ISSN 2218-6204 (online)

World Journal of *Hematology*

World J Hematol 2014 May 6; 3(2): 18-48

Baishideng Publishing Group
www.wjgnet.com

WJH

World Journal of Hematology

A peer-reviewed, online, open-access journal of hematology

Editorial Board

2012-2016

The *World Journal of Hematology* Editorial Board consists of 123 members, representing a team of worldwide experts in hematology. They are from 29 countries, including Argentina (2), Austria (1), Belgium (1), Brazil (1), Canada (1), China (4), Croatian (1), Denmark (1), France (6), Germany (4), Greece (4), India (1), Iran (1), Ireland (1), Israel (2), Italy (11), Japan (9), Luxembourg (1), Mexico (1), Netherlands (7), Norway (2), Romania (1), Singapore (1), South Korea (2), Spain (6), Thailand (1), Turkey (5), United Kingdom (9), and United States (36).

EDITORS-IN-CHIEF

Xiaoyan Jiang, *Vancouver*
Thomas J Kipps, *San Diego*

GUEST EDITORIAL BOARD MEMBERS

Hwei-Fang Tien, *Taipei*

MEMBERS OF THE EDITORIAL BOARD

Argentina

Ricardo Forastiero, *Buenos Aires*
Mirta A Schattner, *Buenos Aires*

Austria

Richard H Moriggl, *Vienna*

Belgium

Xavier Sagaert, *Leuven*

Brazil

Constantino José Fernandes Jr, *São Paulo*

China

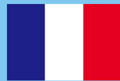
Anskar YH Leung, *Hong Kong*
Raymond HS Liang, *Hong Kong*
Xiao-Yu Tian, *Hong Kong*

Croatian

Mariastefania Antica, *Zagreb*

Denmark

Erik Lerkevang Grove, *Aarhus*


France

Emmanuel Andres, *Strasbourg*
Claude Bagnis, *Marseille*
Bernard Binetruy, *Marseille*
Cyril Fauriat, *Marseille*
Florence Nguyen Khac, *Paris*
Xavier Georges Thomas, *Pierre Benite*

Germany

Arnold Ganser, *Hannover*
Dirk Matthias Hermann, *Essen*
Rory R Koenen, *Munich*
Zhixiong Li, *Hannover*

Greece

Anastasios G Kriebardis, *Athens*
Marie-Christine Kyrtsonis, *Athens*
Gerassimos A Pangalis, *Athens*
Issidora S Papassideri, *Athens*

India

Gurudutta U Gangenahalli, *Delhi*

Iran

Shahram Teimourian, *Tehran*

Ireland

Eva Szegezdi, *Galway*

Israel

Jacob George, *Rehovot*
Avichai Shimoni, *Tel-Hashomer*

Italy

Luca Arcaini, *Pavia*
Vincenzo Casolaro, *Baronissi*
Alessia Colosimo, *Teramo*
Raimondo De Cristofaro, *Rome*
Claudio Fozza, *Sassari*
Edoardo G Giannini, *Genova*
Giampiero La Rocca, *Palermo*
Pier Paolo Piccaluga, *Bologna*
Alessandro Poggi, *Genoa*
Sergio M Siragusa, *Palermo*
Elena Zocchi, *Genova*

Japan

Xian Wu Cheng, *Nagoya*
Seiji Fukuda, *Izumo*
Satoshi Hagiwara, *Yufu*
Shinsaku Imashuku, *Takasago*
Masanobu Kitagawa, *Tokyo*
Tetsuya Nosaka, *Tsu*

Masao Seto, *Nagoya*
Toshiki Shimizu, *Moriguchi*
Masafumi Takahashi, *Shimotsuke*

Luxembourg

Jacques Zimmer, *Luxembourg*

Mexico

Agustin Avilés, *Mexico City*

Netherlands

Miranda Buitenhuis, *Rotterdam*
Roland P Kuiper, *Nijmegen*
Jan Jacques Michiels, *Erasmus*
Gerry AF Nicolaes, *Maastricht*
Pieter Sonneveld, *Rotterdam*
Arnold Spek, *Amsterdam*
Ruurd Torensma, *Nijmegen*

Norway

Brynjar Foss, *Stavanger*
Mikhail Sovershaev, *Tromsø*

Romania

Adriana Georgescu, *Bucharest*

Singapore

Jerry Chan, *Singapore*

South Korea

Jung Weon Lee, *Seoul*
Myung-Geun Shin, *Gwangju*

Spain

Matilde Canelles, *Granada*
Slaven Erceg, *Seville*
Pedro Cosme Redondo Liberal, *Cáceres*
Julian Pardo, *Zaragoza*
Josep-Maria Ribera, *Badalona*
Juan M Zapata, *Madrid*

Thailand

Chirayu Udomsakdi Auewarakul, *Bangkok*

Turkey

Mutay Aslan, *Antalya*
Murat Biteker, *Istanbul*
Taner Demirer, *Ankara*
Selami Kocak Toprak, *Ankara*
Ertan Yetkin, *Mersin*

United Kingdom

Dominique Bonnet, *London*
Olga Tura Ceide, *Edinburgh*
Helen A Ireland, *London*
Charles Henderson Lawrie, *Oxford*
Drew Provan, *London*
Dipak Purshottam Ramji, *Cardiff*
Sabrina Tosi, *Uxbridge*
Shao-An Xue, *London*
Jian guo Zhuang, *Liverpool*

United States

Ivo Abraham, *Tucson*
Julia E Brittain, *Chapel Hill*
Chung-Che Chang, *Houston*
Edward Alan Copelan, *Cleveland*
Zeev Estrov, *Houston*
Steve Fiering, *Lebanon*
Suzanne T Ildstad, *Louisville*
Elias Jabbour, *Houston*
Ming Jiang, *Nashville*
Katsuhiro Kita, *Galveston*
Robert G Lerner, *Valhalla*
Shaoguang Li, *Worcester*
Dazhi Liu, *Sacramento*
Ming-Lin Liu, *Philadelphia*
Surya Nauli, *Toledo*
Steffan Nawrocki, *San Antonio*
Xuyang Peng, *Nashville*
Manuel L Penichet, *Los Angeles*
Luis Francisco Porrata, *Rochester*
Rehan Qayyum, *Baltimore*
L Vijaya Mohan Rao, *San Diego*
Guangwen Ren, *New Brunswick*
Xiaoping Ren, *Cincinnati*
Jatin J Shah, *Houston*
Angus M Sinclair, *Thousand Oaks*
Ali A Sovari, *Chicago*
Christopher A Tormey, *New Haven*
Olga Volpert, *Chicago*
Zack Zhengyu Wang, *Scarborough*
EllenLori Weisberg, *Boston*
Wen-Shu Wu, *Scarborough*
Yi Wu, *Newark*
Feng-Chun Yang, *Indiana*
Karina Yazdanbakhsh, *New York*
Xin-Fu Zhou, *Adelaide*

W J H

World Journal of
Hematology

Contents

Quarterly Volume 3 Number 2 May 6, 2014

EDITORIAL

18 *Ex vivo* expansion of hematopoietic stem and progenitor cells: Recent advances

Kita K, Xiu F, Jeschke MG

REVIEW

29 Anti-CD20 monoclonal antibodies and associated viral hepatitis in hematological diseases

Yang SH, Hsu C, Cheng AL, Kuo SH

CASE REPORT

44 Chronic disseminated candidiasis complicated with a ruptured intracranial fungal aneurysm in ALL

Okawa T, Ono T, Endo A, Takagi M, Nagasawa M

Contents

APPENDIX I-V Instructions to authors

ABOUT COVER

Editorial Board Member of *World Journal of Hematology*, Katsuhiro Kita, PhD, Research Scientist, Department of Biochemistry and Molecular Biology, Mail route 0647, Basic Science Building, The University of Texas Medical Branch, 301 University Boulevard, Galveston, Texas 77555-0144, United States

AIM AND SCOPE

World Journal of Hematology (*World J Hematol*, *WJH*, online ISSN 2218-6204, DOI: 10.5315) 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 experimental, clinical, oncological and transplant hematology, transfusion science, hemostasis and thrombosis, evidence-based medicine, epidemiology and nursing. Priority publication will be given to articles concerning diagnosis and treatment of hematological 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 Hematology is now indexed in Digital Object Identifier.

FLYLEAF I-II Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Huan-Liang Wu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Fang-Fang Ji*

NAME OF JOURNAL
World Journal of Hematology

ISSN
ISSN 2218-6204 (online)

LAUNCH DATE
June 6, 2012

FREQUENCY
Quarterly

EDITOR-IN-CHIEF
Xiaoyan Jiang, MD, PhD, Associate Professor, Medical Genetics, University of British Columbia, Terry Fox Laboratory, British Columbia Cancer Agency, 675 West 10th Ave, Vancouver, BC, V5Z 1L3, Canada

Thomas J Kipps, MD, PhD, Professor of Medicine, University of California, San Diego, Moores Cancer Center, 3855 Health Sciences Drive, MC 0820, La Jolla, CA 92093-0820, United States

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Hematology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: wjhematol@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai,
Hong Kong, China
Fax: +852-6555-7188
Telephone: +852-3177-9906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
May 6, 2014

COPYRIGHT
© 2014 Baishideng Publishing Group Co., Limited. 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 this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2218-6204/g_info_20100722173604.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Hematology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5315/wjh.v3.i2.18

World J Hematol 2014 May 6; 3(2): 18-28
ISSN 2218-6204 (online)
© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

EDITORIAL

Ex vivo expansion of hematopoietic stem and progenitor cells: Recent advances

Katsuhiro Kita, Fangming Xiu, Marc G Jeschke

Katsuhiro Kita, Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, TX 77555, United States
Fangming Xiu, Sunnybrook Research Institute, University of Toronto, Ontario, M5S 2W6, Canada
Marc G Jeschke, Ross Tilley Burn Centre, Sunny brook Health Science Centre and Sunnybrook Research Institute and Division of Plastic Surgery, Department of Surgery, University of Toronto, Toronto, M5S 2W6, Canada
Author contributions: All authors solely contributed to this paper.
Supported by Canadian Institutes of Health Research, No. 123336; CFI Leader's Opportunity Fund, No. 25407; Physicians' Services Incorporated Foundation - Health Research Grant Program (MGJ)
Correspondence to: Katsuhiro Kita, PhD, Research Scientist of Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555, United States. kakitatemp@yahoo.com
Telephone: +1-212-7460293 Fax: +1-212-7468866
Received: April 17, 2012 Revised: December 7, 2013
Accepted: January 17, 2014
Published online: May 6, 2014

Abstract

Hematopoietic stem cells (HSCs) have become the most extensively studied stem cells and HSC-based cellular therapy is promising for hematopoietic cancers and hereditary blood disorders. Successful treatment of patients with HSC cells depends on sufficient number of highly purified HSCs and progenitor cells. However, stem cells are a very rare population no matter where they come from. Thus, *ex vivo* amplification of these HSCs is essential. The heavy demands from more and more patients for HSCs also require industrial-scale expansion of HSCs with lower production cost and higher efficiency. Two main ways to reach that goal: (1) to find clinically applicable, simple and efficient methods (or reagents) to enrich HSCs; (2) to find new developmental regulators and chemical compounds in order to replace the currently used cytokine cocktails for HSCs amplification. In this Editorial review, we would like to introduce the current status of *ex vivo* expansion of HSCs, particularly focusing on enrichment and culture supplements.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Hematopoietic stem cell; *Ex vivo* expansion; Serum-free culture; Cell surface markers; Enrichment; Stem cell isolation

Kita K, Xiu F, Jeschke MG. *Ex vivo* expansion of hematopoietic stem and progenitor cells: Recent advances. *World J Hematol* 2014; 3(2): 18-28 Available from: URL: http://www.wjgnet.com/2218-6204/full/v3/i2/18.htm DOI: http://dx.doi.org/10.5315/wjh.v3.i2.18

INTRODUCTION

The early evidence and definition of hematopoietic stem cells (HSCs) came from studies of people exposed to lethal doses of radiation in 1945 and the pioneering studies by Till and McCullock[1]. But it took until late 1980s and early 1990s for researchers to show the isolation and characterization of HSCs from human and rodents[2-4]. Since then, HSCs have become the most extensively studied stem cells (SCs), and HSC-based cellular therapy is promising to treat hematopoietic cancers, such as leukemia and lymphoma, and hereditary blood disorders such as inherited anemia.

HSCs are a small sub-set of SCs generated by the bone marrow (BM) niche which are essential for self-renewal and derivation of whole blood systems. Therefore, any deficiency of HSCs will cause serious, mostly fatal, outcomes as seen in patients of hematopoietic malignancies treated with high doses of chemotherapy and radiation. To treat patients with these complica-

tions, BM transplantation has been widely used since 1980s. Although whole BM transplantation used to be the only clinically viable option, increased numbers of *ex vivo*-expanded HSCs isolated from either the BM, or peripheral blood or cord blood cells are being used for current transplantation procedures in clinics. Currently, approximately 3000 clinical trials can be found in US with 1/3 of trials focused on the applications of HSCs for leukemia treatment (http://www.clinicaltrials.gov/). Hereafter we use the term HSCs to represent both HSCs and hematopoietic progenitor cells. We should emphasize that cells used in clinical settings are HSC-enriched but do contain progenitors. Thus, the term "HSCs" used throughout this review includes the CD34$^+$ fraction. HSCs only constitute a very minor fraction of the whole blood or BM cells, 1/10000 for BM SCs and 1/100000 for peripheral blood SCs. Thus, it is absolutely essential to develop large-scale culture methods for *ex vivo* expansion. Although HSC culture is the most advanced system among SC culture, further improvement of *ex vivo* culture for HSC expansion is important. Particularly, animal product (*e.g.*, serum)-free cell culture media will be one of the most important factors to be considered. In this short review, we would like to introduce the current status of *ex vivo* culture of HSCs and the most recently emerging reagents. Although culture devices (such as bioreactors) are another important component in *ex vivo* expansion, the main purpose of this Editorial review is to concisely summarize the new advances in enrichment of HSCs and *ex vivo* expansion of HSCs. For additional information on culture devices, we recommend reading Nielsen's excellent review[5]. We also suggest a few recent review articles that nicely and concisely summarized recent advances in the area of *ex vivo* expansion of hematopoietic stem/progenitor cells[6,7].

SOURCES OF HSC

There are potentially three main sources of haematopoietic stem cells for transplantation - BM, peripheral blood stem cells and umbilical cord blood stem cells. Table 1 summarizes advantages and disadvantages of 3 sources. Over the past 40 years, BM transplantation and stem cells isolated from BM has been widely used to treat numerous malignant and nonmalignant diseases. In spite of increasing therapeutic applications utilizing G-CSF to mobilize HSCs into circulation and CD34$^+$ cells are collected[8], BM cell isolation is still a widely accepted HSC source worldwide[9,10]). However, isolation of HSCs from BM is not a comfortable experience for donors, and it carries the risk of infection transmission. Increasing numbers of studies have used umbilical cord blood (UCB) as an alternative source to obtain HSCs. UCB has fewer ethical issues for obtaining cells, rapid availability and reduced stringency for HLA matching[11] make them another promising source of HSC for transplantation. Probably because of lower effective infused cell dose, slower engraftment of UCB-derived HSCs than BM-derived HSCs has been reported[12,13]. In addition, some progenitor cell populations (myeloid progenitors) in UCB were reported to be more chemoresistant[14], which could be beneficial when combined with high-dose chemotherapies. The most recent study describing the isolation of single HSC capable of long-term, multi-lineage engraftment derived from UCB, which suggests UCB cell may contain functionally most primitive HSCs[15]. Although UCB is likely to be more popular as a source to obtain HSCs, further research and careful examinations will certainly be necessary to determine the pros/cons, similarities/differences, and quantity/quality of HSCs isolated from both BM and UCB.

ENRICHMENT OF HSC

As previously mentioned, Weismann's group was the first group demonstrating isolation and purification of HSCs (from mouse BM)[2]. This first study used multiple HSC markers including Thy-1^{low}, Lin$^-$ and Sca-1$^+$, a widely used criteria nowadays, to purify HSCs[2]. Note that Sca-1 is not found in human HSCs. Since HSCs are a very rare population in whole BM cells (1 in 10000-15000 BM cells), enrichment of HSCs certainly is a very important step for effective *ex vivo* expansion of HSCs. CD34 is a widely recognized cell surface marker to enrich HSCs (as reviewed in[16]). Immunoselection based on cell surface CD34 expression is well accepted in clinical settings, although it should be noted that the CD34$^+$ fraction does contain many progenitor cells such as endothelial progenitor cells[17]. Since a CD34$^+$ fraction still consists of crude cell populations, a CD34$^+$CD38$^-$ fraction may contain a higher percentage of primitive HSCs[18]. Therefore, a CD34$^+$CD38$^-$Lin$^-$Sca$^+$ selection criteria may be reasonable for selecting a highly enriched, primitive fraction of HSCs. c-Kit (stem cell factor receptor/CD117)[19,20] and CD133[21,22] are also the markers for the selection, although combination of too many antibodies may not be realistic in clinical settings because of a significant loss of HSCs during purification. We would like to note that these selections would certainly help enrich for higher quality HSCs; however, the above processes are still not enough to identify a nearly pure, HSC population. Most recently, John Dick's group isolated single hematopoietic stem cells[15] whose phenotype is Lin$^-$CD34$^+$CD38$^-$Thy1$^+$CD45RA$^-$Rho^{low}CD49f$^+$ can regenerate the entire hematopoietic system.

It should also be noted that there is evidence showing a considerable number of HSCs can be CD34 negative[23-25]. There are two possibilities accounting for why some studies have shown apparently different trends (cite examples of CD34 negative). First of all, CD34-positive and -negative cells may be interchangeable[26]. This means that HSCs may retain CD34 in intracellular compartments upon induction of cell surface CD34 molecules. Since the majority of anti-CD34 antibodies used for flow cytometric analyses recognize the extracellular domain of CD34, CD34 expression will appear to be negative when intracellular accumulation of CD34 occurs (unless the plasma membrane of the cells are

Table 1 Comparison of the sources for hematopoietic stem cells

	Advantages	Disadvantages
Bone marrow	1 The longest history as a source of HSC 2 Well established procedure	1 High stringency for HLA matching 2 Some complications associated with harvesting 3 Risk of GVHD
Umbilical cord blood	1 Off-the-shelf availability 2 Reduced stringency for HLA matching 3 Low risk of infection/transmission 4 Absence of donor risk	1 Delayed engraftment 2 Lower yield 3 Lack of additional immune cells
Peripheral blood	Compared to BM, 1 More comfortable for donors 2 Better yield with mobilization	1 Extremely low level of HSC (1/100000) without "mobilization" 2 Risk of GVHD

HSC: Hematopoietic stem cell; HLA: Human leukocyte antigen; BM: Bone marrow; GVHD: Graft-versus-host disease.

Table 2 Cell-surface makers of undifferentiated hematopoietic stem cells

Mouse	Human
$CD34^{low/-}$	$CD34^{low/-}$
$Sca\text{-}1^{+}$	$Sca\text{-}1^{+}$
$CD90/Thy\text{-}1^{+/low}$	$CD90/Thy\text{-}1^{+/low}$
$c\text{-}Kit^{+}$	$c\text{-}Kit^{+}$
$CD38^{+}$	$CD38^{-}$
$CD150^{+}$	$CD7^{-}$
Side population (high hoechst-efflux activity)	$CD49f^{+}$
$CD48^{-}$	Rhodamine 123^{low}
$CD244^{-}$	$CD133/AC133^{-}$
Lin^{-}	$CD45RA^{-}$
	Lin^{-}

permeabilized). The exact mechanism why $CD34^{+}$ HSCs and $CD34^{-}$ HSCs have interchangeable phenotypes remains unresolved. In mouse, the most primitive HSCs can be either $CD34^{-}$ or $CD34^{low}$. Nevertheless, human HSCs can be found in $CD34^{+}$ fraction[27] (Table 2). The other potential pitfall is a technical issue related to anti-mouse CD34 monoclonal antibodies (mAbs). There are several anti-mouse CD34 mAbs available to stain cell surface CD34. At least two clones widely used among researchers, clones MEC14.7[28] and HM34[29], have different characteristics in regard to staining. HM34 is reported to be unable to stain CD34 expression on marrow cells by some unknown mechanism. In addition, clone MEC14.7 is known to show relatively low affinity against CD34; therefore, it is recommended to incubate the mAb with samples for a prolonged period of 90-120 min (in contrast, the majority of commonly used protocols in flow cytometry suggest a 30-60 min incubation). Some of the murine HSC studies might not have carefully checked these technical tips requiring careful interpretation of studies reporting $CD34^{-}$ mouse HSCs.

It should likewise be noted that positive selection leaves antibodies attached on the cell surface which may not be favorable in terms of quality control issues. Furthermore, as most recently discussed by Lodish's group[30], the large amount of antibodies used for a clinical scale (10^{9} per unit for cord blood transplantation) will certainly raise the cost for purification of HSCs. Therefore, development of chemical or enzymatic activity-based substrates may be a great advantage to enrich HSCs in clinical settings. Chemical substrates could help lowering production costs, and simplifying quality control compared to mAbs. In addition, substrate-based selection may shorten the time and reduce the labors to prepare a HSC-rich fraction. Interestingly, hematopoietic progenitor cells have been shown to highly express cytosolic aldehyde dehydrogenase (ALDH)[31]. A fluorescent dye-conjugated ALDH substrate, developed by Storms and colleagues[32], enabled them to conduct a relatively simple, ALDH activity-based selection of HSCs and hematopoietic progenitor cells. Prior to this method, side populations selected based on influx of the DNA staining dye, Hoechst 33342 had been reported[33-36]. The combination of Hoechst dye efflux activity and ALDH activity was first shown by Pearce and Bonnet[37]. Their study showed that Hoechst side population (SP) only contained $CD34^{+}$ cells, thus, SP-based selection may not be suitable to enrich certain sub-populations such as $CD34^{-}CD38^{+}$ cells; which suggests that, at least in mouse, SP may not contain the most primitive HSC. Therefore, further studies need to confirm whether SP contains the primitive HSC population in humans. The authors concluded that Hoechst exclusion may not be appropriate for HSC isolation[37]. It was also reported that Hoechst 33342 is even more toxic than the other most commonly used DNA staining dye, 4',6-diamidino-2-phenyl indole dihydrochloride[38], by interrupting DNA topoisomerase I[39]. Topoisomerases play an essential role in cutting damaged DNA during DNA replication. Even temporary inhibition of a topoisomerase, might cause accumulation of unfavorable mutations during *ex vivo* expansion of HSCs; therefore, careful assessment on the long-term effects of the use of Hoechst 33342 must be assessed to pursue an application of Hoechst 33342 for HSC selection. Overall, currently ALDH-based selection may be the best option to sort/enrich HSCs.

Although it is very important to explore the best set of markers to purify the most primitive fraction of HSCs for *ex vivo* expansion and transplantation, significant technical hurdles exist. The combination of many surface antigens to purify HSCs causes a considerable reduction of the number of cells, which may limit the application in clinical settings. The other potentially impor-

tant issue is that maintaining the capacity of self-renewal while preserving the primitive capabilities of these HSCs during expansion of HSCs may require paracrine signals from other less primitive progenitor cells or differentiated leukocytes in culture. As we will describe in the following section, keeping the number of primitive HSCs in HSC-enriched (but still crude) fractions is certainly important. In fact, a simple CD34$^+$ fraction gives cells with enough *ex vivo* expansion capacity[40] to be used in clinical settings. In addition, the most "primitive" HSCs are quiescent, and rapid proliferation of these cells may cause the loss of primitive HSCs[41]. Therefore, rapid expansion of the small number of HSCs may often force HSCs to exit from the resting cycle and lead to HSC exhaustion. It is worthy of note that a nearly pure HSC fraction may not be suitable for large-scale expansion. Another critical issue needs to consider is the long-term repopulation capability of *ex vivo*-expanded fractions. This capability may depend on the number of primitive HSCs after *ex vivo* culture. Therefore, from the clinical stand point, the most important goal in *ex vivo* expansion of HSC may be to develop simple and efficient protocols to obtain HSC-enriched fractions containing a sufficient number of HSCs that would result in satisfactory *in vivo* engraftment after transplantation. Faster recovery of the number of neutrophils in the body seems to be an indicator of successful engraftment of transplanted cells[42], which again justifies the possible advantage of HSC-enriched fractions over nearly pure HSCs. In summary, even great efforts have been made to search the markers for HSCs, it is important to stress that none of the surface markers is entirely specific to the long-term HSCs.

EX VIVO CULTURE MEDIA FOR HSC EXPANSION

Since the limited number of HSCs is the major obstacle in clinical applications of *ex vivo*-expanded HSCs, successful *ex vivo* expansion of HSCs is one of the critical determinants emphasized throughout this review. This is particularly important for adult patients, who require increased units of HSCs in comparison to pediatric patients. The HSC field is relatively well established in comparison to the other SCs, and serum-free culture systems have already been used to expand HSCs. Development of serum-free culture is necessary to avoid the use of animal products such as bovine serum. As mentioned above, it is very important to avoid the use of animal products: (1) to prevent transmission of any possible diseases from animals, such as Creutzfeldt-Jabob disease (caused by Prion protein); (2) to achieve good quality control of expanded cells. In addition, accumulation of animal serum proteins in HSCs might increase the risk of host immune response upon transplantation of *ex vivo* cultured HSCs. The current research has focused on a cytokine cocktail-based culture to allow significant expansion of enriched HSCs.

Another major hurdle in current *ex vivo*-expanded HSCs (or HSC-enriched units) in their clinical applications is that some primitive HSCs are lost during *ex vivo* expansion of HSCs. Since the most primitive HSCs are thought to be in a resting state, the goals to achieve rapid expansion of HSCs and retain primitive HSCs stand at odds to one another. Therefore, to achieve better clinical outcomes (to reduce morbidity and mortality after transplantation of *ex vivo*-expanded cells), it is also essential to improve the number of long-term repopulating cells; *i.e.*, to maintain the number of primitive cells that retain self-renewal capacity. We will address this issue later in the section "Expansion versus *in vivo* reconstitution". Coculture of feeder cells such as a monolayer of mesenchymal stem cells with HSCs may be a better method, since theoretically feeder cell layers should be able to provide a physiologically more relevant environment (stromal cell-HSC interaction and simultaneous feeding of sets of growth factors/cytokines that facilitate expansion of HSCs without unfavorable differentiation). However, coculture with a feeder cell layer is not suitable for large-scale culture, and thus is difficult to translate into clinical settings. Most recently, an attempt to introduce computer simulations was made to help understand complicated paracrine mechanisms involving progenitor cells and differentiated cells in HSC culture[43]. This study may bring a paradigm shift in *ex vivo* cutulre of HSCs. In the following sub-sections, we would like to discuss emerging substrates as well as currently used soluble factors.

Soluble factors (i.e., growth factors and cytokines)

Soluble factors, such as Flt3/Flk2 ligand[44,45], stem cell factor (SCF)[46,47], interleukin-3 (IL-3)[48-50], IL-6[51], and thrombopoietin (Tpo)[52-54] are commonly used as culture supplements for HSCs. Flt3/Flk2 ligand was discovered as a factor promoting proliferation of primitive hematopoietic cells, and[55] is more effective than SCF[56], although SCF probably has the longest history as a supplement. IL-3's effect *in vitro* was first reported by Spivak *et al*[57] However, there is a report showing the suppression of the number of colony-forming cells by IL-3[58], thus, the effect of IL-3 may need careful re-evaluation. This study also showed that IL-3 reduced the reconstituting activity of HSCs in a mouse model system[58]. Other interleukins, such as IL-7 and IL-11, are also included as additional supplements in serum-free culture systems because of their potential to promote HSC proliferation. IL-7 was originally discovered as an interleukin that stimulated proliferation of B cell progenitors in mouse[59]. Although IL-7 alone did not show significant expansion of Lin$^-$ Sca-1$^+$ HSCs, potent synergistic effect on proliferation of Lin$^-$Sca-1$^+$ HSCs was observed when IL-7 was combined with IL-3[60]. IL-11 was cloned as a gene product produced by BM-derived stromal cell lines in 1990[61], and the following study by Ogawa's group showed that IL-11 could also synergistically help expansion of primitive hematopoietic progenitor cells with either IL-3 or IL-4[62].

One interesting study showed that gp130 signaling

(*via* IL-6) synergistically enhanced the effect of Flt3 ligand[63] as well as SCF[64]. Interestingly, IL-6 alone was not enough to trigger this effect[48,63,64]. These findings suggest that gp130 signaling appears to be somewhat supportive in its role of potentiating the effects of SCF and Flt3 ligand as culture supplements. Commonly-used cytokine cocktails often contain IL-6. Sustained activation of the Janus kinase (JAK)-STAT signaling pathway may be an important element in *ex vivo* expansion of HSCs. It is noteworthy that thrombopoietin was reported to activate JAK-STAT signaling and thereby helping to protect CD34$^+$ cells from apoptosis in serum-free media[65]. Moreover, activation of c-Kit and gp130 was shown to synergistically induce thrombopoietin production by cord blood CD34$^+$ cells themselves[66]. It should note that there is a report describing impaired engraftment of murine BM cells cultured in the presence of IL-6[67]. Similar to IL-6, it was reported in the same year that IL-3 reduced the number of colony-forming cells[58], impaired engraftment might be mainly associated with IL-3 in the culture. It was surprising that SCF and some of the interleukins could have adverse effects upon transplantation[67]; although SCF is a well-accepted growth factor for serum-free culture of HSCs or HSC-enriched fractions. In addition to the previously mentioned cytokines, fibroblast growth factors (FGFs) were also found to be effective to help *in vitro* culture of whole blood cells or enriched hematopoietic cells[68,69]. The later study also showed that FGF receptors were not expressed on human CD34$^+$ cells[70]. Thus, the effect of FGF may be controversial.

Recent studies by Lodish have added three endothelial growth factors (angiopoietin-like 5, insulin-like growth factor-binding protein 2, and pleiotrophin) as potential soluble factors that may further help *ex vivo* expansion of HSCs[71].

Although great efforts has been made to improve the rate of HSC engraftment, *ex vivo* cytokine-based expansion protocols may have reached plateau. The use of cytokine cocktails can raise the cost for *ex vivo* expansion of HSCs, too. Therefore, there are a great need of additional factors/molecules in order to support HSC self-renewal and amplification *in vitro*.

Exposure to developmental regulators

It has been suggested recently that some developmentally conserved pathways or transcriptional factors are important in the regulation of the adult stem cell compartment, such as wingless-type (Wnt), Notch, Hox transcription factors and Sonic hedge hog Shh/BMP signaling[72]. Among them, Notch signaling is the most extensively studied. Notch ligand may be the most promising recombinant protein product to assist in *ex vivo* expansion of HSCs. In 1994, CD34$^+$ cells were shown to express high levels of the human homolog of *Drosophila* Notch[73], and this initial observation led to hypothesize the role of Notch signaling in maintenance of undifferentiated status of HSCs. Immobilized Notch ligand (Delta-1) was shown to dramatically increase (up to approximately 100-fold) the number of CD34$^+$ cells[74]. More importantly, the expanded cells enhanced repopulating ability of hematopoietic cells in NOD/SCID mice[74]. The effectiveness of Notch ligand was further confirmed by a follow-up study by the same group[75]. The outcome of their clinical trial is very encouraging, because preliminary data of a phase I clinical trial showed engraftment of the CD34$^+$ cord blood cells has significant advantage over the controls including shorter periods for neutrophil recovery after transplantation[75]. One potential side-effect of Delta-1 is that it has potential to cause density-dependent apoptosis of cells[76], and it is necessary to carefully control ligand density to minimize the loss of cells or unfavorable phenotypic changes during expansion. Interestingly, the most recent study by Rafii's group highlighted the critical role of Notch signaling in the BM microenvironment, where Notch activation by BM endothelium is essential for self-renewal of long-term HSCs[77]. This study clearly indicates that Notch signaling is a naturally-occurring, critical signal for maintenance of HSCs.

Besides Notch 1 ligand, TAT (HIV virus-derived cell permeable peptide)-tagged HOXB4 protein was shown to be effective to expand HSCs *in vitro*[78]. The advantage of TAT-HOXB4 protein over Notch ligands is the lack of a requirement to immobilize it onto the culture apparatus. Overexpression of HOXB4 had been shown to be one of the most potent stimulators of HSC expansion[79,80]; however, recently it was reported that there was high incidence of leukemia in large animals two years after receiving HOXB4-carrying retrovirus-introduced *HSC* gene therapy[81]. Therefore, the use of the plasma membrane-permeable recombinant HOXB4 protein may be a reasonable alternative for *ex vivo* expansion of HSCs. Although Krosl *et al*[78] study showed the effectiveness of recombinant HOXB4, the study did not show long-term repopulating activity of expanded cells. Therefore, further studies examining any potential safety issues caused by HOXB4 would be necessary. Overall, Notch ligands seem to be the most promising peptide-type supplements for HSC expansion at current moment.

Stromal support

In the BM niche, HSCs interact with stromal cells, and direct interaction of HSCs with stromal cells or extracellular matrices in the BM may help maintain small populations of primitive HSCs. Thus, it was quite reasonable to hypothesize that major extracellular matrices in the BM niche facilitated sustained hematopoiesis. In fact, a major extracellular matrix, fibronectin, was found to be perhaps the most important extracellular matrix protein to facilitate proliferation/self-renewal as well as adhesion of HSCs and progenitor cells in the BM niche[82,83]. The initial studies showed that stromal support increased not only gene transduction efficiency but also successfully preserved the ability of human CD34$^+$ cells to sustain long-term hematopoiesis in immune deficient mice[84]. This study also showed that a C-terminal fragment of

fibronectin could help successful long-term engraftment of human HSCs and progenitor cells to bnx/hu mice. This C-terminal fragment contains both CS-1 and RGD(S) domains. The CS-1 domain is known to interact with VLA-4 (integrin α4β1)[85], and interestingly, Verfaillie *et al*[82] reported that primitive progenitors bound to fibronectin CS-1 *via* VLA-4, but expression of VLA-4 was lost upon differentiation of the cells. Immobilized fibronectin peptides were also shown to help *ex vivo* expansion of human cord blood HSCs (CD34$^+$ fraction)[86]. In this study, the authors used several fibronectin peptides including those carrying mutations on binding domains. Although all peptides they used showed significant increase in colony forming units as well as expansion of cells, only the peptide containing the intact CS-1 domain could give successful long-term engraftment of transplanted cells and survival of NOD/SCID mice[86]. Thus, short C-terminal fragments of fibronectin containing the CS-1 domain may be a good coating material for *ex vivo* expansion of HSCs or progenitor cells.

The major disadvantage of these proteins/peptides, except TAT-HOXB4 protein, is their requirement of immobilization onto the surface of cell culture apparatuses. This will increase culture volume as well as cost of industry-scale production of cells; thus, development of suspension culture systems that allow both self-renewal of HSCs and a reasonable level of expansion would be desirable.

Small molecules

It would be desirable to use chemical compounds as supplements for serum-free culture systems, which may significantly help reducproduction costs of HSCs at the industrial scale and at the same time, facilitate efficient *ex vivo* expansion of HSCs.

Most recently, one study has shown that a chemical compound could also be used for *ex vivo* expansion of HSCs[87]. Chemical compounds would be superior to biological compounds because of potentially lower production costs and easier quality control.

These promising chemical compounds include the retinoic acid receptor agonist all-trans retinoic acid, copper chelator tetraethylenepentamine (TEPA), histone deacetylase inhibitors, acryl hydrocarbon receptor antagonist [referred to as StemRegenin1 (SR1)] and, PGE2[72]. Among them, TEPA are the most promising chemical to be used for expansion of human HSCs at current moment. TEPA-supplemented, *ex vivo* cultures of CD34$^+$ cord blood cells significantly increased the number of HSCs and enhanced NOD/SCID repopulating capacity[88]. A phase Ⅰ/Ⅱ clinical trial of TEPA-cultured cord blood cells showed the safety of this approach[89]. The efficiency of TEPA-cultured HSPC is currently under investigation in an ongoing phase Ⅱ/Ⅲ study.

Aryl hydrocarbon receptor antagonists (SR1) were identified as potential drug candidates for promoting *ex vivo* expansion of HSCs by microscopy-based high-throughput screening[87]. The antagonist was subsequently tested with a feedback culture system[43]. Aryl hydrocarbon receptor signaling emerged as an important element in HSC functions[87]. Currently, these compounds require the supplementation of cytokines, and thus further studies will be necessary to apply this strategy for large scale expansion of HSCs without animal serum. Nevertheless, this discovery may eventually pave a way for establishing industrial-scale production of HSCs with chemically defined culture media.

EXPANSION *VS IN VIVO* RECONSTITUTION

Although finding the optimum conditions for clinical-scale *ex vivo* expansion culture is an absolutely important determinant to use HSCs in clinical settings, another important factor in determining the clinical outcomes of HSC transplantation is engraftment and reconstitution of the hematopoietic system in a recipient. Successful engraftment requires repopulation of transplanted cells to irradiated BM, of which success may mainly rely on the ability of transplanted cells to migrate toward BM. (SDF-1/CXCR4 axis). Therefore, it is also essential to test repopulation and engraftment of expanded cells by immunologically incompetent small animal models such as severe combined immunodeficient (SCID) mice. On the other hand, reconstitution of the hematopoietic system requires *de novo* production of hematopoietic lineages from donor-derived HSCs. This function requires self-renewable, primitive HSCs in the BM after transplantation to prevent exhaustion of self-renewable HSCs in BM niche. The CD34$^+$ fraction, which is commonly used in the current clinical settings as a source of HSC-enriched cells, should be T cell-free to avoid graft-versus-host-disease. Successful engraftment is likewise likely to be associated positively with the number of neutrophils present in the body[42].

Before closing this section, we also would like to comment on the quiescence of stem cells under normal conditions. HSCs retain labeled thymidine, indicating replicative quiescence in their niche[41]. We speculate that this may be because HSCs need to maintain longevity and to minimize mutations concomitant with replication errors. Therefore, a line of studies may be necessary to confirm that rapid *ex vivo* expansion of HSCs does not generate and accumulate genetic mutations that can lead to the formation of cancerous cells. In addition, we should always keep in our mind that rapid *ex vivo* expansion of HSCs may often cause the loss of long-term engraftment capacity of HSCs or exhaustion of the most primitive HSC pool. It was demonstrated for more than a decade ago that HSCs proliferate slow *in vivo*[90,91]. Therefore, it is postulated that HSC quiescence is critical in maintaining the stem cell compartment. Weissman's group estimated that approximately 8% of long-term self-renewing HSCs enter the cell cycle per day[91]. However, contradictory results appeared later showing traditionally used DNA labeling dye, BrdU (5-bromo-2-deoxyuridine), may not be specific enough to label slow-dividing cells[92]. More recently, different labeling

strategies have been proposed as alternative methods to trace slowly dividing HSC fractions[93,94]. Proliferation of cells requires the entry to G_1 cycle, thus, it is quite natural to hypothesize that perturbation of cyclin dependent kinase inhibitors may initiate dramatic cell proliferation followed by the exhaustion of HSC pools. Scadden's group showed for the first time, $p21^{cip/waf1}$ deletion eventually causes exhaustion of HSCs in a mouse model system[95].

Since phosphatase and tensin homolog (PTEN)/Akt pathway plays a critical role in cell proliferation, survival and growth, it was predicted that mis-regulation of this pathway could cause problems on hematopoiesis. In fact, PTEN knockout mice study showed decreased numbers of HSCs[96]. PTEN knockout mice also developed leukemia, suggesting there is a critical factors suppressing cell cycle progression. The phenotype of Foxo3a knockout mice is quite interesting. Foxo3a-KO significantly decreased the number of colony forming cells in BM. Age-dependent decrease of HSC pool was also observed. However, Foxo3a-KO little effects on hematopoietic progenitor cells except a decreased number of erythrocytes[97]. More interestingly, distribution of Foxo3a in a cell further implies Foxo3a's specific role in maintaining primitive HSCs; Foxo3a accumulate in inside of a nucleus in a $CD34^-$c-Kit^+Sca-$1^+$$lineage^-$ cell, however, Foxo3a no longer accumulates in a nucleus and translocates to a nuclear envelop in a $CD34^+$c-Kit^+Sca-$1^+$$lineage^-$ cell, which is less primitive than $CD34^-$c-Kit^+Sca-$1^+$$lineage^-$ cells. Thus, it appears that Foxo3a may hold a key role in the maintenance of HSCs in BM.

Although c-Kit may be dispensable for extensive proliferation of HSCs, mice carrying c-Kit mutation (W41/W41) were reported to show approximately 2-fold reduction of long term-HSCs[98]. This study was further reinforced with the result that multipotent progenitor cells were less affected by this mutation, suggesting that c-Kit function may also be very important for maintenance of primitive HSCs for sustained hematopoiesis.

Thus, we should keep in our mind that our attempt to improve *ex vivo* expansion of HSCs by manipulating some signaling pathways could be a "double-edged sword".

CONCLUSION

HSC-based cellular therapy is promising for hematopoietic cancers and hereditary blood disorders. Adaptive transfer of sufficient number of enriched HSCs is the key for successful transplantation. Besides BM, peripheral blood and umbilical cord blood also are the sources of HSCs. Traditionally, BM is the main source of HSCs, but peripheral blood and UCB are increasingly used as sources due to non-invasive harvesting procedures. Soluble growth factors, signaling molecules, small molecules and extracellular matrix support are commonly-used methods to amplify isolated HSCs, but chemical compounds such as TEPA is promising because of its lower cost and easier quality control.

A rapidly increasing number of studies and clinical trials have demonstrated very promising applications of HSCs in clinical settings. Currently, applications of HSCs are often limited to children mostly due to restrictions in the quantity of HSCs that can be obtained. Increased demand of HSC therapies, including those for adult patients, is expected. Therefore, it is necessary to establish large-scale production of HSCs to supply enough units of HSCs in the near future. In addition to cytokine cocktails, most recent studies have added several other options such as recombinant proteins and chemical compounds for *ex vivo* expansion of HSCs. Chemical-based culture would be advantageous, however, it will take some time until it is ready for clinical application.

Using multiple cell surface markers, including CD34, CD38, Thy-1, CD45, c-kit, CD133, CD49f and Lineage cocktail, it is possible to isolate single HSCs with the full capacity to regenerate the entire hematopoietic system. However, it is worthy to note that purification of ultimately primitive HSCs may not be necessary for clinical-scale production of cells. Instead, "HSC-enriched" fractions would be useful because of the possible positive feedback by the other less primitive cells in the culture. Thus, the effective *ex vivo* expansion of HSCs or hematopoietic progenitor cells may require inclusion of precise feedback regulation (*i.e.*, autocrine and paracrine), since the hematopoietic system is a highly complicated system requiring maintenance of a highly ordered hierarchy. Further investigation should develop more promising methods which will substantially enhance generation of HSCs with high efficiency for clinical application.

ACKNOWLEDGMENTS

The authors really appreciate Kimberlee Burckart (Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch) for editing the manuscript.

REFERENCES

1 **TILL JE**, McCULLOCH EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* 1961; **14**: 213-222 [PMID: 13776896 DOI: 10.2307/3570892]

2 **Spangrude GJ**, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. *Science* 1988; **241**: 58-62 [PMID: 2898810 DOI: 10.1126/science.2898810]

3 **Siena S**, Bregni M, Brando B, Ravagnani F, Bonadonna G, Gianni AM. Circulation of CD34+ hematopoietic stem cells in the peripheral blood of high-dose cyclophosphamide-treated patients: enhancement by intravenous recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1989; **74**: 1905-1914 [PMID: 2478216]

4 **Berardi AC**, Wang A, Levine JD, Lopez P, Scadden DT. Functional isolation and characterization of human hematopoietic stem cells. *Science* 1995; **267**: 104-108 [PMID: 7528940 DOI: 10.1126/science.7528940]

5 **Nielsen LK**. Bioreactors for hematopoietic cell culture. *Annu Rev Biomed Eng* 1999; **1**: 129-152 [PMID: 11701485 DOI: 10.1146/annurev.bioeng.1.1.129]

6 **Hai-Jiang W**, Xin-Na D, Hui-Jun D. Expansion of hematopoietic stem/progenitor cells. *Am J Hematol* 2008; **83**: 922-926 [PMID: 18839435 DOI: 10.1002/ajh.21262]
7 **Dahlberg A**, Delaney C, Bernstein ID. Ex vivo expansion of human hematopoietic stem and progenitor cells. *Blood* 2011; **117**: 6083-6090 [PMID: 21436068 DOI: 10.1182/blood-2011-01-283606]
8 **Matsunaga T**, Sakamaki S, Kohgo Y, Ohi S, Hirayama Y, Niitsu Y. Recombinant human granulocyte colony-stimulating factor can mobilize sufficient amounts of peripheral blood stem cells in healthy volunteers for allogeneic transplantation. *Bone Marrow Transplant* 1993; **11**: 103-108 [PMID: 7679596]
9 **Neben S**, Marcus K, Mauch P. Mobilization of hematopoietic stem and progenitor cell subpopulations from the marrow to the blood of mice following cyclophosphamide and/or granulocyte colony-stimulating factor. *Blood* 1993; **81**: 1960-1967 [PMID: 7681707]
10 **Bensinger WI**, Clift RA, Anasetti C, Appelbaum FA, Demirer T, Rowley S, Sandmaier BM, Torok-Storb B, Storb R, Buckner CD. Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony stimulating factor. *Stem Cells* 1996; **14**: 90-105 [PMID: 8820955 DOI: 10.1002/stem.140090]
11 **Harris DT**, Schumacher MJ, Locascio J, Besencon FJ, Olson GB, DeLuca D, Shenker L, Bard J, Boyse EA. Phenotypic and functional immaturity of human umbilical cord blood T lymphocytes. *Proc Natl Acad Sci USA* 1992; **89**: 10006-10010 [PMID: 1438190 DOI: 10.1073/pnas.89.21.10006]
12 **Wagner JE**, Kernan NA, Steinbuch M, Broxmeyer HE, Gluckman E. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. *Lancet* 1995; **346**: 214-219 [PMID: 7616801 DOI: 10.1016/S0140-6736(95)91268-1]
13 **Barker JN**, Davies SM, DeFor T, Ramsay NK, Weisdorf DJ, Wagner JE. Survival after transplantation of unrelated donor umbilical cord blood is comparable to that of human leukocyte antigen-matched unrelated donor bone marrow: results of a matched-pair analysis. *Blood* 2001; **97**: 2957-2961 [PMID: 11342417 DOI: 10.1182/blood.V97.10.2957]
14 **Toren A**, Einat M, Fabian I, Nagler A. Human umbilical cord blood myeloid progenitor cells are relatively chemoresistant: a potential model for autologous transplantations in HIV-infected newborns. *Am J Hematol* 1997; **56**: 161-167 [PMID: 9371528]
15 **Notta F**, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science* 2011; **333**: 218-221 [PMID: 21737740 DOI: 10.1126/science.1201219]
16 **Krause DS**, Fackler MJ, Civin CI, May WS. CD34: structure, biology, and clinical utility. *Blood* 1996; **87**: 1-13 [PMID: 8547630]
17 **Asahara T**, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997; **275**: 964-966 [DOI: 10.1126/science.275.5302.964]
18 **Sakabe H**, Yahata N, Kimura T, Zeng ZZ, Minamiguchi H, Kaneko H, Mori KJ, Ohyashiki K, Ohyashiki JH, Toyama K, Abe T, Sonoda Y. Human cord blood-derived primitive progenitors are enriched in CD34+c-kit- cells: correlation between long-term culture-initiating cells and telomerase expression. *Leukemia* 1998; **12**: 728-734 [PMID: 9593271 DOI: 10.1038/sj.leu.2401001]
19 **Ogawa M**, Matsuzaki Y, Nishikawa S, Hayashi S, Kunisada T, Sudo T, Kina T, Nakauchi H, Nishikawa S. Expression and function of c-kit in hemopoietic progenitor cells. *J Exp Med* 1991; **174**: 63-71 [PMID: 1711568 DOI: 10.1084/jem.174.1.63]
20 **Briddell RA**, Broudy VC, Bruno E, Brandt JE, Srour EF, Hoffman R. Further phenotypic characterization and isolation of human hematopoietic progenitor cells using a monoclonal antibody to the c-kit receptor. *Blood* 1992; **79**: 3159-3167 [PMID: 1375842]
21 **Yin AH**, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 1997; **90**: 5002-5012 [PMID: 9389720]
22 **Miraglia S**, Godfrey W, Yin AH, Atkins K, Warnke R, Holden JT, Bray RA, Waller EK, Buck DW. A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood* 1997; **90**: 5013-5021 [PMID: 9389721]
23 **Jones RJ**, Collector MI, Barber JP, Vala MS, Fackler MJ, May WS, Griffin CA, Hawkins AL, Zehnbauer BA, Hilton J, Colvin OM, Sharkis SJ. Characterization of mouse lymphohematopoietic stem cells lacking spleen colony-forming activity. *Blood* 1996; **88**: 487-491 [PMID: 8695796]
24 **Osawa M**, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 1996; **273**: 242-245 [PMID: 8662508 DOI: 10.1126/science.273.5272.242]
25 **Goodell MA**, Rosenzweig M, Kim H, Marks DF, DeMaria M, Paradis G, Grupp SA, Sieff CA, Mulligan RC, Johnson RP. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat Med* 1997; **3**: 1337-1345 [PMID: 9396603 DOI: 10.1038/nm1297-1337]
26 **Donnelly DS**, Krause DS. Hematopoietic stem cells can be CD34+ or CD34-. *Leuk Lymphoma* 2001; **40**: 221-234 [PMID: 11426544 DOI: 10.3109/10428190109057921]
27 **Shizuru JA**, Negrin RS, Weissman IL. Hematopoietic stem and progenitor cells: clinical and preclinical regeneration of the hematolymphoid system. *Annu Rev Med* 2005; **56**: 509-538 [PMID: 15660525 DOI: 10.1146/annurev.med.54.101601.152334]
28 **Garlanda C**, Berthier R, Garin J, Stoppacciaro A, Ruco L, Vittet D, Gulino D, Matteucci C, Mantovani A, Vecchi A, Dejana E. Characterization of MEC 14.7, a new monoclonal antibody recognizing mouse CD34: a useful reage for identifying and characterizing blood vessels and hematopoietic precursors. *Eur J Cell Biol* 1997; **73**: 368-377 [PMID: 9270880]
29 **Brown J**, Greaves MF, Molgaard HV. The gene encoding the stem cell antigen, CD34, is conserved in mouse and expressed in haemopoietic progenitor cell lines, brain, and embryonic fibroblasts. *Int Immunol* 1991; **3**: 175-184 [PMID: 1709048 DOI: 10.1093/intimm/3.2.175]
30 **Chou S**, Chu P, Hwang W, Lodish H. Expansion of human cord blood hematopoietic stem cells for transplantation. *Cell Stem Cell* 2010; **7**: 427-428 [PMID: 20887947 DOI: 10.1016/j.stem.2010.09.001]
31 **Kastan MB**, Schlaffer E, Russo JE, Colvin OM, Civin CI, Hilton J. Direct demonstration of elevated aldehyde dehydrogenase in human hematopoietic progenitor cells. *Blood* 1990; **75**: 1947-1950 [PMID: 2337669]
32 **Storms RW**, Trujillo AP, Springer JB, Shah L, Colvin OM, Ludeman SM, Smith C. Isolation of primitive human hematopoietic progenitors on the basis of aldehyde dehydrogenase activity. *Proc Natl Acad Sci U S A* 1999; **96**: 9118-9123 [PMID: 10430905 DOI: 10.1073/pnas.96.16.9118]
33 **McAlister I**, Wolf NS, Pietrzyk ME, Rabinovitch PS, Priestley G, Jaeger B. Transplantation of hematopoietic stem cells obtained by a combined dye method fractionation of murine bone marrow. *Blood* 1990; **75**: 1240-1246 [PMID: 1968771]
34 **Leemhuis T**, Yoder MC, Grigsby S, Agüero B, Eder P, Srour EF. Isolation of primitive human bone marrow hematopoietic progenitor cells using Hoechst 33342 and Rhodamine 123. *Exp Hematol* 1996; **24**: 1215-1224 [PMID: 8765497]
35 **Bertoncello I**, Williams B. Hematopoietic stem cell characterization by Hoechst 33342 and rhodamine 123 staining. *Methods Mol Biol* 2004; **263**: 181-200 [PMID: 14976367]
36 **Rossi L**, Challen GA, Sirin O, Lin KK, Goodell MA. Hematopoietic stem cell characterization and isolation. *Methods Mol*

Biol 2011; **750**: 47-59 [PMID: 21618082 DOI: 10.1007/978-1-61779-145-1_3]
37 **Pearce DJ**, Bonnet D. The combined use of Hoechst efflux ability and aldehyde dehydrogenase activity to identify murine and human hematopoietic stem cells. *Exp Hematol* 2007; **35**: 1437-1446 [PMID: 17656008 DOI: 10.1016/j.exphem.2007.06.002]
38 **Park CH**, Kimler BF, Smith TK. Comparison of the supravital DNA dyes Hoechst 33342 and DAPI for flow cytometry and clonogenicity studies of human leukemic marrow cells. *Exp Hematol* 1985; **13**: 1039-1043 [PMID: 4054243]
39 **Chen AY**, Yu C, Bodley A, Peng LF, Liu LF. A new mammalian DNA topoisomerase I poison Hoechst 33342: cytotoxicity and drug resistance in human cell cultures. *Cancer Res* 1993; **53**: 1332-1337 [PMID: 8383008]
40 **Briddell RA**, Kern BP, Zilm KL, Stoney GB, McNiece IK. Purification of CD34+ cells is essential for optimal ex vivo expansion of umbilical cord blood cells. *J Hematother* 1997; **6**: 145-150 [PMID: 9131444]
41 **Zhang J**, Niu C, Ye L, Huang H, He X, Tong WG, Ross J, Haug J, Johnson T, Feng JQ, Harris S, Wiedemann LM, Mishina Y, Li L. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* 2003; **425**: 836-841 [PMID: 14574412 DOI: 10.1038/nature02041]
42 **Fernández MN**, Regidor C, Cabrera R, García-Marco JA, Forés R, Sanjuán I, Gayoso J, Gil S, Ruíz E, Little AM, McWhinnie A, Madrigal A. Unrelated umbilical cord blood transplants in adults: Early recovery of neutrophils by supportive co-transplantation of a low number of highly purified peripheral blood CD34+ cells from an HLA-haploidentical donor. *Exp Hematol* 2003; **31**: 535-544 [PMID: 12829030 DOI: 10.1016/S0301-472X(03)00067-5]
43 **Csaszar E**, Kirouac DC, Yu M, Wang W, Qiao W, Cooke MP, Boitano AE, Ito C, Zandstra PW. Rapid expansion of human hematopoietic stem cells by automated control of inhibitory feedback signaling. *Cell Stem Cell* 2012; **10**: 218-229 [PMID: 22305571]
44 **McKenna HJ**, de Vries P, Brasel K, Lyman SD, Williams DE. Effect of flt3 ligand on the ex vivo expansion of human CD34+ hematopoietic progenitor cells. *Blood* 1995; **86**: 3413-3420 [PMID: 7579445]
45 **Conneally E**, Cashman J, Petzer A, Eaves C. Expansion in vitro of transplantable human cord blood stem cells demonstrated using a quantitative assay of their lympho-myeloid repopulating activity in nonobese diabetic-scid/scid mice. *Proc Natl Acad Sci U S A* 1997; **94**: 9836-9841 [PMID: 9275212 DOI: 10.1073/pnas.94.18.9836]
46 **Gabutti V**, Timeus F, Ramenghi U, Crescenzio N, Marranca D, Miniero R, Cornaglia G, Bagnara GP. Expansion of cord blood progenitors and use for hemopoietic reconstitution. *Stem Cells* 1993; **11** Suppl 2: 105-112 [PMID: 7691315 DOI: 10.1002/stem.5530110818]
47 **Abboud MR**, Xu F, Payne A, Laver J. Effects of recombinant human Steel factor (c-kit ligand) on early cord blood hematopoietic precursors. *Exp Hematol* 1994; **22**: 388-392 [PMID: 7512047]
48 **Kyoizumi S**, Murray LJ, Namikawa R. Preclinical analysis of cytokine therapy in the SCID-hu mouse. *Blood* 1993; **81**: 1479-1488 [PMID: 7680919]
49 **Rossmanith T**, Schröder B, Bug G, Müller P, Klenner T, Knaus R, Hoelzer D, Ottmann OG. Interleukin 3 improves the ex vivo expansion of primitive human cord blood progenitor cells and maintains the engraftment potential of scid repopulating cells. *Stem Cells* 2001; **19**: 313-320 [PMID: 11463951 DOI: 10.1634/stemcells.19-4-313]
50 **Bryder D**, Jacobsen SE. Interleukin-3 supports expansion of long-term multilineage repopulating activity after multiple stem cell divisions in vitro. *Blood* 2000; **96**: 1748-1755 [PMID: 10961873]
51 **Tajima S**, Tsuji K, Ebihara Y, Sui X, Tanaka R, Muraoka K, Yoshida M, Yamada K, Yasukawa K, Taga T, Kishimoto T, Nakahata T. Analysis of interleukin 6 receptor and gp130 expressions and proliferative capability of human CD34+ cells. *J Exp Med* 1996; **184**: 1357-1364 [PMID: 8879208 DOI: 10.1084/jem.184.4.1357]
52 **Schipper LF**, Brand A, Reniers NC, Melief CJ, Willemze R, Fibbe WE. Effects of thrombopoietin on the proliferation and differentiation of primitive and mature haemopoietic progenitor cells in cord blood. *Br J Haematol* 1998; **101**: 425-435 [PMID: 9633882 DOI: 10.1046/j.1365-2141.1998.00737.x]
53 **Ohmizono Y**, Sakabe H, Kimura T, Tanimukai S, Matsumura T, Miyazaki H, Lyman SD, Sonoda Y. Thrombopoietin augments ex vivo expansion of human cord blood-derived hematopoietic progenitors in combination with stem cell factor and flt3 ligand. *Leukemia* 1997; **11**: 524-530 [DOI: 10.1038/sj.leu.2400588]
54 **Piacibello W**, Sanavio F, Garetto L, Severino A, Danè A, Gammaitoni L, Aglietta M. The role of c-Mpl ligands in the expansion of cord blood hematopoietic progenitors. *Stem Cells* 1998; **16** Suppl 2: 243-248 [PMID: 11012196 DOI: 10.1002/stem.5530160727]
55 **Lyman SD**, James L, Vanden Bos T, de Vries P, Brasel K, Gliniak B, Hollingsworth LT, Picha KS, McKenna HJ, Splett RR. Molecular cloning of a ligand for the flt3/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. *Cell* 1993; **75**: 1157-1167 [PMID: 7505204 DOI: 10.1016/0092-8674(93)90325-K]
56 **Shapiro F**, Pytowski B, Rafii S, Witte L, Hicklin DJ, Yao TJ, Moore MA. The effects of Flk-2/flt3 ligand as compared with c-kit ligand on short-term and long-term proliferation of CD34+ hematopoietic progenitors elicited from human fetal liver, umbilical cord blood, bone marrow, and mobilized peripheral blood. *J Hematother* 1996; **5**: 655-662 [PMID: 9117254 DOI: 10.1089/scd.1.1996.5.655]
57 **Spivak JL**, Smith RR, Ihle JN. Interleukin 3 promotes the in vitro proliferation of murine pluripotent hematopoietic stem cells. *J Clin Invest* 1985; **76**: 1613-1621 [PMID: 3932469 DOI: 10.1172/JCI112145]
58 **Yonemura Y**, Ku H, Hirayama F, Souza LM, Ogawa M. Interleukin 3 or interleukin 1 abrogates the reconstituting ability of hematopoietic stem cells. *Proc Natl Acad Sci USA* 1996; **93**: 4040-4044 [PMID: 8633013 DOI: 10.1073/pnas.93.9.4040]
59 **Namen AE**, Lupton S, Hjerrild K, Wignall J, Mochizuki DY, Schmierer A, Mosley B, March CJ, Urdal D, Gillis S. Stimulation of B-cell progenitors by cloned murine interleukin-7. *Nature* 1988; **333**: 571-573 [PMID: 3259677 DOI: 10.1038/333571a0]
60 **Jacobsen FW**, Veiby OP, Skjønsberg C, Jacobsen SE. Novel role of interleukin 7 in myelopoiesis: stimulation of primitive murine hematopoietic progenitor cells. *J Exp Med* 1993; **178**: 1777-1782 [PMID: 7693856 DOI: 10.1084/jem.178.5.1777]
61 **Paul SR**, Bennett F, Calvetti JA, Kelleher K, Wood CR, O'Hara RM, Leary AC, Sibley B, Clark SC, Williams DA. Molecular cloning of a cDNA encoding interleukin 11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. *Proc Natl Acad Sci USA* 1990; **87**: 7512-7516 [PMID: 2145578 DOI: 10.1073/pnas.87.19.7512]
62 **Musashi M**, Clark SC, Sudo T, Urdal DL, Ogawa M. Synergistic interactions between interleukin-11 and interleukin-4 in support of proliferation of primitive hematopoietic progenitors of mice. *Blood* 1991; **78**: 1448-1451 [PMID: 1832057]
63 **Ebihara Y**, Tsuji K, Lyman SD, Sui X, Yoshida M, Muraoka K, Yamada K, Tanaka R, Nakahata T. Synergistic action of Flt3 and gp130 signalings in human hematopoiesis. *Blood* 1997; **90**: 4363-4368 [PMID: 9373247]
64 **Sui X**, Tsuji K, Tanaka R, Tajima S, Muraoka K, Ebihara Y, Ikebuchi K, Yasukawa K, Taga T, Kishimoto T. gp130 and c-Kit signalings synergize for ex vivo expansion of human primitive hemopoietic progenitor cells. *Proc Natl Acad Sci USA* 1995; **92**: 2859-2863 [PMID: 7535932 DOI: 10.1073/

pnas.92.7.2859]
65 **Majka M**, Ratajczak J, Villaire G, Kubiczek K, Marquez LA, Janowska-Wieczorek A, Ratajczak MZ. Thrombopoietin, but not cytokines binding to gp130 protein-coupled receptors, activates MAPKp42/44, AKT, and STAT proteins in normal human CD34+ cells, megakaryocytes, and platelets. *Exp Hematol* 2002; **30**: 751-760 [PMID: 12135673 DOI: 10.1016/S0301-472X(02)00810-X]
66 **Matsui A**, Sato T, Maekawa T, Asano S, Nakahata T, Tsuji K. Glycoprotein 130 and c-kit signals synergistically induce thrombopoietin production by hematopoietic cells. *Int J Hematol* 2000; **72**: 455-462 [PMID: 11197211]
67 **Peters SO**, Kittler EL, Ramshaw HS, Quesenberry PJ. Ex vivo expansion of murine marrow cells with interleukin-3 (IL-3), IL-6, IL-11, and stem cell factor leads to impaired engraftment in irradiated hosts. *Blood* 1996; **87**: 30-37 [PMID: 8547656]
68 **de Haan G**, Weersing E, Dontje B, van Os R, Bystrykh LV, Vellenga E, Miller G. In vitro generation of long-term repopulating hematopoietic stem cells by fibroblast growth factor-1. *Dev Cell* 2003; **4**: 241-251 [PMID: 12586067 DOI: 10.1016/S1534-5807(03)00018-2]
69 **Yeoh JS**, van Os R, Weersing E, Ausema A, Dontje B, Vellenga E, de Haan G. Fibroblast growth factor-1 and -2 preserve long-term repopulating ability of hematopoietic stem cells in serum-free cultures. *Stem Cells* 2006; **24**: 1564-1572 [PMID: 16527900 DOI: 10.1634/stemcells.2005-0439]
70 **Ratajczak MZ**, Ratajczak J, Skorska M, Marlicz W, Calabretta B, Pletcher CH, Moore J, Gewirtz AM. Effect of basic (FGF-2) and acidic (FGF-1) fibroblast growth factors on early haemopoietic cell development. *Br J Haematol* 1996; **93**: 772-782 [PMID: 8703802 DOI: 10.1046/j.1365-2141.1996.d01-1736.x]
71 **Zhang CC**, Kaba M, Iizuka S, Huynh H, Lodish HF. Angiopoietin-like 5 and IGFBP2 stimulate ex vivo expansion of human cord blood hematopoietic stem cells as assayed by NOD/SCID transplantation. *Blood* 2008; **111**: 3415-3423 [PMID: 18202223 DOI: 10.1182/blood-2007-11-122119]
72 **Walasek MA**, van Os R, de Haan G. Hematopoietic stem cell expansion: challenges and opportunities. *Ann N Y Acad Sci* 2012; **1266**: 138-150 [PMID: 22901265]
73 **Milner LA**, Kopan R, Martin DI, Bernstein ID. A human homologue of the Drosophila developmental gene, Notch, is expressed in CD34+ hematopoietic precursors. *Blood* 1994; **83**: 2057-2062 [PMID: 7512837]
74 **Ohishi K**, Varnum-Finney B, Bernstein ID. Delta-1 enhances marrow and thymus repopulating ability of human CD34(+)CD38(-) cord blood cells. *J Clin Invest* 2002; **110**: 1165-1174 [PMID: 12393852 DOI: 10.1172/JCI200216167]
75 **Delaney C**, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat Med* 2010; **16**: 232-236 [PMID: 20081862 DOI: 10.1038/nm.2080]
76 **Delaney C**, Varnum-Finney B, Aoyama K, Brashem-Stein C, Bernstein ID. Dose-dependent effects of the Notch ligand Delta1 on ex vivo differentiation and in vivo marrow repopulating ability of cord blood cells. *Blood* 2005; **106**: 2693-2699 [PMID: 15976178 DOI: 10.1182/blood-2005-03-1131]
77 **Butler JM**, Nolan DJ, Vertes EL, Varnum-Finney B, Kobayashi H, Hooper AT, Seandel M, Shido K, White IA, Kobayashi M, Witte L, May C, Shawber C, Kimura Y, Kitajewski J, Rosenwaks Z, Bernstein ID, Rafii S. Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. *Cell Stem Cell* 2010; **6**: 251-264 [PMID: 20207228 DOI: 10.1016/j.stem.2010.02.001]
78 **Krosl J**, Austin P, Beslu N, Kroon E, Humphries RK, Sauvageau G. In vitro expansion of hematopoietic stem cells by recombinant TAT-HOXB4 protein. *Nat Med* 2003; **9**: 1428-1432 [PMID: 14578881 DOI: 10.1038/nm951]
79 **Antonchuk J**, Sauvageau G, Humphries RK. HOXB4 overexpression mediates very rapid stem cell regeneration and competitive hematopoietic repopulation. *Exp Hematol* 2001; **29**: 1125-1134 [PMID: 11532354 DOI: 10.1016/S0301-472X(01)00681-6]
80 **Antonchuk J**, Sauvageau G, Humphries RK. HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. *Cell* 2002; **109**: 39-45 [PMID: 11955445 DOI: 10.1016/S0092-8674(02)00697-9]
81 **Zhang XB**, Beard BC, Trobridge GD, Wood BL, Sale GE, Sud R, Humphries RK, Kiem HP. High incidence of leukemia in large animals after stem cell gene therapy with a HOXB4-expressing retroviral vector. *J Clin Invest* 2008; **118**: 1502-1510 [PMID: 18357342]
82 **Verfaillie CM**, McCarthy JB, McGlave PB. Differentiation of primitive human multipotent hematopoietic progenitors into single lineage clonogenic progenitors is accompanied by alterations in their interaction with fibronectin. *J Exp Med* 1991; **174**: 693-703 [PMID: 1875168 DOI: 10.1084/jem.174.3.693]
83 **Teixidó J**, Hemler ME, Greenberger JS, Anklesaria P. Role of beta 1 and beta 2 integrins in the adhesion of human CD34hi stem cells to bone marrow stroma. *J Clin Invest* 1992; **90**: 358-367 [PMID: 1379610 DOI: 10.1172/JCI115870]
84 **Nolta JA**, Smogorzewska EM, Kohn DB. Analysis of optimal conditions for retroviral-mediated transduction of primitive human hematopoietic cells. *Blood* 1995; **86**: 101-110 [PMID: 7795215]
85 **Williams DA**, Rios M, Stephens C, Patel VP. Fibronectin and VLA-4 in haematopoietic stem cell-microenvironment interactions. *Nature* 1991; **352**: 438-441 [PMID: 1861722 DOI: 10.1038/352438a0]
86 **Jiang XS**, Chai C, Zhang Y, Zhuo RX, Mao HQ, Leong KW. Surface-immobilization of adhesion peptides on substrate for ex vivo expansion of cryopreserved umbilical cord blood CD34+ cells. *Biomaterials* 2006; **27**: 2723-2732 [PMID: 16376984 DOI: 10.1016/j.biomaterials.2005.12.001]
87 **Boitano AE**, Wang J, Romeo R, Bouchez LC, Parker AE, Sutton SE, Walker JR, Flaveny CA, Perdew GH, Denison MS, Schultz PG, Cooke MP. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science* 2010; **329**: 1345-1348 [PMID: 20688981 DOI: 10.1126/science.1191536]
88 **Peled T**, Landau E, Mandel J, Glukhman E, Goudsmid NR, Nagler A, Fibach E. Linear polyamine copper chelator tetraethylenepentamine augments long-term ex vivo expansion of cord blood-derived CD34+ cells and increases their engraftment potential in NOD/SCID mice. *Exp Hematol* 2004; **32**: 547-555 [PMID: 15183895]
89 **de Lima M**, McMannis J, Gee A, Komanduri K, Couriel D, Andersson BS, Hosing C, Khouri I, Jones R, Champlin R, Karandish S, Sadeghi T, Peled T, Grynspan F, Daniely Y, Nagler A, Shpall EJ. Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial. *Bone Marrow Transplant* 2008; **41**: 771-778 [PMID: 18209724 DOI: 10.1038/sj.bmt.1705979]
90 **Bradford GB**, Williams B, Rossi R, Bertoncello I. Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. *Exp Hematol* 1997; **25**: 445-453 [PMID: 9168066]
91 **Cheshier SH**, Morrison SJ, Liao X, Weissman IL. In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. *Proc Natl Acad Sci USA* 1999; **96**: 3120-3125 [PMID: 10077647 DOI: 10.1073/pnas.96.6.3120]
92 **Kiel MJ**, He S, Ashkenazi R, Gentry SN, Teta M, Kushner JA, Jackson TL, Morrison SJ. Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU. *Nature* 2007; **449**: 238-242 [PMID: 17728714 DOI: 10.1038/nature06115]
93 **Nygren JM**, Bryder D. A novel assay to trace proliferation history in vivo reveals that enhanced divisional kinetics ac-

company loss of hematopoietic stem cell self-renewal. *PLoS One* 2008; **3**: e3710 [PMID: 19002266 DOI: 10.1371/journal.pone.0003710]

94 **Foudi A**, Hochedlinger K, Van Buren D, Schindler JW, Jaenisch R, Carey V, Hock H. Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. *Nat Biotechnol* 2009; **27**: 84-90 [PMID: 19060879 DOI: 10.1038/nbt.1517]

95 **Cheng T**, Rodrigues N, Shen H, Yang Y, Dombkowski D, Sykes M, Scadden DT. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. *Science* 2000; **287**: 1804-1808 [PMID: 10710306 DOI: 10.1126/science.287.5459.1804]

96 **Zhang J**, Grindley JC, Yin T, Jayasinghe S, He XC, Ross JT, Haug JS, Rupp D, Porter-Westpfahl KS, Wiedemann LM, Wu H, Li L. PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature* 2006; **441**: 518-522 [PMID: 16633340 DOI: 10.1038/nature04747]

97 **Miyamoto K**, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, Matsuoka S, Miyamoto T, Ito K, Ohmura M, Chen C, Hosokawa K, Nakauchi H, Nakayama K, Nakayama KI, Harada M, Motoyama N, Suda T, Hirao A. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* 2007; **1**: 101-112 [PMID: 18371339 DOI: 10.1016/j.stem.2007.02.001]

98 **Thorén LA**, Liuba K, Bryder D, Nygren JM, Jensen CT, Qian H, Antonchuk J, Jacobsen SE. Kit regulates maintenance of quiescent hematopoietic stem cells. *J Immunol* 2008; **180**: 2045-2053 [PMID: 18250409]

P- Reviewers: Buitenhuis M, Chang TM, Erceg S
S- Editor: Song XX **L- Editor**: A **E- Editor**: Wu HL

World Journal of Hematology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5315/wjh.v3.i2.29

World J Hematol 2014 May 6; 3(2): 29-43
ISSN 2218-6204 (online)
© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

REVIEW

Anti-CD20 monoclonal antibodies and associated viral hepatitis in hematological diseases

Shih-Hung Yang, Chiun Hsu, Ann-Lii Cheng, Sung-Hsin Kuo

Shih-Hung Yang, Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei 100, Taiwan

Shih-Hung Yang, Chiun Hsu, Ann-Lii Cheng, Sung-Hsin Kuo, Department of Oncology, National Taiwan University Hospital, Taipei 100, Taiwan

Shih-Hung Yang, Chiun Hsu, Ann-Lii Cheng, Sung-Hsin Kuo, Cancer Research Center, National Taiwan University College of Medicine, Taipei 100, Taiwan

Chiun Hsu, Ann-Lii Cheng, Sung-Hsin Kuo, Graduate Institute of Oncology, National Taiwan University College of Medicine, Taipei 100, Taiwan

Ann-Lii Cheng, Department of Internal Medicine, National Taiwan University Hospital, Taipei 100, Taiwan

Author contributions: Yang SH conducted the literature review and drafted the article; Hsu C and Cheng AL wrote the manuscript; Kuo SH revised the content and approved the final version for publication.

Supported by Research grants NSC 101-2321-B-002-032 and NSC 101-2314-B-002-157-MY3 from the National Science Council, Taiwan; NHRI-EX102-10239BI, and NHRI-EX102-10239BI from the National Health Research Institutes, Taiwan; and DOH100-TD-B-111-001 from the Department of Health, Taiwan

Correspondence to: Sung-Hsin Kuo, MD, PhD, Department of Oncology, National Taiwan University Hospital, No. 7, Chung-Shan South Road, Taipei 100,
Taiwan. shkuo101@ntu.edu.tw

Telephone: +886-2-23123456 **Fax:** +886-2-23123456

Received: November 13, 2013 **Revised:** January 27, 2014
Accepted: March 17, 2014
Published online: May 6, 2014

Abstract

Over the past decade, the administration of anti-CD20 monoclonal antibodies such as rituximab has demonstrated various degrees of effectiveness and has improved patients' outcomes during the treatment of autoimmune hematological disorders and hematological malignancies. However, the depletion of B-cells, the distribution of T-cell populations, and the reconstruction of host immunity resulting from the use of anti-CD20 monoclonal antibodies potentially lead to severe viral infections, such as hepatitis B virus (HBV), hepatitis C virus (HCV), parvovirus B19, and herpes viruses, in patients who are undergoing immune therapy or immunochemotherapy. Of these infections, HBV- and HCV-related hepatitis are a great concern in endemic areas because of the high morbidity and mortality rates in untreated patients. As a result, prophylaxis against HBV infection is becoming a standard of care in these areas. Parvovirus B19, a widespread pathogen that causes red blood cell aplasia in immunocompromised hosts, also causes hepatitis in healthy individuals. Recently, its association with hepatitis was recognized in a patient treated with rituximab. In addition, adenovirus, varicella-zoster virus, hepatitis E virus, and rituximab itself have been linked to the occurrence of hepatitis during or after rituximab treatments. The epidemiologies and pathogeneses of these etiologies remain unknown. Because of the increasing use of anti-CD20 monoclonal antibodies for the treatment of hematological malignancies or autoimmune hematological disorders, it is imperative that physicians understand and balance the risks of hepatotropic virus-associated hepatitis against the benefits of using anti-CD20 monoclonal antibodies.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: CD20; Monoclonal antibody; Hepatitis; Hepatitis B virus; Hepatitis C virus

Core tip: Anti-CD20 monoclonal antibodies are widely used for the treatment of hematological malignancies and autoimmune disorders. These agents produce prolonged B-cell depletion and significant immune suppression. In this review, we summarized the clinical use of anti-CD20 monoclonal antibodies and the reports of acute or chronic hepatitis associated with the use of these agents. Most of these hepatitis cases had viral etiologies. We discuss the mechanisms of the hepatitis caused by these drugs. These infections not only interrupted the immunotherapy but are also associated with high mortality and morbidity.

This review may prompt physicians to monitor patients' liver function more closely and to provide adequate prophylaxis while using these agents.

Yang SH, Hsu C, Cheng AL, Kuo SH. Anti-CD20 monoclonal antibodies and associated viral hepatitis in hematological diseases. *World J Hematol* 2014; 3(2): 29-43 Available from: URL: http://www.wjgnet.com/2218-6204/full/v3/i2/29.htm DOI: http://dx.doi.org/10.5315/wjh.v3.i2.29

INTRODUCTION

The nonglycosylated transmembrane phosphoprotein CD20 is a differentiation marker of B cells that was characterized over 30 years ago[1]. CD20 is expressed from early pre-B cells to mature B cells but not in plasma cells[2]. The majority of B-cell lymphomas variably express the CD20 surface marker[3]. CD20 can form multimeric complexes[4,5], interact with B-cell receptors in lipid rafts[5-7], mediate calcium influx[4,7], and regulate the cell cycle and apoptosis[8-11].

Because of the ubiquitous expression of CD20 in normal and malignant B cells, CD20 is an excellent target molecule for the treatment of B cell-related diseases. Rituximab, a genetically engineered chimeric murine/human monoclonal antibody (mAb)-targeting CD20, contains murine light- and heavy-chain variable regions and human constant regions[12]. Rituximab is used to exert cytotoxic effects on B cells by 3 mechanisms: antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated lysis (CDC), and a direct apoptosis-inducing effect on CD20^{+} cells[12,13].

Based on a pivotal trial, rituximab was first approved by the United States Food and Drug Administration in 1997 for relapsed or refractory follicular and low-grade B-cell non-Hodgkin's lymphoma (NHL)[14]. New anti-CD20 mAbs have been developed over the last few years, and these mAbs have been classified into 2 groups (type Ⅰ and type Ⅱ) according to their different activities in inducing CDC, ADCC, apoptosis, and lipid raft redistribution while binding to CD20[15,16]. Because anti-CD20 mAbs have optimized antibody structures and sometimes conjugated radioisotopes, they are used and indicated for the treatment of not only hematological malignancies but also autoimmune diseases[17]. However, following the widespread use of these agents, reports of infections related to B-cell depletion began to appear increasingly. Here, we discuss the clinical applications, the immunocompromising effects, and the association with hepatitis of anti-CD20 mAbs.

APPLICATIONS OF ANTI-CD20 MABS IN HEMATOLOGICAL DISEASES

Malignancies

Rituximab, the first mAb approved for the treatment of malignancies, was initially indicated for relapsed or refractory indolent B-cell NHL[14]. In this pivotal trial, the schedule consisted of 4 weekly doses of 375 mg/m^2. Nearly half of the 166 patients responded, with a projected median time-to-progression of 13 mo. Infusion reactions were the most frequently encountered acute adverse event[14]. The promising response and excellent tolerance to rituximab in indolent B-cell NHL cases were further demonstrated in clinical trials involving extended use and retreatment[18,19]. The excellent single-agent activity of rituximab was demonstrated not only in relapsed or refractory cases but also in newly diagnosed indolent B-cell NHL cases. In a phase Ⅱ trial of single-agent rituximab for patients with low-grade NHL, the initial response rate (RR) after 4 weekly doses of rituximab was 54%, and the RR improved to 64% when rituximab retreatment was administered[20]. In addition to rituximab monotherapy, several studies have demonstrated that the combination of rituximab and chemotherapeutic agents provided high RRs and long time-to-progression in relapsed, refractory, or newly diagnosed indolent B-cell NHL cases[21-25]. To prolong disease control after the initial treatment, maintenance therapies after initial chemotherapy alone[21,26] or rituximab in addition to chemotherapy[21,27,28] have been tested in relapsed, refractory, or newly diagnosed indolent B-cell NHL cases. All of these studies demonstrated considerable improvement in the response duration or progression-free survival[21,26-28]. Moreover, the clinical successes of rituximab were partially recapitulated in diffuse large B-cell lymphoma (DLBCL)[29-35]. The benefits of maintenance therapy were not observed after first-line chemotherapy with or without rituximab or autologous stem-cell transplantation used for treating cases of relapsed DLBCL[34,35]. The difference may reflect the distinct nature of the indolent and aggressive B-cell NHLs.

Because of the clinical benefits demonstrated in rituximab-based regimens, efforts have been made to improve the efficacy of rituximab through the development of new anti-CD20 mAbs or the conjugation of radioisotopes (Table 1). Overall, the administration of yttrium-90 ibritumomab tiuxetan or iodine-131 tositumomab radioimmunotherapy (RIT) has not consistently yielded improved efficacy and survival in relapsed, refractory, or newly diagnosed B-cell NHL cases[36-44]. However, the use of RIT, which offers the theoretical benefits of radiotherapy, can be a viable option for patients who have not responded to prior rituximab treatments or as an alternative or a supplemental method to stem-cell transplantation. Second- and third-generation humanized anti-CD20 mAbs were designed with improved binding affinities for CD20 or the FcγRⅢa receptor for enhanced CDC or ADCC. The efficacy of these agents was also investigated with or without chemotherapy in relapsed, refractory, or newly diagnosed B-cell NHL cases[45-59]. Details regarding these anti-CD20 mAbs are summarized in Table 1.

Autoimmune hematological diseases

The essential mechanism of autoimmune diseases is the

Table 1 Anti-CD20 monoclonal antibodies and associated hepatitis

Antibody	Structure	Clinical applications	Associated hepatitis	Ref.
Rituximab	IgG1, chimeric murine/human mAb	B-cell NHL, RA, SLE, MS, AIHA, TTP, ITP, acquired hemophilia, cryoglobulinemia	HBV, HCV, parvovirus B19, VZV, adenovirus, HEV, drug-related?	[14,18-35,60-65,71, 73,74,78,85,89-93,102-111, 140-147,172,174-179]
Y-90 ibritumomab tiuxetan	IgG1, mouse mAb, conjugated with tiuxetan to yttrium-90	B-cell NHL	HBV	[36-38,112]
I-131 tositumomab	IgG2, mouse mAb, covalently bound to iodine-131	B-cell NHL	None	[39-44]
Ofatumumab	IgG1, human mAb	CLL, B-cell NHL, RA, MS, AIHA	HBV or drug-related?	[45-50,68,114-116]
Veltuzumab	IgG1, humanized mAb	B-cell NHL, ITP	Drug-related?	[51,69]
Ocrelizumab	IgG1, humanized mAb	B-cell NHL, RA, SLE, MS	None	[52]
Obinutuzumab	IgG1, humanized mAb, modified Fc	CLL, B-cell NHL	Drug-related?	[53-57]
PRO131921	IgG1, humanized mAb, modified Fc	CLL, B-cell NHL	None	[58]
Ocaratuzumab	IgG1, humanized mAb, modified Fc	B-cell NHL	None	[59]

mAb: Monoclonal antibody; NHL: Non-Hodgkin lymphoma; RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus; MS: Multiple sclerosis; AIHA: Autoimmune hemolytic anemia; TTP: Thrombotic thrombocytopenic purpura; ITP: Immune thrombocytopenic purpura; DM: Diabetes mellitus; HBV: Hepatitis B virus; HCV: Hepatitis C virus; VZV: Varicella-zoster virus; HEV: Hepatitis E virus; CLL: Chronic lymphocytic leukemia; None: No associated hepatitis reported in hematological autoimmune disorders or malignancies at the time of review (excluding non-hematological diseases).

loss of self-tolerance, which enables the immune system to evoke autoreactive humoral and cellular responses. Elimination of B cells with rituximab is effective in the treatment of autoimmune hematological diseases such as autoimmune hemolytic anemia (AIHA)[60], immune thrombocytopenic purpura (ITP)[61], acquired hemophilia[62], thrombotic thrombocytopenic purpura (TTP)[63], and cryoglobulinemia[64,65]. B cells produce autoantibodies and cytokines, act as antigen-presenting cells, promote naïve CD4$^+$ T-cell differentiation, and affect dendritic cell homeostasis[66]. However, the responses of these autoimmune diseases to the off-label use of rituximab varied widely[67]. The varied results may be partially attributed to the underlying heterogeneity in the etiologies and pathogeneses of these diseases.

The data about the use of non-rituximab anti-CD20 mAbs for the treatment of autoimmune diseases are extremely limited. RIT may also be a valuable option, but its use for the treatment of autoimmune hematological diseases has not been reported. Ofatumumab, approved by the US Food and Drug Administration for treating chronic lymphocytic leukemia (CLL) refractory to fludarabine and alemtuzumab, was demonstrated to be effective in CLL complicated with AIHA[68]. In a phase I trial, veltuzumab demonstrated a RR of 55% in relapsed ITP cases, and long durations of response were observed in some patients[69]. Second- and third-generation humanized anti-CD20 mAbs are being developed for treating nonhematological autoimmune disorders, such as rheumatoid arthritis, systemic lupus erythematosus (SLE), and multiple sclerosis.

HEPATITIS RELATED TO RITUXIMAB AND OTHER ANTI-CD20 MABS

Immune system changes after anti-CD20 mAb treatments

In general, peripheral blood B cells are depleted rapidly and effectively after anti-CD20 mAb treatment. The level of peripheral B cells remains extremely low and recovers gradually until 6 to 12 mo after the last dose of rituximab[61-65,70,71]. The depletion or recovery of B cells is not uniform among the various subsets and locations of B cells and may also depend on the baseline B-cell counts, the nature of the disease, and the dose, duration and type of anti-CD20 mAbs used[36,40,45,46,49-53,55,56,65,71,72]. The recovery of B cells starts with the immature or transitional B cells (CD38$^+$, CD24$^+$, CD10$^+$, CD27$^-$, IgD$^+$) followed by naïve B cells (CD38$^+$, CD27$^-$, IgD$^+$); however, memory B cells (CD27$^+$, CD38$^-$, IgD$^+$) may remain considerably depleted for at least 2 years[73-76]. The pattern of B-cell repopulation is similar between autoimmune disease and B-cell NHL cases[73-76]. The effects of B-cell depletion on plasma immunoglobulin levels and blunted responses to immunization are individualized and heterogeneous among the different diseases treated with rituximab[18,19,21,29,60-65,70,77-80]. Typically, the levels of complements do not change substantially[64,70], but a major increase of C4 levels in the serum was noted after rituximab treatment in type Ⅱ mixed cryoglobulinemia cases[65]. The levels, subsets, and functional status of T cells and natural killer (NK) cells after rituximab treatment are more complex because these factors depend on the expression of CD20 on T and NK cells[74] and the nature of the disease process[17,18,45,46,61,62,64,74,81-87]. Early and persistent reduction of peripheral CD4$^+$/CD40L$^+$ T cells was observed after treatment of SLE with rituximab[84]. The abnormalities of T-cell homeostasis can be reversed after rituximab administration[81-83,87], accompanied by increased CD4$^+$CD25$^+$ regulatory T cells[82,84,85,87], CD8$^+$CD25$^+$ T cells[85], or decreased autoreactive CD4$^+$ T cells[81]. In a mouse model, B-cell depletion inhibited CD4$^+$ but not CD8$^+$ T-cell activation and clonal expansion in response to new exogenous antigens. Therefore, adequate antigen-specific CD4$^+$ T-cell responses still

required the presence of B cells[88]. The limited data on T cell, NK cell, complement, and immunoglobulin levels following treatments with anti-CD20 mAbs other than rituximab were highly similar compared with those with rituximab[36,51-54,69]. Because of the immunodeficiency induced by anti-CD20 mAbs, hepatitis and other infections have become a growing concern since the approval of rituximab.

Hepatitis B virus

Acute hepatitis and even fulminant hepatic failure are a well-documented threat in patients receiving chemotherapy, particularly in lymphoma cases[89]. In a prospective study, 44% (34/78) of hepatitis B virus (HBV; hepatitis B surface antigen, $HBsAg^{+}$) carriers developed some form of hepatitis. Of these cases, 44% (15/34) were attributed to HBV reactivation[89]; 6 of these 15 were patients with lymphoma, and all of them had been treated with adriamycin, cyclophosphamide, vincristine, and prednisolone, also known as the CHOP regimen[89]. In addition, 4 of these 6 patients with lymphoma were seropositive for hepatitis B e antigen (HBeAg) at baseline and developed HBV reactivation sooner than the HBeAg-negative carriers[89]. In addition to those observations in lymphoma cases, HBV reactivation has been frequently reported in other hematological malignancies[90]. Corticosteroids, which are immunosuppressive agents frequently used in hematological malignancies and autoimmune diseases, may increase the risk of liver injury in HBV carriers[90,91]. Steroid-sparing regimens can be used to reduce the risk of HBV reactivation[92]. Additionally, adriamycin, a component of the CHOP regimen, can stimulate the replication of HBV[93]. Because the use of anti-CD20 mAb is common, designing a treatment plan that prevents the risk of HBV reactivation may be more complex than administrating chemotherapy for hematological malignancies or immunosuppressants for autoimmune diseases.

The natural history of HBV infection depends on the interaction of the host immunity, hepatocytes, and viral replication. Chronic HBV infections acquired early in life have 3 phases: the immune tolerance phase, the immune active phase, and the low-replication phase[94-96]. Some inactive HBV carriers (HBeAg seroconversion) can unexpectedly reenter the immune clearance phase and experience HBV reactivation with elevated HBV DNA and/or HBeAg reversion[97]. The incidence of HBV flares varies among studies and may depend on sex, HBV genotype, age at HBeAg seroconversion, and HBV DNA levels[96,97]. In adults, NK cells and type I interferon responses induced by HBV infection were observed before HBV-specific $CD4^{+}$ and $CD8^{+}$ T-cell responses[98]. In chronic HBV infections, HBV-specific $CD8^{+}$ T cells presented HBV antigens regardless of the status of antibody to hepatitis B core antigen (anti-HBc)[99]. Both anti-HBc^{+} patients and inactive HBV carriers exhibited strong memory $CD8^{+}$ T-cell responses[99]. However, the levels of $FoxP3^{+}$, $CD25^{+}$, and $CD4^{+}$ regulatory T cells that inhibit HBcAg-specific responses were also higher in chronic HBV infection cases[100].

HBV replication within the liver and the subsequent spread of virions into the circulation may occur in cancer patients with immunosuppression induced by the diseases or treatments. Hepatitis flares can develop early or late, during immunosuppressive therapy or after its completion. The effects can range from asymptomatic elevation of HBV DNA to fulminant hepatitis. HBV reactivation has been reported not only in patients undergoing chemotherapy or steroid therapy but also in patients undergoing rituximab treatment, regardless of their HBV status[101-104]. In $HBsAg^{+}$ carriers with B-cell NHL, HBV reactivation occurred in 80% (8/10) of patients without prophylaxis[105]. Significantly fewer cases of HBV reactivation were consistently observed in $HBsAg^{+}$ carriers with prophylaxis[101]. In patients with resolved HBV ($HBsAg^{-}$/anti-HBc^{+}) and B-cell NHL, the rituximab-CHOP regimen led to more cases of HBV reactivation (reverse seroconversion or elevated HBV DNA) than the CHOP regimen alone did (23.8% *vs* 0%)[102]. The incidence rates of HBV reactivation and HBV hepatitis flare were reported as 10.4 and 6.4 per person-year in this group of patients, respectively[106]. The timing of HBV reactivation is closely associated with the lymphopenic state. The reconstruction of host immunity induced by rituximab and the recovery of B- and T-lymphocytes after rituximab may result in damage to HBV-infected hepatocytes by cytotoxic T-lymphocytes[90,104,106,107]. The impairment of the immune system appears to be more severe in patients treated with rituximab-CHOP than in those treated with rituximab alone[107].

Rituximab treatments, with or without steroids, were generally well-tolerated by patients with autoimmune hematological disorders such as AIHA[60,108], ITP[61,109], acquired hemophilia[62], TTP[63], and cryoglobulinemia[64,65]. No HBV reactivation was reported, most likely because of the exclusion of patients with positive HBV serology, a limited number of cases, or lesser immunosuppression in these autoimmune diseases compared with hematological malignancies. However, HBV reactivation is a major threat in patients with autoimmune disorders who are undergoing immunosuppressive therapy[110]; therefore, concurrent antiviral treatments are often prescribed to reduce the risk of reactivation[111].

Patients with chronic or resolved HBV receiving RIT or anti-CD20 mAbs are subject to the same or an even greater risk of HBV reactivation, and although these effects were not fully characterized in most prospective trials[36-59], scattered cases have been reported[111,112], both of which were successfully treated with lamivudine[112,113]. The United States Food and Drug Administration announced that physicians should be alert to potential HBV reactivation caused by ofatumumab[114]. Liver toxicities had been reported with ofatumumab with or without chemotherapy in hematological malignancies. However, the data on HBV reactivation in these cases were lacking[45,50,115,116].

HBV is endemic in the Asia-Pacific region with a prevalence of more than 10% in Taiwan, southern China, and certain areas of Southeast Asia[117]. In clinical prac-

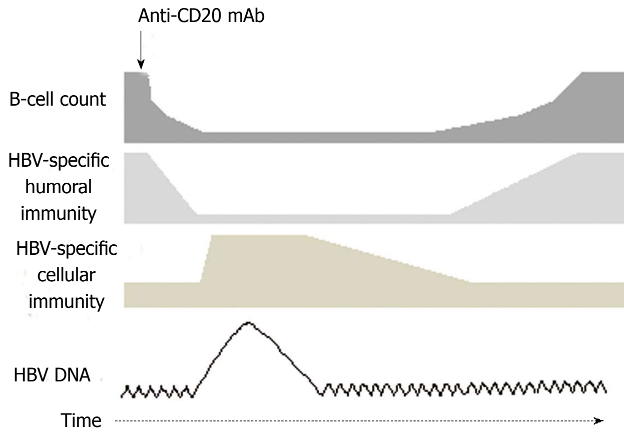


Figure 1 Anti-CD20 monoclonal antibodies and dynamic immunity in hepatitis B virus infection. This illustration summarizes the dynamic changes of hepatitis B virus (HBV) DNA and immune responses during HBV reactivation. Notably, the viral-specific T-cell immunity before and after the administration of anti-CD20 monoclonal antibody (mAb) depends on the carrier status, that is, hepatitis B surface antigen (HBsAg)$^+$ carrier or resolved HBV (anti-HBc$^+$/HBsAg$^-$, anti-HBs$^+$ or $^-$) status. HBV prophylaxis should be considered to maintain low level of HBV replication and prevent HBV-specific T-cell-mediated liver damage. The prophylaxis should be maintained for at least 6 mo after anti-CD20 mAb when HBV-specific T-cell immunity recovers.

tice, a greater than 10-fold increase above the baseline in serum HBV DNA levels, an absolute increase of more than 9 $\log_{10}$ copies/mL, a reappearance of HBV DNA in HBsAg, or a new onset of HBV DNA viremia in HBsAg-negative patients can be considered as an indication of HBV reactivation. In addition, an hepatitis flare is considered if the serum alanine transaminase (ALT) level is 3 times greater than the normal upper limit, or if an ALT level of more than 100 IU/L with a concomitantly increased HBV DNA level of more than 10 times the typical level is detected[89,118]. In high-risk endemic areas, HBV screening must be considered before implementing anti-CD20 mAb treatment[119-123]. In a cost-effectiveness study, screening for HBsAg in all patients about to receive rituximab-CHOP treatment considerably reduced the rate of HBV reactivation and cost the least[124]. The recommended tests for screening include HBsAg, anti-HBc, and anti-HBs (according to the CDC)[122], HBsAg and anti-HBc (AASLD and EASL)[121,125], and HBsAg with or without anti-HBc (ASCO)[123]. A more complex algorithm for screening in different at-risk populations was proposed[126]. Currently, oral agents approved for HBV treatment include lamivudine, adefovir, telbivudine, tenofovir, and entecavir. Based on research findings, the optimal start time and duration of HBV prophylaxis has not yet been determined[119-123]; however, initiating short-term antiviral therapy before starting anti-CD20 mAb treatment appears to be beneficial and safe. In a randomized trial involving HBsAg$^+$ patients with NHL, the prophylactic use of lamivudine reduced considerably the occurrence of HBV reactivation and hepatitis flares[127]. In a meta-analysis, all-cause mortality, HBV reactivation, HBV-related mortality, and interruption of anti-CD20 mAb therapy were considerably reduced with lamivudine prophylaxis[128]. One major problem of lamivudine, telbivudine, and adefovir is that drug resistance and hepatitis flares increase with continuous use[125,129]; therefore, the use of entecavir and tenofovir was suggested for longer duration of prophylaxis[125]. In addition to HBsAg$^+$ patients, HBV prophylaxis must be considered in patients with resolved HBV because the risk of reactivation remains (Figure 1)[106,130]. In a recent retrospective study of HBV reactivation by the Asia Lymphoma Study Group, the authors showed that patients receiving entecavir prophylaxis had a lesser incidence of HBV reactivation than those with lamivudine. Prospective studies to validate these findings are warranted.

Hepatitis C virus

The epidemiology of hepatitis C virus (HCV) differs from that of HBV, particularly because of the existence of a wide variation of HCV genotypes worldwide[131]. The most common types in Taiwan, China, Japan, and Korea are genotypes 1b and 2; by contrast, more diversity exists in North America and Europe[131]. The natural history of HCV infection consists of ramp-up and plateau phases in acute infections and various spontaneous clearances in chronic phases depending on several viral and host factors, including HCV genotypes, host immunity, and the genetic polymorphism of *IFNL3* (*IL-28B*)[131]. The treatment of chronic HCV consists primarily of interferon-α, ribavirin, and protease inhibitors that are accompanied by major toxicities[131].

During acute infections, HCV-specific T cells activated by CD8$^+$ and CD4$^+$ are generated readily against multiple epitopes within 10 wk[132]. In patients with chronic infections and persistent viremia, HCV-specific CD8$^+$ T cells are rarer and responsive to fewer epitopes than in those without viremia, where even HCV-specific CD8$^+$ T cell responses are mounted[132]. In addition, CD8$^+$ T cells are exhausted in chronic HCV with increased expression of programmed death-1 and cytotoxic T-lymphocyte-associated antigen-4[133]. HCV-specific CD8$^+$ T cells are suppressed by increased CD25$^+$ and CD4$^+$ regulatory T cells[134]. In addition to evading CD8$^+$ T-cell responses, HCV also evades CD4$^+$ T-cell responses and humoral immunity with escape mutants because of its error-prone RNA polymerase[135-137].

HCV itself is highly associated with the development of lymphoproliferative disorders, particularly with B-cell NHL with or without mixed cryoglobulinemia[138,139]. HCV-related fulminant hepatitis occurring after chemotherapy with or without corticosteroids was rare in patients with lymphoma[140,141]. Liver dysfunction after chemotherapy was less common in HCV than in HBV patients (18.2% *vs* 75.0%) with hematological malignancies[142]. The incidence of HCV-associated liver dysfunction appeared to be higher in rituximab-containing regimens[143,144]. In addition, increased HCV RNA levels were common during or after rituximab-based chemotherapy and were followed by hepatitis flares and decreased HCV RNA levels in various intervals[144-146]. In contrast to persistent B-cell depletion for several months after rituximab treatments, the HCV viral load and the number of

regulatory T cells were elevated initially, then decreased, indicating that B-cell depletion and HCV-specific T-cell responses participate in the mechanism of HCV reactivation in patients treated with rituximab-based regimens[64,147,148]. Although the elevation of HCV RNA load was not the major problem for lymphoma patients treated with standard courses of immunochemotherapy, the persistent elevation of HCV RNA load and subsequent liver cirrhosis can occur in follicular lymphoma patients treated with rituximab-maintenance therapy[147]. However, these studies evaluated HCV reactivation during and after rituximab regimens according to different criteria. The HCV RNA levels may increase up to 10 times above the baseline in chronic HCV infections[149]. In a retrospective study involving cancer patients, the acute exacerbation of chronic HCV was defined as a 3-fold or greater increase in ALT levels without tumor infiltration within the liver, without the use of hepatotoxic drugs or blood transfusion, and no concomitant systemic infections[150]. In addition, an at least 1 $\log_{10}$ IU/mL increase in HCV RNA levels after treatment with immunosuppressive agents was considered for HCV reactivation[150]. The same criteria may apply for HCV reactivation in anti-CD20 mAb treatments. A simple algorithm for monitoring ALT and HCV RNA in patients undergoing immunosuppressive therapy was proposed[151]. Although rituximab was also widely used in autoimmune hematological disorders such as HCV-related cryoglobulinemia, HCV reactivation with hepatitis flares was rare[65,152,153]. No data were available for RIT and non-rituximab anti-CD20 mAbs.

Traditionally, anti-HCV therapy is not considered in patients receiving immunosuppressive agents because of potential drug-drug interactions, the major side effects of anti-HCV therapy, and the rarity of severe HCV-related hepatitis flares. Currently, there is no consensus regarding the optimal strategy for the treatment and prevention of HCV reactivation in patients undergoing immunosuppressive therapy even though ribavirin with or without interferon-α has been successfully administered to patients with hematological malignancies[154,155].

Parvovirus B19

Parvovirus B19 infections are common infections that spread through respiratory droplets or blood, and seropositivity rates are increasing in people of all ages[156]. The disease spectrum can range from asymptomatic disease to hydrops fetalis, fifth disease, arthropathy, aplastic anemia, autoimmune disorders, meningitis, encephalitis, and even fulminant hepatitis[156,157]. This virus has a tropism for erythroid progenitors in the blood, bone marrow, and fetal liver[156]. In healthy adults, acute infections result in viremia within 2 wk, immediately followed by virus-specific IgM and IgG responses and clearance of the virus in the serum[158]. Humoral immunity appears to be critical for controlling this virus, and patients with immunodeficiency disorders can have chronic infections[159]. Virus-specific $CD8^+$ T cell responses toward multiple epitopes also develop soon after acute infection, and these striking $CD8^+$ T-cell responses may have long durations with continuous viremia[160]. In addition, interferon-γ-secreting, virus-specific $CD62L^+$ and $CD4^+$ T cells developed within 3 mo of acute infection[161].

Parvovirus B19 infection is a major problem in immunocompromised hosts[162-164]. The seropositivity rate was high in cancer patients receiving chemotherapy[162,165], and half of patients exhibited detectable viral DNA in their serum[165]. Several studies have demonstrated that acute parvovirus B19 infections are associated with fever, arthralgia, hepatitis, myocarditis, pneumonia, pancytopenia, and even graft dysfunction[162-164]. Most importantly, immunosuppressive therapies can impair humoral immunity, exposing patients to a high risk of parvovirus B19 infection[164,166]. Several case studies have reported that parvovirus B19 infection-related symptoms can develop in B-cell NHL or immune thrombocytopenia patients treated with rituximab-containing regimens or after being treated with rituximab-containing regimens[167-172]. The major parvovirus B19 infection-related symptom was cytopenia in the erythroid lineage, but neutropenia or thrombocytopenia without anemia occurred in some patients. The onset was preceded by fever and skin eruptions in 2 patients[169,171]. Our group identified the first case with acute hepatitis[172]. Most patients developed the clinical manifestations at least 2 mo after the initiation of rituximab. The patients in 2 cases recovered without treatment, and the others responded positively to intravenous immunoglobulin (IVIG). However, the hepatitis flare in our patient persisted for 7 mo and was paralleled with cytopenia, which was correlated with the recovery of B cells after rituximab treatment[172]. The effects of other anti-CD20 mAbs on parvovirus B19 are unknown.

The diagnosis of parvovirus B19 reactivation or infection is based on serology and viral DNA analysis[156]. However, conducting virus-specific serology may be problematic in immunocompromised hosts[164,166]. The pathogenesis of parvovirus B19-related hepatitis is largely unknown, and either direct cytopathic or indirect immunity-related mechanisms are possible. In addition, the pathology images obtained during liver biopsies are nonspecific. Although viral DNA or RNA can be detected in hepatocytes, the clinical significance remains to be defined[173]. The most reasonable diagnostic sequence may be to exclude the other common hepatotropic viruses first, such as HBV or HCV, and then to analyze serum serology and viral DNA if the tests for hepatotropic viruses are negative. Because liver biopsy is invasive, it must be considered last. The most effective treatment and prevention methods for parvovirus B19-related hepatitis remain unknown; however, IVIG can be used for treating severe cases because of the clinical success achieved in using it in other parvovirus B19-related diseases[156,166-169,171].

Other causes

In addition to HBV, HCV, and parvovirus B19, critical viral infections from cytomegalovirus, varicella-zoster virus (VZV), herpes simplex virus, echovirus, enterovirus,

influenza A virus, or BK/JC virus can occur in lymphoma patients treated with rituximab regimens[174]. Most of them did not induce hepatitis. However, there are reports of 2 cases of hepatitis associated with adenovirus, one case of hepatic necrosis associated with disseminated VZV, and one of chronic hepatitis E virus infection that developed after rituximab treatment[175-178]. These pathogens are rarely connected to the use of rituximab, and the underlying mechanisms of these pathogeneses associated with hepatitis are less characterized. However, the actual incidence rates of these uncommon viral infections in lymphoma patients treated with rituximab regimens may be underestimated. One patient with ITP experienced drug-induced acute hepatitis, and the pathogen was not identified; however, the patient recovered soon after rituximab treatment was stopped[179]. There were also scattered reports of anti-CD20 mAb-related liver function abnormalities in patients with hematological disorders. However, the etiology was most likely related to anti-CD20 mAb[45,50,51,55,69,115,116].

Future in vivo or in vitro studies

There are numerous reports of animal lymphoma models to test the preclinical activity of anti-CD20 mAbs[180-182]. However, the safety data in liver toxicities of these animal models are very limited[180-182]. In addition, no animal or in vitro models of viral hepatitis-induced by anti-CD20 mAbs has been established. It would be very difficult to establish the animal model with viral hepatitis reactivated by anti-CD20 mAbs because most of previous studies used tumor xenografts implanted into mice with severe combined immunodeficiency in their animal model. However, immune-mediated liver damage is important for anti-CD20 mAb-associated viral hepatitis. Most likely, this may work in evaluating the precise mechanism of hepatitis caused by the aforementioned viruses during and after anti-CD20 mAbs alone when using the immunocompetent animal model.

CONCLUSION

This review summarized the clinical use of anti-CD20 mAbs and reports of acute or chronic hepatitis associated with the use of these agents. Most data are from cases where rituximab was used to treat hematological malignancies. The majority of diseases studied were caused by viral infections. These infections must be clinically recognized soon after their occurrence because they not only interrupt immunotherapy but are also associated with high mortality and morbidity. Close monitoring of HBV and HCV infections before and during anti-CD20 mAb treatment is highly recommended in endemic areas. The prophylactic therapy for HBV has become standard of care, but patient selection and the optimal regimen or duration remain to be defined. Physicians must monitor patients being treated with rituximab-based regimens for the risks of hepatotropic viruses, including HBV, HCV, parvovirus B19, and other viruses. Nevertheless, this review has limitations. Some pathogens that rarely induce hepatitis may not be reported in the literature; therefore, the causal relation, epidemiology, and pathogenesis of these pathogens are not the most accurate. Occasionally, the causal association between hepatitis and rituximab is difficult to confirm because of the concurrent use of multiple immunosuppressants or hepatotoxic drugs. The data on non-rituximab anti-CD20 mAbs are limited, likely because most clinical trials employ strict case selection criteria and exclude patients with hepatitis. Therefore, in vivo or in vitro studies are warranted to establish the actual roles of these viruses in the pathogenesis of hepatitis during and after anti-CD20 mAb treatments.

REFERENCES

1 **Stashenko P**, Nadler LM, Hardy R, Schlossman SF. Characterization of a human B lymphocyte-specific antigen. *J Immunol* 1980; **125**: 1678-1685 [PMID: 6157744]

2 **Stashenko P**, Nadler LM, Hardy R, Schlossman SF. Expression of cell surface markers after human B lymphocyte activation. *Proc Natl Acad Sci USA* 1981; **78**: 3848-3852 [PMID: 6973760]

3 **Nadler LM**, Ritz J, Hardy R, Pesando JM, Schlossman SF, Stashenko P. A unique cell surface antigen identifying lymphoid malignancies of B cell origin. *J Clin Invest* 1981; **67**: 134-140 [PMID: 6969730]

4 **Bubien JK**, Zhou LJ, Bell PD, Frizzell RA, Tedder TF. Transfection of the CD20 cell surface molecule into ectopic cell types generates a Ca2+ conductance found constitutively in B lymphocytes. *J Cell Biol* 1993; **121**: 1121-1132 [PMID: 7684739]

5 **Polyak MJ**, Li H, Shariat N, Deans JP. CD20 homo-oligomers physically associate with the B cell antigen receptor. Dissociation upon receptor engagement and recruitment of phosphoproteins and calmodulin-binding proteins. *J Biol Chem* 2008; **283**: 18545-18552 [PMID: 18474602 DOI: 10.1074/jbc.M800784200]

6 **Petrie RJ**, Deans JP. Colocalization of the B cell receptor and CD20 followed by activation-dependent dissociation in distinct lipid rafts. *J Immunol* 2002; **169**: 2886-2891 [PMID: 12218101]

7 **Li H**, Ayer LM, Lytton J, Deans JP. Store-operated cation entry mediated by CD20 in membrane rafts. *J Biol Chem* 2003; **278**: 42427-42434 [PMID: 12920111 DOI: 10.1074/jbc.M308802200]

8 **Golay JT**, Clark EA, Beverley PC. The CD20 (Bp35) antigen is involved in activation of B cells from the G0 to the G1 phase of the cell cycle. *J Immunol* 1985; **135**: 3795-3801 [PMID: 2415587]

9 **Tedder TF**, Forsgren A, Boyd AW, Nadler LM, Schlossman SF. Antibodies reactive with the B1 molecule inhibit cell cycle progression but not activation of human B lymphocytes. *Eur J Immunol* 1986; **16**: 881-887 [PMID: 3091375 DOI: 10.1002/eji.1830160802]

10 **Holder M**, Grafton G, MacDonald I, Finney M, Gordon J. Engagement of CD20 suppresses apoptosis in germinal center B cells. *Eur J Immunol* 1995; **25**: 3160-3164 [PMID: 7489758 DOI: 10.1002/eji.1830251126]

11 **Tedder TF**, Engel P. CD20: a regulator of cell-cycle progression of B lymphocytes. *Immunol Today* 1994; **15**: 450-454 [PMID: 7524522 DOI: 10.1016/0167-5699(94)90276-3]

12 **Reff ME**, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, Newman RA, Hanna N, Anderson DR. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood* 1994; **83**: 435-445 [PMID: 7506951]

13 **Shan D**, Ledbetter JA, Press OW. Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies. *Blood* 1998; **91**: 1644-1652 [PMID: 9473230]
14 **McLaughlin P**, Grillo-López AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol* 1998; **16**: 2825-2833 [PMID: 9704735]
15 **Cragg MS**, Glennie MJ. Antibody specificity controls in vivo effector mechanisms of anti-CD20 reagents. *Blood* 2004; **103**: 2738-2743 [PMID: 14551143 DOI: 10.1182/blood-2003-06-2031]
16 **Beers SA**, Chan CH, French RR, Cragg MS, Glennie MJ. CD20 as a target for therapeutic type I and II monoclonal antibodies. *Semin Hematol* 2010; **47**: 107-114 [PMID: 20350657 DOI: 10.1053/j.seminhematol.2010.01.001]
17 **Avivi I**, Stroopinsky D, Katz T. Anti-CD20 monoclonal antibodies: beyond B-cells. *Blood Rev* 2013; **27**: 217-223 [PMID: 23953071 DOI: 10.1016/j.blre.2013.07.002]
18 **Piro LD**, White CA, Grillo-López AJ, Janakiraman N, Saven A, Beck TM, Varns C, Shuey S, Czuczman M, Lynch JW, Kolitz JE, Jain V. Extended Rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or refractory low-grade or follicular non-Hodgkin's lymphoma. *Ann Oncol* 1999; **10**: 655-661 [PMID: 10442187]
19 **Davis TA**, Grillo-López AJ, White CA, McLaughlin P, Czuczman MS, Link BK, Maloney DG, Weaver RL, Rosenberg J, Levy R. Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: safety and efficacy of retreatment. *J Clin Oncol* 2000; **18**: 3135-3143 [PMID: 10963642]
20 **Hainsworth JD**, Burris HA, Morrissey LH, Litchy S, Scullin DC, Bearden JD, Richards P, Greco FA. Rituximab monoclonal antibody as initial systemic therapy for patients with low-grade non-Hodgkin lymphoma. *Blood* 2000; **95**: 3052-3056 [PMID: 10807768]
21 **van Oers MH**, Klasa R, Marcus RE, Wolf M, Kimby E, Gascoyne RD, Jack A, Van't Veer M, Vranovsky A, Holte H, van Glabbeke M, Teodorovic I, Rozewicz C, Hagenbeek A. Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: results of a prospective randomized phase 3 intergroup trial. *Blood* 2006; **108**: 3295-3301 [PMID: 16873669 DOI: 10.1182/blood-2006-05-021113]
22 **Forstpointner R**, Dreyling M, Repp R, Hermann S, Hänel A, Metzner B, Pott C, Hartmann F, Rothmann F, Rohrberg R, Böck HP, Wandt H, Unterhalt M, Hiddemann W. The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood* 2004; **104**: 3064-3071 [PMID: 15284112 DOI: 10.1182/blood-2004-04-1323]
23 **Marcus R**, Imrie K, Belch A, Cunningham D, Flores E, Catalano J, Solal-Celigny P, Offner F, Walewski J, Raposo J, Jack A, Smith P. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. *Blood* 2005; **105**: 1417-1423 [PMID: 15494430 DOI: 10.1182/blood-2004-08-3175]
24 **Hiddemann W**, Kneba M, Dreyling M, Schmitz N, Lengfelder E, Schmits R, Reiser M, Metzner B, Harder H, Hegewisch-Becker S, Fischer T, Kropff M, Reis HE, Freund M, Wörmann B, Fuchs R, Planker M, Schimke J, Eimermacher H, Trümper L, Aldaoud A, Parwaresch R, Unterhalt M. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood* 2005; **106**: 3725-3732 [PMID: 16123223 DOI: 10.1182/blood-2005-01-0016]
25 **Herold M**, Haas A, Srock S, Neser S, Al-Ali KH, Neubauer A, Dölken G, Naumann R, Knauf W, Freund M, Rohrberg R, Höffken K, Franke A, Ittel T, Kettner E, Haak U, Mey U, Klinkenstein C, Assmann M, von Grünhagen U. Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: an East German Study Group Hematology and Oncology Study. *J Clin Oncol* 2007; **25**: 1986-1992 [PMID: 17420513 DOI: 10.1200/JCO.2006.06.4618]
26 **Hochster H**, Weller E, Gascoyne RD, Habermann TM, Gordon LI, Ryan T, Zhang L, Colocci N, Frankel S, Horning SJ. Maintenance rituximab after cyclophosphamide, vincristine, and prednisone prolongs progression-free survival in advanced indolent lymphoma: results of the randomized phase III ECOG1496 Study. *J Clin Oncol* 2009; **27**: 1607-1614 [PMID: 19255334 DOI: 10.1200/JCO.2008.17.1561]
27 **Forstpointner R**, Unterhalt M, Dreyling M, Böck HP, Repp R, Wandt H, Pott C, Seymour JF, Metzner B, Hänel A, Lehmann T, Hartmann F, Einsele H, Hiddemann W. Maintenance therapy with rituximab leads to a significant prolongation of response duration after salvage therapy with a combination of rituximab, fludarabine, cyclophosphamide, and mitoxantrone (R-FCM) in patients with recurring and refractory follicular and mantle cell lymphomas: Results of a prospective randomized study of the German Low Grade Lymphoma Study Group (GLSG). *Blood* 2006; **108**: 4003-4008 [PMID: 16946304 DOI: 10.1182/blood-2006-04-016725]
28 **Salles G**, Seymour JF, Offner F, López-Guillermo A, Belada D, Xerri L, Feugier P, Bouabdallah R, Catalano JV, Brice P, Caballero D, Haioun C, Pedersen LM, Delmer A, Simpson D, Leppa S, Soubeyran P, Hagenbeek A, Casasnovas O, Intragumtornchai T, Fermé C, da Silva MG, Sebban C, Lister A, Estell JA, Milone G, Sonet A, Mendila M, Coiffier B, Tilly H. Rituximab maintenance for 2 years in patients with high tumour burden follicular lymphoma responding to rituximab plus chemotherapy (PRIMA): a phase 3, randomised controlled trial. *Lancet* 2011; **377**: 42-51 [PMID: 21176949 DOI: 10.1016/S0140-6736(10)62175-7]
29 **Coiffier B**, Haioun C, Ketterer N, Engert A, Tilly H, Ma D, Johnson P, Lister A, Feuring-Buske M, Radford JA, Capdeville R, Diehl V, Reyes F. Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. *Blood* 1998; **92**: 1927-1932 [PMID: 9731049]
30 **Coiffier B**, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, Morel P, Van Den Neste E, Salles G, Gaulard P, Reyes F, Lederlin P, Gisselbrecht C. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346**: 235-242 [PMID: 11807147 DOI: 10.1056/NEJMoa011795]
31 **Pfreundschuh M**, Trümper L, Osterborg A, Pettengell R, Trneny M, Imrie K, Ma D, Gill D, Walewski J, Zinzani PL, Stahel R, Kvaloy S, Shpilberg O, Jaeger U, Hansen M, Lehtinen T, López-Guillermo A, Corrado C, Scheliga A, Milpied N, Mendila M, Rashford M, Kuhnt E, Loeffler M. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. *Lancet Oncol* 2006; **7**: 379-391 [PMID: 16648042 DOI: 10.1016/S1470-2045(06)70664-7]
32 **Pfreundschuh M**, Schubert J, Ziepert M, Schmits R, Mohren

M, Lengfelder E, Reiser M, Nickenig C, Clemens M, Peter N, Bokemeyer C, Eimermacher H, Ho A, Hoffmann M, Mertelsmann R, Trümper L, Balleisen L, Liersch R, Metzner B, Hartmann F, Glass B, Poeschel V, Schmitz N, Ruebe C, Feller AC, Loeffler M. Six versus eight cycles of bi-weekly CHOP-14 with or without rituximab in elderly patients with aggressive CD20+ B-cell lymphomas: a randomised controlled trial (RICOVER-60). *Lancet Oncol* 2008; **9**: 105-116 [PMID: 18226581 DOI: 10.1016/S1470-2045(08)70002-0]

33 **Ketterer N**, Coiffier B, Thieblemont C, Fermé C, Brière J, Casasnovas O, Bologna S, Christian B, Connerotte T, Récher C, Bordessoule D, Fruchart C, Delarue R, Bonnet C, Morschhauser F, Anglaret B, Soussain C, Fabiani B, Tilly H, Haioun C. Phase III study of ACVBP versus ACVBP plus rituximab for patients with localized low-risk diffuse large B-cell lymphoma (LNH03-1B). *Ann Oncol* 2013; **24**: 1032-1037 [PMID: 23235801 DOI: 10.1093/annonc/mds600]

34 **Habermann TM**, Weller EA, Morrison VA, Gascoyne RD, Cassileth PA, Cohn JB, Dakhil SR, Woda B, Fisher RI, Peterson BA, Horning SJ. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. *J Clin Oncol* 2006; **24**: 3121-3127 [PMID: 16754935 DOI: 10.1200/JCO.2005.05.1003]

35 **Gisselbrecht C**, Schmitz N, Mounier N, Singh Gill D, Linch DC, Trneny M, Bosly A, Milpied NJ, Radford J, Ketterer N, Shpilberg O, Dührsen U, Hagberg H, Ma DD, Viardot A, Lowenthal R, Brière J, Salles G, Moskowitz CH, Glass B. Rituximab maintenance therapy after autologous stem-cell transplantation in patients with relapsed CD20(+) diffuse large B-cell lymphoma: final analysis of the collaborative trial in relapsed aggressive lymphoma. *J Clin Oncol* 2012; **30**: 4462-4469 [PMID: 23091101 DOI: 10.1200/JCO.2012.41.9416]

36 **Witzig TE**, Gordon LI, Cabanillas F, Czuczman MS, Emmanouilides C, Joyce R, Pohlman BL, Bartlett NL, Wiseman GA, Padre N, Grillo-López AJ, Multani P, White CA. Randomized controlled trial of yttrium-90-labeled ibritumomab tiuxetan radioimmunotherapy versus rituximab immunotherapy for patients with relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma. *J Clin Oncol* 2002; **20**: 2453-2463 [PMID: 12011122 DOI: 10.1200/JCO.2002.11.076]

37 **Esmaeli B**, McLaughlin P, Pro B, Samaniego F, Gayed I, Hagemeister F, Romaguera J, Cabanillas F, Neelapu SS, Banay R, Fayad L, Wayne Saville M, Kwak LW. Prospective trial of targeted radioimmunotherapy with Y-90 ibritumomab tiuxetan (Zevalin) for front-line treatment of early-stage extranodal indolent ocular adnexal lymphoma. *Ann Oncol* 2009; **20**: 709-714 [PMID: 19150940 DOI: 10.1093/annonc/mdn692]

38 **Morschhauser F**, Radford J, Van Hoof A, Vitolo U, Soubeyran P, Tilly H, Huijgens PC, Kolstad A, d'Amore F, Gonzalez Diaz M, Petrini M, Sebban C, Zinzani PL, van Oers MH, van Putten W, Bischof-Delaloye A, Rohatiner A, Salles G, Kuhlmann J, Hagenbeek A. Phase III trial of consolidation therapy with yttrium-90-ibritumomab tiuxetan compared with no additional therapy after first remission in advanced follicular lymphoma. *J Clin Oncol* 2008; **26**: 5156-5164 [PMID: 18854568 DOI: 10.1200/JCO.2008.17.2015]

39 **Vose JM**, Wahl RL, Saleh M, Rohatiner AZ, Knox SJ, Radford JA, Zelenetz AD, Tidmarsh GF, Stagg RJ, Kaminski MS. Multicenter phase II study of iodine-131 tositumomab for chemotherapy-relapsed/refractory low-grade and transformed low-grade B-cell non-Hodgkin's lymphomas. *J Clin Oncol* 2000; **18**: 1316-1323 [PMID: 10715303]

40 **Kaminski MS**, Tuck M, Estes J, Kolstad A, Ross CW, Zasadny K, Regan D, Kison P, Fisher S, Kroll S, Wahl RL. 131I-tositumomab therapy as initial treatment for follicular lymphoma. *N Engl J Med* 2005; **352**: 441-449 [PMID: 15689582 DOI: 10.1056/NEJMoa041511]

41 **Kaminski MS**, Radford JA, Gregory SA, Leonard JP, Knox SJ, Kroll S, Wahl RL. Re-treatment with I-131 tositumomab in patients with non-Hodgkin's lymphoma who had previously responded to I-131 tositumomab. *J Clin Oncol* 2005; **23**: 7985-7993 [PMID: 16204016 DOI: 10.1200/JCO.2005.01.0892]

42 **Press OW**, Unger JM, Braziel RM, Maloney DG, Miller TP, LeBlanc M, Gaynor ER, Rivkin SE, Fisher RI. A phase 2 trial of CHOP chemotherapy followed by tositumomab/iodine I 131 tositumomab for previously untreated follicular non-Hodgkin lymphoma: Southwest Oncology Group Protocol S9911. *Blood* 2003; **102**: 1606-1612 [PMID: 12738671 DOI: 10.1182/blood-2003-01-0287]

43 **Press OW**, Unger JM, Rimsza LM, Friedberg JW, LeBlanc M, Czuczman MS, Kaminski M, Braziel RM, Spier C, Gopal AK, Maloney DG, Cheson BD, Dakhil SR, Miller TP, Fisher RI. Phase III randomized intergroup trial of CHOP plus rituximab compared with CHOP chemotherapy plus (131)iodine-tositumomab for previously untreated follicular non-Hodgkin lymphoma: SWOG S0016. *J Clin Oncol* 2013; **31**: 314-320 [PMID: 23233710 DOI: 10.1200/JCO.2012.42.4101]

44 **Vose JM**, Carter S, Burns LJ, Ayala E, Press OW, Moskowitz CH, Stadtmauer EA, Mineshi S, Ambinder R, Fenske T, Horowitz M, Fisher R, Tomblyn M. Phase III randomized study of rituximab/carmustine, etoposide, cytarabine, and melphalan (BEAM) compared with iodine-131 tositumomab/BEAM with autologous hematopoietic cell transplantation for relapsed diffuse large B-cell lymphoma: results from the BMT CTN 0401 trial. *J Clin Oncol* 2013; **31**: 1662-1668 [PMID: 23478060 DOI: 10.1200/JCO.2012.45.9453]

45 **Coiffier B**, Lepretre S, Pedersen LM, Gadeberg O, Fredriksen H, van Oers MH, Wooldridge J, Kloczko J, Holowiecki J, Hellmann A, Walewski J, Flensburg M, Petersen J, Robak T. Safety and efficacy of ofatumumab, a fully human monoclonal anti-CD20 antibody, in patients with relapsed or refractory B-cell chronic lymphocytic leukemia: a phase 1-2 study. *Blood* 2008; **111**: 1094-1100 [PMID: 18003886 DOI: 10.1182/blood-2007-09-111781]

46 **Hagenbeek A**, Gadeberg O, Johnson P, Pedersen LM, Walewski J, Hellmann A, Link BK, Robak T, Wojtukiewicz M, Pfreundschuh M, Kneba M, Engert A, Sonneveld P, Flensburg M, Petersen J, Losic N, Radford J. First clinical use of ofatumumab, a novel fully human anti-CD20 monoclonal antibody in relapsed or refractory follicular lymphoma: results of a phase 1/2 trial. *Blood* 2008; **111**: 5486-5495 [PMID: 18390837 DOI: 10.1182/blood-2007-10-117671]

47 **Lemery SJ**, Zhang J, Rothmann MD, Yang J, Earp J, Zhao H, McDougal A, Pilaro A, Chiang R, Gootenberg JE, Keegan P, Pazdur R. U.S. Food and Drug Administration approval: ofatumumab for the treatment of patients with chronic lymphocytic leukemia refractory to fludarabine and alemtuzumab. *Clin Cancer Res* 2010; **16**: 4331-4338 [PMID: 20601446 DOI: 10.1158/1078-0432.CCR-10-0570]

48 **Wierda WG**, Kipps TJ, Dürig J, Griskevicius L, Stilgenbauer S, Mayer J, Smolej L, Hess G, Griniute R, Hernandez-Ilizaliturri FJ, Padmanabhan S, Gorczyca M, Chang CN, Chan G, Gupta I, Nielsen TG, Russell CA. Chemoimmunotherapy with O-FC in previously untreated patients with chronic lymphocytic leukemia. *Blood* 2011; **117**: 6450-6458 [PMID: 21498674 DOI: 10.1182/blood-2010-12-323980]

49 **Czuczman MS**, Fayad L, Delwail V, Cartron G, Jacobsen E, Kuliczkowski K, Link BK, Pinter-Brown L, Radford J, Hellmann A, Gallop-Evans E, DiRienzo CG, Goldstein N, Gupta I, Jewell RC, Lin TS, Lisby S, Schultz M, Russell CA, Hagenbeek A. Ofatumumab monotherapy in rituximab-refractory follicular lymphoma: results from a multicenter study. *Blood* 2012; **119**: 3698-3704 [PMID: 22389254 DOI: 10.1182/blood-2011-09-378323]

50 **Coiffier B**, Radford J, Bosly A, Martinelli G, Barca G, Davies A, Decaudin D, Gallop-Evans E, Padmanabhan-Iyer S, Van Eygen K, Wu KL, Gupta IV, Lin TS, Goldstein N, Jewell RC, Winter P, Lisby S. A multicentre, phase II trial of ofatu-

mumab monotherapy in relapsed/progressive diffuse large B-cell lymphoma. *Br J Haematol* 2013; **163**: 334-342 [PMID: 24032456 DOI: 10.1111/bjh.12537]

51 **Morschhauser F**, Leonard JP, Fayad L, Coiffier B, Petillon MO, Coleman M, Schuster SJ, Dyer MJ, Horne H, Teoh N, Wegener WA, Goldenberg DM. Humanized anti-CD20 antibody, veltuzumab, in refractory/recurrent non-Hodgkin's lymphoma: phase I/II results. *J Clin Oncol* 2009; **27**: 3346-3353 [PMID: 19451441 DOI: 10.1200/JCO.2008.19.9117]

52 **Morschhauser F**, Marlton P, Vitolo U, Lindén O, Seymour JF, Crump M, Coiffier B, Foà R, Wassner E, Burger HU, Brennan B, Mendila M. Results of a phase I/II study of ocrelizumab, a fully humanized anti-CD20 mAb, in patients with relapsed/refractory follicular lymphoma. *Ann Oncol* 2010; **21**: 1870-1876 [PMID: 20157180 DOI: 10.1093/annonc/mdq027]

53 **Salles G**, Morschhauser F, Lamy T, Milpied N, Thieblemont C, Tilly H, Bieska G, Asikanius E, Carlile D, Birkett J, Pisa P, Cartron G. Phase 1 study results of the type II glycoengineered humanized anti-CD20 monoclonal antibody obinutuzumab (GA101) in B-cell lymphoma patients. *Blood* 2012; **119**: 5126-5132 [PMID: 22431570 DOI: 10.1182/blood-2012-01-404368]

54 **Sehn LH**, Assouline SE, Stewart DA, Mangel J, Gascoyne RD, Fine G, Frances-Lasserre S, Carlile DJ, Crump M. A phase 1 study of obinutuzumab induction followed by 2 years of maintenance in patients with relapsed CD20-positive B-cell malignancies. *Blood* 2012; **119**: 5118-5125 [PMID: 22438256 DOI: 10.1182/blood-2012-02-408773]

55 **Salles GA**, Morschhauser F, Solal-Céligny P, Thieblemont C, Lamy T, Tilly H, Gyan E, Lei G, Wenger M, Wassner-Fritsch E, Cartron G. Obinutuzumab (GA101) in patients with relapsed/refractory indolent non-Hodgkin lymphoma: results from the phase II GAUGUIN study. *J Clin Oncol* 2013; **31**: 2920-2926 [PMID: 23835715 DOI: 10.1200/JCO.2012.46.9718]

56 **Morschhauser FA**, Cartron G, Thieblemont C, Solal-Céligny P, Haioun C, Bouabdallah R, Feugier P, Bouabdallah K, Asikanius E, Lei G, Wenger M, Wassner-Fritsch E, Salles GA. Obinutuzumab (GA101) monotherapy in relapsed/refractory diffuse large b-cell lymphoma or mantle-cell lymphoma: results from the phase II GAUGUIN study. *J Clin Oncol* 2013; **31**: 2912-2919 [PMID: 23835718 DOI: 10.1200/JCO.2012.46.9585]

57 **Radford J**, Davies A, Cartron G, Morschhauser F, Salles G, Marcus R, Wenger M, Lei G, Wassner-Fritsch E, Vitolo U. Obinutuzumab (GA101) plus CHOP or FC in relapsed/refractory follicular lymphoma: results of the GAUDI study (BO21000). *Blood* 2013; **122**: 1137-1143 [PMID: 23843495 DOI: 10.1182/blood-2013-01-481341]

58 **Friedberg JW**, Vose J, Kahl BS, Brunvand M, Goy A, Kasamon Y, Brington B, Li J, Ho W, Cheson BD. A Phase I Study of PRO131921, a Novel Anti-CD20 Monoclonal Antibody in Patients with Relapsed/Refractory CD20 Indolent NHL: Correlation Between Clinical Responses and AUC Pharmacokinetics. *ASH Annual Meeting Abstracts* 2009; **114**: 3472

59 **Wayne JL**, Ganjoo KN, Pohlman BL, De Vos S, Flinn IW, Dang NH, Mapara MY, Smith MR, O'Reilly AM, Marulappa SY, Jain VK. Efficacy of ocaratuzumab (AME-133v) in relapsed follicular lymphoma patients refractory to prior rituximab. *ASCO Meeting Abstracts* 2012; **30**: 8081

60 **Barcellini W**, Zaja F, Zaninoni A, Imperiali FG, Battista ML, Di Bona E, Fattizzo B, Consonni D, Cortelezzi A, Fanin R, Zanella A. Low-dose rituximab in adult patients with idiopathic autoimmune hemolytic anemia: clinical efficacy and biologic studies. *Blood* 2012; **119**: 3691-3697 [PMID: 22267606 DOI: 10.1182/blood-2011-06-363556]

61 **Stasi R**, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. *Blood* 2001; **98**: 952-957 [PMID: 11493438 DOI: 10.1182/blood.V98.4.952]

62 **Stasi R**, Brunetti M, Stipa E, Amadori S. Selective B-cell depletion with rituximab for the treatment of patients with acquired hemophilia. *Blood* 2004; **103**: 4424-4428 [PMID: 14996701 DOI: 10.1182/blood-2003-11-4075]

63 **Fakhouri F**, Vernant JP, Veyradier A, Wolf M, Kaplanski G, Binaut R, Rieger M, Scheiflinger F, Poullin P, Deroure B, Delarue R, Lesavre P, Vanhille P, Hermine O, Remuzzi G, Grünfeld JP. Efficiency of curative and prophylactic treatment with rituximab in ADAMTS13-deficient thrombotic thrombocytopenic purpura: a study of 11 cases. *Blood* 2005; **106**: 1932-1937 [PMID: 15933059 DOI: 10.1182/blood-2005-03-0848]

64 **Sansonno D**, De Re V, Lauletta G, Tucci FA, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. *Blood* 2003; **101**: 3818-3826 [PMID: 12506023 DOI: 10.1182/blood-2002-10-3162]

65 **Zaja F**, De Vita S, Mazzaro C, Sacco S, Damiani D, De Marchi G, Michelutti A, Baccarani M, Fanin R, Ferraccioli G. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. *Blood* 2003; **101**: 3827-3834 [PMID: 12560225 DOI: 10.1182/blood-2002-09-2856]

66 **LeBien TW**, Tedder TF. B lymphocytes: how they develop and function. *Blood* 2008; **112**: 1570-1580 [PMID: 18725575 DOI: 10.1182/blood-2008-02-078071]

67 **Barcellini W**, Zanella A. Rituximab therapy for autoimmune haematological diseases. *Eur J Intern Med* 2011; **22**: 220-229 [PMID: 21570637 DOI: 10.1016/j.ejim.2010.12.016]

68 **Nader K**, Patel M, Ferber A. Ofatumumab in rituximab-refractory autoimmune hemolytic anemia associated with chronic lymphocytic leukemia: a case report and review of literature. *Clin Lymphoma Myeloma Leuk* 2013; **13**: 511-513 [PMID: 23726016 DOI: 10.1016/j.clml.2013.02.022]

69 **Liebman HA**, Saleh MN, Bussel JB, Negrea OG, Horne H, Wegener WA, Goldenberg DM. Low-dose anti-CD20 veltuzumab given intravenously or subcutaneously is active in relapsed immune thrombocytopenia: a phase I study. *Br J Haematol* 2013; **162**: 693-701 [PMID: 23829485 DOI: 10.1111/bjh.12448]

70 **Maloney DG**, Grillo-López AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, Janakiraman N, Foon KA, Liles TM, Dallaire BK, Wey K, Royston I, Davis T, Levy R. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* 1997; **90**: 2188-2195 [PMID: 9310469]

71 **Nakou M**, Katsikas G, Sidiropoulos P, Bertsias G, Papadimitraki E, Raptopoulou A, Koutala H, Papadaki HA, Kritikos H, Boumpas DT. Rituximab therapy reduces activated B cells in both the peripheral blood and bone marrow of patients with rheumatoid arthritis: depletion of memory B cells correlates with clinical response. *Arthritis Res Ther* 2009; **11**: R131 [PMID: 19715572 DOI: 10.1186/ar2798]

72 **Kamburova EG**, Koenen HJ, Boon L, Hilbrands LB, effects of rituximab on the proliferation, activation and Joosten I. In vitro differentiation of human B cells. *Am J Transplant* 2012; **12**: 341-350 [PMID: 22070501 DOI: 10.1111/j.1600-6143.2011.03833.x]

73 **Roll P**, Palanichamy A, Kneitz C, Dorner T, Tony HP. Regeneration of B cell subsets after transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. *Arthritis Rheum* 2006; **54**: 2377-2386 [PMID: 16869000 DOI: 10.1002/art.22019]

74 **Leandro MJ**, Cambridge G, Ehrenstein MR, Edwards JC. Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; **54**: 613-620 [PMID: 16447239 DOI: 10.1002/art.21617]

75 **Anolik JH**, Friedberg JW, Zheng B, Barnard J, Owen T, Cushing E, Kelly J, Milner EC, Fisher RI, Sanz I. B cell recon-

stitution after rituximab treatment of lymphoma recapitulates B cell ontogeny. *Clin Immunol* 2007; **122**: 139-145 [PMID: 17008130 DOI: 10.1016/j.clim.2006.08.009]

76 **Abulayha AM**, Tabal SA, Shawesh EI, Elbasir MA, Elbanani AS, Lamami YM, Bredan A. Depletion of peripheral blood B cells with Rituximab and phenotype characterization of the recovering population in a patient with follicular lymphoma. *Leuk Res* 2010; **34**: 307-311 [PMID: 19580998 DOI: 10.1016/j.leukres.2009.06.005]

77 **Tsuda M**, Moritoki Y, Lian ZX, Zhang W, Yoshida K, Wakabayashi K, Yang GX, Nakatani T, Vierling J, Lindor K, Gershwin ME, Bowlus CL. Biochemical and immunologic effects of rituximab in patients with primary biliary cirrhosis and an incomplete response to ursodeoxycholic acid. *Hepatology* 2012; **55**: 512-521 [PMID: 22006563 DOI: 10.1002/hep.24748]

78 **Pescovitz MD**, Torgerson TR, Ochs HD, Ocheltree E, McGee P, Krause-Steinrauf H, Lachin JM, Canniff J, Greenbaum C, Herold KC, Skyler JS, Weinberg A. Effect of rituximab on human in vivo antibody immune responses. *J Allergy Clin Immunol* 2011; **128**: 1295-1302.e5 [PMID: 21908031 DOI: 10.1016/j.jaci.2011.08.008]

79 **Bedognetti D**, Zoppoli G, Massucco C, Zanardi E, Zupo S, Bruzzone A, Sertoli MR, Balleari E, Racchi O, Messina M, Caltabiano G, Icardi G, Durando P, Marincola FM, Boccardo F, Ferrarini M, Ansaldi F, De Maria A. Impaired response to influenza vaccine associated with persistent memory B cell depletion in non-Hodgkin's lymphoma patients treated with rituximab-containing regimens. *J Immunol* 2011; **186**: 6044-6055 [PMID: 21498665 DOI: 10.4049/jimmunol.1004095]

80 **van Vollenhoven RF**, Emery P, Bingham CO, Keystone EC, Fleischmann RM, Furst DE, Tyson N, Collinson N, Lehane PB. Long-term safety of rituximab in rheumatoid arthritis: 9.5-year follow-up of the global clinical trial programme with a focus on adverse events of interest in RA patients. *Ann Rheum Dis* 2013; **72**: 1496-1502 [PMID: 23136242 DOI: 10.1136/annrheumdis-2012-201956]

81 **Eming R**, Nagel A, Wolff-Franke S, Podstawa E, Debus D, Hertl M. Rituximab exerts a dual effect in pemphigus vulgaris. *J Invest Dermatol* 2008; **128**: 2850-2858 [PMID: 18563178 DOI: 10.1038/jid.2008.172]

82 **Stasi R**, Cooper N, Del Poeta G, Stipa E, Laura Evangelista M, Abruzzese E, Amadori S. Analysis of regulatory T-cell changes in patients with idiopathic thrombocytopenic purpura receiving B cell-depleting therapy with rituximab. *Blood* 2008; **112**: 1147-1150 [PMID: 18375792 DOI: 10.1182/blood-2007-12-129262]

83 **Stasi R**, Del Poeta G, Stipa E, Evangelista ML, Trawinska MM, Cooper N, Amadori S. Response to B-cell depleting therapy with rituximab reverts the abnormalities of T-cell subsets in patients with idiopathic thrombocytopenic purpura. *Blood* 2007; **110**: 2924-2930 [PMID: 17548576 DOI: 10.1182/blood-2007-02-068999]

84 **Sfikakis PP**, Souliotis VL, Fragiadaki KG, Moutsopoulos HM, Boletis JN, Theofilopoulos AN. Increased expression of the FoxP3 functional marker of regulatory T cells following B cell depletion with rituximab in patients with lupus nephritis. *Clin Immunol* 2007; **123**: 66-73 [PMID: 17275413 DOI: 10.1016/j.clim.2006.12.006]

85 **Vallerskog T**, Gunnarsson I, Widhe M, Risselada A, Klareskog L, van Vollenhoven R, Malmström V, Trollmo C. Treatment with rituximab affects both the cellular and the humoral arm of the immune system in patients with SLE. *Clin Immunol* 2007; **122**: 62-74 [PMID: 17046329 DOI: 10.1016/j.clim.2006.08.016]

86 **Feuchtenberger M**, Müller S, Roll P, Waschbisch A, Schäfer A, Kneitz C, Wiendl H, Tony HP. Frequency of regulatory T cells is not affected by transient B cell depletion using anti-CD20 antibodies in rheumatoid arthritis. *Open Rheumatol J* 2008; **2**: 81-88 [PMID: 19156222 DOI: 10.2174/1874312900802010081]

87 **Saadoun D**, Rosenzwajg M, Landau D, Piette JC, Klatzmann D, Cacoub P. Restoration of peripheral immune homeostasis after rituximab in mixed cryoglobulinemia vasculitis. *Blood* 2008; **111**: 5334-5341 [PMID: 18292291 DOI: 10.1182/blood-2007-11-122713]

88 **Bouaziz JD**, Yanaba K, Venturi GM, Wang Y, Tisch RM, Poe JC, Tedder TF. Therapeutic B cell depletion impairs adaptive and autoreactive CD4+ T cell activation in mice. *Proc Natl Acad Sci USA* 2007; **104**: 20878-20883 [PMID: 18093919 DOI: 10.1073/pnas.0709205105]

89 **Yeo W**, Chan PK, Zhong S, Ho WM, Steinberg JL, Tam JS, Hui P, Leung NW, Zee B, Johnson PJ. Frequency of hepatitis B virus reactivation in cancer patients undergoing cytotoxic chemotherapy: a prospective study of 626 patients with identification of risk factors. *J Med Virol* 2000; **62**: 299-307 [PMID: 11055239 DOI: 10.1002/1096-9071(200011)]

90 **Takai S**, Tsurumi H, Ando K, Kasahara S, Sawada M, Yamada T, Hara T, Fukuno K, Takahashi T, Oyama M, Onishi H, Tomita E, Takami T, Imawari M, Moriwaki H. Prevalence of hepatitis B and C virus infection in haematological malignancies and liver injury following chemotherapy. *Eur J Haematol* 2005; **74**: 158-165 [PMID: 15654908 DOI: 10.1111/j.1600-0609.2004.00376.x]

91 **Tan J**, Zhou J, Zhao P, Wei J. Prospective study of HBV reactivation risk in rheumatoid arthritis patients who received conventional disease-modifying antirheumatic drugs. *Clin Rheumatol* 2012; **31**: 1169-1175 [PMID: 22544263 DOI: 10.1007/s10067-012-1988-2]

92 **Cheng AL**, Hsiung CA, Su IJ, Chen PJ, Chang MC, Tsao CJ, Kao WY, Uen WC, Hsu CH, Tien HF, Chao TY, Chen LT, Whang-Peng J. Steroid-free chemotherapy decreases risk of hepatitis B virus (HBV) reactivation in HBV-carriers with lymphoma. *Hepatology* 2003; **37**: 1320-1328 [PMID: 12774010 DOI: 10.1053/jhep.2003.50220]

93 **Hsu CH**, Hsu HC, Chen HL, Gao M, Yeh PY, Chen PJ, Cheng AL. Doxorubicin activates hepatitis B virus (HBV) replication in HBV-harboring hepatoblastoma cells. A possible novel mechanism of HBV reactivation in HBV carriers receiving systemic chemotherapy. *Anticancer Res* 2004; **24**: 3035-3040 [PMID: 15517913]

94 **Chu CM**, Karayiannis P, Fowler MJ, Monjardino J, Liaw YF, Thomas HC. Natural history of chronic hepatitis B virus infection in Taiwan: studies of hepatitis B virus DNA in serum. *Hepatology* 1985; **5**: 431-434 [PMID: 3997072]

95 **Ganem D**, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118-1129 [PMID: 15014185 DOI: 10.1056/NEJMra031087]

96 **Liaw YF**, Chu CM. Hepatitis B virus infection. *Lancet* 2009; **373**: 582-592 [PMID: 19217993 DOI: 10.1016/S0140-6736(09)60207-5]

97 **Hsu YS**, Chien RN, Yeh CT, Sheen IS, Chiou HY, Chu CM, Liaw YF. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. *Hepatology* 2002; **35**: 1522-1527 [PMID: 12029639 DOI: 10.1053/jhep.2002.33638]

98 **Dunn C**, Peppa D, Khanna P, Nebbia G, Jones M, Brendish N, Lascar RM, Brown D, Gilson RJ, Tedder RJ, Dusheiko GM, Jacobs M, Klenerman P, Maini MK. Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. *Gastroenterology* 2009; **137**: 1289-1300 [PMID: 19591831 DOI: 10.1053/j.gastro.2009.06.054]

99 **Zerbini A**, Pilli M, Boni C, Fisicaro P, Penna A, Di Vincenzo P, Giuberti T, Orlandini A, Raffa G, Pollicino T, Raimondo G, Ferrari C, Missale G. The characteristics of the cell-mediated immune response identify different profiles of occult hepatitis B virus infection. *Gastroenterology* 2008; **134**: 1470-1481 [PMID: 18355815 DOI: 10.1053/j.gastro.2008.02.017]

100 **Stoop JN**, van der Molen RG, Baan CC, van der Laan LJ,

Kuipers EJ, Kusters JG, Janssen HL. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. *Hepatology* 2005; **41**: 771-778 [PMID: 15791617 DOI: 10.1002/hep.20649]
101 **Kim SJ**, Hsu C, Song YQ, Tay K, Hong XN, Cao J, Kim JS, Eom HS, Lee JH, Zhu J, Chang KM, Reksodiputro AH, Tan D, Goh YT, Lee J, Intragumtornchai T, Chng WJ, Cheng AL, Lim ST, Suh C, Kwong YL, Kim WS. Hepatitis B virus reactivation in B-cell lymphoma patients treated with rituximab: analysis from the Asia Lymphoma Study Group. *Eur J Cancer* 2013; **49**: 3486-3496 [PMID: 23910494 DOI: 10.1016/j.ejca.2013.07.006]
102 **Yeo W**, Chan TC, Leung NW, Lam WY, Mo FK, Chu MT, Chan HL, Hui EP, Lei KI, Mok TS, Chan PK. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J Clin Oncol* 2009; **27**: 605-611 [PMID: 19075267 DOI: 10.1200/JCO.2008.18.0182]
103 **Dervite I**, Hober D, Morel P. Acute hepatitis B in a patient with antibodies to hepatitis B surface antigen who was receiving rituximab. *N Engl J Med* 2001; **344**: 68-69 [PMID: 11187122 DOI: 10.1056/NEJM200101043440120]
104 **Yang SH**, Kuo SH. Reactivation of hepatitis B virus during rituximab treatment of a patient with follicular lymphoma. *Ann Hematol* 2008; **87**: 325-327 [PMID: 17932671 DOI: 10.1007/s00277-007-0396-1]
105 **Pei SN**, Chen CH, Lee CM, Wang MC, Ma MC, Hu TH, Kuo CY. Reactivation of hepatitis B virus following rituximab-based regimens: a serious complication in both HBsAg-positive and HBsAg-negative patients. *Ann Hematol* 2010; **89**: 255-262 [PMID: 19697028 DOI: 10.1007/s00277-009-0806-7]
106 **Hsu C**, Tsou HH, Lin SJ, Wang MC, Yao M, Hwang WL, Kao WY, Chiu CF, Lin SF, Lin J, Chang CS, Tien HF, Liu TW, Chen PJ, Cheng AL; Taiwan Cooperative Oncology Group. Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: A prospective study. *Hepatology* 2013; Epub ahead of print [DOI: 10.1002/hep.26718]
107 **Kurokawa T**, Hase M, Tokuman N, Yoshida T. Immune reconstitution of B-cell lymphoma patients receiving CHOP-based chemotherapy containing rituximab. *Hematol Oncol* 2011; **29**: 5-9 [PMID: 20552573 DOI: 10.1002/hon.947]
108 **Peñalver FJ**, Alvarez-Larrán A, Díez-Martin JL, Gallur L, Jarque I, Caballero D, Díaz-Mediavilla J, Bustelos R, Fernández-Aceñero MJ, Cabrera JR. Rituximab is an effective and safe therapeutic alternative in adults with refractory and severe autoimmune hemolytic anemia. *Ann Hematol* 2010; **89**: 1073-1080 [PMID: 20526716 DOI: 10.1007/s00277-010-0997-y]
109 **Gudbrandsdottir S**, Birgens HS, Frederiksen H, Jensen BA, Jensen MK, Kjeldsen L, Klausen TW, Larsen H, Mourits-Andersen HT, Nielsen CH, Nielsen OJ, Plesner T, Pulczynski S, Rasmussen IH, Rønnov-Jessen D, Hasselbalch HC. Rituximab and dexamethasone vs dexamethasone monotherapy in newly diagnosed patients with primary immune thrombocytopenia. *Blood* 2013; **121**: 1976-1981 [PMID: 23293082 DOI: 10.1182/blood-2012-09-455691]
110 **Droz N**, Gilardin L, Cacoub P, Berenbaum F, Wendling D, Godeau B, Piette AM, Dernis E, Ebbo M, Fautrel B, Le Guenno G, Mekinian A, Bernard-Chabert B, Costedoat-Chalumeau N, Descloux E, Michot JM, Radenne S, Rigolet A, Rivière S, Yvin JL, Thibault V, Thabut D, Pol S, Guillevin L, Mouthon L, Terrier B. Kinetic profiles and management of hepatitis B virus reactivation in patients with immune-mediated inflammatory diseases. *Arthritis Care Res* (Hoboken) 2013; **65**: 1504-1514 [PMID: 23436730 DOI: 10.1002/acr.21990]
111 **Pasquet F**, Combarnous F, Macgregor B, Coppere B, Mausservey C, Ninet J, Hot A. Safety and efficacy of rituximab treatment for vasculitis in hepatitis B virus-associated type II cryoglobulinemia: a case report. *J Med Case Rep* 2012; **6**: 39 [PMID: 22284897 DOI: 10.1186/1752-1947-6-39]
112 **Cil T**, Altintas A, Tuzun Y, Pasa S, Isikdogan A. Hepatitis B virus reactivation induced by Yttrium-90-ibritumomab-tiuxetan. *Leuk Lymphoma* 2007; **48**: 1866-1868 [PMID: 17786727 DOI: 10.1080/10428190701509848)]
113 **Soong YL**, Lee KM, Lui HF, Chow WC, Tao M, Li Er Loong S. Hepatitis B reactivation in a patient receiving radiolabeled rituximab. *Ann Hematol* 2005; **84**: 61-62 [PMID: 15449029 DOI: 10.1007/s00277-004-0948-6]
114 **Mitka M**. FDA: Increased HBV reactivation risk with ofatumumab or rituximab. *JAMA* 2013; **310**: 1664 [PMID: 24150447 DOI: 10.1001/jama.2013.281115]
115 **Ogawa Y**, Ogura M, Suzuki T, Ando K, Uchida T, Shirasugi Y, Tobinai K, Lee JH, Kase M, Katsura K, Hotta T. A phase I/II study of ofatumumab (GSK1841157) in Japanese and Korean patients with relapsed or refractory B-cell chronic lymphocytic leukemia. *Int J Hematol* 2013; **98**: 164-170 [PMID: 23846385 DOI: 10.1007/s12185-013-1393-x]
116 **Cortelezzi A**, Sciumè M, Liberati AM, Vincenti D, Cuneo A, Reda G, Laurenti L, Zaja F, Marasca R, Chiarenza A, Gritti G, Orsucci L, Storti S, Angelucci E, Cascavilla N, Gobbi M, Mauro FR, Morabito F, Fabris S, Piciocchi A, Vignetti M, Neri A, Rossi D, Giannarelli D, Guarini A, Foà R. Bendamustine in combination with ofatumumab in relapsed or refractory chronic lymphocytic leukemia: a GIMEMA Multicenter Phase II Trial. *Leukemia* 2014; **28**: 642-648 [PMID: 24220274 DOI: 10.1038/leu.2013.334]
117 **Chen CJ**, Wang LY, Yu MW. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. *J Gastroenterol Hepatol* 2000; **15** Suppl: E3-E6 [PMID: 10921373 DOI: 10.1046/j.1440-1746.2000.02124.x]
118 **Yeo W**, Johnson PJ. Diagnosis, prevention and management of hepatitis B virus reactivation during anticancer therapy. *Hepatology* 2006; **43**: 209-220 [PMID: 16440366 DOI: 10.1002/hep.21051]
119 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-539 [PMID: 17256718 DOI: 10.1002/hep.21513]
120 **Liaw YF**, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263-283 [PMID: 19669255 DOI: 10.1007/s12072-008-9080-3]
121 **European Association For The Study Of The Liver**. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227-242 [PMID: 19054588 DOI: 10.1016/j.jhep.2008.10.001]
122 **Weinbaum CM**, Williams I, Mast EE, Wang SA, Finelli L, Wasley A, Neitzel SM, Ward JW. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. *MMWR Recomm Rep* 2008; **57**: 1-20 [PMID: 18802412]
123 **Artz AS**, Somerfield MR, Feld JJ, Giusti AF, Kramer BS, Sabichi AL, Zon RT, Wong SL. American Society of Clinical Oncology provisional clinical opinion: chronic hepatitis B virus infection screening in patients receiving cytotoxic chemotherapy for treatment of malignant diseases. *J Clin Oncol* 2010; **28**: 3199-3202 [PMID: 20516452 DOI: 10.1200/JCO.2010.30.0673]
124 **Zurawska U**, Hicks LK, Woo G, Bell CM, Krahn M, Chan KK, Feld JJ. Hepatitis B virus screening before chemotherapy for lymphoma: a cost-effectiveness analysis. *J Clin Oncol* 2012; **30**: 3167-3173 [PMID: 22711851 DOI: 10.1200/JCO.2011.40.7510]
125 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]
126 **Liu CJ**, Chen PJ, Chen DS, Kao JH. Hepatitis B virus reactivation in patients receiving cancer chemotherapy: natural history, pathogenesis, and management. *Hepatol Int* 2011;

Epub ahead of print [PMID: 21670970 DOI: 10.1007/s12072-011-9279-6]
127 **Hsu C**, Hsiung CA, Su IJ, Hwang WS, Wang MC, Lin SF, Lin TH, Hsiao HH, Young JH, Chang MC, Liao YM, Li CC, Wu HB, Tien HF, Chao TY, Liu TW, Cheng AL, Chen PJ. A revisit of prophylactic lamivudine for chemotherapy-associated hepatitis B reactivation in non-Hodgkin's lymphoma: a randomized trial. *Hepatology* 2008; **47**: 844-853 [PMID: 18302293 DOI: 10.1002/hep.22106]
128 **Katz LH**, Fraser A, Gafter-Gvili A, Leibovici L, Tur-Kaspa R. Lamivudine prevents reactivation of hepatitis B and reduces mortality in immunosuppressed patients: systematic review and meta-analysis. *J Viral Hepat* 2008; **15**: 89-102 [PMID: 18184191 DOI: 10.1111/j.1365-2893.2007.00902.x]
129 **Lok AS**, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dienstag JL, Heathcote EJ, Little NR, Griffiths DA, Gardner SD, Castiglia M. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; **125**: 1714-1722 [PMID: 14724824 DOI: 10.1053/j.gastro.2003.09.033]
130 **Huang YH**, Hsiao LT, Hong YC, Chiou TJ, Yu YB, Gau JP, Liu CY, Yang MH, Tzeng CH, Lee PC, Lin HC, Lee SD. Randomized controlled trial of entecavir prophylaxis for rituximab-associated hepatitis B virus reactivation in patients with lymphoma and resolved hepatitis B. *J Clin Oncol* 2013; **31**: 2765-2772 [PMID: 23775967 DOI: 10.1200/JCO.2012.48.5938]
131 **Trembling PM**, Tanwar S, Rosenberg WM, Dusheiko GM. Treatment decisions and contemporary versus pending treatments for hepatitis C. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 713-728 [PMID: 24019151 DOI: 10.1038/nrgastro.2013.163]
132 **Lechner F**, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, Robbins G, Phillips R, Klenerman P, Walker BD. Analysis of successful immune responses in persons infected with hepatitis C virus. *J Exp Med* 2000; **191**: 1499-1512 [PMID: 10790425 DOI: 10.1084/jem.191.9.1499]
133 **Nakamoto N**, Cho H, Shaked A, Olthoff K, Valiga ME, Kaminski M, Gostick E, Price DA, Freeman GJ, Wherry EJ, Chang KM. Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. *PLoS Pathog* 2009; **5**: e1000313 [PMID: 19247441 DOI: 10.1371/journal.ppat.1000313]
134 **Sugimoto K**, Ikeda F, Stadanlick J, Nunes FA, Alter HJ, Chang KM. Suppression of HCV-specific T cells without differential hierarchy demonstrated ex vivo in persistent HCV infection. *Hepatology* 2003; **38**: 1437-1448 [PMID: 14647055 DOI: 10.1016/j.hep.2003.09.026]
135 **Martell M**, Esteban JI, Quer J, Genescà J, Weiner A, Esteban R, Guardia J, Gómez J. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. *J Virol* 1992; **66**: 3225-3229 [PMID: 1313927]
136 **Chang KM**, Rehermann B, McHutchison JG, Pasquinelli C, Southwood S, Sette A, Chisari FV. Immunological significance of cytotoxic T lymphocyte epitope variants in patients chronically infected by the hepatitis C virus. *J Clin Invest* 1997; **100**: 2376-2385 [PMID: 9410918 DOI: 10.1172/JCI119778]
137 **von Hahn T**, Yoon JC, Alter H, Rice CM, Rehermann B, Balfe P, McKeating JA. Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection in vivo. *Gastroenterology* 2007; **132**: 667-678 [PMID: 17258731 DOI: 10.1053/j.gastro.2006.12.008]
138 **Agnello V**, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992; **327**: 1490-1495 [PMID: 1383822 DOI: 10.1056/NEJM199211193272104]
139 **Dal Maso L**, Franceschi S. Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 2078-2085 [PMID: 17119031 DOI: 10.1158/1055-9965.EPI-06-0308]
140 **Vento S**, Cainelli F, Mirandola F, Cosco L, Di Perri G, Solbiati M, Ferraro T, Concia E. Fulminant hepatitis on withdrawal of chemotherapy in carriers of hepatitis C virus. *Lancet* 1996; **347**: 92-93 [PMID: 8538348 DOI: 10.1016/S0140-6736(96)90212-3]
141 **Zuckerman E**, Zuckerman T, Douer D, Qian D, Levine AM. Liver dysfunction in patients infected with hepatitis C virus undergoing chemotherapy for hematologic malignancies. *Cancer* 1998; **83**: 1224-1230 [PMID: 9740089 DOI: 10.1002/(SICI)1097-0142(19980915)]
142 **Kawatani T**, Suou T, Tajima F, Ishiga K, Omura H, Endo A, Ohmura H, Ikuta Y, Idobe Y, Kawasaki H. Incidence of hepatitis virus infection and severe liver dysfunction in patients receiving chemotherapy for hematologic malignancies. *Eur J Haematol* 2001; **67**: 45-50 [PMID: 11553266 DOI: 10.1034/j.1600-0609.2001.067001045.x]
143 **Nosotti L**, D'Andrea M, Pitidis A, Pimpinelli F, Dessanti ML, Pisani F, Vignally P, Petti MC. Hepatitis C virus infection prevalence and liver dysfunction in a cohort of B-cell non-Hodgkin's lymphoma patients treated with immunochemotherapy. *Scand J Infect Dis* 2012; **44**: 70-73 [PMID: 21905952 DOI: 10.3109/00365548.2011.611819]
144 **Ennishi D**, Maeda Y, Niitsu N, Kojima M, Izutsu K, Takizawa J, Kusumoto S, Okamoto M, Yokoyama M, Takamatsu Y, Sunami K, Miyata A, Murayama K, Sakai A, Matsumoto M, Shinagawa K, Takaki A, Matsuo K, Kinoshita T, Tanimoto M. Hepatic toxicity and prognosis in hepatitis C virus-infected patients with diffuse large B-cell lymphoma treated with rituximab-containing chemotherapy regimens: a Japanese multicenter analysis. *Blood* 2010; **116**: 5119-5125 [PMID: 20823454 DOI: 10.1182/blood-2010-06-289231]
145 **Ennishi D**, Terui Y, Yokoyama M, Mishima Y, Takahashi S, Takeuchi K, Okamoto H, Tanimoto M, Hatake K. Monitoring serum hepatitis C virus (HCV) RNA in patients with HCV-infected CD20-positive B-cell lymphoma undergoing rituximab combination chemotherapy. *Am J Hematol* 2008; **83**: 59-62 [PMID: 17712791 DOI: 10.1002/ajh.21022]
146 **Coppola N**, Pisaturo M, Guastafierro S, Tonziello G, Sica A, Iodice V, Sagnelli C, Ferrara MG, Sagnelli E. Increased hepatitis C viral load and reactivation of liver disease in HCV RNA-positive patients with onco-haematological disease undergoing chemotherapy. *Dig Liver Dis* 2012; **44**: 49-54 [PMID: 21885355 DOI: 10.1016/j.dld.2011.07.016]
147 **Tsutsumi Y**, Ichiki K, Shiratori S, Kawamura T, Tanaka J, Asaka M, Imamura M, Masauzi N. Changes in hepatitis C virus antibody titer and viral RNA load in non-Hodgkin's lymphoma patients after rituximab chemotherapy. *Int J Lab Hematol* 2009; **31**: 468-470 [PMID: 18294236 DOI: 10.1111/j.1751-553X.2008.01034.x]
148 **Lake-Bakaar G**, Dustin L, McKeating J, Newton K, Freeman V, Frost SD. Hepatitis C virus and alanine aminotransferase kinetics following B-lymphocyte depletion with rituximab: evidence for a significant role of humoral immunity in the control of viremia in chronic HCV liver disease. *Blood* 2007; **109**: 845-846 [PMID: 17210867 DOI: 10.1182/blood-2006-08-041525]
149 **Halfon P**, Bourlière M, Halimi G, Khiri H, Bertezene P, Portal I, Botta-Fridlund D, Gauthier AP, Jullien M, Feryn JM, Gerolami V, Cartouzou G. Assessment of spontaneous fluctuations of viral load in untreated patients with chronic hepatitis C by two standardized quantitation methods: branched DNA and Amplicor Monitor. *J Clin Microbiol* 1998; **36**: 2073-2075 [PMID: 9650965]
150 **Mahale P**, Kontoyiannis DP, Chemaly RF, Jiang Y, Hwang JP, Davila M, Torres HA. Acute exacerbation and reactivation of chronic hepatitis C virus infection in cancer patients. *J Hepatol* 2012; **57**: 1177-1185 [PMID: 22871500 DOI: 10.1016/

j.jhep.2012.07.031]
151 **Torres HA**, Davila M. Reactivation of hepatitis B virus and hepatitis C virus in patients with cancer. *Nat Rev Clin Oncol* 2012; **9**: 156-166 [PMID: 22271089 DOI: 10.1038/nrclinonc.2012.1]
152 **D'Arena G**, Laurenti L, Capalbo S, D'Arco AM, De Filippi R, Marcacci G, Di Renzo N, Storti S, Califano C, Vigliotti ML, Tarnani M, Ferrara F, Pinto A. Rituximab therapy for chronic lymphocytic leukemia-associated autoimmune hemolytic anemia. *Am J Hematol* 2006; **81**: 598-602 [PMID: 16823816 DOI: 10.1002/ajh.20665]
153 **Dufour JF**, Pradat P, Ruivard M, Hot A, Dumontet C, Broussolle C, Trepo C, Sève P. Severe autoimmune cytopenias in treatment-naive hepatitis C virus infection: clinical description of 16 cases. *Eur J Gastroenterol Hepatol* 2009; **21**: 245-253 [PMID: 19279468 DOI: 10.1097/MEG.0b013e3283249908]
154 **Papaevangelou V**, Varsami M, Papadakis V, Zellos A, Parcharidou A, Papargyri S, Karentzou O, Manolaki N, Roma E, Polychronopoulou S. Hepatitis C treatment concomitant to chemotherapy as "salvage" therapy in children with hematologic malignancies. *Pediatr Infect Dis J* 2010; **29**: 277-280 [PMID: 19949358 DOI: 10.1097/INF.0b013e3181c2115a]
155 **Pellicelli AM**, Zoli V. Role of ribavirin in hepatitis flare in HCV-infected patients with B cell non Hodgkin's lymphoma treated with rituximab-containing regimens. *Dig Liver Dis* 2011; **43**: 501-502 [PMID: 21459059 DOI: 10.1016/j.dld.2010.12.018]
156 **Young NS**, Brown KE. Parvovirus B19. *N Engl J Med* 2004; **350**: 586-597 [PMID: 14762186 DOI: 10.1056/NEJMra030840]
157 **Sokal EM**, Melchior M, Cornu C, Vandenbroucke AT, Buts JP, Cohen BJ, Burtonboy G. Acute parvovirus B19 infection associated with fulminant hepatitis of favourable prognosis in young children. *Lancet* 1998; **352**: 1739-1741 [PMID: 9848349 DOI: 10.1016/S0140-6736(98)06165-0]
158 **Anderson MJ**, Higgins PG, Davis LR, Willman JS, Jones SE, Kidd IM, Pattison JR, Tyrrell DA. Experimental parvoviral infection in humans. *J Infect Dis* 1985; **152**: 257-265 [PMID: 2993431]
159 **Kurtzman GJ**, Ozawa K, Cohen B, Hanson G, Oseas R, Young NS. Chronic bone marrow failure due to persistent B19 parvovirus infection. *N Engl J Med* 1987; **317**: 287-294 [PMID: 3037373 DOI: 10.1056/NEJM198707303170506]
160 **Norbeck O**, Isa A, Pöhlmann C, Broliden K, Kasprowicz V, Bowness P, Klenerman P, Tolfvenstam T. Sustained CD8+ T-cell responses induced after acute parvovirus B19 infection in humans. *J Virol* 2005; **79**: 12117-12121 [PMID: 16140790 DOI: 10.1128/JVI.79.18.12117-12121.2005]
161 **Kasprowicz V**, Isa A, Tolfvenstam T, Jeffery K, Bowness P, Klenerman P. Tracking of peptide-specific CD4+ T-cell responses after an acute resolving viral infection: a study of parvovirus B19. *J Virol* 2006; **80**: 11209-11217 [PMID: 16943301 DOI: 10.1128/JVI.01173-06]
162 **Kuo SH**, Lin LI, Chang CJ, Liu YR, Lin KS, Cheng AL. Increased risk of parvovirus B19 infection in young adult cancer patients receiving multiple courses of chemotherapy. *J Clin Microbiol* 2002; **40**: 3909-3912 [PMID: 12409350]
163 **Heegaard ED**, Schmiegelow K. Serologic study on parvovirus b19 infection in childhood acute lymphoblastic leukemia during chemotherapy: clinical and hematologic implications. *J Pediatr Hematol Oncol* 2002; **24**: 368-373 [PMID: 12142785]
164 **Eid AJ**, Brown RA, Patel R, Razonable RR. Parvovirus B19 infection after transplantation: a review of 98 cases. *Clin Infect Dis* 2006; **43**: 40-48 [PMID: 16758416 DOI: 10.1086/504812]
165 **Li Y**, Dong Y, Jiang J, Yang Y, Liu K, Li Y. High prevelance of human parvovirus infection in patients with malignant tumors. *Oncol Lett* 2012; **3**: 635-640 [PMID: 22740966 DOI: 10.3892/ol.2012.548]
166 **Poplar S**, Allford S, Rooney N. Chronic parvovirus B19 infection leading to red cell aplasia following treatment of Hodgkin lymphoma. *Br J Haematol* 2010; **148**: 671 [PMID: 19681883 DOI: 10.1111/j.1365-2141.2009.07830.x]
167 **Sharma VR**, Fleming DR, Slone SP. Pure red cell aplasia due to parvovirus B19 in a patient treated with rituximab. *Blood* 2000; **96**: 1184-1186 [PMID: 10910942]
168 **Song KW**, Mollee P, Patterson B, Brien W, Crump M. Pure red cell aplasia due to parvovirus following treatment with CHOP and rituximab for B-cell lymphoma. *Br J Haematol* 2002; **119**: 125-127 [PMID: 12358915 DOI: 10.1046/j.1365-2141.2002.03778.x]
169 **Isobe Y**, Sugimoto K, Shiraki Y, Nishitani M, Koike K, Oshimi K. Successful high-titer immunoglobulin therapy for persistent parvovirus B19 infection in a lymphoma patient treated with rituximab-combined chemotherapy. *Am J Hematol* 2004; **77**: 370-373 [PMID: 15558805 DOI: 10.1002/ajh.20200]
170 **Klepfish A**, Rachmilevitch E, Schattner A. Parvovirus B19 reactivation presenting as neutropenia after rituximab treatment. *Eur J Intern Med* 2006; **17**: 505-507 [PMID: 17098597 DOI: 10.1016/j.ejim.2006.05.002]
171 **Hartmann JT**, Meisinger I, Kröber SM, Weisel K, Klingel K, Kanz L. Progressive bicytopenia due to persistent parvovirus B19 infection after immunochemotherapy with fludarabine/cyclophosphamide and rituximab for relapsed B cell lymphoma. *Haematologica* 2006; **91**: ECR49 [PMID: 17194655]
172 **Yang SH**, Lin LW, Fang YJ, Cheng AL, Kuo SH. Parvovirus B19 infection-related acute hepatitis after rituximab-containing regimen for treatment of diffuse large B-cell lymphoma. *Ann Hematol* 2012; **91**: 291-294 [PMID: 21538062 DOI: 10.1007/s00277-011-1238-8]
173 **Wong S**, Young NS, Brown KE. Prevalence of parvovirus B19 in liver tissue: no association with fulminant hepatitis or hepatitis-associated aplastic anemia. *J Infect Dis* 2003; **187**: 1581-1586 [PMID: 12721938 DOI: 10.1086/374781]
174 **Aksoy S**, Harputluoglu H, Kilickap S, Dede DS, Dizdar O, Altundag K, Barista I. Rituximab-related viral infections in lymphoma patients. *Leuk Lymphoma* 2007; **48**: 1307-1312 [PMID: 17613758 DOI: 10.1080/10428190701411441]
175 **Iyer A**, Mathur R, Deepak BV, Sinard J. Fatal adenoviral hepatitis after rituximab therapy. *Arch Pathol Lab Med* 2006; **130**: 1557-1560 [PMID: 17090202]
176 **Ronan BA**, Agrwal N, Carey EJ, De Petris G, Kusne S, Seville MT, Blair JE, Vikram HR. Fulminant hepatitis due to human adenovirus. *Infection* 2014; **42**: 105-111 [PMID: 23979854 DOI: 10.1007/s15010-013-0527-7]
177 **Ollier L**, Tieulie N, Sanderson F, Heudier P, Giordanengo V, Fuzibet JG, Nicand E. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. *Ann Intern Med* 2009; **150**: 430-431 [PMID: 19293084 DOI: 10.7326/0003-4819-150-6-200903170-00026]
178 **Bermúdez A**, Marco F, Conde E, Mazo E, Recio M, Zubizarreta A. Fatal visceral varicella-zoster infection following rituximab and chemotherapy treatment in a patient with follicular lymphoma. *Haematologica* 2000; **85**: 894-895 [PMID: 10942955]
179 **Del Prete CJ**, Cohen NS. A case of rituximab-induced hepatitis. *Cancer Biother Radiopharm* 2010; **25**: 747-748 [PMID: 21204770 DOI: 10.1089/cbr.2010.0806]
180 **Mössner E**, Brünker P, Moser S, Püntener U, Schmidt C, Herter S, Grau R, Gerdes C, Nopora A, van Puijenbroek E, Ferrara C, Sondermann P, Jäger C, Strein P, Fertig G, Friess T, Schüll C, Bauer S, Dal Porto J, Del Nagro C, Dabbagh K, Dyer MJ, Poppema S, Klein C, Umaña P. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood* 2010; **115**: 4393-4402 [PMID: 20194898 DOI: 10.1182/blood-2009-06-225979]

181 **Dalle S**, Reslan L, Besseyre de Horts T, Herveau S, Herting F, Plesa A, Friess T, Umana P, Klein C, Dumontet C. Preclinical studies on the mechanism of action and the anti-lymphoma activity of the novel anti-CD20 antibody GA101. *Mol Cancer Ther* 2011; **10**: 178-185 [PMID: 21220500 DOI: 10.1158/1535-7163.MCT-10-0385]

182 **Goldenberg DM**, Rossi EA, Stein R, Cardillo TM, Czuczman MS, Hernandez-Ilizaliturri FJ, Hansen HJ, Chang CH. Properties and structure-function relationships of veltuzumab (hA20), a humanized anti-CD20 monoclonal antibody. *Blood* 2009; **113**: 1062-1070 [PMID: 18941114 DOI: 10.1182/blood-2008-07-168146]

P- Reviewers: Bensussan A, Lee YY, Shi ZJ **S- Editor**: Gou SX
L- Editor: A **E- Editor**: Wu HL

WJH World Journal of Hematology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5315/wjh.v3.i2.44

World J Hematol 2014 May 6; 3(2): 44-48
ISSN 2218-6204 (online)
© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

CASE REPORT

Chronic disseminated candidiasis complicated with a ruptured intracranial fungal aneurysm in ALL

Teppei Okawa, Toshiaki Ono, Akifumi Endo, Masatoshi Takagi, Masayuki Nagasawa

Teppei Okawa, Toshiaki Ono, Akifumi Endo, Masatoshi Takagi, Masayuki Nagasawa, Department of Developmental Biology, Post Graduate School, Tokyo Medical and Dental University, Tokyo 113-8519, Japan
Teppei Okawa, Masayuki Nagasawa, Department of Hematology Oncology and Immunology, Tokyo Bay Urayasu/Ichikawa Medical Center, Chiba 279-0001, Japan
Author contributions: Okawa T and Nagasawa M designed the report; Okawa T, Ono T, Endo A and Takagi M collected the patient's clinical data; Okawa T, and Nagasawa M analyzed the data and wrote the paper.
Supported by The Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture Japan, No. 24591541 to Masayuki Nagasawa
Correspondence to: Masayuki Nagasawa, MD, PhD, Department of Developmental Biology, Post Graduate School, Tokyo Medical and Dental University, 5-45, Yushima 1-chome, Bunkyo-ku, Tokyo 113-8519, Japan. mnagasawa.ped@tmd.ac.jp
Telephone: +81-3-58035250 Fax: +81-3-58035247
Received: September 30, 2013 Revised: December 23, 2013
Accepted: January 17, 2014
Published online: May 6, 2014

Abstract

An 11-year-old boy with acute lymphocytic leukemia (ALL) contracted disseminated candidiasis during induction therapy, which was complicated with rupture of a fungal cranial aneurysm. Ventricular drainage and coil embolization of a residual aneurysm in combination with intensive antifungal therapy rescued the patient. Although clinical improvement was achieved, high fever and elevated levels of C-reactive protein and β-D-glucan continued for more than 10 mo. One year later, the ALL relapsed during maintenance therapy with methotrexate and 6-mercaptopurine. After salvage chemotherapy, the patient received unrelated bone marrow transplantation (BMT) in a non-complete remission condition and survived. During subsequent chemotherapy and BMT, no recurrence of the fungal infection was observed under the prophylactic anti-fungal therapy with micafungin.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Disseminated candidiasis; β-D-glucan; Fungal cranial aneurysm; Acute lymphocytic leukemia; Bone marrow transplantation

Core tip: Chronic disseminated candidiasis and resulting fungal intracranial aneurysm is a life-threatening complication during the induction therapy of leukemia with a poor survival rate. However, intensive and patient anti-fungal treatment made the patient receive unrelated bone marrow transplantation successfully.

Okawa T, Ono T, Endo A, Takagi M, Nagasawa M. Chronic disseminated candidiasis complicated with a ruptured intracranial fungal aneurysm in ALL. *World J Hematol* 2014; 3(2): 44-48 Available from: URL: http://www.wjgnet.com/2218-6204/full/v3/i2/44htm DOI: http://dx.doi.org/10.5315/wjh.v3.i2.44

INTRODUCTION

Disseminated fungal infection in acute leukemia patients is a serious complication, not only because it is difficult to manage and cure, but it also interferes with continuation and completion of the standard treatment regimen for leukemia, which consequently leads to an unfavorable outcome. We experienced an acute lymphocytic leukemia child who contracted a disseminated candidiasis with a resultant rupture of intracranial fungal aneurysm during the induction chemotherapy. Our clinical experience in this patient is very useful and informative for physicians in this field.

CASE REPORT

An 11-year-old boy with B-precursor acute lymphocytic

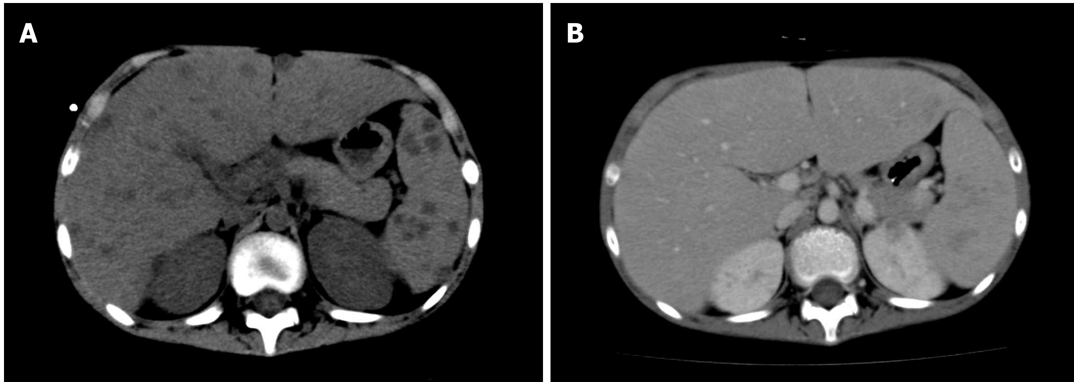


Figure 1 Computer tomography scan images of the liver. Computed tomography (CT) scans of the liver performed on the 51st (upper panel) and 239th d (lower panel) are presented. A: A simple CT scan; B: CT is enhanced.

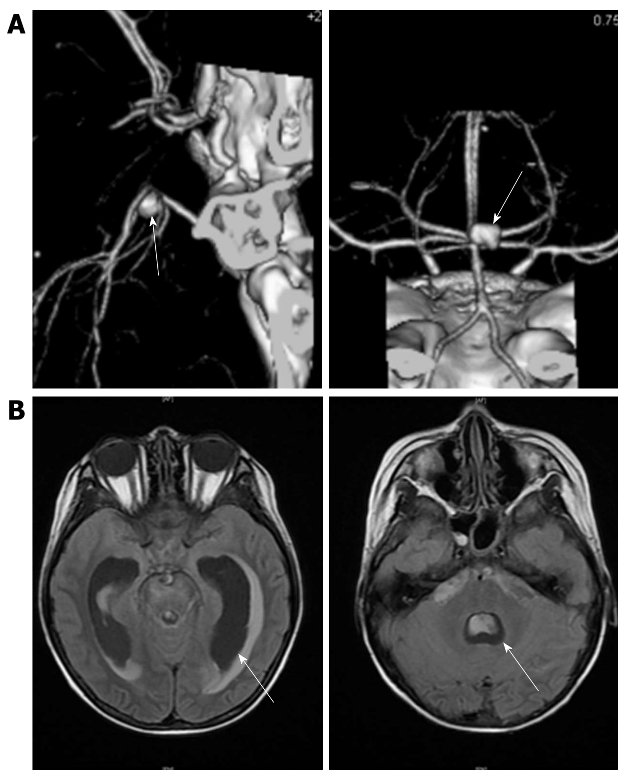


Figure 2 Three-dimensional reconstituted image of the intracranial aneurysm (A) and computer tomography scan (B). A: Arrows indicate intracranial aneurysm; B: Arrows indicate dilated ventricles and subdural hemorrhage.

leukemia (ALL) was admitted to our hospital. After confirming a good response to preceding prednisolone, TCCSG L09-16 (UMIN000003375) high risk (HR) induction chemotherapy was started. Two weeks later, the patient's white blood cell count (WBC) fell below 100/μL and fever was noted on day 19. Serum C-reactive protein (CRP) level was 2.38 mg/dL. Intravenous antibiotic [cefpirome sulfate (CPR)] was started on the same day. On the 22nd d, the patient's β-D-glucan level was below 6.0 pg/mL. Panipenem/betamipron (PAPM/BP) was administered instead of CPR, but changed to meropenem. Micafungin (MCFG; 6 mg/kg per day) was added and induction therapy was discontinued on the 29th d. The values of β-D-glucan and CRP increased to 27.4 pg/mL and 10.52 mg/dL, respectively, on the same day. Granulocyte colony-stimulating factor was started on the 37th day and liposomal amphotericin B (L-AMB; 6 mg/kg per day) was administered instead of MCFG on the 40th day. Whole-body computed tomography (CT) on the 40th day revealed no diagnostic findings. WBC increased to 3500/μL (neutrophils > 90%) on the 44th d. MCFG was re-started on the 48th d in addition to L-AMB. Although a repeated blood culture was negative, serum *Candida* antigen value was greater than 2 ng/mL on the 49th d. On the 51st d, the patient began to complain of an intermittent temporal headache. Whole-body CT on the 54th d revealed multiple lesions in the liver (Figure 1) and flucytosine (5-FC; 150 mg/kg per day) was added. On the 56th day, the patient's consciousness level suddenly dropped and he experienced vomiting and incontinence. CT and magnetic resonance imaging (MRI) (Figure 2) indicated subarachnoid hemorrhage and drainage from both lateral ventricles was performed. The cerebrospinal fluid (CSF) was bloody and negative for bacteria and fungi. On the next day, brain angiography revealed an aneurysm with a diameter of 7 mm on the right basilar artery and coil embolization was successfully performed on the same day.

The patient's condition was stabilized. His consciousness level occasionally fluctuated according to increased ventricular pressure, which rapidly improved on adjustment of ventricular drainage.

Because induction therapy for ALL was discontinued, maintenance therapy consisting of daily 6-mercaptopurine and weekly methotrexate was started on the 76th d. High-grade fever and elevated CRP levels were sustained, although the patient was clinically improving. Unexpectedly, the plasma β-D-glucan level gradually increased to more than 500 pg/mL. A CT scan on the 265th d revealed complete disappearance of multiple lesions in the liver. Five months later, CRP and β-D-glucan levels began to decrease slowly. 5-FC and L-AMB were discontinued on the 265th d and 335th d, respectively (Figure 3). On the 319th d, a ventricle-peritoneal shunt was performed. Ten months later, WBC gradually decreased with the progression of thrombocytopenia. A bone marrow examination on the 356th d confirmed the first relapse of ALL. Although the patient's β-D-glucan

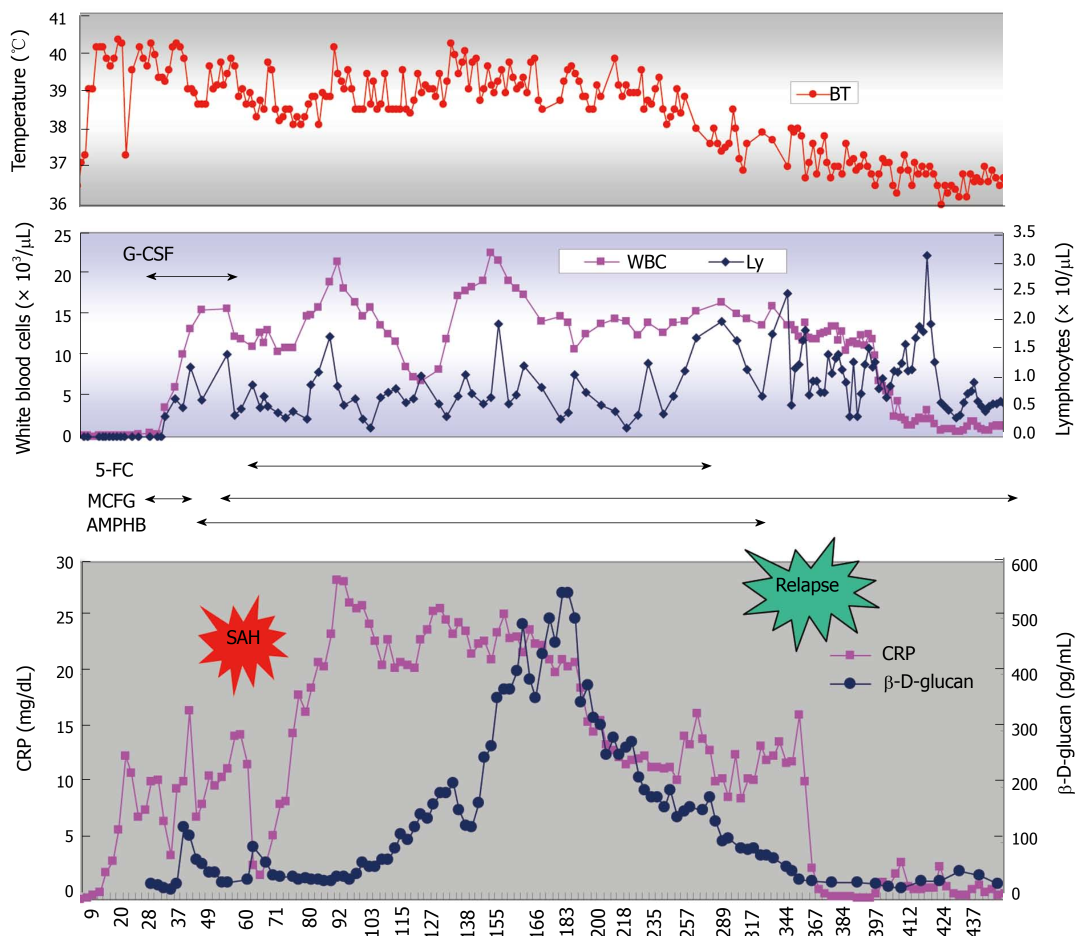


Figure 3 Clinical course of the patient. Initiation of prednisolone was designated as day 1. WBC: White blood cell; 5-FC: 5-flucytosine; MCFG: Micafungin; CRP: C-reactive protein.

level was still above 50 pg/mL, induction therapy based on the BFM95 protocol was started. After being in remission, he relapsed on the 680th d and on the 825th d. He received unrelated bone marrow transplantation (BMT) during non-remission status on the 839th d with a myeloablative conditioning regimen consisting of total body irradiation (TBI; 12 Gy), cyclophosphamide (60 mg/kg × 2) and etoposide (60 mg/kg). Graft versus host disease prophylaxis consisted of tacrolimus and short term methotrexate. Engraftment was on the 16th d and he became free of red cell and platelet cell transfusion on the 15th and 37th d after BMT respectively. He has been in remission for more than 3 mo after BMT. During the subsequent chemotherapy/BMT and thereafter, no recurrence of fungal infection was observed under the prophylactic use of MCFG.

DISCUSSION

Fungal infections account for 4% to 9% of neutropenic infections in patients with hematological malignancy[1]. Additionally, it has been reported that patients with ALL are at the highest risk for invasive candidiasis during the neutropenic period following induction chemotherapy[2]. Disseminated candidiasis is a rare, life-threatening extended form of *Candida* infection and its mortality exceeds 10%-50%[3]. Fungal aneurysms of the intracranial circulation are an extremely rare complication and clinically differ from the more common bacterial "mycotic" aneurysms, which are usually associated with infectious endocarditis[4-6]. Contrary to bacterial "mycotic" aneurysms, fungal aneurysms usually affect the circle of Willis and the proximal arterial tree, and surgical treatment is extremely difficult[6]. Mortality related to fungal aneurysms is extremely high and it exceeds 80%-90% when the aneurysms rupture[7,8]. According to the literature, cases of pediatric fungal aneurysm are very rare and most of them are complicated in patients with chronic mucocutaneous candidiasis, a rare primary immunodeficiency[9-11].

In our case, progression of disseminated candidiasis occurred within 3 wk after chemotherapy, which seems relatively fast. Once disseminated, a great deal of time is needed to treat the fungal infection even when the neutrophil count recovers[12]. It is known that liver lesions are not apparent under agranulocytic conditions because of the lack of inflammation[13]. It may be possible that an increase in neutrophils induces excessive inflammation, which sometimes results in tissue destruction. With intensive anti-fungal chemotherapy and surgical and endovascular treatment, our patient survived through the early critical episode. However, the subsequent clinical course of the patient was difficult and challenging. Once

CRP and β-D-glucan levels decreased after surgery, the CRP level increased again above 20 mg/dL and the β-D-glucan level increased gradually. Additionally, high fever continued for months.

In our case, clinical evidence of disseminated candidiasis was based on elevated β-D-glucan levels, the presence of serum *Candida* antigen and multiple liver lesions. Repeated blood or CSF cultures could not detect *Candida* spp. It has been reported that the positive predictive value of blood cultures for *Candida* is relatively low and the typical findings of a CT scan are clinically valuable for diagnosis with serological data[13]. Although histological and microbiological evidence could not be obtained, it is more likely that intracranial aneurysm was due to disseminated candidiasis from the clinical point of view. Guidelines for disseminated candidiasis recommend fluconazole, amphotericin B, voriconazole or caspofungin[14,15]. MCFG was recently reported to be as effective as caspofungin[16]. Because some strains of *Candida*, such as *C. glabrata* and *C. krusei*, are resistant to fluconazole, we selected a higher dose of MCFG for the first-line therapy. Considering the severity of infection, we added L-AMB to MCFG. Although elevated CRP and β-D-glucan levels with high fever continued as shown in Figure 3, the patient was clinically improving and we continued anti-fungal therapy. It is well known that humans do not produce enzymes that metabolize β-D-glucan. There may be 2 possible explanations for why β-D-glucan was increasing in the middle of the treatment course: one is that the destruction of *Candida* was accelerated at that time and another is that the *Candida* antigen was sequestered from circulation and drained into systemic circulation at that time. In light of the sustained high level of CRP and its later decrease in parallel with β-D-glucan, the former scenario seems to be more likely.

Fortunately, our patient survived and received BMT successfully with no recurrence of candidiasis or other fungal infections under the prophylactic use of MCFG thereafter. He has been in remission for more than 3 mo after BMT, although we have to be careful how long the patient remains in remission. With the recent advance of anti-fungal drugs, disseminated candidiasis is still a challenging complication during the treatment of hematological malignancy and is difficult to manage. However, intensive patient treatment has enabled us to accomplish chemotherapy and BMT successfully even in a high-risk patient with ALL[17].

COMMENTS

Case characteristics

An 11-year-old boy with acute lymphocytic leukemia who suffered from chronic disseminated candidiasis during remission induction chemotherapy.

Clinical diagnosis

Chronic disseminated candidiasis and fungal intracranial aneurysm and its rupture.

Differential diagnosis

Bacterial infection and intracranial hemorrhage due to granulocytopenia and thrombocytopenia

Laboratory diagnosis

Increased β-D-glucan and candida antigen in the serum

Imaging diagnosis

Whole computed tomography scan revealed multiple masses in the liver. Angiography disclosed intracranial aneurysm.

Treatment

Coiled embolization and long-term chemotherapy with combination of multiple anti-fungal drugs

Experiences and lessons

This case report emphasizes the difficulties of management of fungal infection during the chemotherapy of leukemia. Once it has occurred, not only control of fungal infection but also the control of leukemia is disturbed. However, bone marrow transplantation is not a contraindicated choice of therapy.

Peer review

This article is well described and some important messages emerge. This paper will therefore be very useful for hematologists and infectiologists.

REFERENCES

1 **Castagnola E**, Cesaro S, Giacchino M, Livadiotti S, Tucci F, Zanazzo G, Caselli D, Caviglia I, Parodi S, Rondelli R, Cornelli PE, Mura R, Santoro N, Russo G, De Santis R, Buffardi S, Viscoli C, Haupt R, Rossi MR. Fungal infections in children with cancer: a prospective, multicenter surveillance study. *Pediatr Infect Dis J* 2006; **25**: 634-639 [PMID: 16804435 DOI: 10.1097/01.inf.0000220256.69385.2e]

2 **Thaler M**, Pastakia B, Shawker TH, O'Leary T, Pizzo PA. Hepatic candidiasis in cancer patients: the evolving picture of the syndrome. *Ann Intern Med* 1988; **108**: 88-100 [PMID: 3276268 DOI: 10.7326/0003-4819-108-1-88]

3 **Hassan I**, Powell G, Sidhu M, Hart WM, Denning DW. Excess mortality, length of stay and cost attributable to candidaemia. *J Infect* 2009; **59**: 360-365 [PMID: 19744519 DOI: 10.1016/j.jinf.2009.08.020]

4 **Eishi K**, Kawazoe K, Kuriyama Y, Kitoh Y, Kawashima Y, Omae T. Surgical management of infective endocarditis associated with cerebral complications. Multi-center retrospective study in Japan. *J Thorac Cardiovasc Surg* 1995; **110**: 1745-1755 [PMID: 8523887 DOI: 10.1016/S0022-5223(95)70038-2]

5 **Oohara K**, Yamazaki T, Kanou H, Kobayashi A. Infective endocarditis complicated by mycotic cerebral aneurysm: two case reports of women in the peripartum period. *Eur J Cardiothorac Surg* 1998; **14**: 533-535 [PMID: 9860215 DOI: 10.1016/S1010-7940(98)00221-8]

6 **Watson JC**, Myseros JS, Bullock MR. True fungal mycotic aneurysm of the basilar artery: a clinical and surgical dilemma. *Cerebrovasc Dis* 1999; **9**: 50-53 [PMID: 9873163 DOI: 10.1159/000015896]

7 **Hurst RW**, Judkins A, Bolger W, Chu A, Loevner LA. Mycotic aneurysm and cerebral infarction resulting from fungal sinusitis: imaging and pathologic correlation. *AJNR Am J Neuroradiol* 2001; **22**: 858-863 [PMID: 11337328]

8 **Regelsberger J**, Elsayed A, Matschke J, Lindop G, Grzyska U, van den Boom L, Venne D. Diagnostic and therapeutic considerations for "mycotic" cerebral aneurysms: 2 case reports and review of the literature. *Cent Eur Neurosurg* 2011; **72**: 138-143 [PMID: 21830179]

9 **Marazzi MG**, Bondi E, Giannattasio A, Strozzi M, Savioli C. Intracranial aneurysm associated with chronic mucocutaneous candidiasis. *Eur J Pediatr* 2008; **167**: 461-463 [PMID: 17443345 DOI: 10.1007/s00431-007-0490-3]

10 **Leroy D**, Dompmartin A, Houtteville JP, Theron J. Aneurysm associated with chronic mucocutaneous candidiasis during long-term therapy with ketoconazole. *Dermatologica* 1989; **178**: 43-46 [PMID: 2492956 DOI: 10.1159/000248386]

11 **Loeys BL**, Van Coster RN, Defreyne LR, Leroy JG. Fungal intracranial aneurysm in a child with familial chronic mucocutaneous candidiasis. *Eur J Pediatr* 1999; **158**: 650-652 [PMID: 10445344 DOI: 10.1007/s004310051169]

12 **Kontny U**, Walsh TJ, Rossler J, Uhl M, Niemeyer CM. Successful treatment of refractory chronic disseminated candidiasis after prolonged administration of caspofungin in a child with acute myeloid leukemia. *Pediatr Blood Cancer* 2007; **49**: 360-362 [PMID: 16444677 DOI: 10.1002/pbc.20751]
13 **Semelka RC**, Kelekis NL, Sallah S, Worawattanakul S, Ascher SM. Hepatosplenic fungal disease: diagnostic accuracy and spectrum of appearances on MR imaging. *AJR Am J Roentgenol* 1997; **169**: 1311-1316 [PMID: 9353448 DOI: 10.2214/ajr.169.5.9353448]
14 **Ascioglu S**, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, Denning DW, Donnelly JP, Edwards JE, Erjavec Z, Fiere D, Lortholary O, Maertens J, Meis JF, Patterson TF, Ritter J, Selleslag D, Shah PM, Stevens DA, Walsh TJ. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002; **34**: 7-14 [PMID: 11731939 DOI: 10.1086/323335]
15 **Freifeld AG**, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, Raad II, Rolston KV, Young JA, Wingard JR. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the infectious diseases society of america. *Clin Infect Dis* 2011; **52**: e56-e93 [PMID: 21258094]
16 **Spellberg BJ**, Filler SG, Edwards JE. Current treatment strategies for disseminated candidiasis. *Clin Infect Dis* 2006; **42**: 244-251 [PMID: 16355336 DOI: 10.1086/499057]
17 **Masood A**, Sallah S. Chronic disseminated candidiasis in patients with acute leukemia: emphasis on diagnostic definition and treatment. *Leuk Res* 2005; **29**: 493-501 [PMID: 15755501 DOI: 10.1016/j.leukres.2004.10.003]

P- Reviewers: Uderzo C, Zimmer J **S- Editor**: Zhai HH
L- Editor: Roemmele A **E- Edi**

WJH World Journal of Hematology

Online Submissions: http://www.wjgnet.com/esps/
wjhematol@wjgnet.com
www.wjgnet.com

World J Hematol 2014 May 6; 3(2): I-V
ISSN 2218-6204 (online)
© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Hematology (*World J Hematol*, *WJH*, online ISSN 2218-6204, DOI: 10.5315) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

Aim and scope

WJH covers topics concerning experimental, clinical, oncological and transplant hematology, transfusion science, hemostasis and thrombosis, evidence-based medicine, epidemiology and nursing. The current columns of *WJH* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of hematological 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.

WJH is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 42 OA clinical medical journals, including 41 in English, has a total of 15 471 editorial borad members or peer reivewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJH* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in hematology; (12) Brief Articles: To briefly report the novel and innovative findings in hematology; (13) Meta-Analysis: To evaluate the clinical effectiveness in hematology by using data from two or more randomised control trials; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJH*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of hematology; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal

World Journal of Hematology

ISSN

ISSN 2218-6204 (online)

Launch date

June 6, 2012

Frequency

Quarterly

Editor-in-Chief

Xiaoyan Jiang, MD, PhD, Associate Professor, Medical Gen-

etics, University of British Columbia, Terry Fox Laboratory, British Columbia Cancer Agency, 675 West 10th Ave, Vancouver, BC, V5Z 1L3, Canada

Thomas J Kipps, MD, PhD, Professor of Medicine, University of California, San Diego, Moores Cancer Center, 3855 Health Sciences Drive, MC 0820, La Jolla, CA 92093-0820, United States

Editorial office

Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Hematology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjhematol@wjgnet.com
http://www.wjgnet.com

Publisher

Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Telephone: +852-58042046
Fax: +852-31158812
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

Production center

Beijing Baishideng BioMed Scientific Co., Limited
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893

Representative office

USA Office
8226 Regency Drive,
Pleasanton, CA 94588-3144, United States

Instructions to authors

Full instructions are available online at http://www.wjgnet.com/2218-6204/g_info_20100722173604.htm.

Indexed and Abstracted in

Digital Object Identifier.

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing

Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement

In the interests of transparency and to help reviewers assess any potential bias, *WJH* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent

Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights

When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now

available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2218-6204/g_info_20100722173604.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjhematol@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, *e.g.*, Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, *e.g.*, Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present P values where appropriate and must provide relevant data to illustrate how they were obtained, *e.g.*, 6.92 ± 3.86 *vs* 3.61 ± 1.67, P < 0.001), and CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. ^aP <

0.05, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of P values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, *e.g.*, PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2218-6204/g_info_20100723213202.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2218-6204/g_info_20100723213049.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2218-6204/g_info_20100723212803.htm.

Proof of financial support

For papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

Links to documents related to the manuscript

WJH will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Publication fee

WJH is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 698 USD per article. All invited articles are published free of charge.

百世登

Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.