ISSN 2220-3249 (online)

World Journal of *Virology*

World J Virol 2012 December 12; 1(6): 150-183

Baishideng Publishing Group
www.wjgnet.com

World Journal of Virology

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

Editorial Board

2011-2015

The *World Journal of Virology* Editorial Board consists of 137 members, representing a team of worldwide experts in virology. They are from 41 countries, including Argentina (2), Australia (4), Austria (1), Barbados (1), Belgium (1), Brazil (2), Bulgaria (1), Canada (4), Chile (1), China (24), Croatia (1), Egypt (1), Finland (2), France (5), Germany (2), Greece (1), Hungary (1), India (3), Iran (1), Ireland (1), Israel (2), Italy (13), Japan (6), Kazakhstan (1), Mexico (1), Netherlands (3), Pakistan (1), Palestine (1), Poland (1), Portugal (1), Romania (1), Saudi Arabia (1), Singapore (1), Slovakia (1), South Africa (1), Spain (8), Sweden (1), Thailand (4), Turkey (1), United States (28), and Uruguay (1).

Christoph Eisenbach, *Heidelberg*

Greece

Apostolos I Beloukas, *Athens*

Hungary

Krisztián Bányai, *Budapest*

India

Jayanta Bhattacharya, *Pune*
Runu Chakravarty, *Kolkata*
Sibnarayan Datta, *Tezpur*

Iran

Seyed Mojtaba Ghiasi, *Tehran*

Ireland

Carlo Bidoia, *Dublin*

Israel

Irit Davidson, *Bet Dagan*
Murad Ghanim, *Bet Dagan*

Italy

Alberto Alberti, *Sassari*
Gualtiero Alvisi, *Padua*
Massimiliano Berretta, *Aviano*
Franco M Buonaguro, *Naples*
Marco Ciotti, *Rome*
Cristina Costa, *Turin*
Piergiuseppe De Berardinis, *Naples*
Federico De Marco, *Rome*
Massimo De Paschale, *Legnano*
Maurizia Debiaggi, *Pavia*
Paolo Fabris, *Vicenza*
Simone Giannecchini, *Florence*
Roberto Manfredi, *Bologna*

Japan

Masashi Emoto, *Maebashi*
Bin Gotoh, *Otsu*
Kazuyoshi Ikuta, *Suita*
Hiroki Isomura, *Nagoya*
Hideya Kawasaki, *Hamamatsu-shi*
Eiichi N Kodama, *Sendai*

Kazakhstan

Vladimir E Berezin, *Almaty*

Mexico

Juan E Ludert, *Mexico*

Netherlands

Ben Berkhout, *Amsterdam*
Byron EE Martina, *Rotterdam*
Willem JG Melchers, *Nijmegen*

Pakistan

Muhammad Masroor Alam, *Islamabad*

Palestine

Ahamd Y Amro, *Jerusalem*

Poland

Brygida Knysz, *Wroclaw*

Portugal

Celso Cunha, *Lisbon*

Romania

Anda Baicus, *Bucharest*

Saudi Arabia

Ahmed Sayed Abdel-Moneim, *Al-Taif*

Singapore

Ding Xiang Liu, *Singapore*

Slovakia

Gabriela Bukovska, *Bratislava*

South Africa

Huub C Gelderblom, *Durban*

Spain

Alfredo Berzal-Herranz, *Granada*
Rafael Blasco, *Madrid*
Luis Enjuanes, *Madrid*
Jaime Gómez-Laguna, *Córdoba*
Juan Martínez Hernández, *Madrid*
Cecilio López-Galíndez, *Majadahonda*
José A Melero, *Madrid*
Luis Menéndez-Arias, *Madrid*

Sweden

Göran Bucht, *Umeå*

Thailand

Prasert Auewarakul, *Bangkok*
Parin Chaivisuthangkura, *Bangkok*
Wasin Charerntantanakul, *Chiang Mai*
Sasisopin Kiertiburanakul, *Bangkok*

Turkey

Ömer Coşkun, *Ankara*

United States

Judith M Ball, *College Station*
Igor M Belyakov, *Ann Arbor*
Preeti Bharaj, *Orlando*
Alexander A Bukreyev, *Galveston*
Maria G Castro, *Los Angeles*
Vincent N Fondong, *Dover*
Shou-Jiang Gao, *San Antonio*
David R Gretch, *Seattle*
Hailong Guo, *Rochester*
Haitao Guo, *Doylestown*
Young S Hahn, *Charlottesville*
Kuan-Teh Jeang, *Bethesda*
Xia Jin, *Rochester*
Clinton Jones, *Lincoln*
Krishna Mohan V Ketha, *Bethesda*
Prasad S Koka, *San Diego*
Sachin Kumar, *College Park*
Majid Laassri, *Rockville*
Feng Li, *Brookings*
Ling Lu, *Kansas*
Yuanan Lu, *Honolulu*
Paolo Lusso, *Bethesda*
Barry J Margulies, *Towson*
Michael R McConnell, *San Diego*
Mansour Mohamadzadeh, *Chicago*
Thomas P Monath, *Menlo Park*
Jonathan Moorman, *Johnson*

Uruguay

Matías Victoria, *Salto*

World Journal of Virology

Contents

Bimonthly Volume 1 Number 6 December 12, 2012

World Journal of Virology
Volume 1 Number 6 December 12, 2012

Contents

NAME OF JOURNAL
World Journal of Virology

ISSN
ISSN 2220-3249 (online)

LAUNCH DATE
February 12, 2012

FREQUENCY
Bimonthly

EDITING
Editorial Board of *World Journal of Virology*
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: wjv@wjgnet.com
http://www.wjgnet.com

EDITOR-IN-CHIEF
Ling Lu, MD, PhD, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, 3901 Rainbow Blvd, WHE 3020, KS 66160, United States

EDITORIAL OFFICE
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Virology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-8538-1893
E-mail: wjv@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
December 12, 2012

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2220-3249/g_info_20100722180909.htm

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

World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
doi:10.5501/wjv.v1.i6.150

World J Virol 2012 December 12; 1(6): 150-153
ISSN 2220-3249 (online)

EDITORIAL

Evaluation of virus-specific cellular immune response in transplant patients

Cristina Costa, Alda Saldan, Rossana Cavallo

Cristina Costa, Alda Saldan, Rossana Cavallo, Virology Unit, University Hospital San Giovanni Battista di Torino, 10126 Turin, Italy
Author contributions: Costa C and Cavallo R designed and writed the manuscript; Saldan A contributed on method description.
Correspondence to: Cristina Costa, MD, PhD, Virology Unit, University Hospital San Giovanni Battista di Torino, Via Santena 9, 10126 Turin, Italy. cristina.costa@unito.it
Telephone: +39-11-6705630 Fax: +39-11-6705648
Received: August 8, 2011 Revised: August 23, 2012
Accepted: November 7, 2012
Published online: December 12, 2012

Abstract

Virus-specific immune responses have a major impact on the outcome of the infection. Viral agents that are characterized by latency, such as herpesviruses and polyomaviruses, require a continuous immune control to reduce the extent of viral reactivation, as viral clearance cannot be accomplished, independently from the anti-viral treatment. In transplant patients, morbidity and mortality related to viral infections are significantly increased. In fact, the key steps of activation of T-cells are major target for anti-rejection immunosuppressive therapy and anti-viral immune response may be altered when infected cells and cellular effectors of immune response coexist in a transplanted organ. The role of cellular immune response in controlling viral replication and the main methods employed for its evaluation will be discussed. In particular, the main features, including both advantages and limitations, of available assays, including intracellular cytokine staining, major histocompatibility complex - multimer-based assays, Elispot assay, and QuantiFERON test, will be described. The potential applications of these assays in the transplant context will be discussed, particularly in relation to cytomegalovirus and polyomavirus BK infection. The relevance of introducing viro-immunological monitoring, beside virological monitoring, in order to identify the risk profile for viral infections in the transplant patients will allows for define a patient-tailored clinical management, particular in terms of modulation of immunosuppressive therapy and anti-viral administration.

Key words: T-cell; Immune response; Viral replication; Interferon-γ; Transplantation

Peer reviewers: Hailong Guo, DVM, PhD, David H. Smith Center for Vaccine Biology and Immunology, University of Rochester Medical Center, 601 Elmwood Avenue, Box 609, Rochester, NY 14642, United States; Igor M Belyakov, MD, PhD, DSc, University Faculty Member, MNIMBS, Department of Internal Medicine, Graduate Program in Immunology, University of Michigan, School of Medicine, 109 Zina Pitcher Place, BSRB, Room 4039, Ann Arbor, MI 48109, United States

Costa C, Saldan A, Cavallo R. Evaluation of virus-specific cellular immune response in transplant patients. *World J Virol* 2012; 1(6): 150-153 Available from: URL: http://www.wjgnet.com/2220-3249/full/v1/i6/150.htm DOI: http://dx.doi.org/10.5501/wjv.v1.i6.150

INTRODUCTION

Virus-specific immune responses have a major impact on the outcome of the infection. Viral infections are usually followed by the complete clearance of the virus. However, in some cases, immune response is not able to eliminate completely the virus, thus the infection may become persistent. In general, cellular immune response plays a more relevant role in the viral clearance, whereas humoral immune response protects against the reinfection. Immune response may vary according to genetic substrate of the individual and the degree of immunocompetence.

In transplant patients, morbidity and mortality related to viral infections are significantly increased. In fact, anti-rejection immunosuppressive therapy weakens the im-

mune functions and anti-viral immune response may be altered when infected cells and cellular effectors of immune response coexist in a transplanted organ.

In particular, viral agents that are characterized by latency, such as herpesviruses and polyomaviruses, require a continuous immune control to reduce the extent of viral reactivation, as viral clearance cannot be accomplished, independently from the anti-viral treatment. The immunologic control of these viruses in the immunocompromised host is complex and involves both the innate and adaptative immune systems. As regards innate immune system, polymorphisms of Toll-like receptors as well as certain proteins, including complement proteins, and defects in natural killer cells are associated with an increased risk of viral reactivation. Adaptative immune responses of B and T cells are critical in controlling viral replication. In particular, while B cells may be important in the humoral response in that they produce neutralizing antibodies targeting virions, T-cell mediated responses, including both CD4+ and CD8+ T-cells, are crucial components for controlling viral replication of persistent viruses[1]. Several phenotypic markers and functions have been investigated in order to characterize virus-specific CD4+ and CD8+ T cell responses, evidencing a great heterogeneity against different viruses, with functional signatures being probably specific for each virus and being predominantly regulated by the levels of antigen load. In this context, polyfunctional secretion and proliferation [i.e., interferon (IFN)-γ and interleukin-2] of CD4+ and CD8+ T cells play a protective role in terms of antiviral immunity in chronic viral infections[2,3].

METHODS FOR EVALUATING VIRUS-SPECIFIC CELLULAR IMMUNE RESPONSE

Evaluation of virus-specific T-cell responses can be made by different methods (Table 1), including: intracellular cytokine staining (ICS), major histocompatibility complex (MHC)-multimer-based assay, Enzyme-linked Immunospot (ELISPOT) assay and QuantiFERON-cytomegalovirus (CMV) assay (specific for CMV)[4-6]. Beside these virus-specific assays, it is also available a non-specific test, the ImmunKnow assay, that is used to evaluate the overall CD4+ immune response.

Most of these assays have been used in experimental settings and only for some of them commercial kit are available. The majority of the assays are based on the detection of IFN-γ after stimulation with specific viral antigens or viral lysates, the so-called IFN-γ releasing assays (IGRA). Anyway, other markers, including different cytokines (e.g., interleukin-2, tumor necrosis factor-α, *etc.*) can be used. In terms of clinical utility, an ideal test should evaluate both virus-specific CD4+ and CD8+ T-cell immune response from both quantitative (number of virus-specific T cells) and function (number of functional T cells) points of view. Moreover, for an appropriate use in the clinical setting, an assay should be easy and rapid to perform, relatively inexpensive, highly reproducible, and applicable in different routine contexts. At the moment, all the assays present specific advantages and limitations.

Intracellular cytokine staining

Most studies have analyzed virus-specific T-cell responses using ICS for IFN-γ using flow cytometry[4]. Moreover, ICS allows for evaluation of polyfunctionality of T cells[2,3]. Whole blood or isolated peripheral blood mononuclear cells (PBMCs) are stimulated with virus-specific peptides or viral lysates. The assay is not restricted for human leukocyte antigen (HLA) when viral lysate is used and there Is no need to know HLA type. Stimulated cells are stained with monoclonal antibodies directed against IFN-γ. The method is rapid, with a short incubation time and results being available within 24 h; can be performed starting from low blood volume (approximately 1 mL); it allows for identification of CD4+ and CD8+ T cells. The major drawbacks are the need for a flow cytometer and the lack of standardization. Among the advantages, there is the possibility to freeze the cells and send them to reference laboratory for testing.

MHC - multimer-based assays

MHC-multimer-based assays is characterized by the direct staining of peptide-specific T-cells using peptide-conjugated MHC class I tetramers or pentamers[7]. This method evaluates CD8+ T-cell responses, however it is epitope-specific and require knowledge of the patient' s HLA type. As for ICS, there is the need for access to a flow cytometer and the assay is not standardized. Among the advantages, the assay is rapid (1-2 h) and can be performed starting from low blood volume (0.5-1 mL).

Elispot

The ELISPOT assay determines the number of T-cells secreting IFN-γ in specific response to a viral agent. Following stimulation with viral peptides or viral lysates, the produced IFN-γ is captured by a specific antibody, and then quantified using a labeled antibody[8]. The assay evaluates both CD4+ and CD8+ responses and there is no need to know HLA, as well as to use a flow cytometer. There is the possibility to freeze the cells and send them to reference laboratory for testing. Among the diasadvantages, the method requires a relatively high volume of blood (approximately 7-10 mL) and cannot differentiate between CD4+ and CD8+ cells. Although some approaches to standardize the assay, it is still not standardized. Results are available within 24-30 h. A mitogen control assay can determine general T-cell responsiveness. Commercially available kits for virus-specific ELISPOT assays are available (AID, Autoimmun Diagnostika GmbH, Strassberg, Germany).

QuantiFERON-CMV

This is a CMV-specific ELISA-based IFN-γ release assay and is commercially available (Cellestis Inc., Melbourne, Australia). It can be easily performed starting from low blood volume (3 mL) and results are available after

Table 1 Methods for evaluating cellular immune response

Method	Advantages	Limitations
Intracellular cytokine staining	Low volume blood	Flow cytometer required
	Rapid	Not standardized
	Knowledge of HLA not required	
	Identification of CD4+ and CD8+	
MHC multimer staining	Low volume blood	Flow cytometer required
	Rapid	Not standardized
Elispot assay	Identification of CD4+ and CD8+	Not standardized

MHC: Major histocompatibility complex; HLA: Human leukocyte antigen.

30-40 h. It evaluates only CD8+ responses. The assay may yield nonresponse results in the presence of global immunosuppression (nonresponse to mitogen) as in transplant patients. Test sensitivity decreases in lymphopenic patients because an appropriate number of T-cells are required for the production of IFN-γ.

ImmunKnow assay

The Cylex ImmunKnow assay (Cylex Inc., Columbia, MD, US) is a aspecific assay, which is commercially available. It measures the overall immune response and is indicative of immunesuppression. It determines the amount of ATP produced in response to whole-blood stimulation by an aspecific mitogen (phytohemagglutinin).

ROLE OF VIRUS-SPECIFIC CELLULAR IMMUNE RESPONSE IN TRANSPLANT PATIENTS

T-cell responses, including both CD4+ and CD8+, are critically important for controlling viral replication. This has been demonstrated by the use of adoptive immunotherapy for treatment of CMV and EBV infections and by the higher frequency of viral reactivation in patients treated with anti-lymphocyte agents. Early reconstitution of cellular immune response prevents or reduces the duration of the infection, thus avoiding the onset of disease or relapses. On the other hand, a delayed or reduced response represents the pathogenic base for the occurrence of repeated episode of infection and symptomatic disease (in the absence of anti-viral treatment)[7-11]. The role of cellular immune response in the context of organ transplantation has been studied particularly for CMV. In particular, it has been evidenced the basic role played by both CD4+ and CD8+ responses[9]. The CD8+ response is prevalent during the acute phase of infection and it provides for an immediate control of viral replication by the killing of cells in which CMV is replicating. The CD4+ response is fundamental for the long-term maintenance of antiviral control. In a study on solid-organ transplant recipients, it has been demonstrated that stable levels of CMV-specific CD4+ cells correlates with the absence of infectious complications, whereas in patients with unstable levels of specific-CD4+ cells, several episodes of viral reactivation occurred[10]. The median frequency of CMV-specific CD8+ cells was significantly higher in a group of 27 heart and lung transplant patients in cases that did not developed CMV-disease[11]. Moreover, in a study on 73 renal transplant recipients, also the median frequency of CMV-specific CD4+ cells was significantly higher in patients that did not developed CMV disease[12,13]. Gerna and coll. proposed the classification of transplant recipients into two groups in relation to the temporal profile for CMV-specific cellular immune response[14]. The Authors considered a group of early responders, in which reconstitution of cellular immune response occurred within 30 d posttransplantation, and a group of late responders, in which cellular immune response reconstitution occurred at > 30 d posttransplantation and/or was reduced. In these patients, the delay in CD4+ response appeared to be particularly critical[15].

Another virus for which specific cellular response has been investigated is polyomavirus BK. It has been evidenced that healthy BKV-seropositive individuals have CD4+ and CD8+ cells specific for BKV major antigens (including large T antigen and capsid viral protein VP1)[16]. The unbalance between viral replication and BKV-specific cellular immune response should represent the common denominator for the pathogenesis of polyomavirus-associated nephropathy, a viral-associated complication potentially leading to the lost of the transplanted organ and mainly occurring in renal transplant patients[15,16]. It has been evidenced that cellular response to large T antigen and VP1 is significantly higher in patients with decreasing viremia in comparison to those with increasing viremia[16]. Moreover, it seems that the level of immune response is correlated particularly to the administration of some immunosuppressive drugs. For example, in a study on kidney transplant patients *in vivo*, responses were inversely correlated with tacrolimus through levels, but not mycophenolate mofetil, prednisone or the overall immunosuppressive dosing[17]. However, the clinical role of BKV viro-immunological monitoring in renal transplant recipient needs to be further investigated, in particular it is likely that it could represent an approach to modulate immunosuppression.

An interesting issue in the context of protective immunity is represented by the role of mucosal immunization. As mucosal surfaces represent the major entry fro many human pathogens (including HSV, HIV, respiratory viruses, as well as mycobacteria), induction of mucosal

immune system, including both innate and adaptative responses (CD4+ T helper cells, Th17 cells, high avidity CD8+ cytotoxic T lymphocytes, as well as IgA and IgG1 neutralizing antibodies) seem required for an effective protection against pathogens that lead to chronic infections[18,19].

CONCLUSION

The increase in the number of transplant patients and the use of deeply immunosuppressive drugs have lead to the emergence of viral infections that may influence the outcome of these patients. Beside the adoption of protocols for close virological monitoring, several studies have underlined the role of viro-immunological monitoring in the evaluation of patient's risk of infection and disease, decision on treatment by modulating immunosuppression and/or using antiviral, and adoption of other diagnostic strategies. There is no defined method to evaluate the cellular immune response in transplant recipients, as at the moment no assay is standardized and further studies, particularly on procedures, quality controls and references, as well as interpretation of results are required to standardize. However, ELISPOT assay is gaining increasing recognition due to the potential to evaluate both CD4+ and CD8+ responses and other favoring features. Further studies on the clinical role of cellular immune responses are required, in particular with the aim of define modes and temporal profile for viro-immunological monitoring in different transplant contexts and for different viral agents.

REFERENCES

1 **Davenport MP**, Price DA, McMichael AJ. The T cell repertoire in infection and vaccination: implications for control of persistent viruses. *Curr Opin Immunol* 2007; **19**: 294-300
2 **Makedonas G**, Betts MR. Polyfunctional analysis of human t cell responses: importance in vaccine immunogenicity and natural infection. *Springer Semin Immunopathol* 2006; **28**: 209-219
3 **Harari A**, Dutoit V, Cellerai C, Bart PA, Du Pasquier RA, Pantaleo G. Functional signatures of protective antiviral T-cell immunity in human virus infections. *Immunol Rev* 2006; **211**: 236-254
4 **Letsch A**, Scheibenbogen C. Quantification and characterization of specific T-cells by antigen-specific cytokine production using ELISPOT assay or intracellular cytokine staining. *Methods* 2003; **31**: 143-149
5 **Walker JM**, Slifka MK. Longevity of T-cell memory following acute viral infection. *Adv Exp Med Biol* 2010; **684**: 96-107
6 **Kotton CN**, Kumar D, Caliendo AM, Asberg A, Chou S, Snydman DR, Allen U, Humar A. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010; **89**: 779-795
7 **Sester M**, Sester U, Gärtner BC, Girndt M, Meyerhans A, Köhler H. Dominance of virus-specific CD8 T cells in human primary cytomegalovirus infection. *J Am Soc Nephrol* 2002; **13**: 2577-2584
8 **Altman JD**, Moss PA, Goulder PJ, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, Davis MM. Phenotypic analysis of antigen-specific T lymphocytes. Science. 1996. 274: 94-96. *J Immunol* 2011; **187**: 7-9
9 **Czerkinsky CC**, Nilsson LA, Nygren H, Ouchterlony O, Tarkowski A. A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specific antibody-secreting cells. *J Immunol Methods* 1983; **65**: 109-121
10 **Sester U**, Gärtner BC, Wilkens H, Schwaab B, Wössner R, Kindermann I, Girndt M, Meyerhans A, Mueller-Lantzsch N, Schäfers HJ, Sybrecht GW, Köhler H, Sester M. Differences in CMV-specific T-cell levels and long-term susceptibility to CMV infection after kidney, heart and lung transplantation. *Am J Transplant* 2005; **5**: 1483-1489
11 **Bunde T**, Kirchner A, Hoffmeister B, Habedank D, Hetzer R, Cherepnev G, Proesch S, Reinke P, Volk HD, Lehmkuhl H, Kern F. Protection from cytomegalovirus after transplantation is correlated with immediate early 1-specific CD8 T cells. *J Exp Med* 2005; **201**: 1031-1036
12 **Egli A**, Binggeli S, Bodaghi S, Dumoulin A, Funk GA, Khanna N, Leuenberger D, Gosert R, Hirsch HH. Cytomegalovirus and polyomavirus BK posttransplant. *Nephrol Dial Transplant* 2007; **22** Suppl 8: viii72-viii82
13 **Egli A**, Binet I, Binggeli S, Jäger C, Dumoulin A, Schaub S, Steiger J, Sester U, Sester M, Hirsch HH. Cytomegalovirus-specific T-cell responses and viral replication in kidney transplant recipients. *J Transl Med* 2008; **6**: 29
14 **Gerna G**, Lilleri D, Fornara C, Comolli G, Lozza L, Campana C, Pellegrini C, Meloni F, Rampino T. Monitoring of human cytomegalovirus-specific CD4 and CD8 T-cell immunity in patients receiving solid organ transplantation. *Am J Transplant* 2006; **6**: 2356-2364
15 **Comoli P**, Azzi A, Maccario R, Basso S, Botti G, Basile G, Fontana I, Labirio M, Cometa A, Poli F, Perfumo F, Locatelli F, Ginevri F. Polyomavirus BK-specific immunity after kidney transplantation. *Transplantation* 2004; **78**: 1229-1232
16 **Binggeli S**, Egli A, Schaub S, Binet I, Mayr M, Steiger J, Hirsch HH. Polyomavirus BK-specific cellular immune response to VP1 and large T-antigen in kidney transplant recipients. *Am J Transplant* 2007; **7**: 1131-1139
17 **Egli A**, Köhli S, Dickenmann M, Hirsch HH. Inhibition of polyomavirus BK-specific T-Cell responses by immunosuppressive drugs. *Transplantation* 2009; **88**: 1161-1168
18 **Belyakov IM**, Kuznetsov VA, Kelsall B, Klinman D, Moniuszko M, Lemon M, Markham PD, Pal R, Clements JD, Lewis MG, Strober W, Franchini G, Berzofsky JA. Impact of vaccine-induced mucosal high-avidity CD8+ CTLs in delay of AIDS viral dissemination from mucosa. *Blood* 2006; **107**: 3258-3264
19 **Belyakov IM**, Ahlers JD. What role does the route of immunization play in the generation of protective immunity against mucosal pathogens? *J Immunol* 2009; **183**: 6883-6892

S- Editor Wang JL **L- Editor** A **E- Editor** Zheng XM

World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
doi:10.5501/wjv.v1.i6.154

World J Virol 2012 December 12; 1(6): 154-161
ISSN 2220-3249 (online)

REVIEW

Roles of the PI3K/Akt pathway in Epstein-Barr virus-induced cancers and therapeutic implications

Jiezhong Chen

Jiezhong Chen, Illawarra Health and Medical Research Institute, University of Wollongong, Northfields Avenue, NSW 2522, Australia
Author contributions: Chen J solely contributed to this paper.
Correspondence to: Jiezhong Chen, PhD, Illawarra Health and Medical Research Institute, University of Wollongong, Northfields Avenue, NSW 2522, Australia. jiezhong@uow.edu.au
Telephone: +61-2-42213199 Fax: +61-2-42218130
Received: July 13, 2011 Revised: October 16, 2012
Accepted: November 7, 2012
Published online: December 12, 2012

Abstract

Viruses have been shown to be responsible for 10%-15% of cancer cases. Epstein-Barr virus (EBV) is the first virus to be associated with human malignancies. EBV can cause many cancers, including Burkett's lymphoma, Hodgkin's lymphoma, post-transplant lymphoproliferative disorders, nasopharyngeal carcinoma and gastric cancer. Evidence shows that phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) plays a key role in EBV-induced malignancies. The main EBV oncoproteins latent membrane proteins (LMP) 1 and LMP2A can activate the PI3K/Akt pathway, which, in turn, affects cell survival, apoptosis, proliferation and genomic instability *via* its downstream target proteins to cause cancer. It has also been demonstrated that the activation of the PI3K/Akt pathway can result in drug resistance to chemotherapy. Thus, the inhibition of this pathway can increase the therapeutic efficacy of EBV-associated cancers. For example, PI3K inhibitor Ly294002 has been shown to increase the effect of 5-fluorouracil in an EBV-associated gastric cancer cell line. At present, dual inhibitors of PI3K and its downstream target mammalian target of rapamycin have been used in clinical trials and may be included in treatment regimens for EBV-associated cancers.

Key words: Epstein-Barr virus; Latent membrane proteins 1; Latent membrane proteins 2A; Phosphoinositide 3-kinase/protein kinase B; Carcinogenesis; Drug resistance

Peer reviewer: Jaime Gómez-Laguna, DVM, PhD, Researcher, Department of R and D, Centre of Research and Agrifood Quality - CICAP, Ctra de la Canaleja s/n Apdo Correos 105, Pozoblanco, Córdoba, 14400, Spain

Chen J. Roles of the PI3K/Akt pathway in Epstein-Barr virus-induced cancers and therapeutic implications. *World J Virol* 2012; 1(6): 154-161 Available from: URL: http://www.wjgnet.com/2220-3249/full/v1/i6/154.htm DOI: http://dx.doi.org/10.5501/wjv.v1.i6.154

INTRODUCTION

It is now evident that virus-induced cancers account for 10%-15% of all cancer cases[1,2]. Studies of viruses as causes of cancer have played an important role in the elucidation of the mechanisms of carcinogenesis, as indicated by several Nobel Prizes being awarded to scientists in the field of oncoviruses. The initial work to demonstrate that viruses can induce cancer was done by Peyton Rous[3,4]. He identified Rous sarcoma virus as the cause of chicken sarcoma in 1911, and the discovery earned him the 1966 Nobel Prize. The human analogue of the viral oncogene *v-Src* was found and named *c-Src*, which was the first human oncogene[5,6]. This work led to the awarding of a Nobel Prize to John Michael Bishop and Harold E. Varmus in 1989. More recently, Harald zur Hausen identified human papillomavirus (HPV) as the cause of cervical cancer (Nobel Prize, 2008)[7]. This discovery led to the invention of the vaccines Gardasil and Cervarix which can effectively prevent HPV-associated cervical cancer[8,9]. The Epstein-Barr virus (EBV); [also called human herpesvirus 4 (HHV-4)] is the first virus identified (in 1964) to be associated with human cancers[1]. It belongs to the

B-lymphotropic γ-herpesvirus family with a genome consisting of 172 kb of linear double-stranded DNA[1,10,11]. EBV infects both epithelial and B-cells and, thus, can induce both epithelial cancers and lymphoma[12,13]. After EBV infection, there are two viral phases: lytic and latent[14]. In its lytic phase, the virus replicates in epithelial cells, and, in its latent phase, it transforms B-cells.

Cancer is characterized by the loss of the balance between cell proliferation and apoptosis[15-17]. It has been demonstrated that EBV can increase cell proliferation and decrease apoptosis[18]. EBV has been shown to cause several B-cell lymphomas, including Burkitt's lymphoma, Hodgkin's lymphoma and post-transplant lymphoproliferative disorders (PTLDs). This notion is demonstrated by the detection of EBV virus in these cancers, the replication of the virus and its ability to transform B-cells[18,19]. EBV is also closely associated with epithelial cancers. For example, EBV can cause nasopharyngeal carcinoma (NPC), a highly metastatic cancer[20]. The EBV latent membrane proteins (LMP) 1 and LMP2A are frequently detected in NPC[21]. LMP1 may also lead to metastasis of the cancer, as it has been demonstrated that LMP1 can cause epithelial-mesenchymal transition (EMT) *via* transcription factor Snail[22]. Both LMP1 and Snail are correlated with NPC metastasis[22]. Overall, EBV has been shown to be responsible for about 10% of gastric cancers worldwide[23-25]. However, the mechanisms for EBV-induced gastric cancer are not clear.

Many EBV proteins are expressed in the latent phases and are potentially related to carcinogenesis. These proteins include EBV nuclear antigen (EBNA)-1, -2, -3A, -3B, -3C and leader protein, and LMP-1, -2A and -2B[14]. However, the major identified oncoproteins in EBV are LMP1 and LMP2A[20,26]. These proteins can activate multiple signal pathways, such as the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), the mitogen-activated protein kinase (MAPK) and the signal transducer and activator of transcription 3, all of which are important for carcinogenesis[15,27,28]. LMP1 is considered as an analog of the tumor necrosis factor receptor 1, and it can transform human B-lymphocytes and rodent fibroblasts *via* activation of multiple intracellular signal pathways through its two signaling domains, the carboxyl-terminal activating regions 1 and 2 (CTAR1 and CTAR2)[29]. Activated pathways include the nuclear factor κB (NF-κB), PI3K/Akt, Notch, MAPK and Jun N-terminal protein kinase (JNK) signaling pathways[27,30-32]. It has been demonstrated that point mutations in the C-terminal region of the LMP1 cytoplasmic domain can influence the transforming potential of the EBV by reducing the ability of LMP1 to activate PI3K/Akt, NF-κB and AP1[29]. LMP1 is essential for EBV-mediated B-cell transformation and is sufficient to transform several cell lines, such as rodent fibroblasts[33]. A recent study showed that LMP1 expression is regulated by C/EBP in addition to EBNA2[34]. This article will discuss how EBV-expressed proteins activate the PI3K/Akt pathway to cause carcinogenesis in EBV-associated cancers. Although EBV oncogenes can affect many signal pathways, such as NF-κB, MAPK, and JNK, it seems that the PI3K/Akt pathway is the most important. In an LMP1-mediated transformation of rodent fibroblasts, inhibition of PI3K activity by Ly294002 induced apoptosis and inhibited cell growth, however, the NF-κB inhibitor BAY 11-7085 had no such effect[35]. Another study also showed that the PI3K/Akt pathway, but not the MAPK or NF-κB pathways, can account for the LMP-1-induced transformation[36].

ROLE OF PI3K/AKT SIGNAL PATHWAY IN CARCINOGENESIS AND METASTASIS

In 1985, Lewis Cantley initially discovered that PI3K plays an important role in cancer[37-41]. PI3K has now been extensively studied with investigation determining its role in carcinogenesis and the potential use of its inhibitors in the treatment of cancers[42-44]. This kinase phosphorylates the 3' OH position of phosphatidylinositol 4,5-biphosphate (PIP2) and converts it to phosphatidylinositol 3,4,5-triphosphate (PIP3), leading to activation of Akt[45,46], which causes a cascade of cellular signal alterations *via* its downstream target proteins[39].

Many factors, such as insulin, insulin-like growth factor-1, vascular endothelial growth factor, and cytokines interleukin (IL)-6, IL-17 can increase the activity of the PI3K/Akt pathway[6,47-52]. Mutations of genes encoding key components in the pathway have been found to cause the pathway activation in many cancers[38,53]. Many cancer-related viruses can also activate the PI3K/Akt pathway and rely on it for their transformations[38,39]. Such viral oncoproteins include polyoma virus middle-T antigen, Rous sarcoma virus oncoprotein v-Src, HPV oncoproteins E6, E7 and the human T-cell leukemia virus type 1 oncoprotein Tax[54-57]. It has also been demonstrated that the PI3K/Akt pathway plays a critical role in the carcinogenesis of EBV viral oncoproteins[27].

Activated Akt, which is phosphorylated by PDK1, can affect many downstream targets[38,42]. The resulting biological effects include increased genomic instability, increased proliferation, decreased apoptosis and changed cytoskeleton. (Figure 1)[58]. Genomic instability is important for the accumulation of genetic mutations necessary for carcinogenesis[59,60]. Recently, it was reported that constitutively active (CA) Met tyrosine kinase (hepatocyte growth factor receptor) can induce chromosomal instability (CIN), as indicated by increased centrosome counts, multinucleated cells and micronuclei formation[61-63]. While CA-Met increased both phosphorylated Akt and phosphorylated Erk, only phosphorylated Akt is critical in CA-Met-induced CIN. The PI3K inhibitor Ly294002, PTEN (an inhibitor of PI3K), and siRNA against Akt all abolished CA-met mediated CIN[62]. It has also been demonstrated that phosphorylation of Akt can block checkpoint kinase 1 (Chk1), which controls cell cycle progression and maintains genomic stability[61,63,64]. The activation of Chk1 will phosphorylate cdc25A and induce the transient arrest of cells in G1 and S phase before

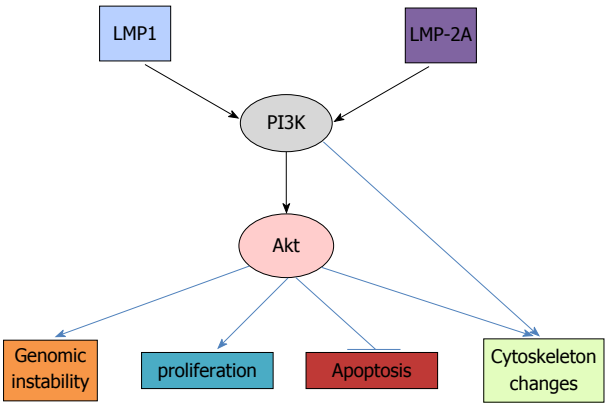

Figure 1 Epstein-Barr virus activates the phosphoinositide 3-kinase/protein kinase B pathway to transform cells. The Epstein-Barr virus latent proteins latent membrane protein (LMP)1 and LMP2A activate the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway, which promotes carcinogenesis by increasing cell proliferation, genomic instability and cytoskeleton changes and by decreasing apoptosis.

the onset of mitosis[65]. The inhibition of Chk1 has been shown to increase double-strand DNA breaks[66].

The activation of Akt can increase cell proliferation and cell size by accelerating the cell cycle and cell metabolism. Akt can phosphorylate glycogen synthase kinase 3β (GSK3β) and, thus, deactivate it, leading to increased cyclin D1 and Myc[67]. Myc is an oncoprotein that upregulates cyclin-dependent kinase 4 (CDK4)[68]. Additionally, the Akt-mediated inhibition of the forkhead protein results in the downregulation of the cell cycle proteins p27 and p21[69], thus promoting cell cycle progression[70]. Both p27 and p21 are G1-checkpoint CDK inhibitors which can promote G1/S transition and thus, accelerate cell cycle[17,71,72]. Another target activated by the activation of Akt is mTORC1, which plays an important role in the carcinogenesis of many cancers, including Burkitt's lymphoma and NPC[73-75]. Phosphorylated Akt blocks TSC1 and 2 (tuberous sclerosis complex 1 and 2) and, thus, activates Rheb (Ras homolog enriched in brain), thereby activating the mTOC1 complex[38]. The mTORC1 is composed of mammalian target of rapamycin (mTOR), regulatory associated protein of mTOR (Raptor), mammalian LST8/G-protein β-subunit like protein (mLST8/GβL), PRAS40 and Deptor[73]. The activation of mTORC1 can increase protein synthesis, cell growth and cell metabolism *via* its downstream targets[76-78]. The mTORC1 increases protein translation by activating the 70 kDa ribosomal S6 kinase (S6K), and inhibiting the elongation-initiation factor 4E binding protein[79,80]. A recent study using phosphoprome-otic technique and new inhibitor Torin1 revealed many more proteins regulated by mTORC1 including protein Grb 10 which feedback inhibits PI3K[76]. Further study may elucidate the roles of these proteins in mTORC1 mediated carcinogenesis.

The activation of Akt can decrease apoptosis by decreasing Fas ligand transcription *via* blocking the forkhead protein and thus affecting FasL-mediated apoptosis[58]. Akt decreases the pro-apoptotic proteins BAD and BAX and increases anti-apoptotic Bcl-xl, Bcl-2 and Mcl1 to promote cell survival[81]. Akt also inhibits the p53 tumor-suppressor, which can cause apoptosis under stimulation of DNA damage or environmental factors[82,83].

Akt can also regulate cytoskeleton, which is important for cell mobility and the metastasis of cancers[84-86]. The p70 S6K, a downstream target of mTORC1, has been demonstrated to promote actin cytoskeleton change to increase cancer cell migration[87]. In addition, PI3K can cause the change of cytoskeleton independent of Akt. It can activate Rac1, which also causes reorganization of actin cytoskeleton[88-90].

INCREASED PI3K/AKT PATHWAY IN EBV-INDUCED CANCERS

Examination of activated PI3K in EBV-associated cancers provides evidence for the critical role of the PI3K/Akt pathway in the carcinogenesis of EBV. Adams *et al*[91] (2009) examined eight cases of post-transplant Hodgkin lymphoma and found that all of them expressed PI3K. Analyses of NPC biopsy samples using microarray and affymetrix assays showed PI3K mediated LMP2A-induced expression of the carcinogenic UDP-glucose dehydrogenase (*UGDH*) gene[92,93]. The overexpression of LMP2A in HEK293 cells increased the expression of *UGDH* which was abolished by the inhibition of the PI3K/Akt pathway[92]. Proteomic analyses of the EBV-infected gastric carcinoma cell line NU-GC-3 [EBV (+)] showed that EBV infection upregulated the phosphorylated Akt[94]. The fact that the increased phosphorylated HSP27 was reduced by treatment with the PI3K inhibitors Ly294002 and wortmannin suggests that EBV infection can upregulate the phosphorylation of HSP27 *via* the PI3K/Akt pathway. In PTLDs, protein microarrays of samples from patients showed that PI3K, mTOR and NF-κB were also dysregulated[95].

The activated PI3K/Akt pathway in EBV-associated cancers have been demonstrated to be mediated by LMP1 and LMP2A. A study showed that LMP1 expression in EBV-infected B-cells induced the production of cellular IL-10, an autocrine growth factor for B cell lymphomas, in a PI3K-dependent manner[96]. In these cell lines, PI3K/Akt pathway is activated and the LMP1-mediated IL-10 production is suppressed by mTORC1 inhibitor rapamycin. It has also been demonstrated that expression of dominant negative forms of LMP1 in EBV-immortalized monocytic and lymphocytic cell lines resulted in decreased Akt and NF-κB activities with increased apoptosis[97]. At present, six identified sequence variants of LMP1 including Alaskan, China 1, China 2, Med+, Med-, and NC have been shown to induce the PI3K/Akt signaling pathway to similar extents after being transformed into Rat-1 fibroblasts, HFK cells and BJAB cells[98]. EBV LMP2A has also been shown to activate PI3K in epithelial cells and to affect differentiation[26]. In epithelial cells, the overexpression of LMP2A of Rhesus lymphocryptovirus (LCV), which is highly homologous to EBV LMP2A activated

the PI3K/Akt pathway, indicated by Akt activation and GSK3β inactivation[26]. LMP2A was shown to act as a B-cell receptor (BCR) signal, which results in B cells exiting the bone marrow and decreases B cell apoptosis in the periphery *via* the activation of PI3K[99].

EBV CAUSES CANCER *VIA* THE ACTIVATION OF THE PI3K/AKT PATHWAY

There are many studies demonstrating that EBV can affect the PI3K/Akt pathway to cause cancers. EBV activation of the PI3K/Akt pathway can increase carcinogenesis *via* multiple downstream targets, including increased genomic instability, cell proliferation, decreased apoptosis and increased cytoskeleton dynamics.

EBV increased genomic instability through the activation of the PI3K/Akt pathway

Genomic stability is important to avoid carcinogenesis and is maintained by the DNA repair system[16,59,60,100-102]. It has been demonstrated that genomic instability plays an important role in EBV-induced cancers[103-107]. In human epithelial cells, LMP1 represses DNA repair *via* the CTAR1-mediated activation of PI3K/Akt pathway[33]. The activated PI3K/Akt pathway resulted in inactivation of FOXO3a, which plays an important role in DNA repair *via* DNA damage-binding protein 1[33]. The critical role of FOXO3a was further demonstrated by the fact that constitutive expression of an active FOXO3a abolished LMP1-mediated repression of DNA repair[33]. Furthermore, a recent study has shown that phosphorylated Akt can block Chk1 to affect genomic instability[62]. This effect may be involved in LMP-1-induced genomic instability and warrants further study.

EBV increased cell proliferation through the activation of the PI3K/Akt pathway

In EBV-immortalized B-cells, also known as lymphoblastoid cell lines, the activation of the PI3K/Akt pathway can promote E2F transcriptional activity to affect the cell cycle and increase proliferation[108]. Inhibition of the PI3K by Ly294002 in these cells reduced both cyclin D2 and cyclin D3, which are two key regulators of cell cycle and increased p27, a cyclin-dependent kinase inhibitor[108]. CTAR1 of LMP1 has been identified to mediate the activation of PI3K signaling and associated induction of cell cycle markers in G1/S transition[30]. This PI3K activating effect was mapped to the TRAF-binding domain within CTAR1. In Rat-1 fibroblast cells, PI3K/Akt has been demonstrated to be a key factor in LMP1 mediated rodent fibroblast transformation[35]. Inhibition of the pathway abolished LMP1-induced cell growth. CTAR1 but not CTAR2 is critical for the activation of the PI3K/Akt pathway and associated cell growth. In human fibroblasts, LMP1 also caused phosphoryaltion of Akt and decreased levels of p27 and thus increased cell proliferation[35]. A study showed that, in an EBV-positive NPC cell line, LMP1 enhanced cell growth and migration through the activation of PI3K/Akt and NF-κB signaling which was reduced by the inhibition of PI3K, Akt, and NF-κB[109]. However, it has been shown that constitutive activation of Akt alone is not sufficient to promote cell growth; NF-κB activation is also required by LMP1 for its effect. Activation of PI3K/Akt and NF-κB has also been demonstrated to increase glucose import which is necessary for increased cell proliferation[110].

EBV decreased apoptosis through the activation of the PI3K/Akt pathway

Several studies have shown that LMP2A can decrease apoptosis *via* the activation of the PI3K/Akt pathway. In LMP2A transgenic mice, peripheral BCR-negative B-cells have CA Ras, an upstream protein of PI3K with correlated increased expression of Bcl-xL, a dowm-stream target protein of PI3K[111]. The specific inhibitors of PI3K and Akt can cause apoptosis of these cells, suggesting the important role of the PI3K/Akt in LMP2A mediated B-cell survival. In an EBV-associated gastric cancer cell line, LMP2A activated PI3K/Akt pathway has been associated with the resistance to apoptosis induced by chemotherapy[112]. In PTLD-derived EBV+ B cell lines, LMP2A increased caspase inhibitor XIAP to block apoptosis *via* the activation of PI3K/Akt pathway[113]. In NPC cell lines, expression of LMP1 activated the PI3K/Akt pathway and its downstream Bcl-2, which in turn suppressed the pro-apoptotic activity of prostate apoptosis response-4[114]. These studies provide sufficient evidence that PI3K/Akt is a key pathway in LMP1 and LMP2A-mediated decreased apoptosis.

EBV increased cytoskeleton dynamics through the activation of the PI3K/Akt pathway

The cytoskeleton plays an important role in carcinogenesis through the control of cell mobility[84-86], and several cancer therapies have been developed targeting the proteins regulating the cytoskeleton[115,116]. The PI3K/Akt pathway has been shown to play a key role in LMP1-induced actin stress-fiber formation[36]. This pathway may be also important in microtubule activity. A study has shown that EBV LMP1 can activate cdc2, which, in turn, phosphorylates Op18/stathmin, a regulator of microtubules[117]. It is possible that this process is mediated by the PI3K/Akt pathway, as Akt has been shown to increase cdc2 activity[118].

INHIBITION OF PI3K FOR THE TREATMENT OF EBV-ASSOCIATED CANCERS

The PI3K/Akt pathway is not only important in carcinogenesis and maintenance of cancer but is important in metastasis and drug resistance to chemotherapy[119-121]. For example, insulin can increase drug resistance *via* this pathway[47,122-124]. Many studies have been performed to

test PI3K/Akt inhibitors and their utilization in combination with chemotherapeutic agents[125-131]. In EBV-associated cancer, the PI3K/Akt pathway is increased, as described above. Thus, the inhibition of the pathway may be effective for the treatment of these cancers. Indeed, some preliminary studies have shown that inhibiting the pathway increased the effect of chemotherapy on EBV-associated cancers.

In an EBV-positive gastric cancer cell line, SNU-719, Ly294002 was tested in combination with 5-fluorouracil (5-FU), a common chemotherapeutic agent[112,132]. In these cells, the use of 5-FU alone increased phosphorylation levels of Akt and NF-κB. The increased activity of the PI3K/Akt is known to cause drug resistance to chemotherapy[120,121]. By contract, the sequential treatment of 5-FU and Ly294002 decreased their levels, as well as bcl-2 expression, and increased the sensitivity of these cancer cells to 5-FU. The therapeutic efficacy of the mTOR inhibitor rapamycin has been demonstrated; it decreased tumor growth and metastasis in a mouse model of EBV-associated Burkitt's lymphoma established by over-expression of both LMP2A and myc[74]. Ly294002 and Akt inhibitor II also induced the apoptosis of EBV-associated NK/T-cell lymphoma cell lines Hank-1 and NK-YS, which have high levels of activated PI3K[133]. NPC is usually treated by radiotherapy, and studies have shown that inhibition of the PI3K/Akt/mTOR pathway can increase the sensitivity of cancer cells to radiotherapy[131]. Thus, it may be useful to apply PI3K inhibitors in the treatment of EBV-associated NPC.

At present, dual inhibitors of PI3K and mTOR including BEZ235, PI-103, SF1126 and XL756 have been developed and some of them are in clinical trials to treat cancers with activated PI3K[38,134,135]. These inhibitors may be ideal compounds to be added into treatment regimens for EBV-associated cancers. Compounds from traditional medicine have been studied to inhibit signaling pathways; specifically, curcumin and flavonoids can inhibit either the PI3K/Akt pathway or its downstream targets cyclooxygenase-2 and NF-κB[136-142]. These compounds could also be tested for their effects on EBV-associated cancers.

CONCLUSION

The PI3K/Akt pathway can be activated by the EBV virus proteins LMP1 and LMP-2A and plays an important role in the carcinogenesis of EBV-associated cancers. This pathway is also known to be involved in drug resistance to chemotherapy. Thus, the inhibition of the pathway may have therapeutic implications for EBV-associated cancers. Indeed, some inhibitors of the PI3K/Akt pathway have been tested in EBV-associated cancer cell lines. At present, dual inhibitors of PI3K and mTOR have been developed and may be useful in the treatment of EBV-associated cancers.

REFERENCES

1 **Moore PS**, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat Rev Cancer* 2010; **10**: 878-889
2 **Martin D**, Gutkind JS. Human tumor-associated viruses and new insights into the molecular mechanisms of cancer. *Oncogene* 2008; **27** Suppl 2: S31-S42
3 **Rous P**. A sarcoma of the fowl transmissible by an agent separable from the tumor cells. *J Exp Med* 1911; **13**: 397-411
4 **Becsei-Kilborn E**. Scientific discovery and scientific reputation: the reception of Peyton Rous' discovery of the chicken sarcoma virus. *J Hist Biol* 2010; **43**: 111-157
5 **Parker RC**, Varmus HE, Bishop JM. Cellular homologue (c-src) of the transforming gene of Rous sarcoma virus: isolation, mapping, and transcriptional analysis of c-src and flanking regions. *Proc Natl Acad Sci USA* 1981; **78**: 5842-5846
6 **Chen J**. Is Src the key to understanding metastasis and developing new treatments for colon cancer? *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 306-307
7 **zur Hausen H**. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002; **2**: 342-350
8 **Schiller JT**, Castellsagué X, Villa LL, Hildesheim A. An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. *Vaccine* 2008; **26** Suppl 10: K53-K61
9 **Zhao KN**, Chen J. Codon usage roles in human papillomavirus. *Rev Med Virol* 2011; **21**: 397-411
10 **Morissette G**, Flamand L. Herpesviruses and chromosomal integration. *J Virol* 2010; **84**: 12100-12109
11 **Odumade OA**, Hogquist KA, Balfour HH. Progress and problems in understanding and managing primary Epstein-Barr virus infections. *Clin Microbiol Rev* 2011; **24**: 193-209
12 **Vereide D**, Sugden B. Insights into the evolution of lymphomas induced by Epstein-Barr virus. *Adv Cancer Res* 2010; **108**: 1-19
13 **Luzuriaga K**, Sullivan JL. Infectious mononucleosis. *N Engl J Med* 2010; **362**: 1993-2000
14 **Petrova M**, Kamburov V. Epstein-Barr virus: silent companion or causative agent of chronic liver disease? *World J Gastroenterol* 2010; **16**: 4130-4134
15 **Chen J**, McMillan NA. Molecular basis of pathogenesis, prognosis and therapy in chronic lymphocytic leukaemia. *Cancer Biol Ther* 2008; **7**: 174-179
16 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674
17 **Abukhdeir AM**, Park BH. P21 and p27: roles in carcinogenesis and drug resistance. *Expert Rev Mol Med* 2008; **10**: e19
18 **Vereide D**, Sugden B. Proof for EBV's sustaining role in Burkitt's lymphomas. *Semin Cancer Biol* 2009; **19**: 389-393
19 **Klein G**, Klein E, Kashuba E. Interaction of Epstein-Barr virus (EBV) with human B-lymphocytes. *Biochem Biophys Res Commun* 2010; **396**: 67-73
20 **Zheng ZM**. Viral oncogenes, noncoding RNAs, and RNA splicing in human tumor viruses. *Int J Biol Sci* 2010; **6**: 730-755
21 **Brooks L**, Yao QY, Rickinson AB, Young LS. Epstein-Barr virus latent gene transcription in nasopharyngeal carcinoma cells: coexpression of EBNA1, LMP1, and LMP2 transcripts. *J Virol* 1992; **66**: 2689-2697
22 **Horikawa T**, Yoshizaki T, Kondo S, Furukawa M, Kaizaki Y, Pagano JS. Epstein-Barr Virus latent membrane protein 1 induces Snail and epithelial-mesenchymal transition in metastatic nasopharyngeal carcinoma. *Br J Cancer* 2011; **104**: 1160-1167
23 **Ryan JL**, Jones RJ, Kenney SC, Rivenbark AG, Tang W, Knight ER, Coleman WB, Gulley ML. Epstein-Barr virus-specific methylation of human genes in gastric cancer cells. *Infect Agent Cancer* 2010; **5**: 27
24 **Fukayama M**. Epstein-Barr virus and gastric carcinoma. *Pathol Int* 2010; **60**: 337-350
25 **Gulley ML**, Pulitzer DR, Eagan PA, Schneider BG. Epstein-Barr virus infection is an early event in gastric carcinogenesis

and is independent of bcl-2 expression and p53 accumulation. *Hum Pathol* 1996; **27**: 20-27
26 **Siler CA**, Raab-Traub N. Rhesus lymphocryptovirus latent membrane protein 2A activates beta-catenin signaling and inhibits differentiation in epithelial cells. *Virology* 2008; **377**: 273-279
27 **Thornburg NJ**, Kulwichit W, Edwards RH, Shair KH, Bendt KM, Raab-Traub N. LMP1 signaling and activation of NF-kappaB in LMP1 transgenic mice. *Oncogene* 2006; **25**: 288-297
28 **Chen J**, Huang XF. The signal pathways in azoxymethane-induced colon cancer and preventive implications. *Cancer Biol Ther* 2009; **8**: 1313-1317
29 **Diduk SV**, Smirnova KV, Pavlish OA, Gurtsevitch VE. Functionally significant mutations in the Epstein-Barr virus LMP1 gene and their role in activation of cell signaling pathways. *Biochemistry* (Mosc) 2008; **73**: 1134-1139
30 **Mainou BA**, Everly DN, Raab-Traub N. Unique signaling properties of CTAR1 in LMP1-mediated transformation. *J Virol* 2007; **81**: 9680-9692
31 **Everly DN**, Kusano S, Raab-Traub N. Accumulation of cytoplasmic beta-catenin and nuclear glycogen synthase kinase 3beta in Epstein-Barr virus-infected cells. *J Virol* 2004; **78**: 11648-11655
32 **Li SS**, Yang S, Wang S, Yang XM, Tang QL, Wang SH. Latent membrane protein 1 mediates the resistance of nasopharyngeal carcinoma cells to TRAIL-induced apoptosis by activation of the PI3K/Akt signaling pathway. *Oncol Rep* 2011; **26**: 1573-1579
33 **Chen YR**, Liu MT, Chang YT, Wu CC, Hu CY, Chen JY. Epstein-Barr virus latent membrane protein 1 represses DNA repair through the PI3K/Akt/FOXO3a pathway in human epithelial cells. *J Virol* 2008; **82**: 8124-8137
34 **Noda C**, Murata T, Kanda T, Yoshiyama H, Sugimoto A, Kawashima D, Saito S, Isomura H, Tsurumi T. Identification and characterization of CCAAT enhancer-binding protein (C/EBP) as a transcriptional activator for Epstein-Barr virus oncogene latent membrane protein 1. *J Biol Chem* 2011; **286**: 42524-42533
35 **Mainou BA**, Everly DN, Raab-Traub N. Epstein-Barr virus latent membrane protein 1 CTAR1 mediates rodent and human fibroblast transformation through activation of PI3K. *Oncogene* 2005; **24**: 6917-6924
36 **Dawson CW**, Tramountanis G, Eliopoulos AG, Young LS. Epstein-Barr virus latent membrane protein 1 (LMP1) activates the phosphatidylinositol 3-kinase/Akt pathway to promote cell survival and induce actin filament remodeling. *J Biol Chem* 2003; **278**: 3694-3704
37 **Zhao L**, Vogt PK. Class I PI3K in oncogenic cellular transformation. *Oncogene* 2008; **27**: 5486-5496
38 **Courtney KD**, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *J Clin Oncol* 2010; **28**: 1075-1083
39 **Engelman JA**. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 2009; **9**: 550-562
40 **Fulda S**. The PI3K/Akt/mTOR pathway as therapeutic target in neuroblastoma. *Curr Cancer Drug Targets* 2009; **9**: 729-737
41 **Vogt PK**, Hart JR, Gymnopoulos M, Jiang H, Kang S, Bader AG, Zhao L, Denley A. Phosphatidylinositol 3-kinase: the oncoprotein. *Curr Top Microbiol Immunol* 2010; **347**: 79-104
42 **Chen J**. Multiple signal pathways in obesity-associated cancer. *Obes Rev* 2011; **12**: 1063-1070
43 **Chen JZ**. Targeted therapy of obesity-associated colon cancer. *Transl Gastrointest Cancer* 2012; **1**: 44-57
44 **Chen JZ**, Wang MB. The roles of miRNA-143 in colon cancer and therapeutic implications. *Transl Gastrointest Cancer* 2012; **1**: 169-174
45 **Engelman JA**, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 2006; **7**: 606-619
46 **Lee JY**, Engelman JA, Cantley LC. Biochemistry. PI3K charges ahead. *Science* 2007; **317**: 206-207
47 **Chen J**, Huang XF, Qiao L, Katsifis A. Insulin caused drug resistance to oxaliplatin in colon cancer cell line HT29. *J Gastrointest Oncol* 2011; **2**: 27-33
48 **Gislette T**, Chen J. The possible role of IL-17 in obesity-associated cancer. *ScientificWorldJournal* 2010; **10**: 2265-2271
49 **Renehan AG**, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* 2004; **363**: 1346-1353
50 **Renehan AG**, Egger M, Minder C, O'Dwyer ST, Shalet SM, Zwahlen M. IGF-I, IGF binding protein-3 and breast cancer risk: comparison of 3 meta-analyses. *Int J Cancer* 2005; **115**: 1006-1007; author reply 1008
51 **Pollak M**. Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 2008; **8**: 915-928
52 **Johnson C**, Han Y, Hughart N, McCarra J, Alpini G, Meng F. Interleukin-6 and its receptor, key players in hepatobiliary inflammation and cancer. *Transl Gastrointest Cancer* 2012; **1**: 58-70
53 **Samuels Y**, Waldman T. Oncogenic mutations of PIK3CA in human cancers. *Curr Top Microbiol Immunol* 2010; **347**: 21-41
54 **Whitman M**, Kaplan DR, Schaffhausen B, Cantley L, Roberts TM. Association of phosphatidylinositol kinase activity with polyoma middle-T competent for transformation. *Nature* 1985; **315**: 239-242
55 **Ling LE**, Druker BJ, Cantley LC, Roberts TM. Transformation-defective mutants of polyomavirus middle T antigen associate with phosphatidylinositol 3-kinase (PI 3-kinase) but are unable to maintain wild-type levels of PI 3-kinase products in intact cells. *J Virol* 1992; **66**: 1702-1708
56 **Fukuda RI**, Tsuchiya K, Suzuki K, Itoh K, Fujita J, Utsunomiya A, Tsuji T. Human T-cell leukemia virus type I tax down-regulates the expression of phosphatidylinositol 3,4,5-trisphosphate inositol phosphatases via the NF-kappaB pathway. *J Biol Chem* 2009; **284**: 2680-2689
57 **Chen JZ**, McMillan N, Gu WY. Intra-tumor injection of lentiviral-vector delivered shRNA targeting human papillomavirus E6 and E7 oncogenes reduces tumor growth in a xenograft cervical cancer model in mice. *J Solid Tumors* 2012; **2**: 4-10
58 **Huang XF**, Chen JZ. Obesity, the PI3K/Akt signal pathway and colon cancer. *Obes Rev* 2009; **10**: 610-616
59 **Thompson SL**, Bakhoum SF, Compton DA. Mechanisms of chromosomal instability. *Curr Biol* 2010; **20**: R285-R295
60 **Murnane JP**. Telomere loss as a mechanism for chromosome instability in human cancer. *Cancer Res* 2010; **70**: 4255-4259
61 **Liu W**, Zhou Y, Reske SN, Shen C. PTEN mutation: many birds with one stone in tumorigenesis. *Anticancer Res* 2008; **28**: 3613-3619
62 **Nam HJ**, Chae S, Jang SH, Cho H, Lee JH. The PI3K-Akt mediates oncogenic Met-induced centrosome amplification and chromosome instability. *Carcinogenesis* 2010; **31**: 1531-1540
63 **Puc J**, Keniry M, Li HS, Pandita TK, Choudhury AD, Memeo L, Mansukhani M, Murty VV, Gaciong Z, Meek SE, Piwnica-Worms H, Hibshoosh H, Parsons R. Lack of PTEN sequesters CHK1 and initiates genetic instability. *Cancer Cell* 2005; **7**: 193-204
64 **Li L**, Ross AH. Why is PTEN an important tumor suppressor? *J Cell Biochem* 2007; **102**: 1368-1374
65 **Bartek J**, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell* 2003; **3**: 421-429
66 **Syljuåsen RG**, Sørensen CS, Hansen LT, Fugger K, Lundin C, Johansson F, Helleday T, Sehested M, Lukas J, Bartek J. Inhibition of human Chk1 causes increased initiation of DNA replication, phosphorylation of ATR targets, and DNA breakage. *Mol Cell Biol* 2005; **25**: 3553-3562
67 **Sears R**, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR.

Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev* 2000; **14**: 2501-2514
68 **Miliani de Marval PL**, Macias E, Rounbehler R, Sicinski P, Kiyokawa H, Johnson DG, Conti CJ, Rodriguez-Puebla ML. Lack of cyclin-dependent kinase 4 inhibits c-myc tumorigenic activities in epithelial tissues. *Mol Cell Biol* 2004; **24**: 7538-7547
69 **Liang J**, Slingerland JM. Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. *Cell Cycle* 2003; **2**: 339-345
70 **Martínez-Gac L**, Alvarez B, García Z, Marqués M, Arrizabalaga M, Carrera AC. Phosphoinositide 3-kinase and Forkhead, a switch for cell division. *Biochem Soc Trans* 2004; **32**: 360-361
71 **Kiyokawa H**, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, Khanam D, Hayday AC, Frohman LA, Koff A. Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). *Cell* 1996; **85**: 721-732
72 **Hsieh FF**, Barnett LA, Green WF, Freedman K, Matushansky I, Skoultchi AI, Kelley LL. Cell cycle exit during terminal erythroid differentiation is associated with accumulation of p27(Kip1) and inactivation of cdk2 kinase. *Blood* 2000; **96**: 2746-2754
73 **Wullschleger S**, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006; **124**: 471-484
74 **Cen O**, Longnecker R. Rapamycin reverses splenomegaly and inhibits tumor development in a transgenic model of Epstein-Barr virus-related Burkitt's lymphoma. *Mol Cancer Ther* 2011; **10**: 679-686
75 **Chen J**, Hu CF, Hou JH, Shao Q, Yan LX, Zhu XF, Zeng YX, Shao JY. Epstein-Barr virus encoded latent membrane protein 1 regulates mTOR signaling pathway genes which predict poor prognosis of nasopharyngeal carcinoma. *J Transl Med* 2010; **8**: 30
76 **Hsu PP**, Kang SA, Rameseder J, Zhang Y, Ottina KA, Lim D, Peterson TR, Choi Y, Gray NS, Yaffe MB, Marto JA, Sabatini DM. The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling. *Science* 2011; **332**: 1317-1322
77 **Yu Y**, Yoon SO, Poulogiannis G, Yang Q, Ma XM, Villén J, Kubica N, Hoffman GR, Cantley LC, Gygi SP, Blenis J. Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. *Science* 2011; **332**: 1322-1326
78 **Yea SS**, Fruman DA. Cell signaling. New mTOR targets Grb attention. *Science* 2011; **332**: 1270-1271
79 **Averous J**, Proud CG. When translation meets transformation: the mTOR story. *Oncogene* 2006; **25**: 6423-6435
80 **Fingar DC**, Salama S, Tsou C, Harlow E, Blenis J. Mammalian cell size is controlled by mTOR and its downstream targets S6K1 and 4EBP1/eIF4E. *Genes Dev* 2002; **16**: 1472-1487
81 **Pastorino JG**, Tafani M, Farber JL. Tumor necrosis factor induces phosphorylation and translocation of BAD through a phosphatidylinositide-3-OH kinase-dependent pathway. *J Biol Chem* 1999; **274**: 19411-19416
82 **Trotman LC**, Pandolfi PP. PTEN and p53: who will get the upper hand? *Cancer Cell* 2003; **3**: 97-99
83 **Chen J**, Huang XF. Activation of p53 for the treatment of cancer. *J Cell Biochem* 2009; **107**: 567-568
84 **Albiges-Rizo C**, Destaing O, Fourcade B, Planus E, Block MR. Actin machinery and mechanosensitivity in invadopodia, podosomes and focal adhesions. *J Cell Sci* 2009; **122**: 3037-3049
85 **Knoblich JA**. Asymmetric cell division: recent developments and their implications for tumour biology. *Nat Rev Mol Cell Biol* 2010; **11**: 849-860
86 **Parsons JT**, Horwitz AR, Schwartz MA. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat Rev Mol Cell Biol* 2010; **11**: 633-643
87 **Ip CK**, Cheung AN, Ngan HY, Wong AS. p70 S6 kinase in the control of actin cytoskeleton dynamics and directed migration of ovarian cancer cells. *Oncogene* 2011; **30**: 2420-2432
88 **Cain RJ**, Vanhaesebroeck B, Ridley AJ. The PI3K p110alpha isoform regulates endothelial adherens junctions via Pyk2 and Rac1. *J Cell Biol* 2010; **188**: 863-876
89 **Huang Q**, Shen HM, Ong CN. Emodin inhibits tumor cell migration through suppression of the phosphatidylinositol 3-kinase-Cdc42/Rac1 pathway. *Cell Mol Life Sci* 2005; **62**: 1167-1175
90 **Reif K**, Nobes CD, Thomas G, Hall A, Cantrell DA. Phosphatidylinositol 3-kinase signals activate a selective subset of Rac/Rho-dependent effector pathways. *Curr Biol* 1996; **6**: 1445-1455
91 **Adams H**, Campidelli C, Dirnhofer S, Pileri SA, Tzankov A. Clinical, phenotypic and genetic similarities and disparities between post-transplant and classical Hodgkin lymphomas with respect to therapeutic targets. *Expert Opin Ther Targets* 2009; **13**: 1137-1145
92 **Pan YR**, Vatsyayan J, Chang YS, Chang HY. Epstein-Barr virus latent membrane protein 2A upregulates UDP-glucose dehydrogenase gene expression via ERK and PI3K/Akt pathway. *Cell Microbiol* 2008; **10**: 2447-2460
93 **Pegtel DM**, Subramanian A, Sheen TS, Tsai CH, Golub TR, Thorley-Lawson DA. Epstein-Barr-virus-encoded LMP2A induces primary epithelial cell migration and invasion: possible role in nasopharyngeal carcinoma metastasis. *J Virol* 2005; **79**: 15430-15442
94 **Fukagawa Y**, Nishikawa J, Kuramitsu Y, Iwakiri D, Takada K, Imai S, Satake M, Okamoto T, Fujimoto M, Okita K, Nakamura K, Sakaida I. Epstein-Barr virus upregulates phosphorylated heat shock protein 27 kDa in carcinoma cells using the phosphoinositide 3-kinase/Akt pathway. *Electrophoresis* 2008; **29**: 3192-3200
95 **Alsayed Y**, Leleu X, Leontovich A, Oton AB, Melhem M, George D, Ghobrial IM. Proteomics analysis in post-transplant lymphoproliferative disorders. *Eur J Haematol* 2008; **81**: 298-303
96 **Lambert SL**, Martinez OM. Latent membrane protein 1 of EBV activates phosphatidylinositol 3-kinase to induce production of IL-10. *J Immunol* 2007; **179**: 8225-8234
97 **Ndour PA**, Brocqueville G, Ouk TS, Goormachtigh G, Morales O, Mougel A, Bertout J, Melnyk O, Fafeur V, Feuillard J, Coll J, Adriaenssens E. Inhibition of latent membrane protein 1 impairs the growth and tumorigenesis of latency II Epstein-Barr virus-transformed T cells. *J Virol* 2012; **86**: 3934-3943
98 **Mainou BA**, Raab-Traub N. LMP1 strain variants: biological and molecular properties. *J Virol* 2006; **80**: 6458-6468
99 **Anderson LJ**, Longnecker R. EBV LMP2A provides a surrogate pre-B cell receptor signal through constitutive activation of the ERK/MAPK pathway. *J Gen Virol* 2008; **89**: 1563-1568
100 **Burrell RA**, Juul N, Johnston SR, Reis-Filho JS, Szallasi Z, Swanton C. Targeting chromosomal instability and tumour heterogeneity in HER2-positive breast cancer. *J Cell Biochem* 2010; **111**: 782-790
101 **Moody CA**, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. *Nat Rev Cancer* 2010; **10**: 550-560
102 **Vineis P**, Schatzkin A, Potter JD. Models of carcinogenesis: an overview. *Carcinogenesis* 2010; **31**: 1703-1709
103 **Ferrasi AC**, Pinheiro NA, Rabenhorst SH, Caballero OL, Rodrigues MA, de Carvalho F, Leite CV, Ferreira MV, Barros MA, Pardini MI. Helicobacter pylori and EBV in gastric carcinomas: methylation status and microsatellite instability. *World J Gastroenterol* 2010; **16**: 312-319
104 **Gruhne B**, Kamranvar SA, Masucci MG, Sompallae R. EBV and genomic instability--a new look at the role of the virus in the pathogenesis of Burkitt's lymphoma. *Semin Cancer Biol* 2009; **19**: 394-400
105 **Gruhne B**, Sompallae R, Masucci MG. Three Epstein-Barr

virus latency proteins independently promote genomic instability by inducing DNA damage, inhibiting DNA repair and inactivating cell cycle checkpoints. *Oncogene* 2009; **28**: 3997-4008
106 **Kamranvar SA**, Gruhne B, Szeles A, Masucci MG. Epstein-Barr virus promotes genomic instability in Burkitt's lymphoma. *Oncogene* 2007; **26**: 5115-5123
107 **Wu CC**, Liu MT, Chang YT, Fang CY, Chou SP, Liao HW, Kuo KL, Hsu SL, Chen YR, Wang PW, Chen YL, Chuang HY, Lee CH, Chen M, Wayne Chang WS, Chen JY. Epstein-Barr virus DNase (BGLF5) induces genomic instability in human epithelial cells. *Nucleic Acids Res* 2010; **38**: 1932-1949
108 **Brennan P**, Mehl AM, Jones M, Rowe M. Phosphatidylinositol 3-kinase is essential for the proliferation of lymphoblastoid cells. *Oncogene* 2002; **21**: 1263-1271
109 **Shair KH**, Schnegg CI, Raab-Traub N. EBV latent membrane protein 1 effects on plakoglobin, cell growth, and migration. *Cancer Res* 2008; **68**: 6997-7005
110 **Sommermann TG**, O'Neill K, Plas DR, Cahir-McFarland E. IKKβ and NF-κB transcription govern lymphoma cell survival through AKT-induced plasma membrane trafficking of GLUT1. *Cancer Res* 2011; **71**: 7291-7300
111 **Portis T**, Longnecker R. Epstein-Barr virus (EBV) LMP2A mediates B-lymphocyte survival through constitutive activation of the Ras/PI3K/Akt pathway. *Oncogene* 2004; **23**: 8619-8628
112 **Shin JY**, Kim JO, Lee SK, Chae HS, Kang JH. LY294002 may overcome 5-FU resistance via down-regulation of activated p-AKT in Epstein-Barr virus-positive gastric cancer cells. *BMC Cancer* 2010; **10**: 425
113 **Hatton O**, Phillips LK, Vaysberg M, Hurwich J, Krams SM, Martinez OM. Syk activation of phosphatidylinositol 3-kinase/Akt prevents HtrA2-dependent loss of X-linked inhibitor of apoptosis protein (XIAP) to promote survival of Epstein-Barr virus+ (EBV+) B cell lymphomas. *J Biol Chem* 2011; **286**: 37368-37378
114 **Lee JW**, Liu PF, Hsu LP, Chen PR, Chang CH, Shih WL. EBV LMP-1 negatively regulates expression and pro-apoptotic activity of Par-4 in nasopharyngeal carcinoma cells. *Cancer Lett* 2009; **279**: 193-201
115 **Dumontet C**, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat Rev Drug Discov* 2010; **9**: 790-803
116 **Kavallaris M**. Microtubules and resistance to tubulin-binding agents. *Nat Rev Cancer* 2010; **10**: 194-204
117 **Lin X**, Liu S, Luo X, Ma X, Guo L, Li L, Li Z, Tao Y, Cao Y. EBV-encoded LMP1 regulates Op18/stathmin signaling pathway by cdc2 mediation in nasopharyngeal carcinoma cells. *Int J Cancer* 2009; **124**: 1020-1027
118 **Katayama K**, Fujita N, Tsuruo T. Akt/protein kinase B-dependent phosphorylation and inactivation of WEE1Hu promote cell cycle progression at G2/M transition. *Mol Cell Biol* 2005; **25**: 5725-5737
119 **Falasca M**. PI3K/Akt signalling pathway specific inhibitors: a novel strategy to sensitize cancer cells to anti-cancer drugs. *Curr Pharm Des* 2010; **16**: 1410-1416
120 **Chen J**. The Src/PI3K/Akt signal pathway may play a key role in decreased drug efficacy in obesity-associated cancer. *J Cell Biochem* 2010; **110**: 279-280
121 **Chen J**, Huang XF, Katsifis A. Activation of signal pathways and the resistance to anti-EGFR treatment in colorectal cancer. *J Cell Biochem* 2010; **111**: 1082-1086
122 **Chen J**, Katsifis A, Hu C, Huang XF. Insulin decreases therapeutic efficacy in colon cancer cell line HT29 via the activation of the PI3K/Akt pathway. *Curr Drug Discov Technol* 2011; **8**: 119-125
123 **Renehan AG**, Dive C. Obesity, insulin and chemoresistance in colon cancer. *J Gastrointest Oncol* 2011; **2**: 8-10
124 **Landriscina M**, Esposito F. Insulin-resistant conditions: A favorable milieu for aggressive drug-resistant malignancies. *J Gastrointest Oncol* 2011; **2**: 11-12
125 **Liu P**, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 2009; **8**: 627-644
126 **Morgan TM**, Koreckij TD, Corey E. Targeted therapy for advanced prostate cancer: inhibition of the PI3K/Akt/mTOR pathway. *Curr Cancer Drug Targets* 2009; **9**: 237-249
127 **Paz-Ares L**, Blanco-Aparicio C, García-Carbonero R, Carnero A. Inhibiting PI3K as a therapeutic strategy against cancer. *Clin Transl Oncol* 2009; **11**: 572-579
128 **Sarker D**, Reid AH, Yap TA, de Bono JS. Targeting the PI3K/AKT pathway for the treatment of prostate cancer. *Clin Cancer Res* 2009; **15**: 4799-4805
129 **Vanhaesebroeck B**, Guillermet-Guibert J, Graupera M, Bilanges B. The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol* 2010; **11**: 329-341
130 **Vanhaesebroeck B**, Vogt PK, Rommel C. PI3K: from the bench to the clinic and back. *Curr Top Microbiol Immunol* 2010; **347**: 1-19
131 **Zaidi SH**, Huddart RA, Harrington KJ. Novel targeted radiosensitisers in cancer treatment. *Curr Drug Discov Technol* 2009; **6**: 103-134
132 **Katkoori VR**, Suarez-Cuervo C, Shanmugam C, Jhala NC, Callens T, Messiaen L, Posey J, Bumpers HL, Meleth S, Grizzle WE, Manne U. Bax expression is a candidate prognostic and predictive marker of colorectal cancer. *J Gastrointest Oncol* 2010; **1**: 76-89
133 **Jeon YK**, Park CH, Kim KY, Li YC, Kim J, Kim YA, Paik JH, Park BK, Kim CW, Kim YN. The heat-shock protein 90 inhibitor, geldanamycin, induces apoptotic cell death in Epstein-Barr virus-positive NK/T-cell lymphoma by Akt down-regulation. *J Pathol* 2007; **213**: 170-179
134 **Manara MC**, Nicoletti G, Zambelli D, Ventura S, Guerzoni C, Landuzzi L, Lollini PL, Maira SM, García-Echeverría C, Mercuri M, Picci P, Scotlandi K. NVP-BEZ235 as a new therapeutic option for sarcomas. *Clin Cancer Res* 2010; **16**: 530-540
135 **Bhende PM**, Park SI, Lim MS, Dittmer DP, Damania B. The dual PI3K/mTOR inhibitor, NVP-BEZ235, is efficacious against follicular lymphoma. *Leukemia* 2010; **24**: 1781-1784
136 **Bisht S**, Maitra A. Systemic delivery of curcumin: 21st century solutions for an ancient conundrum. *Curr Drug Discov Technol* 2009; **6**: 192-199
137 **Tian G**, Guo L, Gao W. Use of compound Chinese medicine in the treatment of lung cancer. *Curr Drug Discov Technol* 2010; **7**: 32-36
138 **Wang H**, Cui Y, Zhao C. Flavonoids of the Genus Iris (Iridaceae). *Curr Drug Discov Technol* 2010; **6**: Epub ahead of print
139 **Wang S**, Penchala S, Prabhu S, Wang J, Huang Y. Molecular basis of traditional Chinese medicine in cancer chemoprevention. *Curr Drug Discov Technol* 2010; **7**: 67-75
140 **Youns M**, Hoheisel JD, Efferth T. Traditional Chinese medicines (TCMs) for molecular targeted therapies of tumours. *Curr Drug Discov Technol* 2010; **7**: 37-45
141 **Choi BH**, Kim CG, Lim Y, Shin SY, Lee YH. Curcumin down-regulates the multidrug-resistance mdr1b gene by inhibiting the PI3K/Akt/NF kappa B pathway. *Cancer Lett* 2008; **259**: 111-118
142 **Chen JZ**. Prevention of obesity-associated colon cancer by (-)-epigallocatechin-3 gallate and curcumin. *Transl Gastrointest Cancer* 2012; **1**: 243-249

S- Editor Wang JL **L- Editor** A **E- Editor** Zheng XM

World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
doi:10.5501/wjv.v1.i6.162

World J Virol 2012 December 12; 1(6): 162-173
ISSN 2220-3249 (online)

REVIEW

Hepatitis viruses and non-Hodgkin's lymphoma: A review

Sibnarayan Datta, Soumya Chatterjee, Rudragoud S Policegoudra, Hemant K Gogoi, Lokendra Singh

Sibnarayan Datta, Soumya Chatterjee, Rudragoud S Policegoudra, Hemant K Gogoi, Lokendra Singh, Biotechnology Division, Defence Research Laboratory, Tezpur, Assam, PIN-784001, India

Author contributions: Datta S and Chatterjee S conceptualized and drafted the manuscript; Policegoudra RS, Gogoi HK and Singh L edited and revised the manuscript.

Correspondence to: Sibnarayan Datta, PhD, Biotechnology Division, Defence Research Laboratory, Post bag No. 2, Tezpur, Assam, PIN-784001, India. sndatta1978@gmail.com
Telephone: +91-3712-258508 Fax: +91-3712-258534
Received: October 9, 2011 Revised: June 6, 2012
Accepted: November 7, 2012
Published online: December 12, 2012

Abstract

Non-Hodgkin's lymphoma (NHL) is among the haematological malignancies with high prevalence worldwide, causing estimated 355 900 new cases and 191 400 deaths in 2008. High prevalence of NHL is documented in economically more developed areas while low prevalence is observed in less developed areas of the globe. A wide array of environmental factors have been reported to be either directly involved or in modifying the risk of NHL development. In addition to these factors, a number of infectious agents, chiefly viruses have also been implicated in the development of NHL. This article reviews the available literature to discuss the role of hepatitis viruses in NHL development, possible mechanisms of lymphomagenesis and also identify the areas in which further research is required to better understand this disease. A brief discussion on the clinical aspects such as classification, staging, treatment approaches have also been included in this article.

Key words: Non-Hodgkin's lymphoma; Hepatitis B virus; Hepatitis C virus; Hepatitis G virus; MiRNA

Peer reviewer: Preeti Bharaj, PhD, 10344 Falcon Parc Blvd, Orlando, FL. PhD, Diabetes and Obesity, Sanford-Burnham MRI, 6400 Sanger Road, Orlando, FL 32827, United States

Datta S, Chatterjee S, Policegoudra RS, Gogoi HK, Singh L. Hepatitis viruses and non-Hodgkin's lymphoma: A review. *World J Virol* 2012; 1(6): 162-173 Available from: URL: http://www.wjgnet.com/2220-3249/full/v1/i6/162.htm DOI: http://dx.doi.org/10.5501/wjv.v1.i6.162

INTRODUCTION

Cancer is a multifaceted disease, and arise mostly due to the changes in the somatic genetic material where, the interaction with the external factors play a very important role, apparently both modifying the effect of each other[1,2]. The disease, according to the GLOBOCAN 2008 data published by the IARC (International Agency for Research on Cancer), is the most important cause of death in developed countries; while second most important cause of death in developing countries and is responsible for about 7.6 million deaths in 2008, worldwide[3].

Cancer is a heterogeneous class of diseases displaying a wide range of pattern, origin site, distribution and malignancy. Of the wide range, lymphomas constitute an important group of cancers of the white blood cells that arise in lymphoid tissues and generally remain localized in lymph nodes or certain locations other than the bone marrow. Lymphomas are broadly separated into two groups, namely Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). The characteristic presence of large, usually multinucleate cells called 'Reed-Sternberg cells' in tumour biopsy samples differentiate HL from NHL[4]. About 80% to 90% NHLs are of B-cell origin, while rest are of T-cell origin[5]. The staging and diagnostic approaches have been reviewed elsewhere[6,7].

NHL is among the haematological malignancies with high prevalence worldwide. NHL ranks 8th and 11th among the most common cancers in men and women respectively, contributing 5.1% of all cancer cases and

2.7% of all cancer deaths[8]. An estimated 355 900 new cases and 191 400 deaths have been attributed to NHL in 2008[3,9]. NHL is more frequent in developed areas, with the highest incidence rates found in Australia/New Zealand, Western, Northern and Southern Europe, and North America, while lowest rates are found in South-Central and Eastern Asia, Eastern Europe and the Caribbean[3,8]. The incidence of NHL is usually low in Africa, but in some sub-Saharan areas (particularly in East Africa) incidence of Burkitt's lymphoma (a subtype of NHL) caused by Epstein-Barr virus (EBV) among children is remarkably high[9]. NHL incidence rates are also increasing in certain developing countries such as Thailand and Uganda, probably due to the acquired immunodeficiency syndrome epidemic.

Worldwide, the occurrence of NHL has been found to be higher in men with age-standardized rate per 100 000 (ASR, standardized to the World Standard Population) of 6.1 as compared to 4.2 for women[3,8]. The ASR of NHL incidence has been found to be 10.3 and 4.2 in males from more developed and less developed areas respectively. The ASR for NHL related mortality in males has been found to be 3.6 and 3.0 in more developed areas and less developed areas respectively. On the other hand, in females, the ASR of NHL incidence and mortality has been found to be 7.0 and 2.2 respectively from more developed areas, compared to incidence and mortality to 2.8 and 1.9 respectively from less developed areas[3,8]. Furthermore, the rise in incidences of NHL has been found to be consistent across the globe which still remains an enigma[10]. As NHL is a group of related yet diverse cancers, originating from different and complex etiologies, the issue of increase in the incidence is poorly understood.

ETIOLOGY

It has already been recognized that the major factors for the development of NHL include genetic alterations/damage to the cells and/or factors that are associated with immunosuppression. Further, NHL tumours have a high rate of genetic alterations like translocations, detectable in up to 90% of NHL cases[6,10,11]. Recently, Lan and colleagues have shown that polymorphisms in the Th1/Th2 cytokine genes may contribute to lymphomagenesis[12]. In addition, compromised immune system may also increase the risk of NHL incidence by allowing cancerous cells to escape the surveillance of immune system as higher rates of NHL is observed in people with inherited or acquired immunodeficiency syndromes and in people receiving immunosuppressive therapy[13,14]. However, genetic predisposition on the higher incidence of NHL among certain families is still a debatable issue[15]. The common genetic changes in NHL, may occur due to varied reasons: rearrangements of Immunoglobulin heavy chain (IgH), Immunoglobulin κ light chain (Igk), T cell receptor β chain (TCRb), T cell receptor γ chain (TCRg); and translocations of BCL-6, C-MYC, *etc.*[6].

Similar to most of the other cancers, several environmental factors have been implicated in the origin of NHL. Exposures to different agricultural chemicals like certain herbicides (phenoxy-, triazine- groups), insecticides (organo-chlorine and organophosphates), and industrial chemicals like polychlorinated biphenyls and polybrominated biphenyl, dioxins, organic solvents seems to be significant risk factors for the onset of the disease[15-17]. It was found that exposed population (like farmers) of these chemicals have higher rates of NHL than non-exposed populace[18,19]. Again, long term exposure of nitrate in drinking water, UV light, X-ray, radionuclides or electromagnetic fields, *etc.* have also been implicated in the development of NHL[15,20]. Further, use of hair dyes, alcohol, tobacco, certain diets, certain immunological conditions may also facilitate in the development of NHL[15]. However, positive correlation among the above mentioned risk factors and the incidence of NHL is still a dubious issue[4,7,8,10,15-20].

During the last 3 decades, the role of infectious agents, mainly viruses, in oncogenesis has become increasingly significant. Approximately 15% to 20% of cancers are associated with viral infections[21]. Apart from the above mentioned genetic and environmental etiologic factors, development of NHL has also been attributed to different viruses[22,23]. For several years EBV has been considered as important cause of NHL. In the later years, human immunodeficiency virus (HIV), hepatitis viruses B, C and G (HBV, HCV, HGV/GBV-C), HTLV-1, HHV 8 and Simian Virus 40 (SV40) have also been implicated in the development of NHL[7,14,15,22]. This review will mainly focus on the hepatitis viruses, HBV, HCV and HGV/GBV-C.

Both the hepatitis viruses, HBV and HCV have been strongly associated with the hepatocellular carcinoma (HCC)[24]. Previously these two viruses were thought to be solely hepatotrophic, but lately their occult lymphotrophic characteristic has been proved in human subjects as well as in animal models[25-27]. Although, the association of HBV and HCV with NHL has been established, it remains the question if these associations simply reflect causal relationships. In this context, Marcucci and Mele, have put forward 3 possibilities to explain these associations- (1) the immunosuppressive effect of the tumor increases the risk of viral infection or reactivation; (2) some previously unknown virus with a similar mode of transmission might trigger the oncogenic signal; and (3) the actual causal relationships between hepatitis viruses and NHL[10]. The authors refuted the first two possibilities and found the third to be correlating with the available literature. The first possibility being ruled out based on the fact that NHL is observed in a number of patients in which immune deficiency is not significant, while the second possibility was refuted for lack of any evidence[10]. Available studies apparently support the third possibility of the oncogenic role of hepatitis viruses in development of NHL[22,23]. However the association of HCV and HBV with the development of NHL is much weaker as compared to the major risk for HCC development caused by HBV and HCV[24,28].

HCV AND NHL

Mostly, the studies on the lymphomagenic role of hepatitis viruses have remained focused on HCV[10]. The simple evidence of an oncogenic role of HCV in NHL came from antiviral therapy studies, which shows, *peginterferon* and *ribavirin* (standard antiviral therapy against HCV) could completely or partially restrict lymphoma in HCV positive, but not in HCV-negative NHL patients[29,30]. More interestingly, in most patients, in a study, it was found that antiviral treatment results in disappearance of Ig heavy chain (IgHC) and t (14;18) translocation[31], suggesting the role of HCV in causing the genetic changes that are associated with NHL.

The association between HCV and NHL is strongest in geographic areas with highest prevalence of the viral infection[15]. In a recent meta-analysis of 15 selected studies, the pooled relative risk (RR) of all NHL among HCV-positive persons was found to be 2.5 (95% CI: 2.1-3.1) in case-control studies and 2.0 (95% CI: 1.8-2.2) in cohort studies[32]. Interestingly, the RR was significantly elevated in geographic areas with high HCV prevalence compared to areas with low HCV prevalence, which correlate well with previous studies from countries with low HCV prevalence, that could not observed any association between HCV and NHL[33,34]. It was suggested that undetectable association in countries with low HCV prevalence, was mainly due to relatively small sample size of HCV positive subjects[35].

HCV is a positive, single-stranded RNA virus of the *Flaviviridae* family[36]. During its replicative cycle it goes through a negative-stranded RNA, but replication does not include a DNA step, hence integration of HCV nucleic acid sequences into the host genome seems improbable, lacking a critical property of classical oncogenic retroviruses[24,35]. The HCV genome produces a single polyprotein that is proteolytically processed by viral and cellular proteases to produce structural (nucleocapsid, E1, E2) and nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B). Studies have demonstrated that NS5A acts as a transcriptional activator, interacts with other proteins and plays a crucial role in hepatocarcinogenesis[37]. In addition, it participates in HCV protein maturation and RNA replication, regulates gene expression in hepatocytes, stimulates cell proliferation, inhibits apoptosis and influences interferon effect[38]. It has been proposed that the E2 protein of HCV may be accountable for chronic antigen-driven polyclonal B-cell proliferation, leading to lymphomagenesis[39]. However, the oncogenic mechanism remains unclear.

HBV AND NHL

In comparison to HCV, the association of HBV with NHL has been studied less thoroughly, despite the fact that the first reports were published almost simultaneously on positive association between these two viruses and NHL[40,41]. The association between HBV and NHL was studied by several authors in both HBV endemic countries (e.g., South Korea, China) and non-endemic countries (e.g., USA, Australia)[40,42-49]. As discussed by Nath and colleagues[50], results of previous retrospective case-control studies have generally supported an association (odds ratios: 1.5-3.6). However, the available data may be an underestimate of the real association between HBV and NHL, because another form of silent HBV infection, known as occult hepatitis B infection has been identified and established in the recent years[25,51].

Occult HBV infections is defined for patients who test negative for the most widely practised HBsAg detection, but carry HBV-DNA in serum or tissues or both[25,51]. Moreover, replication-competent HBV-DNA is supposed to persists in the liver or lymphocytes or in both the compartments for many years or even life long, indicating complete HBV eradication to be an infrequent event[25,52]. HBV DNA has been detected within lymphocytes but whether HBV could directly transform lymphocytes is uncertain, as some studies have not been able to detect HBV in NHL cells[42,44]. In addition, the long incubation period of HBV makes it difficult to precisely estimate the significance of HBV in NHL[10].

HBV is a small partially dsDNA prototype virus of the *Hepadnaviridae* family[53]. During replication, it undergoes transformation into covalently closed circular dsDNA and replicate through an RNA intermediate. It can also integrate into the host genome[54]. HBV is characterized by a genome consisting of 4 overlapping open-reading frames: the *S* gene, encoding envelope proteins; the core gene, encoding the core and "e" proteins; the *P* gene, encoding DNA polymerase; and the "*x*" gene, encoding a transcriptional transactivator. The HBV NS X protein, a key regulatory protein of the virus that modulates viral replication, pathogenesis, interacts with a wide range of cellular proteins including P^{53} has largely been held responsible for the carcinogenic properties of HBV[55].

HGV/GBV-C AND NHL

Discovered lately, HGV/GBV-C are two viral agents that have been shown to be different strains of the same virus based on sequence similarity[56]. Overall, the worldwide prevalence of HGV/GBV-C in blood donors ranges from 0.9% to 10%. HGV/GBV-C is a parenterally transmitted virus, which in most cases occurs in the setting of co-infection of HBV and/or HCV. This coinfection has been attributed to similar modes of transmission, as HBV and HCV. The genome of HGV/ GBV-C is a positive-sense RNA having sequence and organization similar to other viruses belonging to the *Flaviviridae* family. The viral genome contains a continuous open reading frame (ORF) headed by a 458 nucleotide long 5' untranslated region (UTR) followed by a 315 nucleotide long 3' UTR. The ORF encodes a polyprotein of 2873 amino acids with a helicase motif, two chymotrypsin-like protease motifs and an RNA-dependent RNA polymerase motif[56].

Although initially associated with hepatitis, consideration of HGV/ GBV-C primarily as a hepatotropic virus is still under debate[57]. It has been demonstrated that in absence of coinfection with other hepatotropic viruses, liver injury or viral replicative forms are usually not detectable in the liver[58,59]. Since the majority of HGV/ GBV-C positive patients with HCC are also found to be coinfected with either HBV or HCV, it is difficult to assess the true role of HGV in the etiology of HCC[56]. Nevertheless, results from a number of studies clearly indicate a primary lymphotropic nature of GBV-C/HGV as the viral replicative forms (an indicator of active GBV-C/HGV replication) have been detected in circulating lymphocytes, bone marrow, spleen, mononuclear cells and lymph nodes in a proportion of GBV-C/HGV infected patients[57,59-63].

Considering the similarity between HCV and HGV/ GBV-C a similar relationship to the development of lymphoma has been expected[64]. In anticipation, some recent studies have shown the positive correlation between HGV/GBV-C and NHL. Renzo and colleagues reported that, in a series of unselected and untransfused patients in Italy, the prevalence of HGV infections were significantly higher in patients suffering from lymphoproliferative disease compared to healthy subjects[65]. Similar reports on the association of HGV and NHL can also be found from the countries like Germany, Canada, and Greece[64,66-69]. On the contrary, a study from Turkey, suggested neither HCV nor HGV can be linked to NHL[70]. Considering relatively late discovery studies on the exact role of HGV/GBV-C and NHL development are scarce and further studies are needed to firmly conclude about any correlation.

MECHANISM OF VIRUS INDUCED NHL

Based on the results of the association studies, Engels classified known or suspected infectious agents of NHL into three broad groups to explain the mechanism of NHL development[23]. These three mechanisms are schematically shown in Figure 1. First comes the lymphocyte infecting and transforming viruses, which disrupt normal cell functions and promote cell division. Second are those infectious agents that lead to immune deficiency (e.g., HIV) resulting in elevated risk of NHLs. Third group includes certain yet unknown infectious agents that may increase NHL risk through continual immune stimulation and lymphocytes activation. Two other hypothesis were also proposed to explain the mechanism; the "*hit-and-run*" hypothesis, which assumes that an agent significantly initiates oncogenic stimulus in the lymphocyte and disappears till NHL develops, and other "*hygiene*" hypothesis assumes that exposure to common infectious agents in early childhood, modulates NHL risk later in life. Although previous studies seem to support the initial three mechanisms, but the last two hypotheses (*hit-and-run* and *hygiene* hypothesis) are difficult to verify or prove since the oncogenic stimulus/agent is not detectable/lost at the time of NHL diagnosis.

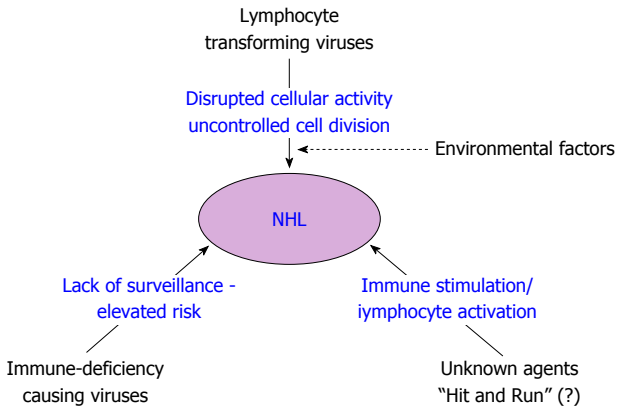

Figure 1 Schematic diagram showing mechanisms for development of non-Hodgkin's lymphoma. NHL: Non-Hodgkin's lymphoma.

Recently different workers have proposed that different individual etiological mechanisms are not mutually exclusive, but development of NHL is a multi causal event[10,27,71,72]. Recently, Marcucci and Mele hypothesised that each of the individual etiological agents provides subliminal oncogenic signals and are thus not strong enough to cause pathogenesis by them alone[10]. However, they suggested that integration of two or more oncogenic signals give rise to a supraliminal composite signal, necessary for lymphomagenesis. To explain the observed geographic discrepancies between incidence rates of NHL and prevalence rates of viral infections, it was suggested that geographic areas with a low prevalence of viral infection may have a high prevalence of a yet undefined environmental factor that may integrate with the viral oncogenic signal in a tissue specific manner[10,73].

Among the three hepatitis viruses, namely HBV, HCV and HGV/GBV-C, mechanism of HCV related NHL development is most widely studied (Figure 2). HCV is considered a classical example of infectious agent causing NHL through persistent immune stimulation, associated with a range of immune-related conditions that can lead to NHL[23,74]. However, a direct oncogenic role of HCV through B-cell infection and deregulation has been proposed since the virus is lymphotropic, but this has never been proved[75]. The observation of HCV viral genome or proteins in only a subset of the neoplastic cells of HCV-associated NHLs, whereas frequently detection of viral genome and proteins in the stromal cells surrounding the neoplastic cells suggest that specific B-cell clones proliferate as a consequence of the chronic antigenic stimulation sustained by HCV. The immunoglobin variable region genes expressed by B-NHL cells from HCV-positive patients show somatic mutations suggestive of an antigen selection process and the amino acid sequences of B-cell receptors in HCV-associated lymphoproliferations has been reported to have a similarity with anti-HCV antibodies[75-78]. Moreover, the histologic presentation of many B-NHL cells from HCV positive patients are characteristic of germinal center (GC) and post-GC B-cells, suggesting the occurrence of lymphom-

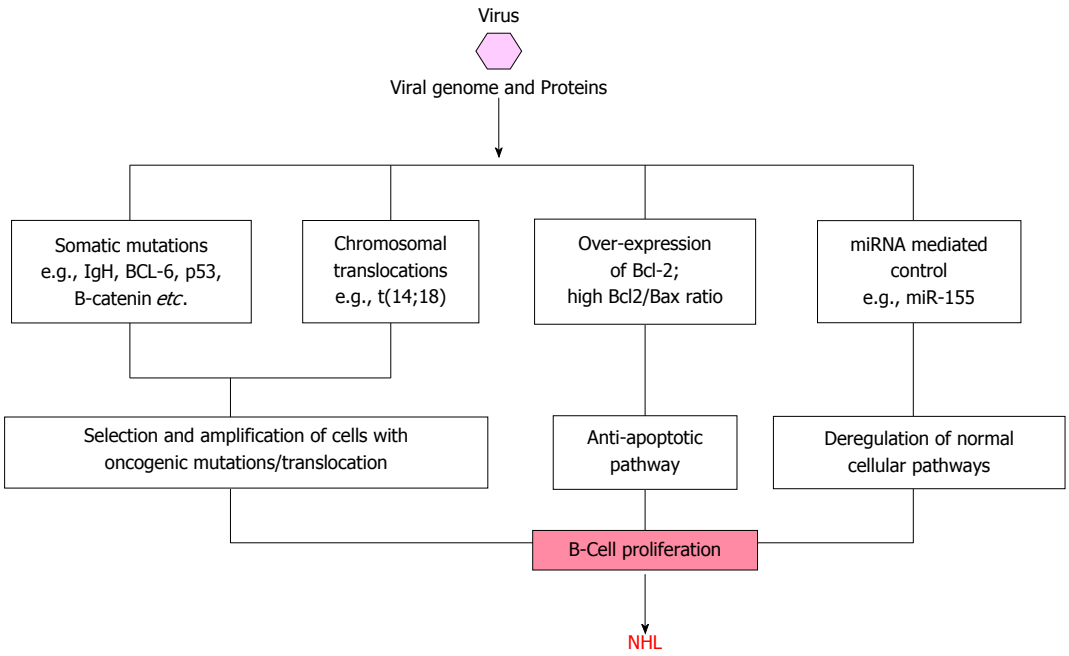

Figure 2 Schematic diagram showing possible modes of transformation of normal cells to non-Hodgkin's lymphoma by viral agents. IgH: Immunoglobulin heavy chain; miRNA: MicroRNAs; NHL: Non-Hodgkin's lymphoma.

agenesis at the time of B-cells proliferation in response to a viral antigen[76].

Neoplastic transformation may also be the result of direct anti-apoptotic pathways activated by HCV within B-cells. In fact, HCV sequences have been detected in lymph node biopsy specimens from patients with B-NHL[79] and the presence of HCV-associated proteins within lymphoma cells has also been demonstrated[80]. Moreover, studies in severe combined immunodeficiency mice have provided evidence of the persistence and low-rate multiplication of HCV infection in human mononuclear cells[81]. Finally, some HCV proteins have been shown to exert anti-apoptotic effects in infected cells in transgenic mice[82,83].

HCV related lymphoproliferation is thought to be due to binding of HCV to receptors on the surface of B-lymphocytes lowering their threshold for antigen response, inducing DNA mutations, increased frequency of abnormal chromosomal translocations associated with NHL[84,85]. The identification of the specific binding of HCV E2 protein to the ubiquitously abundant B-cell surface molecule CD81, has supported the hypothesis that a consistent polyclonal B-cell response to viral antigens favour the development of lymphoproliferative disorders[27,84]. Although HCV can infect lymphocytes, there is lack of evidence for its direct lymphomagenic role[75]. Different studies have showed a significant association between HCV infection and Bcl-2 rearrangement t(14;18 translocation)[85-89]. In these patients, clonal expansion of B-cells harboring translocation t(14;18) was demonstrated, with overexpression of the anti-apoptotic Bcl-2 protein, resulting in higher Bcl-2/bax ratio[87,90]. Fascinatingly, the observation that treatment with interferon-α of HCV positive patients with splenic marginal zone NHL, results in HCV clearance, reduced frequency of translocation *t(14;18)* and regression of NHL clearly implies the causal role of HCV infection in at least certain subsets of NHLs[29,31,90-92]. Different possible modes of NHL causation by viral infection are schematically presented in Figure 2.

Recently, Machida and colleagues have demonstrated that HCV infection resulted in a 5 to 10-fold increase in somatic gene mutation frequency in IgHC, *BCL-6*, *p53*, and β-*catenin* genes of *in vitro* HCV-infected B-cell lines and HCV-associated peripheral blood mononuclear cells, lymphomas, and HCC, and proposed that this mutator phenotype of HCV leads to selection and amplification of deleterious mutations in the protooncogenes or tumor-suppressor genes in tumors[93]. They also suggested two different mechanisms of mutation, based on the observation that nucleotide-substitution pattern of *p53* and β-*catenin* is different from that of IgHC. Very interestingly this mutator function of HCV was found to be unique among oncogenic viruses, as similar amplification of protooncogene mutations in HCV-associated lymphomas and HCCs were not detectable in other types of tumors, lymphomas not related to HCV, HBV-associated HCCs, and HCCs of nonviral origin. Furthermore, the ability of HCV to induce high mutation frequency of cellular genes suggests that HCV may cause tumor formation by a *hit-and-run* mechanism.

Quite obviously, being of multi-factorial aetiology, there are a number of possible mechanisms through which HCV can induce lymphomagenesis, some elucidated, rest remain to be explored. Compared to HCV, HBV was discovered much earlier and its lymphotropic properties have been documented long back. Despite this fact, ironically, HBV related research has not been properly

focused on its lymphomagenic properties. In contrast, HGV/GBV-C being relatively newly discovered, studies are in progress to describe different properties and mechanisms of pathogenesis. It is assumed that being a member of the *Flaviviridae* family, as HCV does, HGV/GBV-C might utilize similar mechanisms.

MicroRNAs AND NHL

MicroRNAs (miRNA) are a group of lately discovered, highly conserved small noncoding RNAs arising from eukaryote genomes, that play an extremely important role in post-transcriptional regulation and are involved in a wide variety of biological pathways[94-96]. They control gene expression through base-pairing with the 3'-UTRs of target mRNAs, inducing mRNA degradation or suppressing translation, depending on the perfection of base-pairing[97-99]. One miRNA is capable of regulating the expression of multiple target genes; computational analyses have indicated that expression of more than 30% human genes is regulated by miRNAs[100]. MiRNAs show a highly tissue specific expression pattern, having important role in organ development, cellular differentiation, homeostasis, immune response, apoptosis and carcinogenesis also[95,101,102]. Very interestingly, apart from eukaryotes, a number of DNA viruses have also been shown to encode miRNAs, which probably help these viruses in modulating host gene expression favourable for its own replication[96]. Among the human DNA viruses, mainly Herpesviruses (EBV; human cytomegalovirus, hCMV; herpes simplex virus, HSV; Kaposi's sarcoma-associated herpesvirus, KHSV, *etc*.), Polyomaviruses, and Adenoviruses (human adenovirus, hAV) have been reported to encode and express miRNAs[96]. Using computational approaches, recently, Jin and colleagues found that HBV (*Hepadnavirus* family, DNA virus) could putatively encode atleast one candidate pre-miRNA, which could target one of its own viral mRNA, but could not target any of the cellular transcripts[103]. However this data remains to be experimentally validated at the cellular level. In contrast, using standard sequencing or advanced sequencing techniques, no viral miRNAs have been identified HPV or in RNA viruses such as Lentiviruses (human T-cell leukemia virus I , HTLV-1) or Flaviviruses (HCV; dengue virus, DENV, *etc*.), which led to a general thought that RNA viruses might not encode or express similar regulatory small RNAs[95,104-106]. Interestingly, recently, Andrew Fire's group studied infection of six different RNA viruses (including HCV, DENV, West Nile viruses, WNV, *etc*.) in 41 experimentally susceptible and resistant host systems, and reported identification of a class of RNA virus-derived small RNAs, termed as "vsRNAs", 99.97% of them showing perfect homology only with the infecting virus genomes. The authors also found that the cellular short RNA apparatus was capable to employ these vsRNAs as vsRNA-primed Ago (Argonaute) complexes, but the degree to which these complexes executed silencing of functional viral RNAs and modulation of cellular miRNAs require further investigations[107].

Whether virus encoded regulatory small RNAs are detectable or not in the infected cells, viral infection has been shown to modulate the host cellular miRNA expression, altering the cellular environment, leading to pathogenesis[96]. Fascinatingly, it is well established that certain viruses such as EBV, Vesicular Stomatitis virus (VSV) induces host miR-155 expression which is one of the typical multifunctional miRNAs involved in B-cell differentiation and proliferation, and has been shown to be overexpressed in Hodgkin and non-Hodgkin lymphomas, chronic lymphocytic leukaemia[95,108-114]. MiR-155 is involved in numerous physiological and pathological processes including innate and adaptive immunity, inflammation and tumorigenesis[115]. It has been shown to be important for immunoglobulin class switching and to prevent potentially oncogenic chromosomal aberrations through regulation of activation induced cytidine deaminase[116,117]. In addition to miR-155, EBV also induces miR-146a expression in B-cells[118]. MiR-146a can target TNF receptor associated factor 6 and Interleukin-1 receptor-associated kinase 1, of the Toll-like Receptor signaling pathway, suggesting a negative-feedback loop to limit innate immune responses[119,120]. Similarly, E6 protein of HPV downregulates the expression of miR-34a (a p53-regulated miRNA) and miR-218, that leads to increase in cell growth and tumorigenicity[121,122]. In contrast, viruses may also induce miRNAs that restrict viral replication. For example, HCV is known to upregulate IFN-β, which induces cellular miR-196, miR-296, miR-351, miR-431, and miR-448, *etc*., which in turn attenuate viral replication and viral accumulation[123].

Apart from modulating the cellular miRNA expression, viruses can even encode mimics of host miRNAs, the later been hypothesized to cause pathogenesis[95,96]. Support to this thought comes from the fact that the chicken oncogenic Marek's disease virus type 1 (MDV-1), expresses a miR-155-mimic, while the non-oncogenic MDV-2 does not express miR-155. Viral mimic of cellular miR-155, known as miR-K12-11 has also been reported from KSHV[124]. More interestingly, viruses have been recognized to utilize certain cellular miRNA for tissue specific infection also. HCV has been demonstrated to exploit a liver tissue specific miR-122 to positively regulate RNA replication[125]. Mammalian miR-122 expression is generally confined to the liver, and it helps maintain liver tissue identity by regulating fatty acid and cholesterol biosynthesis, pathways[95,96]. The liver-specific expression of miR-122 and its positive effect on HCV replication has been associated with hepatic tissue tropism of HCV and it has been hypothesized that tissue specific expression of certain miRNAs subject viruses to selective pressures, and viruses get optimized or evolved for replication in certain tissues (target tissue), while certain other tissues with different miRNAs might pose significant hurdles for viral replication, rendering them non-target tissues for the given virus[95]. This hypothesis has been firmly supported by experimental findings that engineering cellular miRNA target sites in the viral genomes can alter tissue tropism[126]. Even though further investigations are need-

ed to conclude, but this might be a consistent explanation for the compartmentalization of different variants/genotypes of the same virus in different tissues of the same subject, a phenomenon very frequently observed in a number of viral infection[127].

Although the miRNA expression patterns have been widely studied in different human cancers, but similar studies on NHL is relatively scarce, despite its immense importance. Recently some studies have focused on the role of miRNA in development of NHL[128-131]. Generally, up-regulation of miR-155 has been described in NHLs, as well as in several other solid and hematologic malignancies. Another miR-17-92 cluster has been found to be frequently amplified in malignant B-cell lymphomas, and is over-expressed in 65% of B-cell lymphoma patients[132]. MiR-143 and miR-145 expression is shown to be reduced in B-cell malignancies[133]. A recent study showed that enforced expression of the miR-17-92 cluster accelerated MYC induced lymphomagenesis[134]. Unfortunately however, there is hardly any published data on the miRNA expression patterns among NHL patients infected with HBV/HCV or HGV/GBV-C. A recent study clearly demonstrated the existence of HBV- and HCV specific differential cellular miRNAs expression profiles in HBV and HCV infected liver samples as well in Huh 7.5 cell culture models, revealing that entirely different pathways were modulated in HBV or HCV infected liver[135]. These results clearly depict the contribution of the hepatitis viruses (at the focus of this review mainly HBV and HCV) in cellular miRNA deregulation leading to liver pathogenesis. Similarly, it is plausible to assume that similar to hepatocytes, these hepatitis viruses may also alter the miRNA expression patterns in lymphocytes.

CLINICAL AND THERAPEUTICS FOR NHL

NHL comprises diverse subtypes of lymphoproliferative malignancies with distinct epidemiologic, etiologic, morphologic, genetic/molecular, clinical, immunologic and histological features[136,137]. Compared to HL, different NHLs have a higher tendency for extranodal sites. Like most other cancers, prognosis of NHL largely depends on the accuracy of histological classification, stage at detection and response to treatment[138,139]. A number of classification systems have been proposed and updated in the past decades, systems such as Rappaport and Lukes Collins classification system (developed in 1966, modified by Lukes Collins in 1974), Kiel classification system (developed in 1974), Working Formulation (developed in 1982), and the Revised European-American Lymphoid neoplasms classification (REAL, published in 1994) are the important ones. Earlier, classification was solely based on morphological characteristics, but later immunological, cytogenetic and molecular features were incorporated, that facilitate precise classification[138]. Presently, the World Health Organization classification (based on the principles of the REAL system, published in 2001, updated in 2008) is widely accepted and practised[138,140,141]. However, for selecting a therapeutic strategy, Ann Arbor anatomic staging system is presently in practice[138,142,143].

Being highly heterogeneous, different NHL subtypes are associated with a variety of typical and atypical clinical manifestations. However, more than 70% of the patients do not present classic symptoms, and are incidentally diagnosed while undergoing treatment for other nonspecific complaints. Usually NHL may present atypically with fever, fatigue, loss of appetite, drenching night sweats, weight loss, red patchy skin, *etc*. These general symptoms are termed as "B symptoms" and are generally associated with increased cancer "burden" due to delayed detection and poor prognosis. In contrast to Hodgkin's disease, most patients with NHL present with advanced stage Ⅲ or Ⅳ disease. The most typical clinical presentation of NHL is lymphadenopathy or extranodal mass in one or more lymph nodes. NHL involving abdominal lymphatic tissues often present with swelling of belly and lymphoma involving thymus is frequently presented with chest pain, or respiratory problems, *etc*. Sites often involved in NHL include skin thyroid, breast, gastrointestinal tract, brain, and ovaries or testes[138]. NHL may also involve unusual sites, such as epitrochlear or popliteal nodes or Waldeyer's ring (nasopharynx).

Generally, NHLs are divided into two prognostic groups, the indolent lymphomas (mostly nodular or follicular in morphology) have a relatively good prognosis and the aggressive lymphomas have a shorter natural history. Early-stages (Ⅰ and Ⅱ) of indolent lymphomas are effectively treated with radiation therapy, but almost incurable in advanced stages. However, a considerable number of patients with aggressive lymphoma are curable with intensive combination chemotherapy.

Significant improvements in the field of NHL management made in the recent years have resulted in cure of 30% to 60% patients with aggressive NHL. Decision on treatment depends largely on lymphoma sub-type, stage, age and overall health condition of the patient, *etc*. often, in certain cases of indolent lymphomas, treatment delayed till the manifestation, technically known as "watchful waiting". Nevertheless, majority of the patients require treatment consisting of chemotherapy, radiation therapy, and bone marrow/stem cell transplantation, alone or in a combination.

Chemotherapy is the most imperative treatment and often includes anti-cancer drug combinations[138,144,145]. Methotrexate, Doxorubicin Hydrochloride, Chlorambucil, Nelarabine, Tositumomab, Bleomycin, Cyclophosphamide, Liposomal Cytarabine, Pralatrexate, Romidepsin, Rituximab, Vinblastine Sulfate, Vorinostat, *etc*., are some of the drugs used for chemotherapy. Conversely, CHOP (Cyclophosphamide, Hydroxydauno-rubicin/doxorubicin, Oncovin, Prednisone or prednisolone), rituximab with CHOP (R-CHOP), cyclophosphamide, oncovin, procarbazine and prednisone, Etoposide, doxorubicin, vincristine, prednisone, and cycolophosphamide, ifosfamide, carboplatin and etoposide are some of the recipe used for combination chemotherapy. Although chemo-

therapy alone can cure several high-grade lymphomas, but sometimes patients with recurring or drug irresponsive lymphomas are considered for higher dose of chemotherapy followed by autologous bone marrow transplant. The most widespread therapy for NHL is the R-CHOP combination therapy and consists of the CHOP chemotherapy along with Rituximab immunotherapy (a chimeric monoclonal antibody against CD20 protein, principally found on B cells surface), which distinctively targets certain lymphoma cells and selectively kills them.

Apart from chemo-immune therapy, radio-immuno conjugates (radio actives conjugated to monoclonal antibodies) are also used for selective destruction of cancerous cells. This therapy is among the best treatment for NHL, as NHL is highly radiosensitive. In this therapy, Iodine-131 (Tositumomab) or the Yttrium-90 (Ibritumomab tiuxetan) is conjugated to antibodies against CD20, increasing the specificity, while diminishing off-target damage[145]. In addition, conjugation of immunotherapeutic monoclonal antibodies with immunomodulators (such as α-Interferon) is also under clinical trials[145]. Other treatment approaches for NHL, drug regimen, efficacy and results of different clinical trials may be found elsewhere in more details[138,139,144,145]. In addition, novel therapeutic approaches, target pathways and potential small molecule inhibitors for treatment of NHL resistant to conventional treatment have also been reviewed recently[146].

CONCLUSION

The available literature clearly signifies that hepatitis viruses (HBV, HCV and HGV/GBV-C) have strong lymphotropic properties and most of the published data corroborate a causal association between these viruses and NHL. Additionally, prevailing data showing rapid increase in incidences and deaths due to NHL highlight the magnitude of disease burden in developed as well as less developed areas. Despite its significance, hepatitis virus associated NHL remains poorly understood. Although, mechanism of HCV related NHL has been studied in some details, but the other two viruses have remained poorly studied from the perspective of their involvement in NHL development. It is also evident that there is scarcity of data related to miRNA regulation patterns in HBV, HCV and HGV/GBV-C related NHL. The availability of miRNA regulation data might help reveal important facets of lymphomagenic mechanisms. Therefore research efforts focused on hepatitis virus induced NHL is essential to properly understand the virus induced lymphomagenic mechanisms, in order to develop effective intervention strategies and to reduce the disease burden.

REFERENCES

1 **Vogelstein B**, Kinzler KW. The multistep nature of cancer. *Trends Genet* 1993; **9**: 138-141
2 **Dean M**. Cancer as a complex developmental disorder--nineteenth Cornelius P. Rhoads Memorial Award Lecture. *Cancer Res* 1998; **58**: 5633-5636
3 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90
4 Emerging Links between Chronic Disease and Environmental Exposure. Non-Hodgkin's Lymphoma. Physicians for Social Responsibility. Available from: URL: www.psr.org/assets/pdfs/emerging-links-non-hodgkins.pdf
5 **Robbins SL**, Cotran RS, Kumar V. Pathologic basis of disease. 2nd ed. Philadelphia: WB Saunders, 1995
6 **Arber DA**. Molecular diagnostic approach to non-Hodgkin's lymphoma. *J Mol Diagn* 2000; **2**: 178-190
7 **Matasar MJ**, Zelenetz AD. Overview of lymphoma diagnosis and management. *Radiol Clin North Am* 2008; **46**: 175-98, vii
8 **Boffetta P**. Epidemiology of adult non-Hodgkin lymphoma. *Ann Oncol* 2011; **22** Suppl 4: iv27-iv31
9 **American Cancer Society**. Global Cancer Facts & Figures. 2nd ed. Atlanta: American Cancer Society, 2011
10 **Marcucci F**, Mele A. Hepatitis viruses and non-Hodgkin lymphoma: epidemiology, mechanisms of tumorigenesis, and therapeutic opportunities. *Blood* 2011; **117**: 1792-1798
11 **Ye BH**. BCL-6 in the pathogenesis of non-Hodgkin's lymphoma. *Cancer Invest* 2000; **18**: 356-365
12 **Lan Q**, Wang SS, Menashe I, Armstrong B, Zhang Y, Hartge P, Purdue MP, Holford TR, Morton LM, Kricker A, Cerhan JR, Grulich A, Cozen W, Zahm SH, Yeager M, Vajdic CM, Schenk M, Leaderer B, Yuenger J, Severson RK, Chatterjee N, Chanock SJ, Zheng T, Rothman N. Genetic variation in Th1/Th2 pathway genes and risk of non-Hodgkin lymphoma: a pooled analysis of three population-based case-control studies. *Br J Haematol* 2011; **153**: 341-350
13 **Kinlen L**. Immunosuppressive therapy and acquired immunological disorders. *Cancer Res* 1992; **52**: 5474s-5476s
14 **Tirelli U**, Spina M, Gaidano G, Vaccher E, Franceschi S, Carbone A. Epidemiological, biological and clinical features of HIV-related lymphomas in the era of highly active antiretroviral therapy. *AIDS* 2000; **14**: 1675-1688
15 **Fisher SG**, Fisher RI. The epidemiology of non-Hodgkin's lymphoma. *Oncogene* 2004; **23**: 6524-6534
16 **Dich J**, Zahm SH, Hanberg A, Adami HO. Pesticides and cancer. *Cancer Causes Control* 1997; **8**: 420-443
17 **IARC**. Polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans. Lyon: International Agency for Research on Cancer, 1997
18 **Zahm SH**, Blair A. Pesticides and non-Hodgkin's lymphoma. *Cancer Res* 1992; **52**: 5485s-5488s
19 **Pearce N**, Bethwaite P. Increasing incidence of non-Hodgkin's lymphoma: occupational and environmental factors. *Cancer Res* 1992; **52**: 5496s-5500s
20 **Alexander DD**, Mink PJ, Adami HO, Chang ET, Cole P, Mandel JS, Trichopoulos D. The non-Hodgkin lymphomas: a review of the epidemiologic literature. *Int J Cancer* 2007; **120** Suppl 12: 1-39
21 **zur Hausen H**. Viruses in human cancers. *Science* 1991; **254**: 1167-1173
22 **Hjalgrim H**, Engels EA. Infectious aetiology of Hodgkin and non-Hodgkin lymphomas: a review of the epidemiological evidence. *J Intern Med* 2008; **264**: 537-548
23 **Engels EA**. Infectious agents as causes of non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 401-404
24 **McLaughlin-Drubin ME**, Munger K. Viruses associated with human cancer. *Biochim Biophys Acta* 2008; **1782**: 127-150
25 **Michalak TI**. Occult persistence and lymphotropism of hepadnaviral infection: insights from the woodchuck viral hepatitis model. *Immunol Rev* 2000; **174**: 98-111
26 **Pham TN**, Michalak TI. Occult persistence and lymphotropism of hepatitis C virus infection. *World J Gastroenterol* 2008; **14**: 2789-2793
27 **Zignego AL**, Giannini C, Monti M, Gragnani L. Hepatitis C virus lymphotropism: lessons from a decade of studies. *Dig Liver Dis* 2007; **39** Suppl 1: S38-S45
28 **Feitelson MA**, Duan LX. Hepatitis B virus X antigen in the

pathogenesis of chronic infections and the development of hepatocellular carcinoma. *Am J Pathol* 1997; **150**: 1141-1157
29 **Hermine O**, Lefrère F, Bronowicki JP, Mariette X, Jondeau K, Eclache-Saudreau V, Delmas B, Valensi F, Cacoub P, Brechot C, Varet B, Troussard X. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med* 2002; **347**: 89-94
30 **Vallisa D**, Bernuzzi P, Arcaini L, Sacchi S, Callea V, Marasca R, Lazzaro A, Trabacchi E, Anselmi E, Arcari AL, Moroni C, Bertè R, Lazzarino M, Cavanna L. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, B-cell, non-Hodgkin's lymphoma: a multicenter Italian experience. *J Clin Oncol* 2005; **23**: 468-473
31 **Zuckerman E**, Zuckerman T, Sahar D, Streichman S, Attias D, Sabo E, Yeshurun D, Rowe JM. The effect of antiviral therapy on t(14; 18) translocation and immunoglobulin gene rearrangement in patients with chronic hepatitis C virus infection. *Blood* 2001; **97**: 1555-1559
32 **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
33 **Hausfater P**, Cacoub P, Sterkers Y, Thibault V, Amoura Z, Nguyen L, Ghillani P, Leblond V, Piette JC. Hepatitis C virus infection and lymphoproliferative diseases: prospective study on 1,576 patients in France. *Am J Hematol* 2001; **67**: 168-171
34 **Collier JD**, Zanke B, Moore M, Kessler G, Krajden M, Shepherd F, Heathcote J. No association between hepatitis C and B-cell lymphoma. *Hepatology* 1999; **29**: 1259-1261
35 **Negri E**, Little D, Boiocchi M, La Vecchia C, Franceschi S. B-cell non-Hodgkin's lymphoma and hepatitis C virus infection: a systematic review. *Int J Cancer* 2004; **111**: 1-8
36 **Lauer GM**, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52
37 **Shi L**, Zhang SL, Li K, Hong Y, Wang Q, Li Y, Guo J, Fan WH, Zhang L, Cheng J. NS5ATP9, a gene up-regulated by HCV NS5A protein. *Cancer Lett* 2008; **259**: 192-197
38 **Schmitz U**, Tan SL. NS5A--from obscurity to new target for HCV therapy. *Recent Pat Antiinfect Drug Discov* 2008; **3**: 77-92
39 **Gisbert JP**, García-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in B-cell non-Hodgkin's lymphoma: systematic review and meta-analysis. *Gastroenterology* 2003; **125**: 1723-1732
40 **Marcucci F**, Mele A, Spada E, Candido A, Bianco E, Pulsoni A, Chionne P, Madonna E, Cotichini R, Barbui A, De Renzo A, Dore F, Iannitto E, Liso V, Martino B, Montanaro M, Pagano L, Musto P, Rapicetta M. High prevalence of hepatitis B virus infection in B-cell non-Hodgkin's lymphoma. *Haematologica* 2006; **91**: 554-557
41 **Galun E**, Ilan Y, Livni N, Ketzinel M, Nahor O, Pizov G, Nagler A, Eid A, Rivkind A, Laster M. Hepatitis B virus infection associated with hematopoietic tumors. *Am J Pathol* 1994; **145**: 1001-1007
42 **Park SC**, Jeong SH, Kim J, Han CJ, Kim YC, Choi KS, Cho JH, Lee M, Jung HH, Ki SS, Chang YH, Lee SS, Park YH, Lee KH. High prevalence of hepatitis B virus infection in patients with B-cell non-Hodgkin's lymphoma in Korea. *J Med Virol* 2008; **80**: 960-966
43 **Kim JH**, Bang YJ, Park BJ, Yoo T, Kim CW, Kim TY, Heo DS, Lee HS, Kim NK. Hepatitis B virus infection and B-cell non-Hodgkin's lymphoma in a hepatitis B endemic area: a case-control study. *Jpn J Cancer Res* 2002; **93**: 471-477
44 **Chen MH**, Hsiao LT, Chiou TJ, Liu JH, Gau JP, Teng HW, Wang WS, Chao TC, Yen CC, Chen PM. High prevalence of occult hepatitis B virus infection in patients with B cell non-Hodgkin's lymphoma. *Ann Hematol* 2008; **87**: 475-480
45 **Wang F**, Xu RH, Han B, Shi YX, Luo HY, Jiang WQ, Lin TY, Huang HQ, Xia ZJ, Guan ZZ. High incidence of hepatitis B virus infection in B-cell subtype non-Hodgkin lymphoma compared with other cancers. *Cancer* 2007; **109**: 1360-1364
46 **Lim ST**, Fei G, Quek R, Lim LC, Lee LH, Yap SP, Loong S, Tao M. The relationship of hepatitis B virus infection and non-Hodgkin's lymphoma and its impact on clinical characteristics and prognosis. *Eur J Haematol* 2007; **79**: 132-137
47 **Kuniyoshi M**, Nakamuta M, Sakai H, Enjoji M, Kinukawa N, Kotoh K, Fukutomi M, Yokota M, Nishi H, Iwamoto H, Uike N, Nishimura J, Inaba S, Maeda Y, Nawata H, Muta K. Prevalence of hepatitis B or C virus infections in patients with non-Hodgkin's lymphoma. *J Gastroenterol Hepatol* 2001; **16**: 215-219
48 **Anderson LA**, Pfeiffer R, Warren JL, Landgren O, Gadalla S, Berndt SI, Ricker W, Parsons R, Wheeler W, Engels EA. Hematopoietic malignancies associated with viral and alcoholic hepatitis. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 3069-3075
49 **Ulcickas Yood M**, Quesenberry CP, Guo D, Caldwell C, Wells K, Shan J, Sanders L, Skovron ML, Iloeje U, Manos MM. Incidence of non-Hodgkin's lymphoma among individuals with chronic hepatitis B virus infection. *Hepatology* 2007; **46**: 107-112
50 **Nath A**, Agarwal R, Malhotra P, Varma S. Prevalence of hepatitis B virus infection in non-Hodgkin lymphoma: a systematic review and meta-analysis. *Intern Med J* 2010; **40**: 633-641
51 **Bréchot C**, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology* 2001; **34**: 194-203
52 **Rehermann B**, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* 1996; **2**: 1104-1108
53 **Ganem D**, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118-1129
54 **Feitelson MA**, Lee J. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. *Cancer Lett* 2007; **252**: 157-170
55 **Bouchard MJ**, Schneider RJ. The enigmatic X gene of hepatitis B virus. *J Virol* 2004; **78**: 12725-12734
56 **Cheung RC**, Keeffe EB, Greenberg HB. Hepatitis G virus: is it a hepatitis virus? *West J Med* 1997; **167**: 23-33
57 **Radkowski M**, Kubicka J, Kisiel E, Cianciara J, Nowicki M, Rakela J, Laskus T. Detection of active hepatitis C virus and hepatitis G virus/GB virus C replication in bone marrow in human subjects. *Blood* 2000; **95**: 3986-3989
58 **Laskus T**, Radkowski M, Wang LF, Vargas H, Rakela J. Lack of evidence for hepatitis G virus replication in the livers of patients coinfected with hepatitis C and G viruses. *J Virol* 1997; **71**: 7804-7806
59 **Mellor J**, Haydon G, Blair C, Livingstone W, Simmonds P. Low level or absent in vivo replication of hepatitis C virus and hepatitis G virus/GB virus C in peripheral blood mononuclear cells. *J Gen Virol* 1998; **79** (Pt 4): 705-714
60 **Radkowski M**, Wang LF, Cianciara J, Rakela J, Laskus T. Analysis of hepatitis G virus/GB virus C quasispecies and replication sites in human subjects. *Biochem Biophys Res Commun* 1999; **258**: 296-299
61 **Tucker TJ**, Smuts HE, Eedes C, Knobel GD, Eickhaus P, Robson SC, Kirsch RE. Evidence that the GBV-C/hepatitis G virus is primarily a lymphotropic virus. *J Med Virol* 2000; **61**: 52-58
62 **Madejón A**, Fogeda M, Bartolomé J, Pardo M, González C, Cotonat T, Carreño V. GB virus C RNA in serum, liver, and peripheral blood mononuclear cells from patients with chronic hepatitis B, C, and D. *Gastroenterology* 1997; **113**: 573-578
63 **Saito S**, Tanaka K, Kondo M, Morita K, Kitamura T, Kiba T, Numata K, Sekihara H. Plus- and minus-stranded hepatitis G virus RNA in liver tissue and in peripheral blood mono-

nuclear cells. *Biochem Biophys Res Commun* 1997; **237**: 288-291
64 **Ellenrieder V**, Weidenbach H, Frickhofen N, Michel D, Prümmer O, Klatt S, Bernas O, Mertens T, Adler G, Beckh K. HCV and HGV in B-cell non-Hodgkin's lymphoma. *J Hepatol* 1998; **28**: 34-39
65 **De Renzo A**, Persico E, de Marino F, di Giacomo Russo G, Notaro R, di Grazia C, Picardi M, Santoro L, Torella R, Rotoli B, Persico M. High prevalence of hepatitis G virus infection in Hodgkin's disease and B-cell lymphoproliferative disorders: absence of correlation with hepatitis C virus infection. *Haematologica* 2002; **87**: 714-718; discussion 718
66 **Krajden M**, Yu A, Braybrook H, Lai AS, Mak A, Chow R, Cook D, Tellier R, Petric M, Gascoyne RD, Connors JM, Brooks-Wilson AR, Gallagher RP, Spinelli JJ. GBV-C/hepatitis G virus infection and non-Hodgkin lymphoma: a case control study. *Int J Cancer* 2010; **126**: 2885-2892
67 **Giannoulis E**, Economopoulos T, Mandraveli K, Giannoulis K, Nikolaides C, Zervou E, Papageorgiou E, Zoulas D, Tourkantonis A, Giannopoulos G, Fountzilas G. The prevalence of hepatitis C and hepatitis G virus infection in patients with B cell non-Hodgkin lymphomas in Greece: a Hellenic Cooperative Oncology Group Study. *Acta Haematol* 2004; **112**: 189-193
68 **Wiwanitkit V**. Individuals with HGV-RNA are at high risk of B cell non-Hodgkin's lymphoma development. *Asian Pac J Cancer Prev* 2005; **6**: 215-216
69 **Wiwanitkit V**. Hepatitis Virus Infection and Hodgkin's Lymphoma: A Review of the Literature. *Hepatitis Mon* 2007; **7**: 229-231
70 **Kaya H**, Polat MF, Erdem F, Gündogdu M. Prevalence of hepatitis C virus and hepatitis G virus in patients with non-Hodgkin's lymphoma. *Clin Lab Haematol* 2002; **24**: 107-110
71 **Rothman KJ**, Greenland S. Causation and causal inference in epidemiology. *Am J Public Health* 2005; **95** Suppl 1: S144-S150 [PMID: 16030331 DOI: 10.2105/AJPH.2004.059204]
72 **Zignego AL**, Giannini C, Ferri C. Hepatitis C virus-related lymphoproliferative disorders: an overview. *World J Gastroenterol* 2007; **13**: 2467-2478
73 **Stapleton JT**, Chaloner K. GB virus C infection and non-Hodgkin lymphoma: important to know but the jury is out. *Int J Cancer* 2010; **126**: 2759-2761
74 **Pozzato G**, Mazzaro C, Crovatto M, Modolo ML, Ceselli S, Mazzi G, Sulfaro S, Franzin F, Tulissi P, Moretti M. Low-grade malignant lymphoma, hepatitis C virus infection, and mixed cryoglobulinemia. *Blood* 1994; **84**: 3047-3053
75 **Gasparotto D**, De Re V, Boiocchi M. Hepatitis C virus, B-cell proliferation and lymphomas. *Leuk Lymphoma* 2002; **43**: 747-751
76 **De Re V**, De Vita S, Marzotto A, Gloghini A, Pivetta B, Gasparotto D, Cannizzaro R, Carbone A, Boiocchi M. Premalignant and malignant lymphoproliferations in an HCV-infected type II mixed cryoglobulinemic patient are sequential phases of an antigen-driven pathological process. *Int J Cancer* 2000; **87**: 211-216
77 **De Re V**, De Vita S, Marzotto A, Rupolo M, Gloghini A, Pivetta B, Gasparotto D, Carbone A, Boiocchi M. Sequence analysis of the immunoglobulin antigen receptor of hepatitis C virus-associated non-Hodgkin lymphomas suggests that the malignant cells are derived from the rheumatoid factor-producing cells that occur mainly in type II cryoglobulinemia. *Blood* 2000; **96**: 3578-3584
78 **Ivanovski M**, Silvestri F, Pozzato G, Anand S, Mazzaro C, Burrone OR, Efremov DG. Somatic hypermutation, clonal diversity, and preferential expression of the VH 51p1/VL kv325 immunoglobulin gene combination in hepatitis C virus-associated immunocytomas. *Blood* 1998; **91**: 2433-2442
79 **Luppi M**, Grazia Ferrari M, Bonaccorsi G, Longo G, Narni F, Barozzi P, Marasca R, Mussini C, Torelli G. Hepatitis C virus infection in subsets of neoplastic lymphoproliferations not associated with cryoglobulinemia. *Leukemia* 1996; **10**: 351-355
80 **Mele A**, Pulsoni A, Bianco E, Musto P, Szklo A, Sanpaolo MG, Iannitto E, De Renzo A, Martino B, Liso V, Andrizzi C, Pusterla S, Dore F, Maresca M, Rapicetta M, Marcucci F, Mandelli F, Franceschi S. Hepatitis C virus and B-cell non-Hodgkin lymphomas: an Italian multicenter case-control study. *Blood* 2003; **102**: 996-999
81 **Bronowicki JP**, Loriot MA, Thiers V, Grignon Y, Zignego AL, Bréchot C. Hepatitis C virus persistence in human hematopoietic cells injected into SCID mice. *Hepatology* 1998; **28**: 211-218
82 **Majumder M**, Ghosh AK, Steele R, Zhou XY, Phillips NJ, Ray R, Ray RB. Hepatitis C virus NS5A protein impairs TNF-mediated hepatic apoptosis, but not by an anti-FAS antibody, in transgenic mice. *Virology* 2002; **294**: 94-105
83 **Machida K**, Tsukiyama-Kohara K, Seike E, Toné S, Shibasaki F, Shimizu M, Takahashi H, Hayashi Y, Funata N, Taya C, Yonekawa H, Kohara M. Inhibition of cytochrome c release in Fas-mediated signaling pathway in transgenic mice induced to express hepatitis C viral proteins. *J Biol Chem* 2001; **276**: 12140-12146
84 **Pileri P**, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, Abrignani S. Binding of hepatitis C virus to CD81. *Science* 1998; **282**: 938-941
85 **Zignego AL**, Giannelli F, Marrocchi ME, Mazzocca A, Ferri C, Giannini C, Monti M, Caini P, Villa GL, Laffi G, Gentilini P. T(14; 18) translocation in chronic hepatitis C virus infection. *Hepatology* 2000; **31**: 474-479
86 **Zignego AL**, Giannelli F, Marrocchi ME, Giannini C, Gentilini P, Innocenti F, Ferri C. Frequency of bcl-2 rearrangement in patients with mixed cryoglobulinemia and HCV-positive liver diseases. *Clin Exp Rheumatol* 1997; **15**: 711-712
87 **Zignego AL**, Ferri C, Giannelli F, Giannini C, Caini P, Monti M, Marrocchi ME, Di Pietro E, La Villa G, Laffi G, Gentilini P. Prevalence of bcl-2 rearrangement in patients with hepatitis C virus-related mixed cryoglobulinemia with or without B-cell lymphomas. *Ann Intern Med* 2002; **137**: 571-580
88 **Kitay-Cohen Y**, Amiel A, Hilzenrat N, Buskila D, Ashur Y, Fejgin M, Gaber E, Safadi R, Tur-Kaspa R, Lishner M. Bcl-2 rearrangement in patients with chronic hepatitis C associated with essential mixed cryoglobulinemia type II. *Blood* 2000; **96**: 2910-2912
89 **Sasso EH**, Martinez M, Yarfitz SL, Ghillani P, Musset L, Piette JC, Cacoub P. Frequent joining of Bcl-2 to a JH6 gene in hepatitis C virus-associated t(14; 18). *J Immunol* 2004; **173**: 3549-3556
90 **Zuckerman E**, Zuckerman T, Sahar D, Streichman S, Attias D, Sabo E, Yeshurun D, Rowe J. bcl-2 and immunoglobulin gene rearrangement in patients with hepatitis C virus infection. *Br J Haematol* 2001; **112**: 364-369
91 **Giannelli F**, Moscarella S, Giannini C, Caini P, Monti M, Gragnani L, Romanelli RG, Solazzo V, Laffi G, La Villa G, Gentilini P, Zignego AL. Effect of antiviral treatment in patients with chronic HCV infection and t(14; 18) translocation. *Blood* 2003; **102**: 1196-1201
92 **Mazzaro C**, Franzin F, Tulissi P, Pussini E, Crovatto M, Carniello GS, Efremov DG, Burrone O, Santini G, Pozzato G. Regression of monoclonal B-cell expansion in patients affected by mixed cryoglobulinemia responsive to alpha-interferon therapy. *Cancer* 1996; **77**: 2604-2613
93 **Machida K**, Cheng KT, Sung VM, Shimodaira S, Lindsay KL, Levine AM, Lai MY, Lai MM. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. *Proc Natl Acad Sci USA* 2004; **101**: 4262-4267
94 **Ross JS**, Carlson JA, Brock G. miRNA: the new gene silencer. *Am J Clin Pathol* 2007; **128**: 830-836
95 **Gottwein E**, Cullen BR. Viral and cellular microRNAs as determinants of viral pathogenesis and immunity. *Cell Host Microbe* 2008; **3**: 375-387

96 **Skalsky RL**, Cullen BR. Viruses, microRNAs, and host interactions. *Annu Rev Microbiol* 2010; **64**: 123-141
97 **Su C**, Hou Z, Zhang C, Tian Z, Zhang J. Ectopic expression of microRNA-155 enhances innate antiviral immunity against HBV infection in human hepatoma cells. *Virol J* 2011; **8**: 354
98 **Baltimore D**, Boldin MP, O'Connell RM, Rao DS, Taganov KD. MicroRNAs: new regulators of immune cell development and function. *Nat Immunol* 2008; **9**: 839-845
99 **O'Connell RM**, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* 2010; **10**: 111-122
100 **Lewis BP**, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; **120**: 15-20
101 **Lindsay MA**. microRNAs and the immune response. *Trends Immunol* 2008; **29**: 343-351
102 **Bartel DP**, Chen CZ. Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs. *Nat Rev Genet* 2004; **5**: 396-400
103 **Jin WB**, Wu FL, Kong D, Guo AG. HBV-encoded microRNA candidate and its target. *Comput Biol Chem* 2007; **31**: 124-126
104 **Cai X**, Li G, Laimins LA, Cullen BR. Human papillomavirus genotype 31 does not express detectable microRNA levels during latent or productive virus replication. *J Virol* 2006; **80**: 10890-10893
105 **Lin J**, Cullen BR. Analysis of the interaction of primate retroviruses with the human RNA interference machinery. *J Virol* 2007; **81**: 12218-12226
106 **Pfeffer S**, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grässer FA, van Dyk LF, Ho CK, Shuman S, Chien M, Russo JJ, Ju J, Randall G, Lindenbach BD, Rice CM, Simon V, Ho DD, Zavolan M, Tuschl T. Identification of microRNAs of the herpesvirus family. *Nat Methods* 2005; **2**: 269-276
107 **Parameswaran P**, Sklan E, Wilkins C, Burgon T, Samuel MA, Lu R, Ansel KM, Heissmeyer V, Einav S, Jackson W, Doukas T, Paranjape S, Polacek C, dos Santos FB, Jalili R, Babrzadeh F, Gharizadeh B, Grimm D, Kay M, Koike S, Sarnow P, Ronaghi M, Ding SW, Harris E, Chow M, Diamond MS, Kirkegaard K, Glenn JS, Fire AZ. Six RNA viruses and forty-one hosts: viral small RNAs and modulation of small RNA repertoires in vertebrate and invertebrate systems. *PLoS Pathog* 2010; **6**: e1000764
108 **Yin Q**, McBride J, Fewell C, Lacey M, Wang X, Lin Z, Cameron J, Flemington EK. MicroRNA-155 is an Epstein-Barr virus-induced gene that modulates Epstein-Barr virus-regulated gene expression pathways. *J Virol* 2008; **82**: 5295-5306
109 **Wang P**, Hou J, Lin L, Wang C, Liu X, Li D, Ma F, Wang Z, Cao X. Inducible microRNA-155 feedback promotes type I IFN signaling in antiviral innate immunity by targeting suppressor of cytokine signaling 1. *J Immunol* 2010; **185**: 6226-6233
110 **Kluiver J**, Poppema S, de Jong D, Blokzijl T, Harms G, Jacobs S, Kroesen BJ, van den Berg A. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. *J Pathol* 2005; **207**: 243-249
111 **Eis PS**, Tam W, Sun L, Chadburn A, Li Z, Gomez MF, Lund E, Dahlberg JE. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc Natl Acad Sci USA* 2005; **102**: 3627-3632
112 **Metzler M**, Wilda M, Busch K, Viehmann S, Borkhardt A. High expression of precursor microRNA-155/BIC RNA in children with Burkitt lymphoma. *Genes Chromosomes Cancer* 2004; **39**: 167-169
113 **Costinean S**, Zanesi N, Pekarsky Y, Tili E, Volinia S, Heerema N, Croce CM. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc Natl Acad Sci USA* 2006; **103**: 7024-7029
114 **Fulci V**, Chiaretti S, Goldoni M, Azzalin G, Carucci N, Tavolaro S, Castellano L, Magrelli A, Citarella F, Messina M, Maggio R, Peragine N, Santangelo S, Mauro FR, Landgraf P, Tuschl T, Weir DB, Chien M, Russo JJ, Ju J, Sheridan R, Sander C, Zavolan M, Guarini A, Foà R, Macino G. Quantitative technologies establish a novel microRNA profile of chronic lymphocytic leukemia. *Blood* 2007; **109**: 4944-4951
115 **Faraoni I**, Antonetti FR, Cardone J, Bonmassar E. miR-155 gene: a typical multifunctional microRNA. *Biochim Biophys Acta* 2009; **1792**: 497-505
116 **Thai TH**, Calado DP, Casola S, Ansel KM, Xiao C, Xue Y, Murphy A, Frendewey D, Valenzuela D, Kutok JL, Schmidt-Supprian M, Rajewsky N, Yancopoulos G, Rao A, Rajewsky K. Regulation of the germinal center response by microRNA-155. *Science* 2007; **316**: 604-608
117 **Teng G**, Hakimpour P, Landgraf P, Rice A, Tuschl T, Casellas R, Papavasiliou FN. MicroRNA-155 is a negative regulator of activation-induced cytidine deaminase. *Immunity* 2008; **28**: 621-629
118 **Cameron JE**, Yin Q, Fewell C, Lacey M, McBride J, Wang X, Lin Z, Schaefer BC, Flemington EK. Epstein-Barr virus latent membrane protein 1 induces cellular MicroRNA miR-146a, a modulator of lymphocyte signaling pathways. *J Virol* 2008; **82**: 1946-1958
119 **Xiao C**, Rajewsky K. MicroRNA control in the immune system: basic principles. *Cell* 2009; **136**: 26-36
120 **Xia T**, O'Hara A, Araujo I, Barreto J, Carvalho E, Sapucaia JB, Ramos JC, Luz E, Pedroso C, Manrique M, Toomey NL, Brites C, Dittmer DP, Harrington WJ. EBV microRNAs in primary lymphomas and targeting of CXCL-11 by ebv-mir-BHRF1-3. *Cancer Res* 2008; **68**: 1436-1442
121 **Martinez I**, Gardiner AS, Board KF, Monzon FA, Edwards RP, Khan SA. Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells. *Oncogene* 2008; **27**: 2575-2582
122 **Wang X**, Wang HK, McCoy JP, Banerjee NS, Rader JS, Broker TR, Meyers C, Chow LT, Zheng ZM. Oncogenic HPV infection interrupts the expression of tumor-suppressive miR-34a through viral oncoprotein E6. *RNA* 2009; **15**: 637-647
123 **Pedersen IM**, Cheng G, Wieland S, Volinia S, Croce CM, Chisari FV, David M. Interferon modulation of cellular microRNAs as an antiviral mechanism. *Nature* 2007; **449**: 919-922
124 **Skalsky RL**, Samols MA, Plaisance KB, Boss IW, Riva A, Lopez MC, Baker HV, Renne R. Kaposi's sarcoma-associated herpesvirus encodes an ortholog of miR-155. *J Virol* 2007; **81**: 12836-12845
125 **Jopling CL**, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 2005; **309**: 1577-1581
126 **Umbach JL**, Cullen BR. The role of RNAi and microRNAs in animal virus replication and antiviral immunity. *Genes Dev* 2009; **23**: 1151-1164
127 **Datta S**, Panigrahi R, Biswas A, Chandra PK, Banerjee A, Mahapatra PK, Panda CK, Chakrabarti S, Bhattacharya SK, Biswas K, Chakravarty R. Genetic characterization of hepatitis B virus in peripheral blood leukocytes: evidence for selection and compartmentalization of viral variants with the immune escape G145R mutation. *J Virol* 2009; **83**: 9983-9992
128 **Baltic V**, Baltic M, Svircev Z, Jerant-Patic V. microRNA expression in non-Hodgkin's lymphomas. *Arch Oncol* 2008; **16**: 59-68
129 **Fanini F**, Vannini I, Fabbri M. MicroRNAs: tiny players with a big role in the pathogenesis of leukemias and lymphomas. *Hematology Reviews* 2009; **1**: e8
130 **Calin GA**, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**: 857-866
131 **Sandhu SK**, Croce CM, Garzon R. Micro-RNA Expression and Function in Lymphomas. *Adv Hematol* 2011; **2011**: 347137
132 **Ota A**, Tagawa H, Karnan S, Tsuzuki S, Karpas A, Kira S, Yoshida Y, Seto M. Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification

in malignant lymphoma. *Cancer Res* 2004; **64**: 3087-3095
133 **Akao Y**, Nakagawa Y, Kitade Y, Kinoshita T, Naoe T. Down-regulation of microRNAs-143 and -145 in B-cell malignancies. *Cancer Sci* 2007; **98**: 1914-1920
134 **He L**, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM. A microRNA polycistron as a potential human oncogene. *Nature* 2005; **435**: 828-833
135 **Ura S**, Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, Sunakozaka H, Sakai Y, Horimoto K, Kaneko S. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology* 2009; **49**: 1098-1112
136 **Armitage JO**, Berg AR, Purtilo DT. Adult non-Hodgkin's lymphoma. In: Bick RL, editor. Hematology: Clinical and Laboratory Practice. Louis: Mosby-Year Book Inc, 1993: 875-893
137 **Jaffe ES**. Histopathology of the non-Hodgkin's lymphomas and Hodgkin's disease. In: Canellos P, Lister TA, Sklar JL, editors. The lymphomas. Philadelphia: WB Saunders Co., 1998: 77-106
138 **Skarin AT**, Dorfman DM. Non-Hodgkin's lymphomas: current classification and management. *CA Cancer J Clin* 1997; **47**: 351-372
139 **Mounter PJ**, Lennard AL. Management of non-Hodgkin's lymphomas. *Postgrad Med J* 1999; **75**: 2-6
140 **Harris NL**, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, Gatter KC. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; **84**: 1361-1392
141 **Harris NL**, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. The World Health Organization classification of neoplastic diseases of the haematopoietic and lymphoid tissues: Report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Histopathology* 2000; **36**: 69-86
142 **Lister TA**, Crowther D, Sutcliffe SB, Glatstein E, Canellos GP, Young RC, Rosenberg SA, Coltman CA, Tubiana M. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. *J Clin Oncol* 1989; **7**: 1630-1636
143 **Armitage JO**. Staging non-Hodgkin lymphoma. *CA Cancer J Clin* 2005; **55**: 368-376
144 **Cerny T**, Gillessen S. Advances in the treatment of non-Hodgkin's lymphoma. *Ann Oncol* 2002; **13** Suppl 4: 211-216
145 **Vose JM**, Chiu BC, Cheson BD, Dancey J, Wright J. Update on epidemiology and therapeutics for non-Hodgkin's lymphoma. *Hematology Am Soc Hematol Educ Program* 2002; 241-262
146 **Mahadevan D**, Fisher RI. Novel therapeutics for aggressive non-Hodgkin's lymphoma. *J Clin Oncol* 2011; **29**: 1876-1884

S- Editor Wang JL **L- Editor** A **E- Editor** Zheng XM

World Journal of Virology

Online Submissions: http://www.wjgnet.com/esps/
wjv@wjgnet.com
doi:10.5501/wjv.v1.i6.174

World J Virol 2012 December 12; 1(6): 174-183
ISSN 2220-3249 (online)

REVIEW

Antiviral treatment to prevent chronic hepatitis B or C-related hepatocellular carcinoma

Li-Ping Chen, Jun Zhao, Yan Du, Yi-Fang Han, Tong Su, Hong-Wei Zhang, Guang-Wen Cao

Li-Ping Chen, Yan Du, Yi-Fang Han, Tong Su, Hong-Wei Zhang, Guang-Wen Cao, Department of Epidemiology, the Second Military Medical University, Shanghai 200433, China
Jun Zhao, Department of Hepatobiliary Surgery, the Third Affiliated Hospital of the Second Military Medical University, Shanghai 200438, China
Author contributions: Chen LP and Zhao J collected the data from the published literature, drafted the first version and contributed equally to the work; Du Y, Han YF, Su T and Zhang HW critically read the draft; Cao GW presented the study conception, supervised data collection and drafted subsequent versions of this manuscript.
Supported by National Natural Scientific Foundation of China, No. 81025015 and 91129301
Correspondence to: Guang-Wen Cao, MD, PhD, Professor, Chairman, Department of Epidemiology, the Second Military Medical University, 800 Xiangyin Rd., Shanghai 200433, China. gcao@smmu.edu.cn
Telephone: +86-21-81871060 Fax: +86-21-81871060
Received: October 28, 2011 Revised: June 18, 2012
Accepted: November 7, 2012
Published online: December 12, 2012

Abstract

Antiviral treatment is the only option to prevent or defer the occurrence of hepatocellular carcinoma (HCC) in patients chronically infected with hepatitis B virus (HBV) or hepatitis C virus (HCV). The approved medication for the treatment of chronic HBV infection is interferon-α (IFNα) and nucleos(t)ide analogues (NAs), including lamivudine, adefovir dipivoxil, telbivudine, entecavir and tenofovir disoproxil fumarate. IFNα is the most suitable for young patients with less advanced liver diseases and those infected with HBV genotype A. IFNα treatment significantly decreases the overall incidence of HBV-related HCC in sustained responders. However, side effects may limit its long-term clinical application. Orally administered NAs are typically implemented for patients with more advanced liver diseases. NA treatment significantly reduces disease progression of cirrhosis and therefore HCC incidence, especially in HBV e antigen-positive patients. NA-resistance due to the mutations in HBV polymerase is a major limiting factor. Of the NA resistance-associated mutants, A181T mutant significantly increases the risk of HCC development during the subsequent course of NA therapy. It is important to initiate treatment with NAs that have a high genetic barrier to resistance, to counsel patients on medication adherence and to monitor virological breakthroughs. The recommended treatment for patients with chronic HCV infection is peg-IFN plus ribavirin that can decrease the occurrence of HCC in those who achieve a sustained virological response and have not yet progressed to cirrhosis. IFN-based treatment is reserved for patients with decompensated cirrhosis who are under evaluation of liver transplantation to reduce post-transplant recurrence of HCV. More effective therapeutic options such as direct acting antiviral agents will hopefully increase the response rate in difficult-to-treat patients with HCV genotype 1. However, the risk of HCC remains in cirrhotic patients (both chronic HBV and HCV infection) if treatment is initiated after cirrhosis is established. Future research should focus on investigating new agents, especially for those patients with hepatic decompensation or post-transplantation.

Key words: Hepatitis B virus; Hepatitis C virus; Hepatocellular carcinoma; Antiviral therapy; Interferon; Nucleos(t)ide analogues; Virological response

Peer reviewer: Gualtiero Alvisi, PhD, Department of Infectious Diseases, Heidelberg University, INF345, Heidelberg 69121, Germany

Chen LP, Zhao J, Du Y, Han YF, Su T, Zhang HW, Cao GW. Antiviral treatment to prevent chronic hepatitis B or C-related hepatocellular carcinoma. *World J Virol* 2012; 1(6): 174-183 Available from: URL: http://www.wjgnet.com/2220-3249/full/v1/i6/174.htm DOI: http://dx.doi.org/10.5501/wjv.v1.i6.174

INTRODUCTION

Hepatocellular carcinoma (HCC), one of the most common and aggressive malignancies, is the third leading cause of cancer-related deaths worldwide[1]. Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) affect over 400 million and 170 million individuals respectively, together contributing to 75%-80% of global HCC[2]. The incidence of HCC has a wide geographical variation due to the heterogeneous penetration of the main causal factors within a given population[3]. In HCC-endemic areas, such as Asia and Africa, chronic HBV infection is the principal etiological factor. While in the West and Japan, chronic HCV infection plays a predominant role. Chronic HBV and HCV are progressive diseases; the dynamic process involving the interplay between the hepatitis viruses and host inflammatory factors contributes to the development of advanced liver diseases such as HCC. The most effective measure to avert HCC is to prevent HBV and HCV infection. Hepatitis B vaccination for newborns has led to a substantial reduction in the incidence of HCC in HBV endemic regions, while no vaccine is currently available for HCV. For individuals who are chronically infected with HBV or HCV, antiviral therapy is the only option for the prevention of HCC. In chronic hepatitis B or C patients without cirrhosis, antiviral therapy may prevent the occurrence of HCC by slowing the progression of liver diseases and possibly reversing liver damage[4]. In patients with advanced fibrosis or cirrhosis, eradication or oppression of HBV or HCV does not remove this risk, but can control the complications and gain the time to prepare for liver transplantation[5]. Postoperative antiviral therapy also improves the prognosis of HBV/HCV-related HCC[6,7]. Therefore, the treatment might be of greater benefit if patients are treated earlier and adhere to medications during the course of chronic HBV or HCV infection[8]. Several safe and effective medications have been approved. Decision to start or defer treatment should take into consideration the stage of liver disease, initial virus replication status, adverse effects, drug resistance and costs of the treatment. Moreover, the response should be closely monitored so that the treatment can be modified in a timely fashion.

ANTIVIRAL THERAPY AND PREVENTION OF HBV-RELATED HCC

The natural course of chronic HBV infection consists of 4 phases, namely immune tolerant, immune clearance, inactive (carrier) and reactivation phases. The immune tolerance phase [or hepatitis B e antigen (HBeAg)-positive chronic hepatitis B] is characterized by the presence of HBeAg, normal serum alanine aminotransferase (ALT) and high HBV DNA levels. Most patients in this phase have minimal liver injury and little or no fibrosis[9]. The immune clearance phase is characterized by the presence of HBeAg, high serum HBV DNA levels, persistent or intermittent elevation of ALT and active inflammation in the liver. During this phase, some patients undergo spontaneous HBeAg seroconversion which occurs at a rate of 10%-20% per year and others may experience recurrent hepatitis flares and even progress to cirrhosis or hepatic decompensation. The inactive (carrier) phase is characterized by the absence of HBeAg, presence of HBe antibody (anti-HBe), persistently normal ALT levels and low or undetectable levels of serum HBV DNA. Patients in this phase have a favorable prognosis[10]. The reactivation phase (or HBeAg-negative chronic hepatitis B) is characterized by the absence of HBeAg, presence of anti-HBe, intermittently or persistently elevated serum HBV DNA and ALT levels, and active inflammation in the liver. Patients in this phase are usually older and have more advanced liver disease than those in other phases[11]. However, patients vary regarding which phases they go through. This is largely influenced by the HBV genotype and host immune status. Previous studies have demonstrated that the presence of HBeAg and persistently high serum HBV DNA levels are risk factors for the occurrence of cirrhosis and HCC[12-14].

Moreover, chronic infection with HBV genotype C is more likely to cause liver cirrhosis than genotype B[15]. Chronic infection with genotype C (C2) is related to HBV-associated HCC, especially in cirrhotic patients aged > 50 years, whereas HBV B2 infection is related to high prevalence of HCC in non-cirrhotic young patients and HCC recurrence after resection[16]. In addition, precore or core promoter HBV variants that occur in most patients can prevent or decrease the production of HBeAg[11]. Different HBV subgenotypes have distinct patterns of mutations. We and others have found that serum HBV load (> 10^4 copies/mL) and viral mutations in the enhancer Ⅱ/basal core promoter (EnhⅡ/BCP) regions (such as C1653T, T1753V, A1762T/G1764A, T1674C/G and C1766T/T1768A) and in the precore/core gene (such as G1899A, C2002T, A2159G, A2189C and G2203A/T), as well as in the preS region (such as T53C, preS2 start codon mutation, preS1 deletion, C2964A, A2962G, C3116T and C7A) are significantly associated with the occurrence of HCC[17-22]. Reduction of $CD8^+$ T cell epitopes in HBV to evade immune clearance is one of the most common ways of these mutations. It remains to be evaluated if the HCC-associated HBV mutants are still sensitive to the antiviral treatment.

Clinical assessment of the treatment response relies on intermediate outcomes, including a decrease in levels of serum HBV DNA, HBeAg seroconversion, loss of HBsAg, normalization of ALT levels and a decrease in hepatic inflammation. The ultimate goal of the antiviral therapy is to obtain clinical benefits by reducing complications, including HCC. However, treatment choices mainly depend on the degree of viral replication and disease progression. Other factors, such as the patient's age, HBeAg status, family history of HCC, occupational requirement and need for immunosuppressive or cancer chemotherapy, could also influence the decision to start or defer antiviral treatment. In patients with life-threaten-

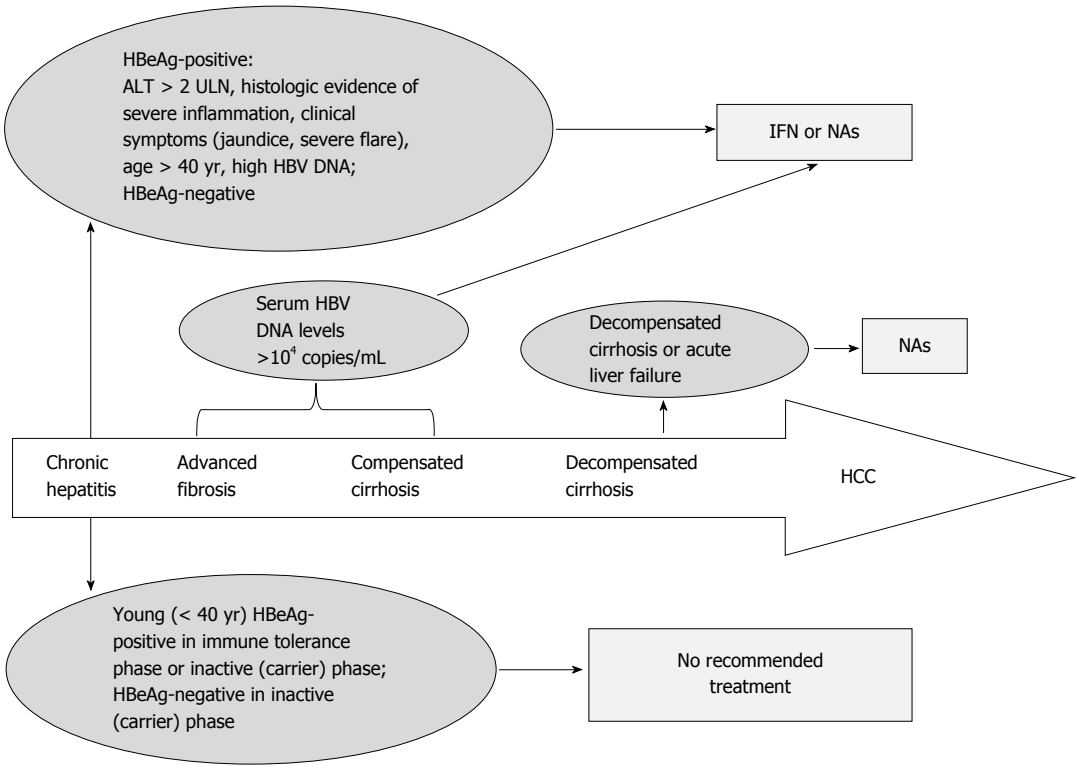

Figure 1 Flowchart of therapy choice for patients with chronic hepatitis B virus infection. ALT: Alanine aminotransferase; HBeAg: Hepatitis B e antigen; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; IFN: Interferon; NAs: Nucleos(t)ide analogues; ULN: Upper limit of normal.

ing liver diseases, such as acute liver failure, decompensated cirrhosis or severe exacerbation of chronic hepatitis B, antiviral treatment should be initiated as soon as possible in order to stabilize liver function and prepare for liver transplantation[23]. In patients with advanced fibrosis or compensated cirrhosis (those whose laboratory tests indicate normal hepatic function and no evidence of portal hypertension), antiviral treatment should be initiated when serum HBV DNA levels > 10^4 copies/mL because the risks of cirrhosis and HCC increase when serum HBV DNA reaches or exceeds this level[13,24]. HBeAg-positive chronic hepatitis patients who have ALT levels persistently twice the normal upper limit or with clinical and/or histological evidence of severe inflammation should be considered for treatment. Patients older than 40 years should be treated if HBeAg remains positive and the serum HBV DNA level is still high, regardless of ALT levels. Given that sustained spontaneous remission is rare, patients who have HBeAg-negative chronic hepatitis should also be considered for treatment. However, it is not recommended to initiate antiviral therapy for young HBeAg-positive patients (< 40 years) in the immune tolerance phase because most have little or no fibrosis and a favorable prognosis during follow-up of up to 10 years[25]. Another reason for deferring treatment is that antiviral treatment is less efficacious during this phase due to the low likelihood of treatment-related HBeAg seroconversion. Continued monitoring is necessary for timely initiation of treatment if patients fail to undergo spontaneous HBeAg seroconversion. Treatment may also be deferred in the inactive (carrier) phase because no evidence supports the hypothesis that antiviral therapy will alter the outcome of patients who are truly in this phase. However, the decision should only be made on the premise that patients have been observed for at least one year of HBV DNA and ALT levels tested on three or four occasions (Figure 1).

Currently, interferon-α (IFNα) and nucleos(t)ide analogues (NAs) are the two main categories of medications approved for the treatment of chronic hepatitis B. IFNα has an immunomodulatory activity that can lead to a higher rate of HBeAg seroconversion and HBsAg loss[12,13]. Treatment with IFNα may significantly decrease overall HCC incidence in sustained responders, especially in Asians[26-28]. IFNα may be used in carefully selected patients with compensated cirrhosis. It is most appropriate for young patients, particularly among HBeAg-positive patients who have a genotype A infection. However, it is contraindicated in patients with decompensated cirrhosis to avoid sepsis and liver failure. It is also not used in patients with severe exacerbations of chronic hepatitis B or acute liver failure, and in those undergoing immunosuppressive or cancer chemotherapy. The major advantages of IFNα-based treatment include short treatment duration and a sustained off-treatment response once achieved. However, low probability of achieving a response and high costs as well as side-effects may limit its long-time clinical use.

Treatment with NAs can also result in a significantly lower incidence of HCC compared to untreated patients

but it does not completely eliminate the risk of HCC, particularly in patients with pre-existing cirrhosis. Hence, patients with chronic hepatitis B require careful surveillance for HCC, even when they are undergoing antiviral therapy[29,30]. Another disadvantage of NA treatment is the long-term continuous treatment course, resulting in antiviral drug resistance[31]. The choice of treatment should consider the initial HBV-DNA levels, HBV genotype/subgenotype, age of the patient and any contraindication to NA treatment. NAs are most appropriate for patients who have decompensated liver diseases or contraindications to IFN, and those who are willing to commit to a long duration of treatment. The treatment should be initiated with NAs with a high genetic barrier to resistance (that is, a low potential for drug resistance).

IFNα

Two forms of IFNα are currently available: conventional IFNα and the pegylated, long-acting formulation (PEG-IFN). The introduction of PEG-IFN mainly impacts tolerability. It allows for weekly injections compared to the daily or three times/week schedules of conventional IFNα administration, while maintaining similar antiviral efficacy. Long-term follow-up of patients treated with conventional IFNα therapy shows that responders have a decreased incidence of decompensated cirrhosis or HCC and improved overall survival compared with non-responders[32]. A retrospective analysis of PEG-IFN in patients with HBeAg-positive hepatitis showed that factors associated with response to treatment included high ALT, low HBV DNA, female sex, older age and the absence of previous IFN therapy. Patients with the best outcomes were those with genotype A and high ALT or low HBV DNA, and those with genotypes B or C and both high ALT and low HBV DNA[33]. A recent meta-analysis of 14 trials suggested that PEG-IFN facilitated HBsAg clearance or seroconversion in chronic hepatitis B patients. According to an assessment conducted in 24 wk after completion of a 1 year course of PEG-IFN, approximately 30% of HBeAg-positive patients achieved HBeAg seroconversion and undetectable serum HBV DNA, and 15% of HBeAg-negative patients had normalized ALT levels[34]. Compared with the placebo, a 1 year course of PEG-IFN therapy resulted in a greater HBV DNA decline in HBeAg-seropositive patients (32% *vs* 11%)[35]. A large study evaluated long-term outcomes of IFNα therapy in HBeAg seropositive patients by comparing 233 IFNα-treated patients with 233 well-matched untreated controls and the cumulative incidences at the end of 15 years of follow-up (median 6.8 years, range 1.1-16.5 years) in the IFNα-treated patients *vs* the controls were: HBeAg seroconversion 74.6% *vs* 51.7% (P = 0.031); HBsAg seroclearance 3% *vs* 0.4% (P = 0.03); cirrhosis 17.8% *vs* 33.7% (P = 0.041); and HCC 2.7% *vs* 12.5% (P = 0.011)[26]. Another meta-analysis with a total of 2742 subjects pooled from 12 studies has shown that the risk of HCC in patients treated by IFNα is reduced by 34% (RR = 0.66, 95%CI: 0.48-0.89) and the benefit is more significant among patients with early cirrhosis than among those without cirrhosis[23]. These data indicated that the main advantages of IFN are durable administration course and a high rate of HBsAg loss and HBeAg seroconversion, particularly among HBeAg-positive patients who have a genotype A infection compared with patients who are infected with other HBV genotypes. However, the adverse effects of IFNα, including initial flu-like illness, fatigue, bone marrow suppression and exacerbation of autoimmune illnesses, should be closely monitored.

NAs

There are five NAs approved for the treatment of chronic hepatitis B: lamivudine, adefovir dipivoxil, telbivudine, entecavir and tenofovir disoproxil. We have summarized the pros and cons of each NA for the treatment of chronic HBV infection in Table 1.

In patients who do not respond to IFNα, an oral sequential therapy with NAs is preferable because of its predictable efficacy and minimal side-effects. It has been demonstrated that a 1 year course of NA treatment results in high rates of undetectable serum HBV DNA, normalization of ALT levels and increase in liver function, but low rates of HBsAg loss in chronic hepatitis B patients with either positive or negative HBeAg[36,37]. Extending the duration of NA treatment to more than 1 year can increase rates of HBeAg seroconversion to 40%-50%, but rates of HBsAg loss remain below 10% in patients with HBeAg-positive chronic hepatitis B patients after 5 years of treatment[38,39]. In a randomized controlled trial, 651 HBsAg-positive patients with compensated liver diseases were allocated into two groups: receiving 100 mg/d lamivudine (n = 436) or receiving placebo (n = 215). HCC occurred in 3.9% of those in the lamivudine group and 7.4% of those in the placebo group (HR = 0.49, 95%CI: 0.25-0.99, P = 0.047) after a median treatment duration of 32.4 mo (ranging from 0 to 42 mo)[40]. A meta-analysis pooling 5 studies (n = 2289) compared the incidence of HCC in patients with and without NA treatment. It found that the incidence of HCC was reduced by 78% (RR = 0.22, 95%CI: 0.10-0.50) in the treatment arm, especially in HBeAg-positive patients[27]. Similarly, a recent meta analysis pooling 3881 patients with NA treatment and 534 untreated controls from 21 studies has concluded that NA treatment is associated with a lower incidence of HCC (2.8% *vs* 6.4%, P = 0.003)[29].

Current approved NAs act primarily by inhibiting the reverse transcription of the pregenomic HBV RNA to the first strand of HBV DNA rather than directly inhibiting cccDNA. Therefore, viral relapse is common after treatment. In addition, inadequate or slow decline in serum HBV DNA levels during the first 12-24 wk of NA treatment is associated with an increased risk of antiviral drug resistance during continued therapy. Patients receiving NAs with a low genetic barrier to resistance, such as lamivudine and telbivudine, should receive additional therapy if initial viral decline is inadequate, while patients

Table 1 Pros and cons of each nucleos(t)ide analogue therapy for the treatment of chronic hepatitis B infection

Nucleos(t)ide analogue	Regimen	Pros	Cons
Lamivudine	100 mg daily	First licensed agent Well established safety and efficacy record Lowest cost	Highest incidence of resistant mutations of M204V/I substitution (20% at year 1, 70% at year 5) Adverse effects including hepatitis flare ups, hepatic decompensation and even death
Adefovir dipivoxil	10 mg daily	Low drug resistance rate, and no cross resistance with other nucleos(t)ide analogs	Incidence of resistant mutations of N236T and/or A181V substitution (29% at year 5) Adverse effects including renal tubular acidosis with hypophosphataemia when treatment is prolonged
Telbivudine	600 mg daily	Higher seroconversion rate	Incidence of resistant mutations of M204I mutation (5% at year 1) Adverse effects including myopathy and neuropathy
Entecavir	1.0 mg daily	Anti-HBV effect Lowest rate of resistance	Incidence of resistant mutations of T184G or M250V (1.2% at year 5) (I169T and M250V, or T184G and S202I if also lamivudine-resistant) Most expensive
Tenofovir disoproxil	300 mg daily	More potent in reducing HBV load in patients with prior failure or resistance to lamivudine and/or adefovir	No resistant mutations reported at year 3

HBV: Hepatitis B virus.

who receive NAs that have a high genetic barrier to resistance, including entecavir and tenofovir disoproxil, may remain on the same drug if serum HBV DNA levels continue to decline[41,42].

Lamivudine 100 mg/d is the first approved NA to treat chronic hepatitis B and has been extensively used for more than a decade with an excellent safety record. Lamivudine treatment can reduce disease progression of HBV-related cirrhosis, resulting in approximately a 50% decrease in HCC incidence. Such efficacy is achieved despite emergence of drug resistance in approximately 50% of cases[28]. Long-term therapy with lamivudine leads to viral breakthrough in some patients, owing to the emergence of viral mutation harboring a M204V or I substitution in the YMDD motif[10]. M204V/I is the most frequently encountered lamivudine-resistant mutant. L180M mutation usually concurrently occurs with M204V mutation. Another mutation, A181T, exists in a substantial proportion of lamivudine-resistant patients. More importantly, the emergence of A181T mutant significantly increases the risk of HCC development in lamivudine-resistant patients during the subsequent course of antiviral therapy[43,44]. The rate of lamivudine resistance is 24% after 1 year and approximately 70% after 5 years[45]. Furthermore, lamivudine-resistance causes the attenuation of HBV suppression, hepatitis flare ups, hepatic decompensation and even death, thereby posing a serious clinical challenge[46]. Because of the overlap between the S and polymerase genes of HBV, a great proportion of patients carrying A181T mutation also possess sW172* nonsense mutation, resulting in truncation of the pre-S/S reading frames. This partially explains why chronic hepatitis B patients who fail to NA treatment at the late stage are more likely to develop HCC compared to those who respond to the treatment. Despite this, lamivudine is still widely used in several countries, mostly because of its low cost. Treatment of patients with lamivudine-resistance includes the addition of adefovir dipivoxil or tenofovir disoproxil and entecavir rescue treatment. A daily dose of 10 mg of adefovir dipivoxil is not an ideal first-line NA therapy because of its low potency. A proportion (20%-50%) of patients fails to achieve even a 10^2-fold reduction in serum HBV DNA. Although adefovir dipivoxil has been widely used in lamivudine-resistant HBV infection, up to 25% of patients fail to achieve a satisfactory response and 30% of naïve patients develop adefovir dipivoxil resistance in 5 years[47]. Furthermore, adefovir mutations harboring a N236T and/or A181V substitution emerge more frequently in lamivudine-resistant patients than in treatment-naïve patients[48-50]. Entecavir rescue monotherapy can be adopted as a treatment option for patients with resistance to both lamivudine and adefovir dipivoxil[51,52]. A clinical trial studying the long-term efficacy of entecavir therapy with 146 patients has shown that among patients with up to 5 years of continuous entecavir 0.5 or 1.0 mg therapy, 94% resulted in HBV DNA reduction to < 300 copies/mL and 80% achieve normalization of ALT levels, while the HBeAg seroconversion and decrease in HBsAg rates are only 23% and 1.4%, respectively[39]. Entecavir monotherapy may be efficacious in adefovir dipivoxil-refractory chronic hepatitis B patients with prior lamivudine-resistance if these patients have an early virological response to the monotherapy at 12 wk. Entecavir-resistance is rare in treatment-naïve patients, even with long-term therapy, but the cumulative probability of genotypic entecavir resistance with a combination of substitutions I169T and M250V, or T184G and S202I, in lamivudine-resistant patients increases up to 51% after 5 years of treatment[41,53]. A recent meta-analysis has demonstrated that a combination therapy with lamivudine and adefovir dipivoxil is more effective and produces long-lasting effects than switching to entecavir monotherapy in treating chronic hepatitis B patients with lamivudine resistance. However, taking into account the

practical benefits and the limitations of adefovir dipivoxil, individualized therapy will be needed in patients with a prior history of lamivudine-resistant infections[54]. Entecavir rescue therapy for 96 wk is less efficacious in patients with lamivudine/adefovir dipivoxil-refractory HBV, particularly in those who have an initial HBV DNA of > 10^7 copies/mL. Patients who achieve a HBV DNA level of < 10^4 copies/mL and a normalized ALT level should continue, rather than stop, entecavir therapy[55]. Telbivudine is as potent as entecavir. The therapeutic response to telbivudine is superior to that of lamivudine in HBeAg-positive and HBeAg-negative patients. In HBeAg-positive patients, telbivudine has better outcomes compared to lamivudine in terms of nondetectable viremia, HBeAg loss and viral resistance[56]. Resistance to telbivudine is associated with a signature M204I mutation in viral polymerase[37,57]. Tenofovir disoproxil appears to be safe and effective in patients with prior resistance to lamivudine and adefovir dipivoxil and becomes the optimal choice of antiviral treatment[58,59]. The cost-effectiveness of switching to tenofovir disoproxil or adding tenofovir disoproxil to ongoing lamivudine in lamivudine-resistance patients is still debatable. Adefovir dipivoxil-resistant mutants are usually susceptible to lamivudine, telbivudine or entecavir and they may also be sensitive to tenofovir disoproxil, depending on the mutation pattern, while telbivudine or the rare entecavir resistance strains are usually sensitive to adefovir dipivoxil and tenofovir disoproxil[43,60]. Despite their high initial potency, 1 year of therapy with NAs does not usually lead to sustained off-therapy responses and therefore treatments usually last for several years or even longer. Nowadays, entecavir and tenofovir disoproxil are recommended as first-line treatments because of their higher potency and lower risk of resistance compared to lamivudine, adefovir or telbivudine[43,44]. Although it is difficult to compare entecavir and tenofovir disoproxil since no comparison studies have been conducted, tenofovir disoproxil monotherapy appears to be superior to entecavir monotherapy in multidrug-resistant HBV.

ANTIVIRAL THERAPY AND PREVENTION OF HCV-RELATED HCC

Cirrhosis is the strongest risk factor for HCC among patients with chronic HCV infection. Antiviral therapy to inhibit and even eradicate HCV can result in decreasing hepatic necroinflammation and, over time, causes reversal of fibrosis and eventually decreases the risk of HCC[61]. The standard of care in patients with chronic hepatitis C consists of a 24 to 48 wk course of PEG-IFNα2a or PEG-IFNα2b in combination with the guanosin analog ribavirin. This therapy leads to a sustained virological response (SVR) of 42%-52%, 65%-85% and 76%-82% of those infected with HCV genotype 1, HCV genotypes 4, 5 or 6, and HCV genotypes 2 or 3, respectively[62,63]. A clinical trial following-up 150 patients for 5 years has shown that the clinical, virological, biochemical and histological outcomes of patients with SVR are favorable and recovery of normal or nearly normal liver architecture is possible[64]. The standard of care can decrease the risk of HCC, although the effect is predominantly evident in patients who achieve SVR and in those who have not yet progressed to cirrhosis[65,66]. However, several studies have demonstrated that, for patients with advanced fibrosis or cirrhosis, the risk of developing HCC remains even if a SVR is achieved, highlighting the importance of continued surveillance in these patients[67,68]. A recent meta-analysis pooled data from 20 studies (4700 patients with HCV-related cirrhosis) and compared untreated patients with those given IFNα alone or combined with ribavirin treatment and it showed a reduced risk of HCC in the treatment group (RR = 0.43, 95%CI: 0.33-0.56). Another meta-analysis using data from 14 studies (n = 3310) indicated that patients achieving a SVR had a lower incidence of HCC (RR = 0.35, 95%CI: 0.26-0.46) compared with nonresponders and the maximum benefits were observed in those treated with ribavirin-based regimens (RR=0.25, 95%CI: 0.14-0.46)[65]. Due to the potential anti-tumoral, anti-angiogenic and anti-fibrotic roles of IFNα, maintaining IFNα therapy might decrease the risk of HCC in patients who fail to achieve SVR. However, several large-scale randomized controlled trials with long (3-4 years) and extended (up to 5 years) follow-up time have shown that low-dose PEG-IFN in patients with advanced fibrosis or cirrhosis have minimal or even no benefit on overall clinical outcomes[69-71]. Although the treatment of HCV chronic infection with the standard of care therapy can eradicate HCV in 40%-90% of patients, approximately 10%-15% of patients have to discontinue the treatment due to adverse effects. The adverse effects, ranging from mild to moderate in severity, impact most organ systems and can cause serious and even life-threatening toxicity, such as psychological disturbances, poor appetite, skin rash, infection, anemia and leukopenia[72]. Accordingly, patients should be closely monitored for adverse effects during treatment.

Although the standard of care therapy will probably continue for some years, more effective therapeutic options with shorter treatment durations are being introduced to increase the response rate in difficult-to-treat patients (mainly infected with genotype 1) and reduce the impact of HCV infection and related complications. So far, intensive efforts have been made to develop different compounds that specifically target the replication cycle of the virus. These direct-acting antiviral agents (DAAs) act by directly inhibiting the NS3/4A serine protease (which processes the HCV polyprotein to generate mature viral proteins), the NS5B polymerase (which replicates the viral RNA genome) and the NS5A phosphoprotein (which functions as a part of the replicase complex)[73-76]. The new standard of care for patients with chronic hepatitis C is to add nonstructural (NS) 3/4A protease inhibitors boceprevir or telaprevir to the Peg-IFNα plus ribavirin regimen. The recent sixty-first annual meeting of the American Association for the Study of Liver Diseases (AASLD) provided an overview of the pipeline of these

novel drugs. Numerous other protease inhibitors, as well as nucleoside and non-nucleoside inhibitors of the RNA-dependent RNA NS5B polymerase and inhibitors of the NS5A protein, are also under evaluation currently. These can achieve higher SVR rates in previously untreated patients infected with HCV genotype 1 and provide successful medical care for those who have failed treatment under current standard of care[77]. In a recent phase Ⅱ study, 465 chronic hepatitis C patients with poor response to PEG-IFNα plus ribavirin therapy were allocated to the triple therapy group and the control group. It was found that the triple therapy significantly increased SVR rates in these difficult-to-treat patients compared with the controls (53% *vs* 14%)[78]. Remarkably, in a group of HCV patients treated for 24 wk with this triple therapy followed by another 24 wk of PEG-IFNα2b plus ribavirin, the SVR rates for patients who had previously relapsed was 76% and up to 39% for those who did not response to the standard therapy. Another report of a phase Ⅱ study indicated that adding boceprevir to the standard treatment leads to a notably increased SVR (75% *vs* 38%) in treatment-naive patients who were infected with HCV genotype 1[79]. The combination of three agents, PEG-IFNα, ribavirin and HCV protease inhibitor, is able to increase SVR rates substantially. The potency and safety of the two first generation HCV protease inhibitors are also confirmed in large phase Ⅲ studies. In the treatment-naive patients infected with HCV genotype 1, the SVR rates are 75% with the addition of telaprevir *vs* 44% with the standard therapy and 68% with the addition of boceprevir *vs* 40% with the standard therapy. Although the upcoming triple therapy regimens including telaprevir or boceprevir may be different, this strategy means that in treatment-naive patients, treatment duration will be reduced to 24 or 28 wk for the patients with a rapid viral response, those with a negative result of serum HCV RNA after 4 wk of exposure to an HCV protease inhibitor. In addition, the single nucleotide polymorphisms around the gene encoding interleukin 28B (*IL28B*) have been identified as key predictive factors[80]. In treatment-naïve HCV-1 patients treated with PEG-IFN and ribavirin, among host and viral factors associated with SVR, combination of *IL28B* genotypes and rapid viral response monitoring seems to provide a high predictive value of treatment outcome, particularly in the context of emerging therapies and DDAs[81]. Furthermore, a study combining a nucleoside polymerase inhibitor (RG7128) and danoprevir led to an average of 5.1 log reduction of plasma HCV RNA levels within 14 d[82]. Overall, this treatment is not only an important step towards an IFN-free regimen, but also reflects the high genetic barrier to resistance associated with nucleoside polymerase inhibitors. Co-administration of different classes of DAAs, combined with or without PEG-IFNα and/or ribavirin, might make HCV RNA suppression possible in most individuals who are infected with HCV genotype 1, including those who are not responsive to PEG-IFNα[73]. However, it is still unclear whether the novel agents will be useful in the most difficult-to-treat patients, such as those with advanced or decompensated liver diseases or after liver transplantation. Furthermore, DAAs also need to be developed for other HCV genotypes. Therefore, characterizing resistance to DAAs and the combination of antiviral agents with different resistance profiles in clinical trials are the best strategies to prevent the emergence of drug-resistant mutants and thereby maximize SVR rate.

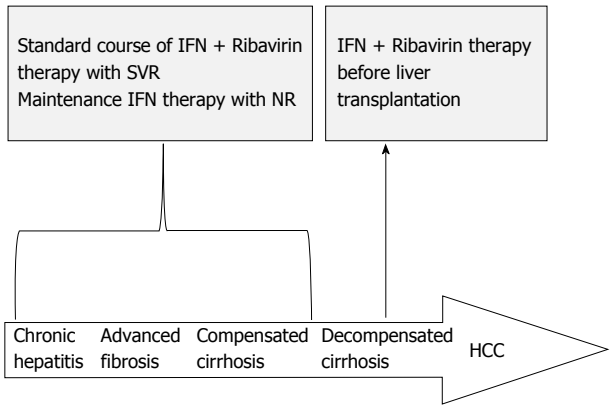

Figure 2 Flowchart of therapy choice for patients with chronic hepatitis C virus infection. HCC: Hepatocellular carcinoma; IFN: Interferon; NR: No response; SVR: Sustained virological response.

However, given the adverse effects, it remains uncertain whether patients with decompensated cirrhosis could tolerate HCV eradication treatment and therefore should be treated to prevent progression of decompensation. The AASLD recommends those patients to be referred for consideration of liver transplantation[83]. Eradication of HCV before liver transplantation could reduce post-transplant recurrence of HCV, especially in patients infected with HCV genotypes other than genotype 1[84]. Thus, treatment should be reserved for those patients awaiting liver transplantation. It is recommended that patients with decompensated cirrhosis initiate treatment at a low dose of IFN-based therapy. However, the treatment should be administered with caution since it is still unclear whether the novel agents will be effective in those most difficult-to-treat patients[79,85]. In addition, use of hematological growth factors can improve the life quality of treated patients and manage treatment-induced cytopenias (Figure 2).

CONCLUSION

In summary, antiviral treatment of chronic hepatitis B or chronic hepatitis C is so far the only option to prevent HCC. Treatment of chronic hepatitis B with IFNα may significantly decrease overall incidence of HCC in sustained responders, while the adverse effects may limit its long-term application. Orally administered NAs significantly reduce disease progression of liver cirrhosis, resulting in up to a 78% decrease in HCC incidence, especially in HBeAg-positive patients. In patients with life-threatening liver diseases, antiviral treatment should be initiated

as soon as possible in order to stabilize liver function and prepare for liver transplantation. Long-term continuous treatment with NAs results in antiviral drug resistance due to the mutations in HBV polymerase. Of the NA resistance-associated mutants, A181T mutant significantly increases the risk of HCC in lamivudine-resistant patients during the subsequent courses of antiviral therapy. In addition, it remains to be explored if the HCC-associated HBV mutants, whose emergence is likely to be selected by virus-host interaction during carcinogenesis, are sensitive to the antiviral therapy.

The recommended treatment for patients with chronic HCV infection is PEG-IFN plus ribavirin which can decrease HCC incidence in those who achieve SVR and have not yet progressed to cirrhosis. Patients with decompensated cirrhosis are under evaluation of liver transplantation, because achievement of a SVR is possible in these patients but does not forestall the disease progression. IFN and ribavirin therapy, therefore, should be reserved in these patients to prevent post-transplant recurrence of HCV. More effective therapeutic options such as DDAs are promising in increasing the response rate of difficult-to-treat patients with HCV genotype 1.

There is a great need to develop safer, more effective and affordable antiviral therapies. To optimize treatment responses, appropriate therapy should be initiated at the proper time. Patients must be educated about the importance of treatment compliance. The response to antiviral treatment should be closely monitored so that the therapy can be modified when the initial one fails. HCC risk remains in cirrhotic patients (both HBV and HCV infection) if treatment is initiated after cirrhosis is established and close monitoring is needed. Future research should focus on investigating the use of new agents, especially for patients with hepatic decompensation or after transplantation.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90
2 **Yang JD**, Roberts LR. Hepatocellular carcinoma: A global view. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 448-458
3 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917
4 **Lok AS**. Does antiviral therapy for hepatitis B and C prevent hepatocellular carcinoma? *J Gastroenterol Hepatol* 2011; **26**: 221-227
5 **Fink SA**, Jacobson IM. Managing patients with hepatitis-B-related or hepatitis-C-related decompensated cirrhosis. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 285-295
6 **Chan AC**, Chok KS, Yuen WK, Chan SC, Poon RT, Lo CM, Fan ST. Impact of antiviral therapy on the survival of patients after major hepatectomy for hepatitis B virus-related hepatocellular carcinoma. *Arch Surg* 2011; **146**: 675-681
7 **Miao RY**, Zhao HT, Yang HY, Mao YL, Lu X, Zhao Y, Liu CN, Zhong SX, Sang XT, Huang JF. Postoperative adjuvant antiviral therapy for hepatitis B/C virus-related hepatocellular carcinoma: a meta-analysis. *World J Gastroenterol* 2010; **16**: 2931-2942
8 **Chotiyaputta W**, Peterson C, Ditah FA, Goodwin D, Lok AS. Persistence and adherence to nucleos(t)ide analogue treatment for chronic hepatitis B. *J Hepatol* 2011; **54**: 12-18
9 **Andreani T**, Serfaty L, Mohand D, Dernaika S, Wendum D, Chazouillères O, Poupon R. Chronic hepatitis B virus carriers in the immunotolerant phase of infection: histologic findings and outcome. *Clin Gastroenterol Hepatol* 2007; **5**: 636-641
10 **Manno M**, Cammà C, Schepis F, Bassi F, Gelmini R, Giannini F, Miselli F, Grottola A, Ferretti I, Vecchi C, De Palma M, Villa E. Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years. *Gastroenterology* 2004; **127**: 756-763
11 **Chotiyaputta W**, Lok AS. Hepatitis B virus variants. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 453-462
12 **Yang HI**, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174
13 **Chen CJ**, Yang HI, Su J, Jen CL, You SL, Lu SN, Huang GT, Iloeje UH. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65-73
14 **Wu CF**, Yu MW, Lin CL, Liu CJ, Shih WL, Tsai KS, Chen CJ. Long-term tracking of hepatitis B viral load and the relationship with risk for hepatocellular carcinoma in men. *Carcinogenesis* 2008; **29**: 106-112
15 **Yin JH**, Zhao J, Zhang HW, Xie JX, Li WP, Xu GZ, Shen J, Dong HJ, Zhang J, Wang L, Han JK, Wang HY, Cao GW. HBV genotype C is independently associated with cirrhosis in community-based population. *World J Gastroenterol* 2010; **16**: 379-383
16 **Yin J**, Zhang H, Li C, Gao C, He Y, Zhai Y, Zhang P, Xu L, Tan X, Chen J, Cheng S, Schaefer S, Cao G. Role of hepatitis B virus genotype mixture, subgenotypes C2 and B2 on hepatocellular carcinoma: compared with chronic hepatitis B and asymptomatic carrier state in the same area. *Carcinogenesis* 2008; **29**: 1685-1691
17 **Yin J**, Xie J, Liu S, Zhang H, Han L, Lu W, Shen Q, Xu G, Dong H, Shen J, Zhang J, Han J, Wang L, Liu Y, Wang F, Zhao J, Zhang Q, Ni W, Wang H, Cao G. Association between the various mutations in viral core promoter region to different stages of hepatitis B, ranging of asymptomatic carrier state to hepatocellular carcinoma. *Am J Gastroenterol* 2011; **106**: 81-92
18 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082
19 **Yin J**, Xie J, Zhang H, Shen Q, Han L, Lu W, Han Y, Li C, Ni W, Wang H, Cao G. Significant association of different preS mutations with hepatitis B-related cirrhosis or hepatocellular carcinoma. *J Gastroenterol* 2010; **45**: 1063-1071
20 **Zhu Y**, Jin Y, Guo X, Bai X, Chen T, Wang J, Qian G, Groopman JD, Gu J, Li J, Tu H. Comparison study on the complete sequence of hepatitis B virus identifies new mutations in core gene associated with hepatocellular carcinoma. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2623-2630
21 **Xie JX**, Zhao J, Yin JH, Zhang Q, Pu R, Lu WY, Zhang HW, Wang HY, Cao GW. Association of novel mutations and haplotypes in the preS region of hepatitis B virus with hepatocellular carcinoma. *Front Med China* 2010; **4**: 419-429
22 **Liu S**, Xie J, Yin J, Zhang H, Zhang Q, Pu R, Li C, Ni W, Wang H, Cao G. A matched case-control study of hepatitis B virus mutations in the preS and core promoter regions associated independently with hepatocellular carcinoma. *J Med Virol* 2011; **83**: 45-53
23 **Fontana RJ**, Hann HW, Perrillo RP, Vierling JM, Wright T, Rakela J, Anschuetz G, Davis R, Gardner SD, Brown NA. Determinants of early mortality in patients with decompensated chronic hepatitis B treated with antiviral therapy. *Gastroenterology* 2002; **123**: 719-727
24 **Iloeje UH**, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predict-

ing cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130**: 678-686
25 **Hui CK**, Leung N, Yuen ST, Zhang HY, Leung KW, Lu L, Cheung SK, Wong WM, Lau GK. Natural history and disease progression in Chinese chronic hepatitis B patients in immune-tolerant phase. *Hepatology* 2007; **46**: 395-401
26 **Lin SM**, Yu ML, Lee CM, Chien RN, Sheen IS, Chu CM, Liaw YF. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. *J Hepatol* 2007; **46**: 45-52
27 **Sung JJ**, Tsoi KK, Wong VW, Li KC, Chan HL. Meta-analysis: Treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2008; **28**: 1067-1077
28 **Lim SG**, Mohammed R, Yuen MF, Kao JH. Prevention of hepatocellular carcinoma in hepatitis B virus infection. *J Gastroenterol Hepatol* 2009; **24**: 1352-1357
29 **Papatheodoridis GV**, Lampertico P, Manolakopoulos S, Lok A. Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t)ide therapy: a systematic review. *J Hepatol* 2010; **53**: 348-356
30 **Thursz M**, Brown A. Can antiviral therapy of chronic hepatitis B prevent the development of hepatocellular carcinoma? *Gut* 2011; **60**: 1025-1026
31 **Sonneveld MJ**, Janssen HL. Chronic hepatitis B: peginterferon or nucleos(t)ide analogues? *Liver Int* 2011; **31** Suppl 1: 78-84
32 **van Zonneveld M**, Honkoop P, Hansen BE, Niesters HG, Darwish Murad S, de Man RA, Schalm SW, Janssen HL. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. *Hepatology* 2004; **39**: 804-810
33 **Buster EH**, Hansen BE, Lau GK, Piratvisuth T, Zeuzem S, Steyerberg EW, Janssen HL. Factors that predict response of patients with hepatitis B e antigen-positive chronic hepatitis B to peginterferon-alfa. *Gastroenterology* 2009; **137**: 2002-2009
34 **Li WC**, Wang MR, Kong LB, Ren WG, Zhang YG, Nan YM. Peginterferon alpha-based therapy for chronic hepatitis B focusing on HBsAg clearance or seroconversion: a meta-analysis of controlled clinical trials. *BMC Infect Dis* 2011; **11**: 165
35 **Hansen BE**, Rijckborst V, Ter Borg MJ, Janssen HL. HBV DNA suppression in HBeAg-positive chronic hepatitis B patients treated with peginterferon or placebo. *J Med Virol* 2011; **83**: 1917-1923
36 **Leung N**. Treatment of HBeAg-positive chronic hepatitis B with nucleos(t)ide analogues. *Liver Int* 2011; **31** Suppl 1: 85-89
37 **Papatheodoridis GV**. Treatment of HBeAg-negative chronic hepatitis B patients with nucleos(t)ide analogues. *Liver Int* 2011; **31** Suppl 1: 95-103
38 **Marcellin P**, Chang TT, Lim SG, Sievert W, Tong M, Arterburn S, Borroto-Esoda K, Frederick D, Rousseau F. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2008; **48**: 750-758
39 **Chang TT**, Lai CL, Kew Yoon S, Lee SS, Coelho HS, Carrilho FJ, Poordad F, Halota W, Horsmans Y, Tsai N, Zhang H, Tenney DJ, Tamez R, Iloeje U. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2010; **51**: 422-430
40 **Liaw YF**, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; **351**: 1521-1531
41 **Tenney DJ**, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, Wichroski MJ, Xu D, Yang J, Wilber RB, Colonno RJ. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009; **49**: 1503-1514
42 **Snow-Lampart A**, Chappell B, Curtis M, Zhu Y, Myrick F, Schawalder J, Kitrinos K, Svarovskaia ES, Miller MD, Sorbel J, Heathcote J, Marcellin P, Borroto-Esoda K. No resistance to tenofovir disoproxil fumarate detected after up to 144 weeks of therapy in patients monoinfected with chronic hepatitis B virus. *Hepatology* 2011; **53**: 763-773
43 **Papatheodoridis GV**, Deutsch M. Resistance issues in treating chronic hepatitis B. *Future Microbiol* 2008; **3**: 525-538
44 **European Association For The Study Of The Liver**. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227-242
45 **Lai CL**, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, Brown N, Woessner M, Boehme R, Condreay L. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. *Clin Infect Dis* 2003; **36**: 687-696
46 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-539
47 **Hadziyannis SJ**, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, Marcellin P, Lim SG, Goodman Z, Ma J, Brosgart CL, Borroto-Esoda K, Arterburn S, Chuck SL. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. *Gastroenterology* 2006; **131**: 1743-1751
48 **Yeon JE**, Yoo W, Hong SP, Chang YJ, Yu SK, Kim JH, Seo YS, Chung HJ, Moon MS, Kim SO, Byun KS, Lee CH. Resistance to adefovir dipivoxil in lamivudine resistant chronic hepatitis B patients treated with adefovir dipivoxil. *Gut* 2006; **55**: 1488-1495
49 **Schildgen O**, Sirma H, Funk A, Olotu C, Wend UC, Hartmann H, Helm M, Rockstroh JK, Willems WR, Will H, Gerlich WH. Variant of hepatitis B virus with primary resistance to adefovir. *N Engl J Med* 2006; **354**: 1807-1812
50 **Lee YS**, Suh DJ, Lim YS, Jung SW, Kim KM, Lee HC, Chung YH, Lee YS, Yoo W, Kim SO. Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. *Hepatology* 2006; **43**: 1385-1391
51 **Sherman M**, Yurdaydin C, Simsek H, Silva M, Liaw YF, Rustgi VK, Sette H, Tsai N, Tenney DJ, Vaughan J, Kreter B, Hindes R. Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. *Hepatology* 2008; **48**: 99-108
52 **Suzuki F**, Toyoda J, Katano Y, Sata M, Moriyama M, Imazeki F, Kage M, Seriu T, Omata M, Kumada H. Efficacy and safety of entecavir in lamivudine-refractory patients with chronic hepatitis B: randomized controlled trial in Japanese patients. *J Gastroenterol Hepatol* 2008; **23**: 1320-1326
53 **Kay A**, Zoulim F. Hepatitis B virus genetic variability and evolution. *Virus Res* 2007; **127**: 164-176
54 **Sheng YJ**, Liu JY, Tong SW, Hu HD, Zhang DZ, Hu P, Ren H. Lamivudine plus adefovir combination therapy versus entecavir monotherapy for lamivudine-resistant chronic hepatitis B: a systematic review and meta-analysis. *Virol J* 2011; **8**: 393
55 **Kwak MS**, Choi JW, Lee JS, Kim KA, Suh JH, Cho YS, Won SY, Park BK, Lee CK. Long-term efficacy of entecavir therapy in chronic hepatitis B patients with antiviral resistance to lamivudine and adefovir. *J Viral Hepat* 2011; **18**: e432-e438
56 **Liaw YF**, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL, Heathcote EJ, Manns M, Bzowej N, Niu J, Han SH, Hwang SG, Cakaloglu Y, Tong MJ, Papatheodoridis G, Chen Y, Brown NA, Albanis E, Galil K, Naoumov NV. 2-Year GLOBE trial results: telbivudine Is superior to lamivudine in patients with chronic hepatitis B. *Gastroenterology* 2009; **136**: 486-495
57 **Tillmann HL**, McHutchison JG. Telbivudine versus lamivudine in patients with chronic hepatitis B. *N Engl J Med* 2008; **358**: 1517; author reply 1517-1518
58 **Patterson SJ**, George J, Strasser SI, Lee AU, Sievert W, Nicoll AJ, Desmond PV, Roberts SK, Locarnini S, Bowden S, Angus

PW. Tenofovir disoproxil fumarate rescue therapy following failure of both lamivudine and adefovir dipivoxil in chronic hepatitis B. *Gut* 2011; **60**: 247-254
59 **van Bömmel F**, de Man RA, Wedemeyer H, Deterding K, Petersen J, Buggisch P, Erhardt A, Hüppe D, Stein K, Trojan J, Sarrazin C, Böcher WO, Spengler U, Wasmuth HE, Reinders JG, Möller B, Rhode P, Feucht HH, Wiedenmann B, Berg T. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. *Hepatology* 2010; **51**: 73-80
60 **Locarnini S**, Mason WS. Cellular and virological mechanisms of HBV drug resistance. *J Hepatol* 2006; **44**: 422-431
61 **Cammà C**, Di Bona D, Schepis F, Heathcote EJ, Zeuzem S, Pockros PJ, Marcellin P, Balart L, Alberti A, Craxì A. Effect of peginterferon alfa-2a on liver histology in chronic hepatitis C: a meta-analysis of individual patient data. *Hepatology* 2004; **39**: 333-342
62 **Hoofnagle JH**, Seeff LB. Peginterferon and ribavirin for chronic hepatitis C. *N Engl J Med* 2006; **355**: 2444-2451
63 **Antaki N**, Craxi A, Kamal S, Moucari R, Van der Merwe S, Haffar S, Gadano A, Zein N, Lai CL, Pawlotsky JM, Heathcote EJ, Dusheiko G, Marcellin P. The neglected hepatitis C virus genotypes 4, 5 and 6: an international consensus report. *Liver Int* 2010; **30**: 342-355
64 **George SL**, Bacon BR, Brunt EM, Mihindukulasuriya KL, Hoffmann J, Di Bisceglie AM. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; **49**: 729-738
65 **Singal AK**, Singh A, Jaganmohan S, Guturu P, Mummadi R, Kuo YF, Sood GK. Antiviral therapy reduces risk of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis. *Clin Gastroenterol Hepatol* 2010; **8**: 192-199
66 **Craxì A**, Cammà C. Prevention of hepatocellular carcinoma. *Clin Liver Dis* 2005; **9**: 329-346, viii
67 **Bruno S**, Stroffolini T, Colombo M, Bollani S, Benvegnù L, Mazzella G, Ascione A, Santantonio T, Piccinino F, Andreone P, Mangia A, Gaeta GB, Persico M, Fagiuoli S, Almasio PL. Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; **45**: 579-587
68 **Morgan TR**, Ghany MG, Kim HY, Snow KK, Shiffman ML, De Santo JL, Lee WM, Di Bisceglie AM, Bonkovsky HL, Dienstag JL, Morishima C, Lindsay KL, Lok AS. Outcome of sustained virological responders with histologically advanced chronic hepatitis C. *Hepatology* 2010; **52**: 833-844
69 **Di Bisceglie AM**, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, Lok AS, Bonkovsky HL, Morgan TR, Ghany MG, Morishima C, Snow KK, Dienstag JL. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008; **359**: 2429-2441
70 **Lok AS**, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Goodman ZD. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; **136**: 138-148
71 **Lok AS**, Everhart JE, Wright EC, Di Bisceglie AM, Kim HY, Sterling RK, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, Morgan TR. Maintenance peginterferon therapy and other factors associated with hepatocellular carcinoma in patients with advanced hepatitis C. *Gastroenterology* 2011; **140**: 840-849; quiz e12
72 **Sulkowski MS**, Cooper C, Hunyady B, Jia J, Ogurtsov P, Peck-Radosavljevic M, Shiffman ML, Yurdaydin C, Dalgard O. Management of adverse effects of Peg-IFN and ribavirin therapy for hepatitis C. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 212-223
73 **Hofmann WP**, Zeuzem S. A new standard of care for the treatment of chronic HCV infection. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 257-264
74 **Melnikova I**. Hepatitis C--pipeline update. *Nat Rev Drug Discov* 2011; **10**: 93-94
75 **Bruno R**, Cima S, Maiocchi L, Sacchi P. Forthcoming challenges in the management of direct-acting antiviral agents (DAAs) for hepatitis C. *Dig Liver Dis* 2011; **43**: 337-344
76 **Ciesek S**, Manns MP. Hepatitis in 2010: the dawn of a new era in HCV therapy. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 69-71
77 **Manns MP**, Foster GR, Rockstroh JK, Zeuzem S, Zoulim F, Houghton M. The way forward in HCV treatment--finding the right path. *Nat Rev Drug Discov* 2007; **6**: 991-1000
78 **McHutchison JG**, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, Zeuzem S, Reesink HW, Garg J, Bsharat M, George S, Kauffman RS, Adda N, Di Bisceglie AM. Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010; **362**: 1292-1303
79 **Kwo PY**, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, Galati JS, Gordon SC, Ravendhran N, Rossaro L, Anderson FH, Jacobson IM, Rubin R, Koury K, Pedicone LD, Brass CA, Chaudhri E, Albrecht JK. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376**: 705-716
80 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401
81 **Thompson AJ**, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterology* 2010; **139**: 120-9.e18
82 **Gane EJ**, Roberts SK, Stedman CA, Angus PW, Ritchie B, Elston R, Ipe D, Morcos PN, Baher L, Najera I, Chu T, Lopatin U, Berrey MM, Bradford W, Laughlin M, Shulman NS, Smith PF. Oral combination therapy with a nucleoside polymerase inhibitor (RG7128) and danoprevir for chronic hepatitis C genotype 1 infection (INFORM-1): a randomised, double-blind, placebo-controlled, dose-escalation trial. *Lancet* 2010; **376**: 1467-1475
83 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374
84 **Everson GT**, Trotter J, Forman L, Kugelmas M, Halprin A, Fey B, Ray C. Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. *Hepatology* 2005; **42**: 255-262
85 **Sarrazin C**, Rouzier R, Wagner F, Forestier N, Larrey D, Gupta SK, Hussain M, Shah A, Cutler D, Zhang J, Zeuzem S. SCH 503034, a novel hepatitis C virus protease inhibitor, plus pegylated interferon alpha-2b for genotype 1 nonresponders. *Gastroenterology* 2007; **132**: 1270-1278

S- Editor Wang JL **L- Editor** Roemmele A **E- Editor** Zheng XM

World Journal of Virology

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

World J Virology 2012 December 12; 1(6): I
ISSN 2220-3249 (online)

Acknowledgments to reviewers of *World Journal of Virology*

We acknowledge our sincere thanks to our reviewers. Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of our World Series Journals. Both the editors of the journals and authors of the manuscripts submitted to the journals are grateful to the following reviewers for reviewing the articles (either published or rejected) over the past period of time.

Alexander A Bukreyev, PhD, Professor, Galveston National Laboratory, Center for Biodefense and Emerging Infectious Diseases, Department of Pathology, 301 University Boulevard, University of Texas Medical Branch, Galveston, TX 77555-0609, United States

Anda Baicus, MD, PhD, Lecturer, Senior Scientific Researcher, Head of the Enteric Viral infections Laboratory, Cantacuzino National Institute of Research-Development for Microbiology and Immunology, Spl. Independentei 103, Sect.5, 050096, Bucharest, Romania

Eiichi N Kodama, MD, PhD, Assistant Professor, Department of Internal Medicine, Division of Emerging Infectious Diseases, Tohoku University School of Medicine, Building 1 Room 515, 2-1 Seiryo, Aoba-ku, Sendai 980-8575, Japan

Gualtiero Alvisi, PhD, Department of Infectious Diseases, Heidelberg University, INF345, Heidelberg 69121, Germany

Hailong Guo, DVM, PhD, David H. Smith Center for Vaccine Biology and Immunology, University of Rochester Medical Center , 601 Elmwood Avenue, Box 609, Rochester , NY 14642, United States

Hsin-Wei Chen, Associate Investigator, Vaccine Research and Development Center, National Health Research Institutes, No. 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, China

Ian M Mackay, PhD, Qpid Laboratory, Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Herston Road, Herston Q4029, Australia

Jayanta Bhattacharya, PhD, Scientist D, Department of Molecular Virology, National AIDS Research Institute, 73G, MIDC, Bhosari, Pune 411026, India

José A Melero, PhD, Director, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, 28220 Madrid, Spain

Luis Menéndez-Arias, Professor, Centro de Biología Molecular "Severo Ochoa", c/Nicolás Cabrera, 1, Campus de Cantoblanco, 28049 Madrid, Spain

Majid Laassri, PhD, Staff Scientist, Office of Vaccines Research and Review, CBER, FDA, 1401 Rockville Pike, HFM-470, Rockville, MD 20852-1448, United States

Maurizia Debiaggi, PhD, Department of Morphological Sciences, Microbiology Unit, University of Pavia, via Brambilla 74, 27100 Pavia, Italy

Pablo Daniel Ghiringhelli, PhD, Director Ligbcm-Avi, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Saenz Peña 352, B1876BXD Bernal, Argentina

Parin Chaivisuthangkura, PhD, Associate Professor, Department of Biology, Faculty of Science, Srinakharinwirot University, Sukhumvit 23, Wattana, Bangkok 10110, Thailand

Roberto Manfredi, MD, Associate Professor of Infectious Diseases, University of Bologna, c/o Infectious Diseases, S. Orsola Hospital, Via Massarenti 11, I-40138 Bologna, Italy

Preeti Bharaj, PhD, Diabetes and Obesity, Sanford-Burnham MRI, 6400 Sanger Road, Orlando, FL 32827, United States

Samia Ahmed Kamal, PhD, Virology Department, Animal Health Research Institute, Nadi Al- Said street, Beside Ministry of Agriculture, Dokki, Cairo, Egypt

Seyed Mojtaba Ghiasi, Assistant Professor, Arboviruses and Viral Hemorrhagic Fevers Laboratory, Pasteur Institute of Iran, No. 69, 12 Farvardin St, Pasteur Ave, Pasteur Sq, Tehran 1316943551, Iran

Sibnarayan Datta, PhD, Scientist, Biotechnology Division, Defence Research Laboratory, Post Bag no.2, Tezpur, Assam 784 001, India

World Journal of Virology

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

World J Virol 2012 December 12; 1(6): I
ISSN 2220-3249 (online)

Events Calendar 2012

January 13-14, 2012
Innovation in Severe Acute Respiratory Infections
Sitges, Spain

January 20, 2012
Exploiting Bacteriophages for Bioscience, Biotechnology and Medicine
Welwyn Garden City,
United Kingdom

January 20-22, 2012
International Science Symposium on HIV and Infectious Diseases
Chennai, India

February 26-29, 2012
10th American Society for Microbiology Biodefense and Emerging Diseases Research Meeting
Washington, DC, United States

March 11-15, 2012
8th International Symposium on Pneumococci and Pneumococcal Diseases
Foz de Iguaçu City, Brazil

March 12-16, 2012
Vaccine World Summit India
Hyderabad, India

March 14-17, 2012
22nd Annual Meeting of the German Society for Virology
Essen, Germany

March 21-26, 2012
2012 HIV Vaccines
Keystone, CO, United States

March 21-26, 2012
Viral Immunity and Host Gene Influence
Keystone, CO, United States

March 26-31, 2012
Cell Biology of Virus Entry, Replication and Pathogenesis
Whistler, BC, Canada

March 26-31, 2012
Frontiers in HIV Pathogenesis, Therapy and Eradication
Whistler, BC, Canada

April 20-21, 2012
2012 Molecular Virology Workshop
Daytona Beach, FL, United States

April 22-25, 2012
2012 28th Clinical Virology Symposium
Daytona Beach, FL, United States

April 24-27, 2012
European Molecular Biology Organization Workshop - Antigen presentation and processing
Amsterdam, Netherlands

May 19-22, 2012
European Molecular Biology Organization and European Molecular Biology Laboratory Symposium - New perspectives on immunity to infection
Heidelberg, Germany

June 11-16, 2012
Antiviral RNAi: From Molecular Biology Towards Applications
Pultusk, Poland

June 28-29, 2012
7th International Workshop on Hepatitis C - Resistance and New Compounds
Cambridge, MA, United States

July 16-20, 2012
European Molecular Biology Organization Conference Series - Viruses of microbes: From exploration to applications in the -omics era
Brussels, Belgium

July 19–20, 2012
7th International Workshop on HIV Transmission - Principles of Intervention
Washington, DC, United States

July 21-25, 2012
31st Annual Meeting of American Society for Virology
Madison, WI, United States

July 30 - August 1, 2012
3rd Annual Symposia of Hepatitis Virus
Guangzhou, China

August 20-22, 2012
2nd World Congress on Virology
Las Vegas, NV, United States

September 7-9, 2012
Viral Hepatitis Congress 2012
The Johann Wolfgang Goethe University, Frankfurt, Germany

October 18-20, 2012
2nd World Congress on Controversies in the Management of Viral Hepatitis (C-Hep)
Berlin, Germany

November 28 -December 1, 2012
6th Asian Congress of Paediatric Infectious Diseases
Colombo, Sri Lanka

World Journal of Virology

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

World J Virol 2012 December 12; 1(6): I-V
ISSN 2220-3249 (online)

GENERAL INFORMATION

World Journal of Virology (*World J Virol*, *WJV*, online ISSN 2220-3249, DOI: 10.5501) is a bimonthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 137 experts in virology from 41 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJV* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJV* is an OA journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJV* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

WJV aims to report rapidly new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of virology. *WJV* covers topics concerning arboviral infections, bronchiolitis, central nervous system viral diseases, DNA virus infections, encephalitis, eye infections, fatigue syndrome, hepatitis, meningitis, opportunistic infections, pneumonia, RNA virus infections, sexually transmitted diseases, skin diseases, slow virus diseases, tumor virus infections, viremia, zoonoses, and virology-related traditional medicine, and integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of virology-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs.

Columns

The columns in the issues of *WJV* will include: (1) Editorial: To introduce and comment on the substantial advance and its importance in the fast-developing areas; (2) Frontier: To review the most representative achievements and comment on the current research status in the important fields, and propose directions for the future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (6) Review: To systemically review the most representative progress and unsolved problems in the major scientific disciplines, comment on the current research status, and make suggestions on the future work; (7) Original Articles: To originally report the innovative and valuable findings in virology; (8) Brief Articles: To briefly report the novel and innovative findings in virology; (9) Case Report: To report a rare or typical case; (10) Letters to the Editor: To discuss and make reply to the contributions published in *WJV*, or to introduce and comment on a controversial issue of general interest; (11) Book Reviews: To introduce and comment on quality monographs of virology; and (12) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on the research in virology.

Name of journal

World Journal of Virology

ISSN

ISSN 2220-3249 (online)

Editor-in-Chief

Ling Lu, MD, PhD, Department of Pathology and Laboratory

Medicine, University of Kansas Medical Center, Kansas City, 3901 Rainbow Blvd, WHE 3020, KS 66160, United States

Editorial office
World Journal of Virology
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjv@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-85381891
Fax: +86-10-85381893

Indexed and abstracted in
Digital Object Identifier

Published by
Baishideng Publishing Group Co., Limited

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, *WJV* 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/2220-3249/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjv@wjgnet.com, or by telephone: +86-10-85381891. 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: +86-10-85381891 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJV*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no less than 256 words) and structured abstracts (no less than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no less than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no less than 140 words); RESULTS (no less than 294 words): 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, $P < 0.001$; 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.

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. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/2220-3249/g_info_20100725072755.htm.

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. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. 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 printed and 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. $^{a}P < 0.05$, $^{b}P < 0.01$ 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 pres-

sure, 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/2220-3249/g_info_20100725073806.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

Editorial: http://www.wjgnet.com/2220-3249/g_info_20100725071851.htm

Frontier: http://www.wjgnet.com/2220-3249/g_info_20100725071932.htm

Topic highlight: http://www.wjgnet.com/2220-3249/g_info_20100725072121.htm

Observation: http://www.wjgnet.com/2220-3249/g_info_20100725072232.htm

Guidelines for basic research: http://www.wjgnet.com/2220-3249/g_info_20100725072344.htm

Guidelines for clinical practice: http://www.wjgnet.com/2220-3249/g_info_20100725072543.htm

Review: http://www.wjgnet.com/2220-3249/g_info_20100725072656.htm

Original articles: http://www.wjgnet.com/2220-3249/g_info_20100725072755.htm

Brief articles: http://www.wjgnet.com/2220-3249/g_info_20100725072920.htm

Case report: http://www.wjgnet.com/2220-3249/g_info_20100725073015.htm

Letters to the editor: http://www.wjgnet.com/2220-3249/g_info_20100725073136.htm

Book reviews: http://www.wjgnet.com/2220-3249/g_info_20100725073214.htm

Guidelines: http://www.wjgnet.com/2220-3249/g_info_20100725073300.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

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 B 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 or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2220-3249/g_info_20100725073726.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/2220-3249/g_info_20100725073445.htm.

Proof of financial support

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

Links to documents related to the manuscript

WJV 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.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

WJV 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, 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: 600 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.