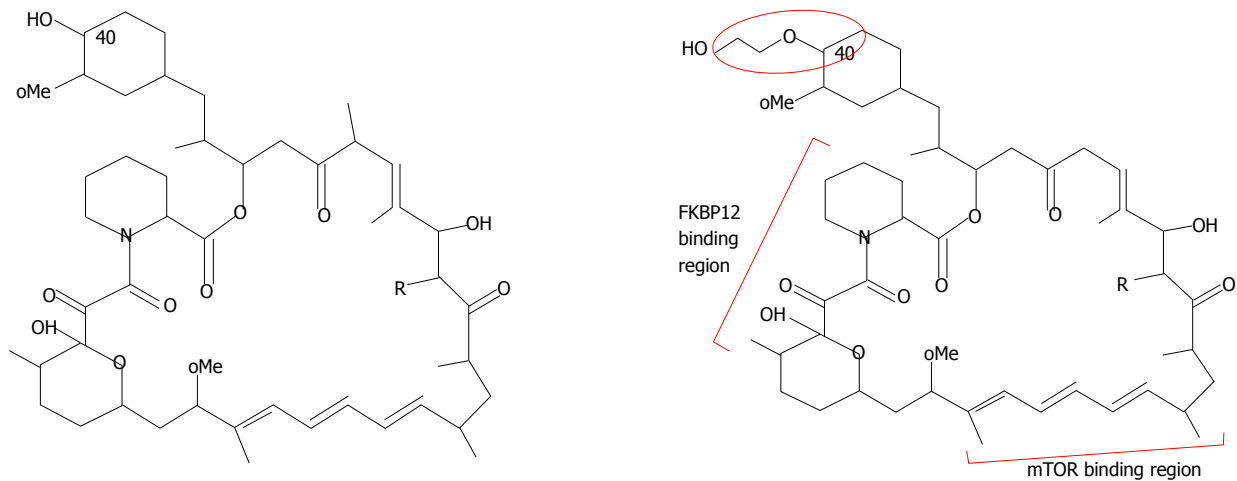
Ref.	Treatment group	Time (d) from transplant EVR was initiated	Key inclusion and exclusion criteria	n	Follow-up period (mo)	Efficacy	Mean improvement in eGFR (mL/min per 1.73 m ²)	Safety
Fischer <i>et al</i> ^[13] 2012 (PROTECT Study)	EVR + eliminate CNI by month 4 (EVR C ₀ 5-12 ng/mL, if with CsA, EVR C ₀ 8-12 ng/mL) Control: FK or CsA	from day 30 and by day 56	Inclusion: No rejection 2 wk before study, renal function > 50 mL/min Exclusion: Severe systemic infections, total cholesterol ≥ 9 mmol/L, TG > 8.5 mmol/L, significant renal dysfunction (eGFR < 50 mL/min)	101 102	12	BPAR, graft loss or death: 20.8% vs 20.4% (P = 1.0)	7.8 (P = 0.021)	No HAT, no increased risk of delayed wound healing. Higher incidence of infections, leukopenia, hyperlipidemia, anemia, proteinuria and arterial hypertension in the EVR group
Sterneck <i>et al</i> ^[14] 2014 (PROTECT Study, extended to 36 mo)	Same as above	From day 30 and by day 56		41 40	36	BPAR, graft loss and death: 19.5% vs 2.5% (P = 0.029) at month 11 (baseline) BPAR, graft loss and death: 4.9% vs 5.0% (P = 1.0) at month 36	9.4 (P = 0.053)	Peripheral edema and back pain were significantly higher in EVR group
Sterneck <i>et al</i> ^[15] 2016 (PROTECT Study, extended to 59 mo)	Same as above	From day 30 and by day 56		41 40	59	BPAR, graft loss and death: 9.8% vs 7.5% (P = 1.0) from month 11 to month 59	11.4 (P = 0.021)	Peripheral edema and back pain were significantly higher in EVR group
De Simone <i>et al</i> ^[16] 2012 (H2304 Study)	EVR + low FK (EVR C ₀ 3-8 ng/mL and FK C ₀ 3-5 ng/mL) FK elimination (EVR C ₀ 3-8 ng/mL till month 4 then 6-10 ng/mL thereafter and FK elimination started at month 4 when EVR C ₀ 6-10 ng/mL achieved Control: FK (C ₀ 8-12 ng/mL until month 4 and C ₀ 6-10 ng/mL thereafter)	Day 30	Inclusion: eGFR ≥ 30 mL/min, FK trough ≥ 8 ng/mL. Patent hepatic artery and veins, absence of rejection Exclusion: HCC not fulfill Milan criteria, receipt of antibody induction therapy proteinuria ≥ 1 g/24 h	245 231 243	12	BPAR, graft loss or death: 6.5% in EVR group vs 9.5% in control group (P < 0.001)	8.5 (P < 0.001)	Higher incidence of proteinuria, acute renal failure, hyperlipidemia, neutropenia, peripheral edema, stomatitis/mouth ulceration, and thrombocytopenia in the EVR group
Saliba <i>et al</i> ^[17] 2013 (H2304 Study, extended to 24 mo)	EVR + low FK (EVR C ₀ 3-8 ng/mL and FK C ₀ 3-5 ng/mL)	Day 30		245 243	24	BPAR, graft loss or death: 10.3% in EVR group vs 12.5% in control group (P = 0.452)	6.7 (P = 0.002)	No increased risk of wound healing. Higher incidence of proteinuria, acute renal failure, hyperlipidemia, neutropenia, peripheral edema, stomatitis/mouth ulceration, and thrombocytopenia in the EVR group
Fischer <i>et al</i> ^[18] 2015 (H2304 Study, extended to 36 mo)	Same as above	Day 30		106 125	36	BPAR, graft loss and death: 11.5% vs 14.6% (P = 0.334)	8.5 (P = 0.005)	Higher drop-out rate due to ADR and incidence of hyperlipidemia in EVR group

ADR: Adverse drug reaction; BPAR: Biopsy proven acute rejection; C₀: Trough level; CNI: Calcineurin inhibitor; CsA: Cyclosporine; EVR: Everolimus; FK: Tacrolimus; eGFR: Based on Modification of Diet in Renal Disease (MDRD) 4.

Table 2 Outcomes of everolimus-based immunosuppressant as maintenance for It recipients in prospective RCT

Ref.	Treatment group	Time (mo) from transplant surgery EVR was initiated	Key inclusion and exclusion criteria	n	Follow-up period (mo)	Efficacy	Mean improvement in CrCl (mL/min)	Safety
De Simone <i>et al</i> ^[19] 2009 (RESCUE Study)	EVR with CNI reduction or elimination (EVR C ₀ 3-8 ng/mL, FK C ₀ 3-5 ng/mL or EVR C ₀ 6-12 ng/mL with FK elimination Control: Standard exposure of FK or CsA	12 to 60 mo	Inclusion: CrCl ≤ 60 mL/min and ≥ 20 mL/min Exclusion: Renal dysfunction not due to CNI toxicity, proteinuria ≥ 1 g/24 h, acute rejection < 6 mo, hepatitis C infection need active antiviral therapy	72 73	12	BPAR, graft loss or death: 8.3% in EVR group vs 4.1% in control group	-1.1 (<i>P</i> = 0.463) at month 6	Higher incidence of hyperlipidemia, mouth ulceration, increased hepatitis C virus viral titer, dry skin, eczema, and rash in the EVR group

BPAR: Biopsy proven acute rejection; C₀: Trough level; CNI: Calcineurin inhibitor; CrCl: Creatinine clearance (based on Cockcroft-Gault formula); CsA: Cyclosporine; EVR: Everolimus; FK: Tacrolimus.

**Figure 2 Molecular structure of sirolimus and everolimus.**

group as compared to standard CNI. In its subsequent study, 81 patients were further followed-up till 3 years. A significant difference in renal function continued to be seen between the EVR with CNI-withdrawal vs the control group at month 35 from randomization, mainly due to the progressive deterioration of renal function in the standard CNI group^[14]. Recently, the 5-year follow-up on these same 81 patients has been published, reporting a continued improved trend in renal function in the EVR-treated group (eGFR improved by 11.4 mL/min, *P* = 0.021) with comparable treatment failure rates (9.8% in EVR group vs 7.5% in standard CNI group, *P* = 1.000) in both groups^[15].

In another open-label multi-center prospective RCT, H2304, 719 patients were randomised to receive EVR (EVR C₀ 3-8 ng/mL) with reduced FK dosing (FK C₀ 3-5 ng/mL) (*n* = 245) or control standard FK dosing (FK C₀ 8-12 ng/mL till month 4 then C₀ 6-10 ng/mL thereafter) (*n* = 243) or FK elimination (EVR C₀ 3-8 ng/mL till month 4 then 6-10 ng/mL thereafter, FK elimination from month 4 when EVR C₀ 6-10 ng/mL achieved) (*n* = 231) at 1 mo post liver transplant^[16]. Steroid was

initiated at time of transplant up till at least 6 mo from transplant while MMF was discontinued at the time of randomization. Recruitment to FK elimination group was terminated prematurely due to higher (19.5%) treated BPAR (tBPAR) episodes as compared to 6.5% and 9.5% of tBPAR in the EVR with reduced FK and control group, which clustered around the time of FK elimination at 4 mo post-randomization. At the end of both the first and second year, subjects in the EVR with reduced FK group had improved renal function significantly with comparable primary efficacy (tBPAR, graft loss and death) but a higher incidence of adverse effects (mainly hyperlipidemia, neutropenia, peripheral edema and stomatitis/mouth ulceration) than controls^[16,17]. At the end of the third year, improvement in renal function was consistently significant in EVR with reduced FK group (*n* = 106) with comparable tBPAR rates and adverse effects as compared to the standard FK group (*n* = 125)^[18].

The third Phase III study of interest, RESCUE, provides evidence for converting to EVR 1 year post-LT (Table 2)^[19]. In this 6 mo open-label multi-center

prospective RCT, 154 patients were followed-up for 12 mo. The studied group, EVR with CNI reduction or elimination was compared to standard CNI with or without MMF, azathioprine or steroid in both groups. While all concurrent immunosuppressants were kept the same in control group, MMF was discontinued at day 1 in the EVR group. Despite no graft loss, BPAR in EVR group at month 12 (4.2%) were higher than standard CNI group (1.4%). Furthermore, the improvement of renal function in the studied group was not statistically significant at 12-mo follow up.

EFFICACY OF EVR IN LT

De novo therapy

In the PROTECT study, the efficacy of EVR in LT was still doubtful with conflicting results. Initial results reported comparable composite BPAR, graft loss and death rates in the EVR-treated (20.8%) and the control (20.4%) group, up to month 11 of follow-up^[13]. A similar comparable trend for its composite end-points in the extension study (from month 11 to month 35) results were reported at the end of 35 mo, despite a difference at baseline between both groups^[14]. Treatment efficacy with EVR was difficult to analyse due to high discontinuation rates of drugs used in both groups due to adverse drug reactions (49.5% in EVR group and 38.2% in control CNI group). The discontinuation of CNI by end of month 4 could have compromised efficacy of immunosuppressive therapy. This similar finding was reported in the H2304 Study where efficacy failure (BPAR) in the FK elimination group was significantly higher (19.9%) as compared to control group (10.7%), $P = 0.005$. Hence, EVR monotherapy is not recommended in LT and EVR should instead be used in combination with CNI. EVR efficacy in *de novo* LT, and hence, United States FDA and Europe EMEA approval is based on results from De Simone's landmark H2304 study (Table 1)^[16]. The reported outcomes of BAPR, graft loss and death in the treatment group were non-inferior to the control group on FK alone. In the post-hoc analysis for H2304 study, incidence of tBPAR was lower in those aged < 60 years and hepatitis C virus (HCV)-negative^[20].

Based on phase III studies (Table 1), EVR is approved for use 30 d from LT. However, there is emerging data on the safety and efficacy of EVR initiation within 30 d from LT. Masetti *et al.*^[21]'s prospective, single-center randomized trial described early initiation of EVR at day 10 from LT in 52 patients (EVR C_0 6-10 ng/mL till day 30, then C_0 8-12 ng/mL (when CsA was discontinued from day 30) till month 6 and then C_0 6-10 ng/mL thereafter) vs standard CsA in 26 patients (CsA C_0 225 \pm 25 ng/mL till day 30, 200 \pm 25 ng/mL till month 6 and 150 \pm 25 ng/mL thereafter)^[21]. There was no difference in BPAR nor patient survival rates in both groups. The study concluded that early withdrawal of CsA and early EVR use in *de novo* LT recipients significantly improved renal function (eGFR 87.7 mL/min

in EVR group vs 59.9 mL/min in standard CsA group) and reduced incidence of chronic kidney disease (CKD) stage ≥ 3 (15.4% in EVR group vs 52.2% in CsA group, $P = 0.005$) at 1 year post-LT.

In a single-center prospective cohort study, safety of EVR use in the early post-LT was evaluated in 43 living donor LT recipients^[22]. All patients received basiliximab, steroid, FK and MMF as immunosuppressive therapy where steroid was discontinued after 2 wk from transplant and FK was maintained at C_0 of 8-10 ng/mL. EVR was introduced from low dose of 0.25 mg BD and titrated to 0.5 mg BD to achieve C_0 of 3-5 ng/mL while FK was kept at C_0 of 6-8 ng/mL. Mean time for EVR initiation was 12 \pm 8 d (range: 4-20 d) from transplant where 33 patients were initiated within the 1st week, 9 patients within the 2nd week and 1 patient on day 20. EVR was continued for an average of 97 d (range: 26-190 d) from transplant. The mean follow up was 9 \pm 6 mo (range: 3-15 mo) till discontinuation of EVR or death. No acute rejection episodes were reported.

In a retrospective study, Gastaca *et al.*^[23] reported 92.7% patient survival rates at 1 year post-LT for 28 patients who had EVR initiated early post-LT (median 14 d) where 85.7% was in combination with MMF or enteric-coated mycophenolate sodium and steroid. Nonetheless, more concrete data is warranted for EVR initiation within 30 d from LT.

Maintenance therapy

The efficacy data for EVR as maintenance immunosuppression in LT is sparse; with only one RCT to date (Table 2). De Simone *et al.*^[19]'s RCT reported results of conversion from CNI-based to EVR-based maintenance immunosuppression after 12 mo and up till 60 mo post-LT. Although the composite endpoint of BPAR, graft loss and death was low overall in both groups, it was double in the EVR group (8.3%) as compared to control group (4.1%)^[19].

In another prospective cohort study by De Simone *et al.*^[24], 40 patients were converted to EVR at mean of 45.5 \pm 31.2 mo from transplant and CNI was tapered by 50% every week and withdrawn over 4 wk with or without MMF or azathioprine and steroid. Concurrent MMF or azathioprine was discontinued at day 1 of conversion while steroid was remained unchanged in the EVR group. Indications for conversion to EVR included deterioration of renal function (90.0%), CNI-associated peripheral neuropathy (7.5%) and CNI-associated microangiopathy (2.5%). Despite a 100% patient and graft survival rate at 12 mo post-conversion, the incidence of BPAR was 15% and 4 of the patients (10%) had to be switched back to CNI for this reason.

Castroagudín *et al.*^[25] analysed impact on renal function post conversion to EVR at mean 62.4 \pm 36.6 mo from LT in 21 patients with CKD. Twenty patients (95%) were able to have CNI completely withdrawn. From a baseline eGFR of 42.1 \pm 8.7 mL/min, renal function improved to eGFR 49.8 \pm 10.3 mL/min at the end of 360 d from conversion.

In a retrospective study, Saliba *et al.*^[26] described 240 patients who were successfully converted to EVR at median of 3 years from transplant with a low overall rejection rate (1.6%). At 12 mo post conversion, 61% of patients had CNI discontinued. Mean EVR C_0 was 7.3 ng/mL and 8.1 ng/mL at month 1 and 12 post conversion respectively while mean EVR C_0 was higher (8.8 ng/mL) in 40 patients who were kept on EVR monotherapy at month 12. Immunosuppression therapy was in combination with or without MMF and steroid in both groups. Renal function was markedly improved in patients who were converted within the first year from transplant ($n = 68$) as compared to conversion after 1 year from transplant ($n = 172$), calculated creatinine clearance 12.5 mL/min vs 5.5 mL/min based on Cockcroft-Gault formula.

In another retrospective study, 477 patients were recruited and 157 (33%) were converted to EVR for indication of renal dysfunction at median of 24 mo^[27]. Significant improvement of renal function was observed in patients who were converted to EVR within 1 year from transplant but not in patients who were converted after 5 years from transplant. Of note, in patients who were converted in between 1-5 years from transplant, the improvement in renal function was only appreciable at month 3 and 6 but did not persist at 12 mo post conversion. Overall graft rejection rate was 5.9% which mostly occurred at 3 and 6 mo post conversion.

VALUE OF EVR IN LT

Renoprotective effect

Long-term renoprotective benefits of EVR in LT have been demonstrated in the H2304 study^[16-18]. Based on the results, De Simone *et al.*^[16] showed that EVR with reduced FK dose is as efficacious as the FK standard regimen in the control group and improved patients' eGFR by 8.5 mL/min at month 12. The improvement trend in eGFR continued to be seen at month 24 (eGFR improved by 6.7 mL/min) and at month 36 (eGFR improved by 8.5 mL/min) of follow-up (Table 1). However, it can also be argued that the H2304 study had unintentionally recruited a majority of patients (72.3%) with better baseline renal functions of eGFR ≥ 60 mL/min, with mean baseline of 80.8 mL/min in EVR group and 78.9 mL/min in control group. Similar high baseline eGFR (78.0 mL/min and 74.9 mL/min in EVR and control groups respectively) were also seen in the PROTECT study. In the RESCUE study, baseline eGFR was 51.0 mL/min and 50.3 mL/min in EVR and control groups. Clearly, results in these studies should not be generalised to LT recipients with eGFR < 50 mL/min, where similar benefits might be doubtful. This was reaffirmed with the H2304 *post-hoc* analysis which showed that renal improvement was not observed in patients with eGFR of 30 to < 55 mL/min^[20]. This analysis suggested that EVR renoprotective effect was observed particularly in patients aged < 60 years, female gender, HCV-negative and in those with baseline

eGFR of 55 to < 70 mL/min.

The FK dose in the control group of the H2304 study was maintained at target C_0 of 8-12 ng/mL until month 4 and then tapered to target C_0 of 6-10 ng/mL for the remainder of the study. The FK C_0 in EVR group was targeted to be 3-5 ng/mL from 1 mo post-LT, though majority of patients maintained levels slightly above 5 ng/mL from month 3 onwards till month 12 of study period. Hence, the addition of EVR early post-LT allowed tapering of FK safely without an increased risk of rejection. The decrease in renal impairment was possibly contributed by the reduced CNI level. It has been proven that CNI minimization strategy improves renal function in LT recipients^[28-30].

Before EVR was started in the H2304 study, majority (70%) of patients were also on mycophenolate mofetil (MMF) which was discontinued according to protocol. It would seem logical in clinical practice to have another non-CNI immunosuppressant, in combination with reduced CNI doses. In fact, the combination of MMF with reduced CNI has been a strategy which many clinicians adopt to minimize CNI nephrotoxicity^[29,30]. A case-control study described 20 patients on *de novo* EVR plus MMF and steroids without CNI in comparison to 31 controls of FK plus MMF and steroids^[31]. The eGFR in both groups were not statistically different at the end of 1- and 2-years follow-up but a 35% of rejection rate at 2 years from LT in EVR group was reported and attributed to difficulty achieving target drug levels. There is no head-to-head RCT comparing EVR-based and MMF-based LT immunosuppression regimes to date.

On the other hand, the evidence for EVR renoprotective effect in conversion after 6 mo post-LT is lacking. The RESCUE Study which showed an increased composite outcome of BPAR, graft loss and death demonstrated an improvement of eGFR by only 1.1 mL/min at the end of 1 year. In this study, the conversion to EVR occurred at mean 3.3 ± 1.7 years from transplant. In a retrospective observational study, Saliba *et al.*^[26] found the improvement in renal function to be greater when conversion to EVR was within first year post-LT (eGFR increased from 77.5 to 90.0 mL/min, $P = 0.04$) vs those who were converted beyond 1 year post-LT (eGFR increased from 59.1 to 64.6 mL/min, $P = 0.01$)^[26]. The findings Castroagudín *et al.*^[25] reported in a retrospective study echoed the less remarkable renal improvement (eGFR improved by 7.7 mL/min at month 12) when conversion to EVR occurred at 5.2 ± 3.1 years from transplant.

Thus, the best time point for conversion to EVR for optimal renoprotective effect is still unclear and further studies are warranted. Although it would appear, from current available data, that earlier conversion (within 12 mo post-LT) is better than late conversion (beyond 3 years post-LT); and that renal protective effects are more prominent with mild renal impairment (eGFR > 60 mL/min) rather than with moderate-severe renal impairment (eGFR < 55 mL/min).

Alternative for CNI-induced neurotoxicity

Bilbao *et al.*^[32] reported a retrospective analysis on the use of EVR in 10 patients who experienced FK-neurotoxicity requiring the discontinuation of FK in the first 3 mo post-LT. Seven of the patients were converted to everolimus in the first month post-LT and the remaining 3 were converted in the second or third month.

However, within 80 d post conversion, graft rejection occurred in 4 of the 10 patients, all of whom were on triple immunosuppression (*i.e.*, EVR plus MMF plus steroids) at the time of graft rejection. All 4 patients subsequently had CNI (3 with FK, 1 with CsA) re-introduced, without recurrence of neurotoxicity. The findings suggest EVR use enables a temporary withdrawal of CNI in managing CNI-induced neurotoxicity. Re-introduction of CNI may be prudent after resolution of neurotoxicity in view of high rejection rates, especially within first 3 mo post-transplant, when EVR is not used in combination with CNI. Furthermore, in patients with acute rejection, the introduction of CsA or re-introduction of FK may be possible because the risk of further neurologic complications may be low.

Prevention of hepatocellular carcinoma recurrence

EVR has proven efficacy against breast cancer, renal cell carcinoma, neuroendocrine tumours and subependymal giant cell astrocytoma^[33]. There is no data on *de novo* hepatocellular carcinoma (HCC). The HCC recurrence rate post-LT is 8%-20%, with most occurring within the first 2 years post-LT^[34,35]. There is no RCT on EVR for the prevention of HCC recurrence. In Jeng *et al.*^[22]'s single-centre prospective non-randomised study, HCC recurred in 7% of the patients using EVR. In retrospective studies, it has been observed that EVR has no HCC recurrence post-LT during a mean follow-up of 11.2 ± 6.8 mo in 44 patients and 48 mo (range: 11-76 mo) in 21 patients respectively^[26,36]. In a systematic review, LT recipients who were on mTOR inhibitors (sirolimus or EVR) had lower HCC recurrence rates^[37]. However, the follow up period varied widely among the groups on CNI (42 mo), sirolimus (30 mo) and EVR (19 mo). No mortality data was presented in this review.

Hepatitis C and liver fibrosis

In an open-label multi-center randomised study, conversion to EVR delayed histological fibrosis progression in 43 LT recipients with HCV recurrence as compared to FK-based immunosuppressive therapy^[38]. However, this potential benefit was not observed in the extended H2304 study, where no significant difference in histological fibrosis scores between the EVR and control groups was reported at the end of 3 years of follow-up^[16]. Hence, more studies are warranted to confirm EVR benefit in delaying liver fibrosis progression of hepatitis C.

ADVERSE EFFECTS OF EVR

The most common adverse effects of EVR use in LT

recipients are infections (50.6%), hyperlipidaemia (23.7%), hypertension (18.0%), peripheral oedema (17.6%), leukopenia (14.3%), and wound healing impairment (11.0%)^[8,9]. In a phase II study, the incidence of adverse effects was higher in patients with higher daily EVR doses, especially > 4 mg/d^[11]. In *de novo* LT, the discontinuation of EVR due to adverse effects was higher in the EVR group, 25.7%, which was nearly double of the control (14.1%) in this study^[16]. The common adverse effects that led to EVR discontinuation were proteinuria, delayed wound healing, pancytopenia, leukopenia and thrombocytopenia.

In maintenance therapy, 22% of patients discontinued EVR due to adverse effects while no patients in the control group discontinued study medication^[18]. The adverse effects that led to EVR discontinuation included leukopenia, proteinuria, thrombotic microangiopathy, elevation in hepatic enzymes, increased HCV viral load, hypertriglyceridemia, renal impairment, interstitial lung disease, pneumonitis, pulmonary fibrosis and stomatitis. In a study on 94 patients converting to EVR at mean of 5 years from transplant, as many as 70% of patients experienced adverse reaction and 16% required EVR to be discontinued despite mean EVR C₀ level being at only 6 ng/mL^[39].

Hepatic artery thrombosis

In February 2013, the United States Food and Drug Administration included a warning of hepatic artery thrombosis (HAT) in the EVR product insert. Most of the reported cases of HAT in the presence of mTOR inhibition occurred within the first 30 d from transplant surgery, leading to graft loss or death. Therefore, in most EVR studies, randomization was started only 30 d after transplant surgery.

One case of HAT was reported in the H2304 study, occurring in a subject who had a prior history of HAT before randomization. There were no reports of HAT in the PROTECT nor RESCUE studies. In the RESCUE study, although an adverse effect of thrombotic microangiopathy was reported, there were no specific details of its incidence nor eventual outcomes. Combination of EVR and CNI has also been reported to increase the risk of thrombotic microangiopathy elsewhere^[40].

In Masetti *et al.*^[21]'s study on the early use of EVR within the first 30 d of LT, no HAT was reported with EVR use. This is in contrast to the control CsA group which had 2 (7.6%) patients with HAT and 2 (7.6%) patient with hepatic artery stenosis. Although there was a significant higher rate of hepatic stenosis and thrombosis in CsA group, it is important to note number of patients in CsA group ($n = 26$) was just half of patients in EVR group ($n = 52$).

In another prospective cohort study, no HAT was reported with EVR use in 43 patients in the early (33 patients within week 1, 9 patients within week 2 and 1 at day 20 from transplant) post-LT period^[22]. Similarly, in a retrospective study, no HAT was observed in 28

patients when EVR was initiated at median of 14 (range 4-24) d^[23].

Impaired wound healing

Wound healing is an important care issue for post-transplant surgery. Sirolimus has as high as 36% incidence rate of impaired wound healing^[41]. Furthermore, it was reported that mTOR inhibitor is an independent risk factor for incisional hernia in LT^[42]. Impairment of wound healing between EVR and control group was presented in the PROTECT study (3.0% vs 4.9% at 11 mo) and H2304 study (11.0% vs 7.9% at 1 year, RR = 1.40, 95%CI: 0.80, 2.45; 11.0% vs 8.3% at 2 year, $P = 0.36$)^[13,16,17]. Both studies also reported an increase of incisional hernia with EVR exposure, although the difference did not reach statistical significance in either study^[14,17]. Similarly, Masetti *et al.*^[21] reported a non-significant increase in incisional hernia in the EVR group. The findings in these studies were similar to the not statistically significant increase of wound healing impairment in renal transplant patients using EVR as compared to standard CNI reported by Nashan *et al.*^[43].

Generally, impaired wound healing rates with EVR use ranges from 11%-35%^[41]. Despite the lack of statistical significance, further analyses or studies to guide the optimal time for initiation of EVR in LT are warranted, especially when EVR is used in combination with other immunosuppressants that may delay healing process as well.

Infection

The risk of infection is of concern in post-transplant care and the higher incidence of infection with EVR should not be overlooked. In the H2304 study, the overall incidence of any infection at 1 year was not statistically significant^[16]. However, there was an increase for any serious infection (13.9% in EVR group vs 7.9% in control, RR = 1.76, 95%CI: 1.03, 3.00) which included pneumonia and hepatitis C. The overall incidence of any infection was also comparable between EVR and control groups in the 2-year and 3-year follow up (56.3% vs 51.7% and 70.8% vs 64.0% respectively) period, without a significant difference in the rates of serious infections.

In the PROTECT study, Kaplan-Meier survival plot showed the occurrence of any infection was higher in the EVR group as compared to standard CNI group (79.5% vs 68.3%, $P = 0.050$) at 11 mo from randomization particularly oral herpes, sinusitis and wound infection^[13]. In the RESCUE study, 31.9% of patients in EVR group vs 21.9% in standard CNI group experienced infections which included stomatitis, herpes simplex, bronchitis and urinary tract infections^[19]. Of note, it also reported a significant increase in HCV viral load in their EVR group (6.9%, $P = 0.028$) as compared to none in the control group. Although statistical difference was unknown, the authors also reported 15.3% (EVR group) in contrast to 1.4% (standard CNI group)

of infections being related to studied drug.

Incidence of infection was the same in both EVR and control groups, 46.2% in Masetti's study^[21]. In a retrospective observational study, infection was 60.7% with *de novo* EVR use in 28 patients. On the other hand, only 1 case of infection was reported in a single-center prospective study^[22]. There was no clear definition of infection and the disparity could possibly be due to different definition among various studies.

Stomatitis

Stomatitis incidence was significantly higher (10.6%, 26.4%) in EVR group as compared to standard CNI group (1.2%, 0%) at 1-year and 2 year follow up of the H2304 ($P < 0.001$) and RESCUE ($P < 0.010$) studies^[17,19]. Stomatitis has also been reported as one of the common adverse effects when EVR was used as maintenance immunosuppressive therapy^[22,24]. Management strategies for stomatitis include the use of local anesthetic, intralesional and topical steroid to control stomatitis and reduce pain^[41,44,45]. EVR has also been used as an alternative for renal transplant recipients who experienced sirolimus-induced stomatitis^[46].

In general, mTOR inhibitor-associated stomatitis is generally not severe ($< 5\%$ is Grade 3 or 4)^[41]. However, if nutrition status is compromised due to poor oral intake secondary to stomatitis, dose reduction or even withdrawal may be warranted.

Peripheral edema

mTOR inhibitor adverse effect of peripheral edema may be related to its anti-lymphangiogenic effect, leading to lymphedema and capillary leak which may not be reversible^[41]. In all 3 main phase III studies, peripheral edema was reported to be significantly higher in EVR group in comparison to control group. In the PROTECT study, peripheral edema was consistently higher in EVR group (26.8%) vs in standard CNI group (12.5%), $P = 0.162$ at month 11^[13]. The incidence of peripheral edema continued to increase in the extension study period from month 11-35 (22% in EVR group vs 5% in standard CNI group, $P = 0.048$) and from month 11-59 (31.7% in EVR group vs 7.5% in standard CNI group, $P = 0.011$)^[14,15]. In H2304 study, 17.6% and 22.4% in EVR group as compared to 10.8% and 14.9% in the standard CNI group experienced peripheral edema at 1-year (RR = 1.63, 95%CI: 1.03, 2.56) and at 2-year ($P = 0.036$) respectively^[16,17]. Similar trend was observed in the RESCUE study, with the incidence of peripheral oedema 5.6% in EVR group and 1.4% in the standard CNI group^[19]. Nonetheless, peripheral edema was not reported as one of the adverse effects that led to drug discontinuation in all above studies.

Proteinuria

It is unclear how mTOR inhibitors influence glomerulus permeability and cause proteinuria^[41]. Nonetheless, as proteinuria is an indicator of kidney injury and strong predictor for cardiovascular events, this adverse effect

Table 3 Recommendation for everolimus use in liver transplantation recipients

Indication and regimen	Renoprotective benefit EVR in combination with CNI to allow CNI dose reduction Management of CNI neurotoxicity EVR allows temporary withdrawal of CNI till resolution of neurotoxicity
Patients	LT recipients with renal function > 60 mL/min LT recipients proteinuria < 1 g/24 h
Timing	<i>De novo</i> therapy: Initiate EVR at 1 mo from transplant Maintenance therapy: Introduce EVR within 1 yr from transplant CNI neurotoxicity: Stop CNI and initiate EVR immediately

CNI: Calcineurin inhibitor; EVR: Everolimus; LT: Liver transplantation.

warrants clinical attention. Patients with proteinuria (≥ 1 g/24 h) were excluded in H2304 and RESCUE study^[15,18]. At the end of 1 year of H2304 study, 2.9% in EVR group developed proteinuria as compared to 0.4% in standard CNI group (RR = 6.89, 95%CI: 0.85, 55.54)^[16]. In the subsequent follow up year, proteinuria was the most frequent adverse event that resulting in discontinuation of EVR (3.3%) in contrast to standard CNI group (0.4%)^[17]. In the RESCUE study, 2 out of 16 patients required discontinuation of EVR due to proteinuria, while no patients required drug discontinuation in the standard CNI group for the same reason^[19]. Similarly, incidence of proteinuria was significantly higher in EVR group (9.9%) as compared to the standard CNI group (2.0%) at month 11 in the PROTECT study^[13]. A similar trend in their extension study up to month 59 was seen, albeit without statistical difference^[15].

The characteristic and long-term outcomes of patients experiencing proteinuria, which could possibly guide patient selection and risk-benefit consideration to use EVR in LT, are lacking.

Hyperlipidemia

Hyperlipidemia is one of the most common adverse effects of mTOR inhibitors^[41]. From phase II studies, a trend of dose-dependent hyperlipidemia was observed^[11]. Masetti *et al*^[21] reported significant increase in the incidence of hyperlipidemia but not hypertriglyceridemia with EVR use. In H2304, 23.3% in EVR group vs 17.8% in standard CNI group required lipid-lowering therapy ($P = 0.944$) at the end of 1 year and the incidence of hyperlipidemia was significantly higher (26.9% in EVR group vs 11.6% in standard CNI, $P < 0.001$) at the end of 2 years^[16,17]. In the PROTECT study, EVR use was associated with an increased incidence of hyperlipidemia as compared to controls (11.9% vs 2.0%, $P < 0.05$) at month 11^[13].

Although cardiovascular risk in LT is lower than renal and cardiac transplant, cardiovascular disease is still one of the leading causes of morbidity^[47]. Undoubtedly, there is a range of effective lipid-lowering therapy in managing hyperlipidemia, and it is prudent to always weigh cardiovascular risks over the benefits before initiation or conversion to EVR.

RECOMMENDATION

A working group has recently consolidated recommendations for EVR use in LT based on consensus and experiences^[48]. It provides some guidance while more outcome data is warranted to establish a comprehensive guideline for EVR use in LT. Based on current available data discussed in this review, EVR is an appropriate immunosuppressant for LT recipients as listed in Table 3.

The increased risk of adverse effects could offset the benefit of EVR particularly in preserving renal function. Although it has been mentioned that dose reduction was exercised in managing EVR adverse effects, but there were no details on the methods or outcomes^[24,41,49]. Patient selection and strategies to reduce and minimise adverse effects will be key in determining the success of EVR use in LT.

CONCLUSION

EVR could be a viable alternative immunosuppressant in LT recipients who are at risk of renal impairment. Initiating EVR early (from 30 d post-LT and before eGFR < 55 mL/min) post-transplant allows CNI reduction and thus reduces CNI nephrotoxicity. Future research to strengthen EVR initiation, switch, or combination strategies and cost-effectiveness analyses would be important.

REFERENCES

- 1 Kim WR, Lake JR, Smith JM, Skeans MA, Schladt DP, Edwards EB, Harper AM, Wainright JL, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2013 Annual Data Report: liver. *Am J Transplant* 2015; **15** Suppl 2: 1-28 [PMID: 25626341 DOI: 10.1111/ajt.13197]
- 2 Moini M, Schilsky ML, Tichy EM. Review on immunosuppression in liver transplantation. *World J Hepatol* 2015; **7**: 1355-1368 [PMID: 26052381 DOI: 10.4254/wjh.v7.i10.1355]
- 3 Jain A, Singhal A, Fontes P, Mazariegos G, DeVera ME, Cacciarelli T, Lopez RC, Sindhi R, Humar A, Marsh JW. One thousand consecutive primary liver transplants under tacrolimus immunosuppression: a 17- to 20-year longitudinal follow-up. *Transplantation* 2011; **91**: 1025-1030 [PMID: 21378604 DOI: 10.1097/TP.0b013e3182129215]
- 4 Busuttil RW, Farmer DG, Yersiz H, Hiatt JR, McDiarmid SV, Goldstein LI, Saab S, Han S, Durazo F, Weaver M, Cao C, Chen T, Lipshutz GS, Holt C, Gordon S, Gornbein J, Amersi F, Ghobrial RM. Analysis of long-term outcomes of 3200 liver transplantations

- over two decades: a single-center experience. *Ann Surg* 2005; **241**: 905-916; discussion 916-918 [PMID: 15912040 DOI: 10.1097/01.sla.0000164077.77912.98]
- 5 **Ojo AO**, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, Arndorfer J, Christensen L, Merion RM. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931-940 [PMID: 12954741 DOI: 10.1056/NEJMoa021744]
 - 6 **von Wichert G**, Jehle PM, Hoeflich A, Koschnick S, Dralle H, Wolf E, Wiedenmann B, Boehm BO, Adler G, Seufferlein T. Insulin-like growth factor-I is an autocrine regulator of chromogranin A secretion and growth in human neuroendocrine tumor cells. *Cancer Res* 2000; **60**: 4573-4581 [PMID: 10969809]
 - 7 **Kirchner GI**, Meier-Wiedenbach I, Manns MP. Clinical pharmacokinetics of everolimus. *Clin Pharmacokinet* 2004; **43**: 83-95 [PMID: 14748618 DOI: 10.2165/00003088-200443020-00002]
 - 8 **Gabardi S**, Baroletti SA. Everolimus: a proliferation signal inhibitor with clinical applications in organ transplantation, oncology, and cardiology. *Pharmacotherapy* 2010; **30**: 1044-1056 [PMID: 20874042 DOI: 10.1592/phco.30.10.1044]
 - 9 Certican (Everolimus) Product Insert. Novartis, Mar 2013
 - 10 **Niioaka T**, Kagaya H, Saito M, Inoue T, Numakura K, Yamamoto R, Akamine Y, Habuchi T, Satoh S, Miura M. Influence of everolimus on the pharmacokinetics of tacrolimus in Japanese renal transplant patients. *Int J Urol* 2016; **23**: 484-490 [PMID: 26990259 DOI: 10.1111/iju.13081]
 - 11 **Shipkova M**, Hesselink DA, Holt DW, Billaud EM, van Gelder T, Kunicki PK, Brunet M, Budde K, Barten MJ, De Simone P, Wieland E, López OM, Masuda S, Seger C, Picard N, Oellerich M, Langman LJ, Wallemacq P, Morris RG, Thompson C, Marquet P. Therapeutic Drug Monitoring of Everolimus: A Consensus Report. *Ther Drug Monit* 2016; **38**: 143-169 [PMID: 26982492 DOI: 10.1097/FTD.0000000000000260]
 - 12 **Levy G**, Schmidli H, Punch J, Tuttle-Newhall E, Mayer D, Neuhaus P, Samuel D, Nashan B, Klempnauer J, Langnas A, Calmus Y, Rogiers X, Abecassis M, Freeman R, Sloof M, Roberts J, Fischer L. Safety, tolerability, and efficacy of everolimus in de novo liver transplant recipients: 12- and 36-month results. *Liver Transpl* 2006; **12**: 1640-1648 [PMID: 16598777 DOI: 10.1002/lt.20707]
 - 13 **Fischer L**, Klempnauer J, Beckebaum S, Metselaar HJ, Neuhaus P, Schemmer P, Settmacher U, Heyne N, Clavien PA, Muehlbacher F, Morard I, Wolters H, Vogel W, Becker T, Sterneck M, Lehner F, Klein C, Kazemier G, Pascher A, Schmidt J, Rauchfuss F, Schnitzbauer A, Nadalin S, Hack M, Ladenburger S, Schlitt HJ. A randomized, controlled study to assess the conversion from calcineurin-inhibitors to everolimus after liver transplantation--PROTECT. *Am J Transplant* 2012; **12**: 1855-1865 [PMID: 22494671 DOI: 10.1111/j.1600-6143.2012.04049.x]
 - 14 **Sterneck M**, Kaiser GM, Heyne N, Richter N, Rauchfuss F, Pascher A, Schemmer P, Fischer L, Klein CG, Nadalin S, Lehner F, Settmacher U, Neuhaus P, Gotthardt D, Loss M, Ladenburger S, Paulus EM, Mertens M, Schlitt HJ. Everolimus and early calcineurin inhibitor withdrawal: 3-year results from a randomized trial in liver transplantation. *Am J Transplant* 2014; **14**: 701-710 [PMID: 24502384 DOI: 10.1111/ajt.12615]
 - 15 **Sterneck M**, Kaiser GM, Heyne N, Richter N, Rauchfuss F, Pascher A, Schemmer P, Fischer L, Klein CG, Nadalin S, Lehner F, Settmacher U, Gotthardt D, Loss M, Ladenburger S, Wimmer P, Dworak M, Schlitt HJ. Long-term follow-up of five yr shows superior renal function with everolimus plus early calcineurin inhibitor withdrawal in the PROTECT randomized liver transplantation study. *Clin Transplant* 2016; **30**: 741-748 [PMID: 27160359 DOI: 10.1111/ctr.12744]
 - 16 **De Simone P**, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, Saliba F, Jonas S, Sudan D, Fung J, Fischer L, Duvoux C, Chavin KD, Koneru B, Huang MA, Chapman WC, Foltys D, Witte S, Jiang H, Hexham JM, Junge G. Everolimus with reduced tacrolimus improves renal function in de novo liver transplant recipients: a randomized controlled trial. *Am J Transplant* 2012; **12**: 3008-3020 [PMID: 22882750 DOI: 10.1111/j.1600-6143.2012.04212.x]
 - 17 **Saliba F**, De Simone P, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, Jonas S, Sudan D, Fischer L, Duvoux C, Chavin KD, Koneru B, Huang MA, Chapman WC, Foltys D, Dong G, Lopez PM, Fung J, Junge G. Renal function at two years in liver transplant patients receiving everolimus: results of a randomized, multicenter study. *Am J Transplant* 2013; **13**: 1734-1745 [PMID: 23714399 DOI: 10.1111/ajt.12280]
 - 18 **Fischer L**, Saliba F, Kaiser GM, De Carlis L, Metselaar HJ, De Simone P, Duvoux C, Nevens F, Fung JJ, Dong G, Rauer B, Junge G. Three-year Outcomes in De Novo Liver Transplant Patients Receiving Everolimus With Reduced Tacrolimus: Follow-Up Results From a Randomized, Multicenter Study. *Transplantation* 2015; **99**: 1455-1462 [PMID: 26151607 DOI: 10.1097/TP.0000000000000555]
 - 19 **De Simone P**, Metselaar HJ, Fischer L, Dumortier J, Boudjema K, Hardwigen J, Rostaing L, De Carlis L, Saliba F, Nevens F. Conversion from a calcineurin inhibitor to everolimus therapy in maintenance liver transplant recipients: a prospective, randomized, multicenter trial. *Liver Transpl* 2009; **15**: 1262-1269 [PMID: 19790150 DOI: 10.1002/lt.21827]
 - 20 **De Simone P**, Saliba F, Dong G, Escrig C, Fischer L. Do patient characteristics influence efficacy and renal outcomes in liver transplant patients receiving everolimus? *Clin Transplant* 2016; **30**: 279-288 [PMID: 26717035 DOI: 10.1111/ctr.12687]
 - 21 **Masetti M**, Montalti R, Rompianesi G, Codeluppi M, Gerring R, Romano A, Begliomini B, Di Benedetto F, Gerunda GE. Early withdrawal of calcineurin inhibitors and everolimus monotherapy in de novo liver transplant recipients preserves renal function. *Am J Transplant* 2010; **10**: 2252-2262 [PMID: 20486905 DOI: 10.1111/j.1600-6143.2010.03128.x]
 - 22 **Jeng LB**, Thorat A, Hsieh YW, Yang HR, Yeh CC, Chen TH, Hsu SC, Hsu CH. Experience of using everolimus in the early stage of living donor liver transplantation. *Transplant Proc* 2014; **46**: 744-748 [PMID: 24767339 DOI: 10.1016/j.transproceed.2013.11.068]
 - 23 **Gastaca M**, Bilbao I, Jimenez M, Bustamante J, Dopazo C, Gonzalez R, Charco R, Santoyo J, Ortiz de Urbina J. Safety and Efficacy of Early Everolimus When Calcineurin Inhibitors Are Not Recommended in Orthotopic Liver Transplantation. *Transplant Proc* 2016; **48**: 2506-2509 [PMID: 27742336 DOI: 10.1016/j.transproceed.2016.08.027]
 - 24 **De Simone P**, Carrai P, Precisi A, Petrucci S, Baldoni L, Balzano E, Ducci J, Caneschi F, Coletti L, Campani D, Filippini F. Conversion to everolimus monotherapy in maintenance liver transplantation: feasibility, safety, and impact on renal function. *Transpl Int* 2009; **22**: 279-286 [PMID: 19054383 DOI: 10.1111/j.1432-2277.2008.00768.x]
 - 25 **Castroagudín JF**, Molina E, Romero R, Otero E, Tomé S, Varo E. Improvement of renal function after the switch from a calcineurin inhibitor to everolimus in liver transplant recipients with chronic renal dysfunction. *Liver Transpl* 2009; **15**: 1792-1797 [PMID: 19938140 DOI: 10.1002/lt.21920]
 - 26 **Saliba F**, Dharancy S, Lorho R, Conti F, Radenne S, Neau-Cransac M, Hurtova M, Hardwigen J, Calmus Y, Dumortier J. Conversion to everolimus in maintenance liver transplant patients: a multicenter, retrospective analysis. *Liver Transpl* 2011; **17**: 905-913 [PMID: 21384525 DOI: 10.1002/lt.22292]
 - 27 **Bilbao I**, Salcedo M, Gómez MA, Jimenez C, Castroagudín J, Fabregat J, Almohalla C, Herrero I, Cuervas-Mons V, Otero A, Rubin A, Miras M, Rodrigo J, Serrano T, Crespo G, De la Mata M, Bustamante J, Gonzalez-Dieguez ML, Moreno A, Narvaez I, Guilera M. Renal function improvement in liver transplant recipients after early everolimus conversion: A clinical practice cohort study in Spain. *Liver Transpl* 2015; **21**: 1056-1065 [PMID: 25990257 DOI: 10.1002/lt.24172]
 - 28 **Farkas SA**, Schnitzbauer AA, Kirchner G, Obed A, Banas B, Schlitt HJ. Calcineurin inhibitor minimization protocols in liver transplantation. *Transpl Int* 2009; **22**: 49-60 [PMID: 19121146 DOI: 10.1111/j.1432-2277.2008.00796.x]
 - 29 **Pageaux GP**, Rostaing L, Calmus Y, Duvoux C, Vanlemmens C, Hardwigen J, Bernard PH, Barbotte E, Vercambre L, Bismuth M, Puche P, Navarro F, Larrey D. Mycophenolate mofetil in combination with reduction of calcineurin inhibitors for chronic renal dysfunction after liver transplantation. *Liver Transpl* 2006; **12**: 1755-1760 [PMID: 17133564 DOI: 10.1002/lt.20903]

- 30 **Hao JC**, Wang WT, Yan LN, Li B, Wen TF, Yang JY, Xu MQ, Zhao JC, Wei YG. Effect of low-dose tacrolimus with mycophenolate mofetil on renal function following liver transplantation. *World J Gastroenterol* 2014; **20**: 11356-11362 [PMID: 25170222 DOI: 10.3748/wjg.v20.i32.11356]
- 31 **Jiménez-Pérez M**, González Grande R, Rando Muñoz FJ, de la Cruz Lombardo J, Muñoz Suárez MA, Fernández Aguilar JL, Pérez Daga JA, Santoyo-Santoyo J, Manteca González R, Rodrigo López JM. Everolimus plus mycophenolate mofetil as initial immunosuppression in liver transplantation. *Transplant Proc* 2015; **47**: 90-92 [PMID: 25645779 DOI: 10.1016/j.transproceed.2014.11.005]
- 32 **Bilbao I**, Dopazo C, Castells L, Lazaro J, Caralt M, Sapisochin G, Charco R. Immunosuppression based on everolimus in liver transplant recipients with severe early post-transplantation neurotoxicity. *Transplant Proc* 2014; **46**: 3104-3107 [PMID: 25420835 DOI: 10.1016/j.transproceed.2014.10.001]
- 33 Afinitor (Everolimus) prescribing information. Novartis, Jul 2015
- 34 **Clavien PA**, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13**: e11-e22 [PMID: 22047762 DOI: 10.1016/S1470-2045(11)70175-9]
- 35 **Dugum MF**, Zein NN. Liver Transplantation for Hepatocellular Carcinoma. *Clinical Liver Disease* 2016; **7**: 36-39 [DOI: 10.1002/cld.531]
- 36 **Cholongitas E**, Goulis I, Theocariou E, Antoniadis N, Fouzas I, Giakoustidis D, Imvrios G, Gioulema O, Papanikolaou V, Akriviadis E, Vasiliadis T. Everolimus-based immunosuppression in liver transplant recipients: A single-center experience. *Hepatol Int* 2014; **8**: 137-145 [DOI: 10.1007/s12072-013-9492-6]
- 37 **Cholongitas E**, Mamou C, Rodríguez-Castro KI, Burra P. Mammalian target of rapamycin inhibitors are associated with lower rates of hepatocellular carcinoma recurrence after liver transplantation: a systematic review. *Transpl Int* 2014; **27**: 1039-1049 [PMID: 24943720 DOI: 10.1111/tri.12372]
- 38 **Villamil FG**, Gadano AC, Zingale F, Perez R, Gil O, Yantorno S, Mastai R, Cairo FO, Otero AB, Dong G, Lopez P. Fibrosis progression in maintenance liver transplant patients with hepatitis C recurrence: a randomised study of everolimus vs. calcineurin inhibitors. *Liver Int* 2014; **34**: 1513-1521 [PMID: 25453134]
- 39 **Vallin M**, Guillaud O, Morard I, Gagnieu MC, Mentha G, Adham M, Morelon E, Boillot O, Giostra E, Dumortier J. Tolerability of everolimus-based immunosuppression in maintenance liver transplant recipients. *Clin Transplant* 2011; **25**: 660-669 [PMID: 21158921 DOI: 10.1111/j.1399-0012.2010.01370.x]
- 40 **Ventura-Aguilar P**, Campistol JM, Diekmann F. Safety of mTOR inhibitors in adult solid organ transplantation. *Expert Opin Drug Saf* 2016; **15**: 303-319 [PMID: 26667069 DOI: 10.1517/14740338.2016.1132698]
- 41 **Kaplan B**, Qazi Y, Wellen JR. Strategies for the management of adverse events associated with mTOR inhibitors. *Transplant Rev (Orlando)* 2014; **28**: 126-133 [PMID: 24685370 DOI: 10.1016/j.tre.2014.03.002]
- 42 **Montali R**, Mimmo A, Rompianesi G, Serra V, Cautero N, Ballarin R, De Ruvo N, Cunningham Gerring R, Enrico Gerunda G, Di Benedetto F. Early use of mammalian target of rapamycin inhibitors is an independent risk factor for incisional hernia development after liver transplantation. *Liver Transpl* 2012; **18**: 188-194 [PMID: 21987434 DOI: 10.1002/lt.22445]
- 43 **Nashan B**, Citterio F. Wound healing complications and the use of mammalian target of rapamycin inhibitors in kidney transplantation: a critical review of the literature. *Transplantation* 2012; **94**: 547-561 [PMID: 22941182 DOI: 10.1097/TP.0b013e3182551021]
- 44 **Ji YD**, Aboalela A, Villa A. Everolimus-associated stomatitis in a patient who had renal transplant. *BMJ Case Rep* 2016; **2016**: bcr2016217513 [PMID: 27797804 DOI: 10.1136/bcr-2016-217513]
- 45 **Vermeulen T**, Rodríguez IE, Vrints CJ, Conraads V. Severe stomatitis complicating immune-suppressive switch after cardiac transplantation. *Acta Chir Belg* 2010; **110**: 339-341 [PMID: 20690519]
- 46 **Ram R**, Swarnalatha G, Neela P, Dakshinamurthy KV. Sirolimus-induced aphthous ulcers which disappeared with conversion to everolimus. *Saudi J Kidney Dis Transpl* 2008; **19**: 819-820 [PMID: 18711306]
- 47 **Luca L**, Westbrook R, Tsochatzis EA. Metabolic and cardiovascular complications in the liver transplant recipient. *Ann Gastroenterol* 2015; **28**: 183-192 [PMID: 25830307]
- 48 **De Simone P**, Fagioli S, Cescon M, De Carlis L, Tisone G, Volpes R, Cillo U. Use of Everolimus in Liver Transplantation: Recommendations From a Working Group. *Transplantation* 2017; **101**: 239-251 [PMID: 27495768 DOI: 10.1097/TP.0000000000001438]
- 49 **Dumortier J**, Dharancy S, Calmus Y, Duvoux C, Durand F, Salamé E, Saliba F. Use of everolimus in liver transplantation: The French experience. *Transplant Rev (Orlando)* 2016; **30**: 161-170 [PMID: 27083870 DOI: 10.1016/j.tre.2015.12.003]

P- Reviewer: Carter WG, Demonacos C **S- Editor:** Ji FF

L- Editor: A **E- Editor:** Lu YJ



Prospective Study

MicroRNAs and clinical implications in hepatocellular carcinoma

Amal Ahmed Mohamed, Zainab A Ali-Eldin, Tamer A Elbedewy, Magdy El-Serafy, Fatma A Ali-Eldin, Hossameldin AbdelAziz

Amal Ahmed Mohamed, Biochemistry Department, National Hepatology and Tropical Medicine Research Institute, Cairo 11796, Egypt

Zainab A Ali-Eldin, Hossameldin AbdelAziz, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, Cairo 11331, Egypt

Tamer A Elbedewy, Department of Internal Medicine, Faculty of Medicine, Tanta University, Tanta 31111, Egypt

Magdy El-Serafy, Department of Tropical Medicine, Faculty of Medicine, Cairo University, Cairo 11796, Egypt

Fatma A Ali-Eldin, Department of Tropical Medicine, Faculty of Medicine, Ain Shams University, Cairo 11331, Egypt

Author contributions: Mohamed AA designed the research; Ali-Eldin ZA, Elbedewy TA and Ali-Eldin FA performed the clinical part of the research; Mohamed AA performed the biochemical part of the work; Elbedewy TA performed statistical analysis of the data; Ali-Eldin ZA, Elbedewy TA and Ali-Eldin FA wrote the paper; El-Serafy M and AbdelAziz H revised the paper.

Institutional review board statement: The study was approved.

Informed consent statement: All study participants, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: None.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Dr. Amal Ahmed Mohamed, Assistant Professor of Biochemistry and Molecular Biology, Biochemistry Department, National Hepatology and Tropical Medicine Research Institute, 10 Kasar El Eini Street, Cairo 11796, Egypt. amalahmedhcp@yahoo.com
Telephone: +20-2-23649005
Fax: +20-2-23649005

Received: February 14, 2017

Peer-review started: February 17, 2017

First decision: April 20, 2017

Revised: May 31, 2017

Accepted: June 19, 2017

Article in press: June 20, 2017

Published online: August 18, 2017

Abstract

AIM

To assess the role of some circulating miRNAs (miR-23a, miR-203, miR338, miR-34, and miR-16) as tumor markers for diagnosis of hepatocellular carcinoma (HCC).

METHODS

One hundred and seventy-one subjects were enrolled, 57 patients with HCC, 57 patients with liver cirrhosis (LC) and 57 healthy subjects as control group. Severity of liver disease was assessed by Child Pugh score. Tumor staging was done using Okuda staging system. Quantification of Micro RNA (miR-23a, miR-203, miR338, miR-34, and miR-16) was performed.

RESULTS

All studied miRNA showed significant difference between HCC and cirrhotic patients in comparison to

healthy control. miR-23a showed statistically significant difference between HCC and cirrhotic patients being higher in HCC group than cirrhotic. miR-23a is significantly higher in HCC patients with focal lesion size equal or more than 5 cm, patients with multiple focal lesions and Okuda stage III. At cutoff value $\geq 2^{10}$, miR-23a showed accuracy 79.3% to diagnose HCC patients with sensitivity 89.47% and specificity about 64.91%. At cut off level ≥ 200 ng/mL, serum alpha fetoprotein had 73.68% sensitivity, 52.63% specificity, 43.75% PPV, 80% NPV for diagnosis of HCC.

CONCLUSION

MicroRNA 23a can be used as a screening test for early detection of HCC. Also, it is related to larger size of tumour, late Okuda staging and multiple hepatic focal lesions, so it might be a prognostic biomarker.

Key words: Hepatocellular carcinoma; MicroRNA; Liver cirrhosis; MiR-23a

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: MicroRNA is promising as diagnostic and prognostic biomarkers. miR-23a can be used in screening of hepatocellular carcinoma (HCC) and it gives better results than alpha fetoprotein. It is also related to more progressive HCC so it can be used as predictor of prognosis.

Mohamed AA, Ali-Eldin ZA, Elbedewy TA, El-Serafy M, Ali-Eldin FA, AbdelAziz H. MicroRNAs and clinical implications in hepatocellular carcinoma. *World J Hepatol* 2017; 9(23): 1001-1007 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v9/i23/1001.htm> DOI: <http://dx.doi.org/10.4254/wjh.v9.i23.1001>

INTRODUCTION

MicroRNAs (miRNA) are small, non-coding RNAs that negatively regulate gene expression at the post-transcriptional level^[1]. miRNA is known to regulate the cell cycle, apoptosis and metastasis^[2]. Aberrant miRNA expression contributes to tumorigenesis and cancer progression^[3]. miRNA is involved in various biological processes that underlie hepatic tumor formation^[1]. Murakami *et al.*^[4] was the first to report that hepatic malignancy exhibited an abnormal expression pattern of miRNAs as the dysregulation of miRNA expression has been identified as a common characteristic of liver cancer. Later on, a number of studies have confirmed that miRNAs possess important regulatory roles in hepatocarcinogenesis and malignant transformation^[5].

Circulating miRNAs that are released from cancerous tissues are stable and readily available for clinical analysis, and therefore may be useful for the first-line detection of cancer^[6]. Studies concerning

miRNAs appear to show a novel perspective for cancer diagnosis and treatment^[2].

Hepatocellular carcinoma (HCC) is characterized by significant morbidity and high mortality rates worldwide^[7]. Because of the difficulty of clinical diagnosis at the early stage, only 30%-40% of cases can undergo curative resection^[8]. As there are currently no reliable tumor markers or imaging technologies that can accurately diagnose early HCC, the use of circulating miRNAs as a potential tool for HCC detection has become an emerging area of study^[6,9]. Many circulating microRNAs were evaluated in liver diseases including miR-122, miR-21, miR-34a, miR-221, miR-23a, miR-216, miR-155, miR-186, miR-150, miR-130b, and miR-214^[10]. Our aim is to detect the possibility of using some circulating miRNAs as tumor markers for diagnosis of HCC namely miR-23a, miR-203, miR-338-3p, miR-34, and miR-16.

MATERIALS AND METHODS

This prospective cross sectional study included 171 subjects divided into 3 groups: Group I comprising 57 patients with HCC, Group II comprising 57 patients with liver cirrhosis, Group III 57 healthy subjects as a control group.

Informed written consent was obtained from all participants prior to enrollment in the study and approved by ethical committee of Faculty of Medicine, Tanta University. Patients with other cancers or meta-static liver cancer were excluded. All patients were submitted to detailed history and clinical assessment. Liver cirrhosis was diagnosed on the basis of history, clinical examination, laboratory findings, and abdominal ultrasonography. Severity of liver disease was assessed by Child Pugh score^[11]. HCC was diagnosed by abdominal ultrasonography, abdominal triphasic computed tomography and serum Alpha fetoprotein (AFP). Tumor characteristics were detected including tumor size, focal lesion number, site, and portal vein invasion. Tumor staging was done using Okuda staging system^[12].

Fasting venous blood samples (5 mL) were collected by trained laboratory technicians. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin levels and creatinine were measured by using SynchronCX4 clinical system. Serum alpha-fetoprotein levels and viral status (HCV-Ab and HBs Ag) were estimated by serological techniques (Axyam System, Abbott Laboratories). Prothrombin time measurements were performed for all patients; normal time was 12 s [100% concentration and International normalization ratio (INR) of 1]. Complete blood count was done using Automatic blood cell counter model PCE-210N (ERMA INC).

RNA isolation

Total RNA was isolated according to the instructions of the supplier and was further purified using an RN easy

Table 1 Demographic data and Child Pugh scoring of the studied groups *n* (%)

Variable(s)	Group I HCC group (<i>n</i> = 57)	Group II Cirrhotic group (<i>n</i> = 57)	Group III Control group (<i>n</i> = 57)	<i>P</i> value
Gender				
Male	37 (64.91)	39 (68.42)	40 (70.18)	0.8289
Female	20 (35.09)	18 (31.58)	17 (29.82)	
Age (mean ± SD) (yr)	55.9 ± 5.194	54.88 ± 9.907	54.3 ± 6.34	0.5087
Child Pugh classification				
A	10 (17.54)	15 (26.32)	-	0.527
B	18 (31.58)	16 (28.07)	-	
C	29 (50.88)	26 (45.61)	-	

HCC: Hepatocellular carcinoma.

Table 2 Imaging characteristics of hepatocellular carcinoma cases

Variables	<i>n</i> (%)
Size (mean ± SD) (range) cm	7.61 ± 3.037 (3.2-14)
Portal veins thrombosis	
Yes	10 (17.54)
No	47 (82.46)
No. of focal lesions	
Single	32 (56.14)
Multiple	25 (43.86)
Site of focal lesions	
Right lobe	32 (56.14)
Left lobe	17 (29.82)
Both lobes	8 (14.04)
Okuda stage	
I	7 (12.28)
II	23 (40.35)
III	27 (47.37)

mini kit (Qiagen, Valencia, CA, United States).

Quantitative real-time PCR

Quantification of Micro RNA was performed using Taq Man Gene Expression (Applied Biosystems Inc, Foster City, CA, United States). RNAU6 was used as house-keeping gene (endogenous reference cDNA) for all micro RNA in this study. Fractional threshold cycles (CT) were expressing the initial concentration of target sequence. Relative mRNA quantification was calculated using the arithmetic formula $2^{-\Delta CT}$, where ΔCT is the difference between the CT of a given target cDNA and an endogenous reference cDNA. Thus, this value yields the amount of the target normalized to an endogenous reference.

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 23 software (SPSS Inc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean, standard deviation and median. Comparison of continuous data between two groups was made by using unpaired *t* test for parametric data and Mann-Whitney test for non-parametric data. Comparison of continuous data between more than two groups was made by using one way ANOVA for parametric data and Kruskal-Wallis test for nonparametric data. χ^2 test was used for comparison between categorical data. Receiving

operating characteristic (ROC) analysis curves and the corresponding area under the curve were calculated for providing the accuracy of the microRNAs and AFP, in diagnosis of HCC. ROC curve was used for estimation of sensitivity (*i.e.*, true positive rate), specificity (*i.e.*, true negative rate), positive predictive value (PPV), negative predictive value (NPV) and cutoff values showing the best equilibrium between sensitivity and specificity were evaluated. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant).

RESULTS

The demographic data and Child-Pugh scoring of the studied groups are shown in Table 1. Symptoms were elicited by 85.96% of the HCC. Out of the recruited patients, 73.68%, 14.04% and 12.28% were HCV patients, HBV patients and non-HCV non-HBV respectively. Regarding Okuda staging system, 12.28% of HCC patients presented in stage I, 40.35% of HCC patients presented in stage II and 47.37% of HCC patients presented in stage III. Imaging showed that all HCC occurred on top of cirrhosis, ascites was present in 82.46% of the HCC patients and portal vein thrombosis was found in 17.54%. Focal lesions were single in 56.14% of cases, affected the right lobe in 56.14% of cases and their size ranged from 3.2 to 14 cm with a mean of 7.61 ± 3.037 as shown in Table 2. Comparison between all studied groups as regard liver functions tests, and other investigations are shown in Table 3.

Regarding miRNA values, the tested miR-23a, miR-203, miR-338, miR-34, and miR-16 showed a statistically significant difference between patients group I and II vs group III. It was found that 23a, 34 and 16 microRNAs were significantly higher in HCC group and cirrhotic group when compared with the control group; but 203 and 338 microRNAs were significantly lower in HCC group and cirrhotic group when compared with the control group. But only miRNA 23a showed statistically significant difference between group I and II, being higher in the HCC group than the cirrhotic group as shown in Table 4.

When miRNAs were studied according to the focal lesion characteristics, it was found that 23a microRNA

Table 3 Laboratory characteristics among the studied groups

Variable(s)	Group I	Group II	Group III	P	P1	P2	P3
	HCC group (n = 57) mean ± SD	Cirrhotic group (n = 57) mean ± SD	Control group (n = 57) mean ± SD				
ALT (U/L)	60.75 ± 32.63	59.04 ± 68.74	30.18 ± 5.48	< 0.0001	< 0.05	< 0.001	< 0.001
AST (U/L)	86.7 ± 35.1	66.77 ± 32.07	32.79 ± 7.2	< 0.0001	< 0.01	< 0.001	< 0.001
Total bilirubin (mg/dL)	4.48 ± 4.7	5.2 ± 5.59	0.77 ± 0.18	< 0.0001	> 0.05	< 0.001	< 0.001
Serum albumin (g/dL)	2.54 ± 0.38	2.72 ± 0.53	4.05 ± 0.47	< 0.0001	> 0.05	< 0.001	< 0.001
INR	1.48 ± 0.3	1.54 ± 0.72	0.99 ± 0.07	< 0.0001	> 0.05	< 0.001	< 0.001
Serum α-feto protein (ng/mL)	1418.55 ± 2953.2	41.61 ± 15.78	5.8 ± 1.65	< 0.0001	> 0.05	< 0.001	< 0.001
Serum creatinine (mg/dL)	2.2 ± 1.77	1.64 ± 1.23	0.95 ± 0.16	< 0.0001	> 0.05	< 0.001	< 0.001
Hemoglobin (g/dL)	9.72 ± 1.22	10.02 ± 0.89	12.62 ± 1.1	< 0.0001	> 0.05	< 0.001	< 0.001
Platelet (× 10 ⁹ /L)	98.33 ± 30.83	102.32 ± 33.24	220.93 ± 53.14	< 0.0001	> 0.05	< 0.001	< 0.001
Total leucocytic count (× 10 ⁹ /L)	3.17 ± 0.47	3.39 ± 0.50	6.83 ± 2	< 0.0001	> 0.05	< 0.001	< 0.001

Table 4 MicroRNAs levels among the studied groups

Variable(s)	Group I	Group II	Group III	P	P1	P2	P3
	HCC group (n = 57) Median (range)	Cirrhotic group (n = 57) Median (range)	Control group (n = 57) Median (range)				
MicroRNA 23a	2 ¹⁴ (2 ¹ -2 ¹⁸)	2 ¹¹ (2 ¹ -2 ¹⁸)	2 ⁶ (2 ⁰ -2 ¹⁹)	< 0.0001	< 0.05	< 0.001	< 0.001
MicroRNA 34	2 ¹⁴ (2 ² -2 ²⁰)	2 ¹¹ (2 ⁴ -2 ¹⁸)	2 ⁵ (2 ⁰ -2 ¹⁹)	< 0.0001	> 0.05	< 0.001	< 0.001
MicroRNA 203	2 ⁶ (2 ¹ -2 ¹⁹)	2 ⁶ (2 ⁰ -2 ¹⁹)	2 ¹⁴ (2 ¹ -2 ²⁵)	< 0.0001	> 0.05	< 0.001	< 0.001
MicroRNA 338	2 ⁶ (2 ¹ -2 ¹⁹)	2 ⁶ (2 ¹ -2 ¹⁹)	2 ¹² (2 ⁹ -2 ¹⁷)	< 0.0001	> 0.05	< 0.001	< 0.001
MicroRNA 16	2 ¹⁴ (2 ¹ -2 ²⁵)	2 ¹³ (2 ⁴ -2 ¹⁸)	2 ⁵ (2 ⁰ -2 ¹⁹)	< 0.0001	> 0.05	< 0.001	< 0.001

P1: Group I vs II; P2: Group I vs III; P3: Group II vs III.

Table 5 Comparison between microRNAs levels in relation to tumour characteristics, α-feto protein level, Okuda staging, and the etiology of liver cirrhosis

MicroRNA 16			MicroRNA 338			MicroRNA 203			MicroRNA 34			MicroRNA 23a			n	Variable(s)	
P-value	Range	Median	P-value	Range	Median	P-value	Range	Median	P-value	Range	Median	P-value	Range	Median			
0.3910	2 ⁴ -2 ¹⁷	2 ¹²	0.3890	2 ⁴ -2 ¹⁹	2 ⁷	0.2571	2 ² -2 ¹⁷	2 ⁴	0.0558	2 ⁴ -2 ¹⁵	2 ¹³	0.0008 ^a	2 ¹ -2 ¹³	2 ¹¹	11	Less than 5 cm	Size of focal lesions
	2 ¹ -2 ²⁵	2 ¹⁴		2 ¹ -2 ¹⁵	2 ⁵		2 ¹ -2 ¹⁹	2 ⁶		2 ² -2 ²⁰	2 ¹⁵		2 ³ -2 ¹⁸	2 ¹⁴	46	Equal or more 5 cm	
0.3026	2 ³ -2 ²¹	2 ¹⁴	0.5352	2 ¹ -2 ¹⁹	2 ⁶	0.5040	2 ² -2 ¹⁹	2 ⁶	0.8975	2 ⁴ -2 ²⁰	2 ¹⁴	0.0001 ^a	2 ¹ -2 ¹⁸	2 ¹¹	32	Single	No. of focal lesions
	2 ¹ -2 ²⁵	2 ¹⁴		2 ² -2 ¹⁵	2 ⁶		2 ¹ -2 ⁹	2 ⁶		2 ² -2 ¹⁹	2 ¹⁵		2 ¹⁴ -2 ¹⁸	2 ¹⁵	25	multiple	
0.9581	2 ² -2 ¹⁸	2 ¹⁴	0.9247	2 ² -2 ⁹	2 ⁶	0.7767	2 ³ -2 ⁹	2 ⁶	0.8255	2 ² -2 ¹⁹	2 ¹⁴	0.0795	2 ¹⁰ -2 ¹⁷	2 ¹⁵	10	Present	Portal vein thrombosis
	2 ¹ -2 ²⁵	2 ¹⁴		2 ¹ -2 ¹⁹	2 ⁶		2 ¹ -2 ¹⁹	2 ⁶		2 ² -2 ²⁰	2 ¹⁴		2 ¹ -2 ¹⁸	2 ¹³	47	Absent	
0.8881	2 ² -2 ²⁵	2 ¹⁴	0.8295	2 ¹ -2 ¹⁹	2 ⁶	0.0807	2 ¹ -2 ¹⁷	2 ⁷	0.3367	2 ² -2 ²⁰	2 ¹⁵	0.9736	2 ¹ -2 ¹⁸	2 ¹⁴	36	Less than 200 ng/mL	AFP level
	2 ¹ -2 ²¹	2 ¹⁴		2 ¹ -2 ¹⁵	2 ⁶		2 ¹ -2 ¹⁹	2 ⁵		2 ⁷ -2 ¹⁹	2 ¹⁴		2 ³ -2 ¹⁷	2 ¹⁴	21	Equal or more 200 ng/mL	
0.9347	2 ⁵ -2 ¹⁷	2 ¹⁴	0.1145	2 ⁴ -2 ⁹	2 ⁷	0.4488	2 ³ -2 ¹⁷	2 ⁶	0.4396	2 ⁴ -2 ¹⁵	2 ¹⁴	0.0001 ^a	2 ⁴ -2 ¹³	2 ¹¹	7	Stage I	Okuda stage
	2 ³ -2 ²¹	2 ¹⁴		2 ¹ -2 ¹⁹	2 ⁴		2 ² -2 ¹⁹	2 ⁷		2 ⁹ -2 ²⁰	2 ¹⁴		2 ¹ -2 ¹⁸	2 ¹¹	23	Stage II	
	2 ¹ -2 ²⁵	2 ¹⁴		2 ² -2 ¹⁵	2 ⁷		2 ¹ -2 ⁹	2 ⁵		2 ² -2 ¹⁹	2 ¹⁴		2 ¹¹ -2 ¹⁸	2 ¹⁵	27	Stage III	
0.1603	2 ³ -2 ²⁵	2 ¹⁴	0.4405	2 ¹ -2 ¹⁹	2 ⁶	0.4007	2 ¹ -2 ¹⁹	2 ⁷	0.7827	2 ⁴ -2 ²⁰	2 ¹⁴	0.5433	2 ¹ -2 ¹⁸	2 ¹⁴	42	HCV	Etiology of liver cirrhosis
	2 ¹ -2 ¹⁵	2 ¹¹		2 ¹ -2 ⁹	2 ⁶		2 ⁴ -2 ¹⁵	2 ⁵		2 ¹⁰ -2 ¹⁷	2 ¹⁴		2 ³ -2 ¹⁵	2 ¹⁴	8	HBV	
	2 ⁵ -2 ¹⁶	2 ¹⁴		2 ⁴ -2 ⁹	2 ⁷		2 ³ -2 ⁶	2 ⁴		2 ² -2 ¹⁹	2 ¹⁵		2 ¹⁰ -2 ¹⁷	2 ¹⁴	7	None	

^aThe first column is variable but is here in this copy the last column. HCV: Hepatitis C virus; HBV: Hepatitis B virus.

levels were higher in patients with focal lesion size equal to or more than 5 cm, in patients with multiple focal lesions; and in Okuda stage III as shown in Table 5.

At a cut off level of 200 ng/mL, serum AFP in the studied patients had 73.68% sensitivity, 52.63% specificity, 43.75% PPV, and 80% NPV for the diagnosis of HCC. At a cut off level of 2¹⁰ct, serum microRNA 23a had 89.47% sensitivity, 64.91%

specificity, 56.04% PPV, and 92.5% NPV for diagnosis of HCC as shown in Table 6 and Figures 1 and 2.

DISCUSSION

microRNA is differentially expressed in development of different types of malignancies, including hepatic malignancy^[13], which suggests that microRNAs may

Table 6 Sensitivity, specificity, positive prediction value, negative prediction value and accuracy of microRNAs and α -feto protein

Variable(s)	Cutoff value	Sensitivity%	Specificity%	Positive predictive value (PPV%)	Negative predictive value (NPV%)	Accuracy
MicroRNA 23a	$\geq 2^{10}$	89.47%	64.91%	56.04%	92.5%	79.3%
MicroRNA 34	$\geq 2^{10}$	89.47%	55.26%	50%	91.3%	79.3%
MicroRNA 203	$\geq 2^4$	80.7%	10.53%	31.08%	52.17%	29.6%
MicroRNA 338	$\geq 2^5$	63.16%	16.67%	27.48%	47.5%	26.4%
MicroRNA 16	$\geq 2^{10}$	87.72%	57.89%	51.02%	90.4%	75.7%
α -feto protein	≥ 200	73.68%	52.63%	43.75%	80%	78.5%

PPV: Positive predictive value; NPV: Negative predictive value.

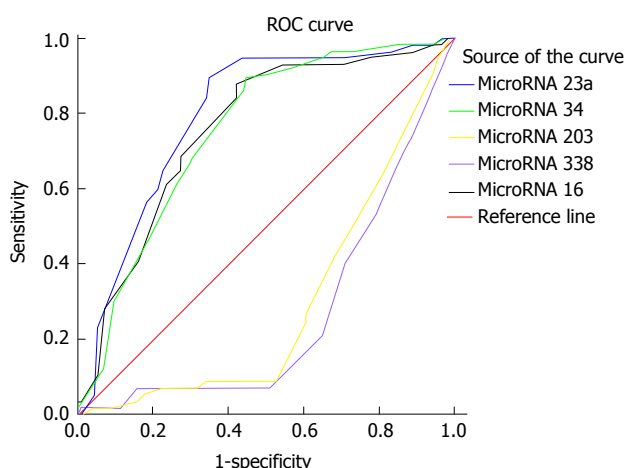


Figure 1 Receiving operating characteristic curve of microRNAs. ROC: Receiving operating characteristic.

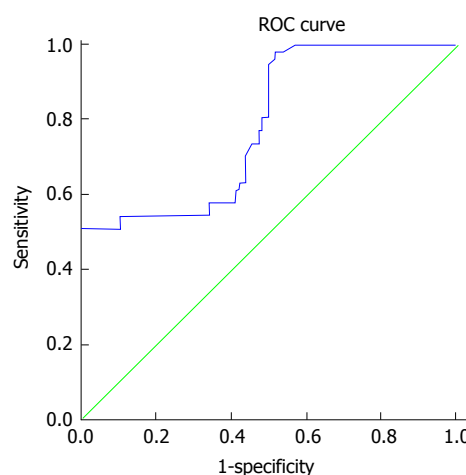


Figure 2 Receiving operating characteristic curve of α -feto protein. ROC: Receiving operating characteristic.

have a role in carcinogenesis as new oncogenes or tumor-suppressor genes. The presence of microRNAs in serum was first reported in 2008 in cases with large B cell lymphoma^[14]. They could be a potential biomarker for diagnosis of tumors^[15]. The present study was designed to evaluate the role of some microRNA (23a, 34, 203, 338 and 16) in early diagnosis of HCC. To fulfill this aim 57 HCC patients, 57 patients with liver cirrhosis and 57 healthy controls were enrolled.

In the present work, serum microRNA 23a level was significantly higher in the HCC group in comparison to other groups. Also, it is significantly higher in liver cirrhosis group in comparison to healthy controls. A similar result was obtained by Li *et al.*^[16]. They reported up regulation of microRNA 23a in HCC patients in comparison to liver cirrhosis patients and healthy control. This up-regulation in liver cirrhosis than healthy controls and further up-regulation in HCC patients in comparison to viral hepatitis patients suggests a role of microRNA 23a in the pathogenesis of HCC. A study on resected human HCC tissues found that microRNA-23a down-regulates the expression of interferon regulatory factor-1 in HCC cells^[17].

Serum microRNA 34 and microRNA 203 levels were similarly elevated in HCC and liver cirrhosis groups. Many authors found that microRNA 34 expression is increased in hepatic fibrosis^[18], HCV infection^[19], alcoholic liver disease^[20], NAFLD^[21,22] and HCC tissues^[23-25].

On the contrary, studies on microRNA 203 in HCC tissues found that microRNA 203 is down-regulated in HCC tissue^[26,27]. Moreover, studies correlate this down-regulation with recurrence of HCC in liver transplantation^[26] and bad prognosis in HCC patients^[27]. None of these studies was done on serum; they assessed tissue level of HCC in resected HCC tissues.

Serum microRNA 338 and microRNA 16 levels are similarly reduced in HCC patients and liver cirrhosis patients. In line with these findings, many studies concluded reduced tissue expression of miR-338-3p in different types of cancers^[28-30]. Studies on HCC showed that miR-338-3p/miR-338-3p was significantly down-regulated in HCC tissues and cell lines compared to the corresponding matched adjacent normal tissues^[31,32].

On the contrary, other researchers found that circulating microRNA 338 level increased in HCC patients than liver cirrhosis and controls^[33] but their study has a limitation of small sample size (37 HCC patients, 29 cirrhosis patients, and 31 healthy controls).

Serum microRNA 23a level at cutoff value $\geq 2^{10}$ showed accuracy of 79.3% to differentiate HCC patients from cirrhotic patients and healthy control with high sensitivity about 90%, specificity about 65%, PPV 56% and NPV 92.9. These values are better than those elicited by alpha fetoprotein. The later at a cut off level of 200 ng/mL, had 73.68% sensitivity, 52.63% specificity, 43.75% PPV, and 80% NPV for

the diagnosis of HCC. So, serum microRNA 23a can be used as a screening test to diagnose HCC as it showed high sensitivity. To the best of our knowledge no previous studies elicited such finding. A single study that used combination of 13 microRNA including 23a found that HCC on top of chronic HBV infection could be differentiated from chronic HCV infection and healthy control^[16]. MicroRNA 23a levels were significantly higher in patients with focal lesion 5 cm or more in size, patients with multiple focal lesions; and Okuda stage III when compared with patients with less advanced HCC disease. Thus it could be used as a prognostic biomarker.

Other studied microRNA factors showed insignificant difference between HCC and liver cirrhosis patients, so they cannot be used as diagnostic markers of HCC. In conclusion, microRNA 23a can be used as a screening test for early detection of HCC. Also, it is related to larger size of tumour, late Okuda staging and multiple hepatic focal lesions, so it might be a prognostic biomarker. Validation study on large scale is needed to confirm these results.

COMMENTS

Background

MicroRNAs are small, non-coding RNAs that negatively regulate gene expression at the post transcriptional level including cell cycle, apoptosis and metastasis. Aberrant miRNA expression contributes to tumorigenesis and cancer progression. Number of studies have confirmed that miRNAs possess important regulatory roles in hepatocarcinogenesis and malignant transformation. Hepatocellular carcinoma (HCC) is characterized by significant morbidity and high mortality rates. Because of the difficulty of early clinical diagnosis, only 30%-40% of cases can undergo curative resection. Circulating miRNAs released from cancerous tissues are stable and readily available for clinical analysis and appear to show a novel perspective for cancer diagnosis.

Research frontiers

Many microRNAs have been studied as biomarkers for diagnosis of malignancies. Yet, role of microRNA in early diagnosis of HCC is not confirmed.

Innovations and breakthroughs

This work demonstrates that miR-23a can be used in screening of liver cancer and it gives better results than alpha fetoprotein. This work showed first demonstration that microRNA 23a could be used as a promising biomarker for HCC patients, even though large scale examination is required. This manuscript would provide important clues for the development of microRNA as biomarkers for HCC.

Applications

MicroRNA 23a can be used as a screening test for early detection of HCC. Also, it is related to larger size of tumour, late Okuda staging and multiple hepatic focal lesions, so it might be a prognostic biomarker. Validation study on large scale is needed to confirm these results.

Peer-review

This manuscript would provide important clues for the development of microRNA as biomarkers for HCC.

REFERENCES

1 Gong J, Zhang JP, Li B, Zeng C, You K, Chen MX, Yuan Y,

- Zhuang SM. MicroRNA-125b promotes apoptosis by regulating the expression of Mcl-1, Bcl-w and IL-6R. *Oncogene* 2013; **32**: 3071-3079 [PMID: 22824797 DOI: 10.1038/onc.2012.318]
- 2 Gong J, He XX, Tian A. Emerging role of microRNA in hepatocellular carcinoma (Review). *Oncol Lett* 2015; **9**: 1027-1033 [PMID: 25663852 DOI: 10.3892/ol.2014.2816]
- 3 Zhu Z, Zhang X, Wang G, Zheng H. Role of MicroRNAs in Hepatocellular Carcinoma. *Hepat Mon* 2014; **14**: e18672 [PMID: 25337143 DOI: 10.5812/hepatmon.18672]
- 4 Murakami Y, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, Shimotohno K. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene* 2006; **25**: 2537-2545 [PMID: 16331254 DOI: 10.1038/sj.onc.1209283]
- 5 Ji J, Yamashita T, Budhu A, Forgues M, Jia HL, Li C, Deng C, Wauthier E, Reid LM, Ye QH, Qin LX, Yang W, Wang HY, Tang ZY, Croce CM, Wang XW. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. *Hepatology* 2009; **50**: 472-480 [PMID: 19585654 DOI: 10.1002/hep.22989]
- 6 Qi J, Wang J, Katayama H, Sen S, Liu SM. Circulating microRNAs (cmRNAs) as novel potential biomarkers for hepatocellular carcinoma. *Neoplasia* 2013; **60**: 135-142 [PMID: 23259781]
- 7 World Health Organization. GLOBOCAN Estimated Cancer Incidence, Mortality and Prevalence Worldwide, 2012
- 8 Zhou J, Yu L, Gao X, Hu J, Wang J, Dai Z, Wang JF, Zhang Z, Lu S, Huang X, Wang Z, Qiu S, Wang X, Yang G, Sun H, Tang Z, Wu Y, Zhu H, Fan J. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 4781-4788 [PMID: 22105822 DOI: 10.1200/JCO.2011.38.2697]
- 9 Chang-Hao Tsao S, Behren A, Cebon J, Christophi C. The role of circulating microRNA in hepatocellular carcinoma. *Front Biosci (Landmark Ed)* 2015; **20**: 78-104 [PMID: 25553441]
- 10 Callegari E, Elamin BK, Sabbioni S, Gramantieri L, Negrini M. Role of microRNAs in hepatocellular carcinoma: a clinical perspective. *Onco Targets Ther* 2013; **6**: 1167-1178 [PMID: 24039437 DOI: 10.2147/OTT.S36161]
- 11 Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649 [PMID: 4541913]
- 12 Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, Nakajima Y, Ohnishi K. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. *Cancer* 1985; **56**: 918-928 [PMID: 2990661]
- 13 Ventura A, Jacks T. MicroRNAs and cancer: short RNAs go a long way. *Cell* 2009; **136**: 586-591 [PMID: 19239879 DOI: 10.1016/j.cell.2009.02.005]
- 14 Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultonwood J, Wainscoat JS, Hatton CS, Harris AL. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol* 2008; **141**: 672-675 [PMID: 18318758 DOI: 10.1111/j.1365-2141.2008.07077.x]
- 15 Huang FY, Wong DK, Seto WK, Lai CL, Yuen MF. Estradiol induces apoptosis via activation of miRNA-23a and p53: implication for gender difference in liver cancer development. *Oncotarget* 2015; **6**: 34941-34952 [PMID: 26439986 DOI: 10.18632/oncotarget.5472]
- 16 Li LM, Hu ZB, Zhou ZX, Chen X, Liu FY, Zhang JF, Shen HB, Zhang CY, Zen K. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res* 2010; **70**: 9798-9807 [PMID: 21098710 DOI: 10.1158/0008-5472.CAN-10-1001]
- 17 Yan Y, Liang Z, Du Q, Yang M, Geller DA. MicroRNA-23a downregulates the expression of interferon regulatory factor-1 in hepatocellular carcinoma cells. *Oncol Rep* 2016; **36**: 633-640 [PMID: 27279136 DOI: 10.3892/or.2016.4864]
- 18 Li WQ, Chen C, Xu MD, Guo J, Li YM, Xia QM, Liu HM, He J, Yu HY, Zhu L. The rno-miR-34 family is upregulated and targets ACSL1 in dimethylnitrosamine-induced hepatic fibrosis in rats.

- FEBS J* 2011; **278**: 1522-1532 [PMID: 21366874 DOI: 10.1111/j.1742-4658.2011.08075.x]
- 19 **Cermelli S**, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. *PLoS One* 2011; **6**: e23937 [PMID: 21886843 DOI: 10.1371/journal.pone.0023937]
 - 20 **Meng F**, Glaser SS, Francis H, Yang F, Han Y, Stokes A, Staloch D, McCarra J, Liu J, Venter J, Zhao H, Liu X, Francis T, Swendsen S, Liu CG, Tsukamoto H, Alpini G. Epigenetic regulation of miR-34a expression in alcoholic liver injury. *Am J Pathol* 2012; **181**: 804-817 [PMID: 22841474 DOI: 10.1016/j.ajpath.2012.06.010]
 - 21 **Cheung O**, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, Kellum JM, Min H, Luketic VA, Sanyal AJ. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. *Hepatology* 2008; **48**: 1810-1820 [PMID: 19030170 DOI: 10.1002/hep.22569]
 - 22 **Yamada H**, Suzuki K, Ichino N, Ando Y, Sawada A, Osakabe K, Sugimoto K, Ohashi K, Teradaira R, Inoue T, Hamajima N, Hashimoto S. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. *Clin Chim Acta* 2013; **424**: 99-103 [PMID: 23727030 DOI: 10.1016/j.cca.2013.05.021]
 - 23 **Bernardi C**, Soffientini U, Piacente F, Tonetti MG. Effects of microRNAs on fucosyltransferase 8 (FUT8) expression in hepatocarcinoma cells. *PLoS One* 2013; **8**: e76540 [PMID: 24130780 DOI: 10.1371/journal.pone.0076540]
 - 24 **Chiu LY**, Kishnani PS, Chuang TP, Tang CY, Liu CY, Bali D, Koeberl D, Austin S, Boyette K, Weinstein DA, Murphy E, Yao A, Chen YT, Li LH. Identification of differentially expressed microRNAs in human hepatocellular adenoma associated with type I glycogen storage disease: a potential utility as biomarkers. *J Gastroenterol* 2014; **49**: 1274-1284 [PMID: 24129885 DOI: 10.1007/s00535-013-0890-2]
 - 25 **Dang Y**, Luo D, Rong M, Chen G. Underexpression of miR-34a in hepatocellular carcinoma and its contribution towards enhancement of proliferating inhibitory effects of agents targeting c-MET. *PLoS One* 2013; **8**: e61054 [PMID: 23593387 DOI: 10.1371/journal.pone.0061054]
 - 26 **Chen HY**, Han ZB, Fan JW, Xia J, Wu JY, Qiu GQ, Tang HM, Peng ZH. miR-203 expression predicts outcome after liver transplantation for hepatocellular carcinoma in cirrhotic liver. *Med Oncol* 2012; **29**: 1859-1865 [PMID: 21786180 DOI: 10.1007/s12032-011-0031-9]
 - 27 **Liu Y**, Ren F, Rong M, Luo Y, Dang Y, Chen G. Association between underexpression of microRNA-203 and clinicopathological significance in hepatocellular carcinoma tissues. *Cancer Cell Int* 2015; **15**: 62 [PMID: 26109910 DOI: 10.1186/s12935-015-0214-0]
 - 28 **Guo B**, Liu L, Yao J, Ma R, Chang D, Li Z, Song T, Huang C. miR-338-3p suppresses gastric cancer progression through a PTEN-AKT axis by targeting P-REX2a. *Mol Cancer Res* 2014; **12**: 313-321 [PMID: 24375644 DOI: 10.1158/1541-7786.MCR-13-0507]
 - 29 **Li P**, Chen X, Su L, Li C, Zhi Q, Yu B, Sheng H, Wang J, Feng R, Cai Q, Li J, Yu Y, Yan M, Liu B, Zhu Z. Epigenetic silencing of miR-338-3p contributes to tumorigenicity in gastric cancer by targeting SSX2IP. *PLoS One* 2013; **8**: e66782 [PMID: 23826132 DOI: 10.1371/journal.pone.0066782]
 - 30 **Chen X**, Pan M, Han L, Lu H, Hao X, Dong Q. miR-338-3p suppresses neuroblastoma proliferation, invasion and migration through targeting PREX2a. *FEBS Lett* 2013; **587**: 3729-3737 [PMID: 24140344 DOI: 10.1016/j.febslet.2013.09.044]
 - 31 **Wang G**, Sun Y, He Y, Ji C, Hu B, Sun Y. MicroRNA-338-3p inhibits cell proliferation in hepatocellular carcinoma by target forkhead box P4 (FOXP4). *Int J Clin Exp Pathol* 2015; **8**: 337-344 [PMID: 25755720]
 - 32 **Chen JS**, Liang LL, Xu HX, Chen F, Shen SL, Chen W, Chen LZ, Su Q, Zhang LJ, Bi J, Zeng WT, Li W, Ma N, Huang XH. miR-338-3p inhibits epithelial-mesenchymal transition and metastasis in hepatocellular carcinoma cells. *Oncotarget* 2016; Epub ahead of print [PMID: 27331410 DOI: 10.18632/oncotarget.10138]
 - 33 **Chen Y**, Chen J, Liu Y, Li S, Huang P. Plasma miR-15b-5p, miR-338-5p, and miR-764 as Biomarkers for Hepatocellular Carcinoma. *Med Sci Monit* 2015; **21**: 1864-1871 [PMID: 26119771 DOI: 10.12659/MSM.893082]

P- Reviewer: Yu DY **S- Editor:** Ji FF **L- Editor:** A
E- Editor: Li D



Association of autoimmune hepatitis type 1 in a child with Evans syndrome

Chaowapong Jarasvaraparn, Hamayun Imran, Abdul Siddiqui, Felicia Wilson, David A Gremse

Chaowapong Jarasvaraparn, Hamayun Imran, Abdul Siddiqui, Felicia Wilson, Department of Pediatrics, University of South Alabama, Mobile, AL 36604, United States

David A Gremse, Division of Pediatric Gastroenterology, Hepatology and Nutrition, University of South Alabama, Mobile, AL 36604, United States

Author contributions: All authors contributed to the acquisition of data, writing, and revision of this manuscript.

Institutional review board statement: This case report was exempt from the Institutional Review Board standards at University of South Alabama.

Informed consent statement: Our patient's legal guardian provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors have no conflicts of interest to disclose.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: David A Gremse, MD, FAAP, FACG, Department of Pediatrics, University of South Alabama, 1601 Center St, Mobile, AL 36604, United States. dgregm@health.southalabama.edu
Telephone: +1-251-4343919

Received: February 10, 2017

Peer-review started: February 15, 2017

First decision: April 17, 2017

Revised: June 27, 2017

Accepted: July 7, 2017

Article in press: July 10, 2017

Published online: August 18, 2017

Abstract

Autoimmune hepatitis (AIH) is a progressive liver disease that is often associated with extrahepatic autoimmune disorders. Evans syndrome (ES) is a rare autoimmune disorder, which is characterized by immune thrombocytopenia and autoimmune hemolytic anemia. Association of AIH with ES is rare, especially in children. We report a 3-year-old female with a past medical history of ES who presented with jaundice and significant transaminitis due to AIH type 1. She required multiple treatments with steroids as well as azathioprine, intravenous immunoglobulin and a course of rituximab.

Key words: Evans syndrome; Autoimmune hepatitis type 1; Child

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: We report a 3-year-old female with a past medical history of Evans syndrome (ES) who presented with jaundice and significant transaminitis due to autoimmune hepatitis (AIH) type 1. To our knowledge, this is a rare association of concurrent AIH and ES in a child who responded well to rituximab. The patient also demonstrated short-term response to intravenous immunoglobulin, methylprednisolone, azathioprine and oral prednisone. We conclude that ES may evolve over a period of several months therefore evaluation for associated autoimmune conditions should be considered in these patients.

Jarasvaraparn C, Imran H, Siddiqui A, Wilson F, Gremse DA. Association of autoimmune hepatitis type 1 in a child with Evans

syndrome. *World J Hepatol* 2017; 9(23): 1008-1012 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v9/i23/1008.htm> DOI: <http://dx.doi.org/10.4254/wjh.v9.i23.1008>

INTRODUCTION

Autoimmune hepatitis (AIH) is characterized by chronic necroinflammatory liver disease of unknown cause, circulating non organ-specific autoantibodies and increased levels of immunoglobulin G. The epidemiology of pediatric AIH is unknown. Most patients are diagnosed before the age of 18 years and 75% are girls- the peak incidence being prior to puberty. Currently two types of AIH are recognized according to seropositivity for smooth muscle and/or anti-nuclear antibody for AIH type 1 or liver kidney microsomal antibody and/or to a liver cytosol antigen for AIH type 2. AIH type 1 accounts for two-thirds of the cases and presents usually during adolescence, whereas AIH type 2 presents at a younger age especially during infancy^[1]. Liver biopsies remain essential for diagnosis and evaluation of disease severity in patients with AIH. In children, AIH often presents acutely and has a more aggressive course than in adults^[2]. If left untreated, it generally progresses rapidly to cirrhosis and liver failure.

Evans syndrome (ES) is a rare autoimmune disease, which is characterized by immune thrombocytopenia (ITP) and autoimmune hemolytic anemia (AIHA). Both diseases are mediated by autoantibodies, though in some cases it is considered a T-lymphocyte disorder. It was first described in 1951. The incidence of ES in children has not been calculated^[3]. ES has a chronic and relapsing course, and patients usually depend on prolonged immunosuppressive treatments. ES is more difficult to treat and has a higher mortality than AIHA alone^[4]. In approximately half of those diagnosed with ES, no other immune disorder is recognized but in rest of the patients it may be a manifestation of systemic lupus erythematosus, common variable immune deficiency^[5], autoimmune lymphoproliferative disorder^[6] or another immune disorders^[7]. The first line of treatment is intravenous immunoglobulin or steroids. The second-line immunosuppressive therapies are rituximab, azathioprine, cyclosporine and mycophenolate mofetil. ES in children should be considered a severe disease because the risk of life threatening hemorrhage is greater than in classic ITP^[3].

AIH cases have been reported concomitantly with extrahepatic immune disorders such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, chronic thyroiditis, ulcerative colitis, celiac disease, connective tissue disorder, proliferative glomerulonephritis, Myasthenia Gravis or ITP. Association with ES is rare, especially in children. We report a child with past medical history of ES who presented with jaundice and significant transaminitis due to AIH

type 1.

CASE REPORT

A previously healthy two-year-old African American female presented with a two-month history of epistaxis and easy bruising. She was admitted to the University of South Alabama Children's and Women's hospital in May 2015. Examination was remarkable for few healing bruises without hepatosplenomegaly. Laboratory tests (Table 1) showed hemoglobin of 8.4 g/dL, mean corpuscular volume of 75 fL, white blood cell count of 14800/ μ L, platelet count of 61000/ μ L and reticulocyte count of 8%. Her aspartate aminotransferase (AST) was 387 IU/L and alanine aminotransferase was 449 IU/L. Coagulation studies were normal and the viral panels including anti-HAV-IgM, HBsAg, anti-HBc, anti-HCV, CMV-IgM, EBV-VCA-IgG, EBV-VCA-IgM, EB early Ag, EBnA, Parvovirus, and HSV-IgM were negative. Further laboratory evaluations yielded a negative anti-nuclear antibody, positive antiplatelet antibody and direct Coomb's test positive for both IgG and anti-complement factor 3 antibody. Thus, a diagnosis of ES was made. After treatment with single 1 g/kg dose of intravenous immunoglobulin (IVIg) followed by oral prednisone at 2 mg/kg per day, her hemoglobin improved from 8.4 to 10.9 g/dL and corticosteroids were discontinued but she was lost to follow-up over-time. She was hospitalized a few times for intravenous antibiotics due to a bacterial pneumonia and acute bacterial sinusitis. Her ES remained stable during this time. Immune work up showed normal immunoglobulin levels. (Immunoglobulin G 1.090 mg/dL, Immunoglobulin A 114 mg/dL, Immunoglobulin M 86 mg/dL and Immunoglobulin E 148 kU/L), normal absolute lymphocyte counts and sub-set population (including CD3, CD4, CD8, CD56, no double negative T cells) *via* flow cytometry without evidence of autoimmune lymphoproliferative disorder.

One year later, she developed jaundice and pruritus, hepatomegaly with a liver span of 13-cm and increased echogenicity without gallstones on abdominal ultrasound. Her laboratory findings included AST 547 IU/L, alanine transaminase (ALT) 600 IU/L, albumin 2.6 g/dL, total protein 7.9 g/dL, total bilirubin 10.2 mg/dL and direct bilirubin 8.8 mg/dL, prothrombin time (PT) 13.5 s, partial thromboplastin time (aPTT) 31 s, International Normalized Ratio (INR) 1.02, positive anti-nuclear antibody (1:40), positive smooth muscle antibody (1:40), positive F actin antibody (39 units) and elevated total serum IgG (1090 mg/dL). The anti-liver-kidney-microsome antibody, anti-HAV-IgM, HBsAg, anti-HBc and anti-HCV were all negative. The serum alpha-1-antitrypsin and ceruloplasmin concentrations were normal. Prior to percutaneous liver biopsy, she received packed red blood cell (for associated AIHA flare with Hb 4.9 g/dL and reticulocyte count 44%) and fresh frozen plasma. Her pre-biopsy hemoglobin was

Table 1 Laboratory tests during the disease course

Laboratory tests	0 mo	12 mo	13 mo	14 mo	16 mo
Hemoglobin (g/dL)	8.4	4.9	9.6	9.8	14
Reticulocyte count (%)	8	44	34.2	32	4.7
Platelet (cells/ μ L)	61000	187000	303000	327000	502000
Albumin (g/dL)	2.7	2.6	3.5	3.7	4.1
Aspartate aminotransferase (IU/L)	387	547	45	49	87
Alanine transaminase (IU/L)	449	600	51	188	104
Total bilirubin (mg/dL)	0.8	10.2	1.3	0.5	0.4

0 mo: Diagnosis of Evans syndrome; 12 mo: Diagnosis of autoimmune hepatitis; 13 mo: One month after treatment of methylprednisolone and oral prednisolone; 14 mo: Prior to rituximab; 16 mo: Present.

11.5 g/dL with platelet count 101000 /m μ L, PT 10.9 s, INR 1.0, and aPTT 31 s. She received high doses of intravenous methylprednisolone (30 mg/kg per day for 3 d) and oral ursodiol after percutaneously liver biopsy due to suspected AIH type 1. She was discharged with oral prednisone therapy after liver biopsy. Before discharge, her AST was 677 IU/L and ALT 1094 IU/L.

Liver biopsy revealed interface hepatitis with a mixed inflammatory infiltrate including lymphoid cells, eosinophils, neutrophils, histiocytic cells and plasma cells in addition to periportal fibrosis with rare portal-portal septa (stage 2 fibrosis) along with canalicular and hepatocytic cholestasis, indicating AIH. One month later after a high dose of methylprednisolone and oral prednisone, her AST improved to 194 IU/L and ALT to 424 IU/L. Shortly after, she was started on oral azathioprine at a dose of 1.5 mg/kg per day. Currently (4 mo after diagnosis of AIH), her AIH is controlled very well with oral azathioprine and oral prednisone, her present AST is 87 IU/L and ALT is 104 IU/L.

During her hospitalization for AIH, she also had a flare up of ES, with a drop in hemoglobin to 4.9 g/dL and elevated reticulocyte count up to 44% but stable normal platelet counts. She eventually received intravenous rituximab 375 mg/m² every week as an outpatient for four doses and she is currently on a replacement IVIg course once a month for six months. Her present labs show hemoglobin of 14 g/dL, reticulocyte count of 4.7% and a normal white blood cells and platelets count. She has not been hospitalized since starting rituximab and IVIg for 7 mo.

DISCUSSION

This report describes an unusual case of ES and AIH type 1 in a child. The diagnosis of ES preceded that of AIH for over a year. Patients with ES have a relapse rate of 74%, with a median delay of eight months (41 d to 9.5 years). Among those, 52% relapse with ITP and AIHA, 40% with ITP alone and 8% with AIHA alone^[3]. In a French study ES was found to be secondary to an underlying disease in 10% of patients. No secondary disease was diagnosed over the entire course of study in 30% of children^[3]. In addition, 60% of patients with ES demonstrated other associated immune manifestations

such as autoimmunity and lymphoproliferation. This suggests that ES occurs within the context of a poorly understood autoimmune dysfunction^[3]. Tokgoz *et al*^[8] published a case report of a 12-year-old female presenting with ES, AIH and nephrotic syndrome. She differed from our patient by having lymphopenia, leukopenia, low IgA, IgG and IgM levels; low CD3, CD4, CD8 and low TCR alpha/beta expression. Finally, she was diagnosed with CD3 γ (gamma) deficiency. CD3 chain deficiency is a heterogeneous group of immunodeficiencies responsible for a small proportion of Severe Combine Immune Deficiency (SCID). Our patient had a history of recurrent infections but her immunoglobulin levels were not low, CD3, CD4 and CD8 were also unremarkable. Flow cytometry also showed no evidence of autoimmune lymphoproliferative disorder. Therefore, our patient demonstrated AIH and ES without evidence of CD3 γ deficiency.

Patients with ES are difficult to manage. Although ES may initially respond well to corticosteroids, it usually runs a chronic course with intermittent exacerbations. Interestingly, the effectiveness of rituximab for adults in ES has been established in a number of cases^[9,10]. The effects of a weekly infusion with rituximab for four weeks would be effective for up to one year^[11]. Experience with the use of Rituximab for treatment of concurrent ES and AIH is limited, especially in children. Carey *et al*^[11] reported successful treatment of refractory AIH and ES with rituximab in an adult. Rituximab has been explored in children for a number of hematologic conditions including treatment of AIHA, ITP, factors VIII and IX inhibitors in patients with hemophilia, post-transplant lymphoproliferative disease, Burkitt's lymphoma and so on. It is overall well tolerated except for occasional symptoms of chills, fever, headache, occasional dyspnea, nausea, pruritus, angioedema, and/or hypotension^[12].

Lastly, long-term treatment of pediatric AIH is usually required, with roughly 20% of AIH type 1 patients able to discontinue therapy successfully^[11]. Interestingly, our case had elevated levels of immunoglobulin G during diagnosis of AIH type 1. Immunoglobulin G is usually raised at presentation in both types of AIH, although 15% of AIH types 1 and 25% of AIH type 2 have normal levels^[13].

To our knowledge, this case report is a rare concurrent association of AIH and ES in a child who responded well to rituximab. The patient also demonstrated short-term response to IVIg, methylprednisolone, azathioprine and oral prednisone. We conclude that ES may evolve over a period of several months therefore evaluation for associated autoimmune conditions should be considered periodically in these patients. Most of the published literature consists of either case reports or small case series. International collaboration is essential in order to better understand the association and treatment of ES and AIH in children and adults.

COMMENTS

Case characteristics

A 2-year-old African American female with past medical history of Evans syndrome (ES) presented with jaundice and significant transaminitis.

Clinical diagnosis

A two-month history of epistaxis and easy bruising at diagnosis of ES and one year later she developed jaundice, pruritus, and hepatomegaly.

Differential diagnosis

Viral hepatitis, cholelithiasis, alpha-1-antitrypsin deficiency, Wilson's disease, glycogen storage disease or congenital hepatic fibrosis.

Laboratory diagnosis

Aspartate aminotransferase 547 IU/L, alanine transaminase 600 IU/L, albumin 2.6 g/dL, total protein 7.9 g/dL, total bilirubin 10.2 mg/dL and direct bilirubin 8.8 mg/dL, prothrombin time 13.5 s, partial thromboplastin time 31 s, International Normalized Ratio 1.02, positive anti-nuclear antibody (1:40), positive smooth muscle antibody (1:40), positive F actin antibody (39 units) and elevated total serum IgG (1090 mg/dL). The anti-liver-kidney-microsome antibody, anti-HAV-IgM, HBsAg, anti-HBc and anti-HCV were all negative. The serum alpha-1-antitrypsin and ceruloplasmin concentrations were normal.

Imaging diagnosis

Abdominal ultrasound showed a liver span of 13-cm and increased echogenicity without gallstones.

Pathological diagnosis

Liver biopsy revealed interface hepatitis with a mixed inflammatory infiltrate including lymphoid cells, eosinophils, neutrophils, histiocytic cells and plasma cells in addition to periportal fibrosis with rare portal-portal septa (stage 2 fibrosis) indicating autoimmune hepatitis (AIH).

Treatment

High doses of methylprednisolone (30 mg/kg per day for 3 d) and then oral prednisone, oral ursodiol, oral azathioprine, intravenous immunoglobulin and intravenous rituximab.

Related reports

AIH is characterized by chronic necroinflammatory liver disease of unknown cause, circulating non organ-specific autoantibodies and increased levels of immunoglobulin G. AIH cases have been reported concomitantly with extrahepatic immune disorders. Association with ES is rare, especially in children.

Term explanation

ES may evolve over a period of several months therefore evaluation for associated autoimmune conditions should be considered periodically even if negative initially, especially AIH.

Experiences and lessons

This is the rare case report of concurrent AIH and ES in a child who responded well to rituximab. The patient also demonstrated short-term response to intravenous immunoglobulin, methylprednisolone, azathioprine and oral prednisone. International collaboration is essential in order to better understand the association and treatment of ES and AIH in children and adults.

Peer-review

Authors report an interesting case of 3-year-old child with ES associated with type 1 AIH.

REFERENCES

- 1 **Mieli-Vergani G**, Heller S, Jara P, Vergani D, Chang MH, Fujisawa T, González-Peralta RP, Kelly D, Mohan N, Shah U, Murray KF. Autoimmune hepatitis. *J Pediatr Gastroenterol Nutr* 2009; **49**: 158-164 [PMID: 19561543 DOI: 10.1097/MPG.0b013e3181a1c265]
- 2 **Mieli-Vergani G**, Vergani D. Autoimmune paediatric liver disease. *World J Gastroenterol* 2008; **14**: 3360-3367 [PMID: 18528933 DOI: 10.3748/wjg.14.3360]
- 3 **Aladjidi N**, Fernandes H, Leblanc T, Vareliette A, Rieux-Laucat F, Bertrand Y, Chambost H, Pasquet M, Mazingue F, Guitton C, Pellier I, Roqueplan-Bellmann F, Armari-Alla C, Thomas C, Marie-Cardine A, Lejars O, Fouyssac F, Bayart S, Lutz P, Piguat C, Jeziorski E, Rohrlach P, Lemoine P, Bodet D, Paillard C, Couillaud G, Millot F, Fischer A, Pélér Y, Leverger G. Evans Syndrome in Children: Long-Term Outcome in a Prospective French National Observational Cohort. *Front Pediatr* 2015; **3**: 79 [PMID: 26484337 DOI: 10.3389/fped.2015.00079]
- 4 **Norton A**, Roberts I. Management of Evans syndrome. *Br J Haematol* 2006; **132**: 125-137 [PMID: 16398647 DOI: 10.1111/j.1365-2141.2005.05809.x]
- 5 **Savaşan S**, Warrier I, Buck S, Kaplan J, Ravindranath Y. Increased lymphocyte Fas expression and high incidence of common variable immunodeficiency disorder in childhood Evans' syndrome. *Clin Immunol* 2007; **125**: 224-229 [PMID: 17936685 DOI: 10.1016/j.jclim.2007.08.010]
- 6 **Seif AE**, Manno CS, Sheen C, Grupp SA, Teachey DT. Identifying autoimmune lymphoproliferative syndrome in children with Evans syndrome: a multi-institutional study. *Blood* 2010; **115**: 2142-2145 [PMID: 20068224 DOI: 10.1182/blood-2009-08-239525]
- 7 **Stepensky P**, Rensing-Ehl A, Gather R, Revel-Vilk S, Fischer U, Nabhani S, Beier F, Brümendorf TH, Fuchs S, Zenke S, Firat E, Pessach VM, Borkhardt A, Rakhmanov M, Keller B, Warnatz K, Eibel H, Niedermann G, Elpeleg O, Ehl S. Early-onset Evans syndrome, immunodeficiency, and premature immunosenescence associated with tripeptidyl-peptidase II deficiency. *Blood* 2015; **125**: 753-761 [PMID: 25414442 DOI: 10.1182/blood-2014-08-593202]
- 8 **Tokgoz H**, Caliskan U, Keles S, Reisli I, Guin IS, Morgan NV. Variable presentation of primary immune deficiency: two cases with CD3 gamma deficiency presenting with only autoimmunity. *Pediatr Allergy Immunol* 2013; **24**: 257-262 [PMID: 23590417 DOI: 10.1111/pai.12063]
- 9 **Koulova L**, Alexandrescu D, Dutcher JP, O'Boyle KP, Eapen S, Wiernik PH. Rituximab for the treatment of refractory idiopathic thrombocytopenic purpura (ITP) and thrombotic thrombocytopenic purpura (TTP): report of three cases. *Am J Hematol* 2005; **78**: 49-54 [PMID: 15609292 DOI: 10.1002/ajh.20243]
- 10 **Rückert A**, Glimm H, Lübbert M, Grüllich C. Successful treatment of life-threatening Evans syndrome due to antiphospholipid antibody syndrome by rituximab-based regimen: a case with long-term follow-up. *Lupus* 2008; **17**: 757-760 [PMID: 18625656 DOI: 10.1177/0961203307087876]
- 11 **Carey EJ**, Somaratne K, Rakela J. Successful rituximab therapy in refractory autoimmune hepatitis and Evans syndrome. *Rev Med Chil* 2011; **139**: 1484-1487 [PMID: 22446656 DOI: 10.4067/

S0034-98872011001100015]

- 12 **Giulino LB**, Busnel JB, Neufeld EJ; Pediatric and Platelet Immunology Committees of the TMH Clinical Trial Network. Treatment with rituximab in benign and malignant hematologic disorders in children. *J Pediatr* 2007; **150**: 338-344, 344.e1 [PMID:

17382107 DOI: 10.1016/j.jpeds.2006.12.038]

- 13 **Gregorio GV**, Portmann B, Reid F, Donaldson PT, Doherty DG, McCartney M, Mowat AP, Vergani D, Mieli-Vergani G. Auto-immune hepatitis in childhood: a 20-year experience. *Hepatology* 1997; **25**: 541-547 [PMID: 9049195 DOI: 10.1002/hep.510250308]

P- Reviewer: Carbone M, He ST, Shi Z **S- Editor:** Ji FF
L- Editor: A **E- Editor:** Li D





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

