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AASLD/NIAID NIH Corner

Antiviral Therapy Against Hepatitis Viruses: Understanding and Managing Drug Resistance

Sunday, November 4, 2007
John B. Hynes Convention Center, Ballroom B

Course Directors:
Rajen Koshy, PhD and Anna S. F. Lok, MD

Target Audience

This course is intended for hepatologists and virologists with an interest in antiviral therapy of chronic hepatitis B and C, the diagnosis of antiviral resistance, and the management of patients with resistant virus infection.

Description

This symposium, presented in collaboration with the American Association for the Study of Liver Diseases (AASLD) and the U.S. National Institute of Allergy and Infectious Diseases (NIAID), an Institute of the National Institutes of Health (NIH), will address issues important to physicians/health care providers who treat patients with chronic hepatitis, and investigators conducting research on antiviral resistance. A panel of experts (hepatologists, infectious disease specialists, and virologists) will discuss basic and clinical issues including standardization of nomenclature and assays relating to antiviral resistance, detection and monitoring of antiviral resistance, and management of patients with antiviral-resistant HBV. The panel will also identify research gaps and provide directions for future research.

Goals and Objectives

- To clarify the nomenclature of antiviral-resistant HBV, clinical criteria for diagnosis of antiviral resistance, and assays used to detect antiviral-resistant mutations.
- To discuss the monitoring of HBV infected patients receiving antiviral therapy for antiviral resistance, and the management of patients with antiviral-resistant HBV.
- To discuss these issues in the context of new drugs for the treatment of hepatitis C virus infection
- To provide an interactive forum for researchers and NIH to discuss future research initiatives and international collaboration.

Continuing Medical Education

The American Association for the Study of Liver Diseases (AASLD) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

The AASLD designates this educational activity for a maximum of 3 *AMA PRA Category 1 Credits*[™]. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Disclosure of Faculty and NIH Liaison Committee Relationships

It is the policy of the American Association for the Study of Liver Diseases (AASLD) that program audiences for all AASLD sponsored, co-sponsored, and jointly-sponsored activities be informed, prior to their participation, of all relevant financial relationships or other relationships within the last 12 months, of all faculty and activity planning committee members. AASLD has identified and resolved all conflicts of interest prior to program implementation.

In addition, faculty is asked to make a reasonable effort to identify for the participant any discussion of off-label or investigative use or application of a product or device that may occur during the educational presentation.

Speakers and NIH Liaison Committee members provided the following information (* Denotes NIH Liaison Committee members)

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Nothing to disclose

Di Bisceglie, Adrian M., MD *

Nothing to disclose

Diehl, Anna Mae, MD *

Grant/Research: GlaxoSmithKline, Axcan Pharma

Doo, Edward, MD

Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Ghany, Marc, MD

Nothing to disclose

Content of presentation does include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Koshy, Rajen, PhD

Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Kuiken, Carla, PhD

Nothing to disclose

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

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Consultant/Advisor: Evivar Pty Ltd, Gilead, Pharmasset, BMS

Major Stockholder: Pharmasset - Other: Melbourne Health, BMS

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

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Nothing to disclose

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Consultant/Advisor: Gilead, Idenix, Novartis, Innogenetics, Abbott

Speakers' Bureau: Gilead, BMS, Idenix, Novartis, Abbott

Content of presentation does not include discussion of off-label/investigative use of medicine(s), medical devices or procedure(s)

Antiviral Therapy Against Hepatitis Viruses: Understanding and Managing Drug Resistance

Session 1:	Chairs: Rajen Koshy, PhD and Jean-Michel Pawlotsky MD
1:00 – 1:15pm	NIAID Goals in Viral Hepatitis Research Rajen Koshy, PhD
1:15 – 1:30pm	NIDDK Funding of Viral Hepatitis Research Edward Doo, MD
1:30 – 1:45pm	Standardized Nomenclature and Management of Antiviral-resistant HBV Marc Ghany, MD
1:45 – 2:00pm	Genotypic and Phenotypic Assays to Detect Antiviral-resistant Mutations Fabien Zoulim, MD
2:00 – 2:15pm	Break
Session 2:	Chairs: Jake Liang, MD and Masashi Mizokami, MD
2:15 – 2:30pm	Antiviral Resistance to New HCV Treatments John G. McHutchison, MD
2:30 – 2:50 pm	Panel Discussion: The Management of Patients with Antiviral Resistance
2:50 – 3:05 pm	Database for Antiviral Resistance Monitoring Stephen Locarnini, MD, PhD
3:05 – 3.30pm	NIH HCV and HIV Database Carla Kuiken, PhD
3:20 – 4:00pm	Panel Discussion: The Monitoring of Antiviral Resistance, Databases, Collaborations, and Funding Jean-Michel Pawlotsky, Jake Liang, Jay Hoofnagle, Fabien Zoulim, Masashi Mizokami, Carla Kuiken, John G. McHutchison, Yun-Fan Liaw, Rajen Koshy Moderators: Anna S.F. Lok and Stephen Locarnini

NIAID Goals in Viral Hepatitis Research

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NIAID Goals in Viral Hepatitis Research

The mission of the NIAID is to conduct and support basic and applied research to better understand, treat, and ultimately prevent infectious, immunologic, and allergic diseases. The hepatitis viruses, which cause both acute and chronic liver infections, are associated with a number of serious and fatal diseases in developed as well as developing countries. Approximately 8-10 % of the world's population is chronically infected with hepatitis B and C and thereby at high risk for the development of cirrhosis, liver failure and primary liver cancer; coinfections with HCV and HBV in HIV infected patients considerably exacerbate liver disease. Hepatitis E virus infections, which are endemic in most of the developing world, are associated with significant mortality in pregnant women and there are concerns of its potential zoonotic spread. Hepatitis A virus, involved in recent outbreaks of infection in the US, is listed as a category B priority pathogen, as part of the NIAID biodefense research agenda. Hence, the study of viral hepatitis is of great concern to the NIAID.

The NIAID has long fostered multidisciplinary research which covers the range from epidemiology to molecular virology and immunology. The NIAID is particularly interested in understanding the immunological relationship between virus and host in order to accelerate the development of immune therapies and vaccines for hepatitis B and C viruses. NIAID has established Cooperative Research Centers for the study of hepatitis C; these specialized Centers facilitate vital collaborations between clinicians, virologist and immunologists, both within the Centers and in the wider research community. A major strength of these Centers is their access to various clinically well characterized patient cohorts, critical to validating the clinical relevance of observations in experimental in vitro systems and animal models. There have recently been spectacular advances in developing much needed research tools for the study of HCV and, consequently, in a greater understanding of HCV biology. Much of this has come from investigators in these Centers.

The NIAID has recently established partnerships with companies to develop vaccines for HCV using novel and promising platforms. For candidate vaccines already in late development, the NIAID provides possibilities for early phase clinical trials; various critical resources may be provided in NIAID-supported trials, including protocol development, monitoring, and data analysis and management.

The NIAID also focuses on antiviral drug development, through grants to small businesses. Contract facilities are available for in vitro screening of compounds in drug discovery programs against HBV and HCV, and to test selected compounds in suitable animal models – woodchuck and transgenic mouse models for HBV, and the newly developed Scid-Alb-UpA mouse model for HCV. Additional resources include genome and immunology databases for HCV.

Through these several and diverse programs, the NIAID maintains a very strong commitment towards the amelioration of the burden of viral hepatitis.

NIDDK Funding of Viral Hepatitis Research

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Viral hepatitis remains a significant public health issue with approximately 2.7 million and 1.25 million persons in the United States with hepatitis C and hepatitis B, respectively. Investigative support for viral hepatitis research has recently been coordinated across the various Institutes and Centers of the National Institutes of Health through the Liver Disease Subcommittee of the Digestive Diseases Interagency Coordinating Committee. The first charge of the Liver Disease Subcommittee was the publication of the Trans-NIH Action Plan for Liver Disease Research which highlighted the current status of liver disease research into sixteen chapters. The significance of viral hepatitis is highlighted with a chapter devoted to the liver disease caused by these viruses. Furthermore, the viral hepatitis chapter identified current research challenges as well as several research priorities that would significantly advance the field.

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) supports an array of conferences and investigative activities for viral hepatitis research and offers a range of funding mechanisms for investigator initiated research proposals. Current and recently supported clinical studies include the ViraHep-C, HALT-C, Peds-C, and the HBV-OLT studies. Additionally, other large clinical research consortia supported by the NIDDK with significant viral hepatitis components include the A2ALL and the SynCH hepatitis C studies. This fall, the NIDDK intends to release a Request for Applications for the establishment of the Hepatitis B Clinical Research Network in order to address several research issues and priorities delineated in the Trans-NIH Action Plan for Liver Disease Research as well as from the recently held Management of Chronic HBV:2006 meeting. In October 20-22, 2008, through the auspices of the Office of Medical Applications of Research and the NIDDK, a consensus conference on the management of hepatitis B will be held in the Washington, DC area.

Although the current budgetary climate has constrained support, the proportion of funded viral hepatitis grants remains a reflection of the general liver grant application pool. The majority of Research Project Grants to the NIDDK seeking R01 and R21 support for proposals are investigator initiated and not in response to a specific Funding Opportunity Announcement (Program Announcement). Although the NIDDK supports a considerable proportion of viral hepatitis research activities, several other institutes and centers also have significant viral hepatitis research grant portfolios illustrating the coordinated efforts of the NIH community through the Trans-NIH Action Plan for Liver Disease Research.

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9. Peds-C web site: <http://www.peds-c.org/>
10. A2ALL web site: <http://www.nih-a2all.org/contact.asp>
11. SyNCH web site: <http://www.synchtrials.org/>

Standardized Nomenclature and Management of Antiviral-Resistant HBV

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Management of chronic hepatitis B (CHB) has improved significantly in the last decade due to the availability of nucleos(t)ide analogues (NAs). In comparison to interferons, these agents are orally administered, safe, well tolerated and very effective at suppressing HBV DNA replication. However, sustained virological suppression is not maintained after withdrawal of NAs and long-term, indefinite therapy is usually required.¹⁻⁵ Unfortunately, long-term use of NAs is associated with the development of antiviral drug resistance. Emergence of drug resistance is usually associated with loss of clinical response, hepatitis flares and even death.^{6,7} Prevention and management of antiviral resistance is one of the biggest challenges facing practitioners who care for patients with CHB. Therefore, a workshop was held at the National Institutes of Health and a working group convened, with one of the goals being to standardize the nomenclature of antiviral resistance, which would allow for comparison of results among different trials and lead to progress in the management of antiviral resistance.^{8,9}

Resistance is typically classified as *primary*, *secondary* and *clinical* and as *genotypic* or *phenotypic*.⁹ *Primary antiviral treatment failure* or non-response refers to the inability of the antiviral agent to decrease HBV DNA by $\geq 1 \log_{10}$ IU/ml after the first 6 months of therapy. Primary treatment failure is important because it may be associated with a higher rate of developing antiviral resistance. *Secondary antiviral treatment failure* or *virological breakthrough* is defined as a $\geq 1 \log_{10}$ IU/ml increase in serum HBV DNA level from a nadir in two consecutive samples 1 month apart in a patient who has responded and has been compliant with antiviral medications.¹⁰ *Clinical or biochemical breakthrough* refers to an elevation in serum aminotransferase level during treatment in a patient who has achieved initial normalization.

Genotypic antiviral resistance refers to the substitution of one or more nucleotides in the target (HBV polymerase gene) of the antiviral agent that has been previously demonstrated to be associated with virological breakthrough. *Primary drug resistant mutations* cause an amino acid change that leads to conformational changes in the target, which results in reduced susceptibility to the antiviral agent, while *secondary* or *compensatory mutations* are ones that restore functional defects in the enzyme caused by the primary mutation. Secondary mutations generally do not arise in the absence of primary mutations and do not lead to antiviral resistance on their own. Confirmation of genotypic resistance is based upon *in vitro phenotypic analysis*. *In vitro phenotype testing* is based on the determination of changes to the effective concentration of the drug required to inhibit 50% of the target (HBV polymerase) (IC_{50}) relative to the "wild-type" reference HBV.

All patients receiving NA therapy should be monitored for the emergence of anti-viral resistance. Measurement of serum HBV DNA is the best marker to follow and a reasonable frequency is every 3 months. If non-compliance is excluded, testing for known antiviral resistance mutations should be performed at the time of virological breakthrough.

Development of antiviral resistance is related to the pre-treatment serum HBVDNA level, pre-existing viral mutations, immune status of the host, potency and genetic barrier to resistance. Of the currently approved agents, rates of resistance are highest with lamivudine (~70% at 5 years) and lowest with entecavir (<1% at 4 years) in NA-naïve patients. Management of antiviral resistance depends on the prior history of anti-viral therapy and the virologic response to these treatments, the pattern of mutations detected at time of virologic breakthrough and the cross-resistance profile of the various NAs against the resistance HBV isolate. The optimal management of anti-viral resistance has not been determined and remains under investigation. Three approaches are available¹¹: 1) Continue the anti-viral agent if HBV DNA levels are low, serum ALT is normal and there is no underlying cirrhosis or immunosuppression; 2) Immediately switch or add another anti-viral agent if viral rebound or hepatitis flare is present and in all patients with cirrhosis or immunosuppression and; 3) withdraw therapy if the patient did not warrant therapy initially such as an inactive carrier.

In general, agents from the same class have similar resistance profiles and therefore, sequential use should be avoided.¹² Recent data suggest that changing therapy when virologic breakthrough is first detected is more effective than delaying therapy until HBV DNA levels rebound or biochemical breakthrough occurs.¹³ Additionally, emerging data advocates adding therapy rather than switching therapy as lower rates of resistance have been reported with this approach and sequential monotherapy has been associated with multi-drug resistant HBV.^{14, 15} Ultimately, the goal should be to prevent the development of antiviral resistance by judicious prescription of antiviral therapy, reinforcing the need for compliance with the prescribed regimen, and to avoid sequential monotherapy, which may lead to the development of multi-drug resistant HBV. Future goals should be to develop more potent agents with better resistance profiles and drugs that target different aspects of the HBV lifecycle.

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Standardized Nomenclature and Management of Antiviral-Resistant HBV

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Genotypic and Phenotypic Assays to Detect Antiviral Resistant HBV Mutants

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HBV resistance to antivirals is becoming a major clinical issue because of the increasing number of patients being treated and exposed to the risk of selection of drug resistant mutants. However, more and more drugs are available, as well as better monitoring tools, that allow the early detection of treatment failure and an early treatment adaptation. Besides HBV DNA quantification assays, genotypic and phenotypic assays provide important clinical information on the cause of treatment failure, the nature of the resistant mutants and the choice of drug to adapt patients' treatment.

Because of their nature, these assays can be seen either as clinical tools or as important research tools to generate relevant information for treatment recommendation in case of treatment failure.

Genotypic assays rely on HBV genome sequence analysis in the viral polymerase domain, the main target of nucleoside analogs. Two types of assays can be used in the clinic: sequencing of the viral polymerase using in-house or commercial assays and line probe assays.

The first type of assay provides the potential advantage of detecting any new mutation, but the methodology remains tedious. When using in-house assays, caution should be taken regarding standardization of procedures and the potential pitfalls in terms of sensitivity, viral genome domain analyzed etc... Direct sequencing of PCR products is believed to be not very sensitive in terms of the minimal viral load allowing the detection of the mutations, and in terms of the capacity to detect minor mutants within the viral quasi-species.

Specific probe assays are easier to handle, but they can detect only known mutations for which specific probes have been designed. However, they are believed to be more sensitive than direct sequencing with a lower limit of detection and they can detect minor mutants in the viral population .

The use of one or the other assays depends on the local facility of each site and on the specific goal of the search : 1) in clinical practice both assays can be used ; 2) for clinical research purposes, if the goal is to detect known mutations both assays can be used, but when looking for new mutations, sequencing is mandatory.

Cloning of the viral genome and the sequencing of clones allows the study of the viral dynamics in vivo and provides indirect information on the viral fitness of the mutants and their role in treatment failure. Other assays relying on the DNA chip technology are being developed. They rely on a re-sequencing approach. They have the potential to be a very powerful tool as the whole viral genome can be analyzed in one set of experiments, providing information not only on the viral polymerase gene but also on other clinically relevant regions, i.e. the pre-core region and viral genotypes. Other assays have been reported for the quantitative detection of

specific mutants by specific real time PCR. However these assays face the general problem of setting up experimental conditions for each new mutation described.

Several **phenotypic assays** have been developed in the past 5 years to study the replication capacity and drug susceptibility of mutants selected during therapy. The first assays were based on polymerase gene fragment exchange or on site directed mutagenesis in HBV expression vectors. More recent assays have taken into account the fact that mutations outside of the domain of interest may be involved in drug resistance, for instance by restoring replication capacity to the primary resistance mutation (compensatory resistance mutation). These assays rely either on the cloning of the whole HBV genome followed by its transfection into hepatoma cell lines. Other assays rely on the cloning of the whole viral polymerase gene as a subgenome. Other assays are based on the amplification of the whole HBV genome and its transfection without a cloning step. All these assays have advantages and pitfalls. The cloning procedure is tedious but it provides permanent material for the study. The assays based on transfection without cloning are easier to handle but the viral genome material needs to be amplified for each experiment and may be lost over time. All these are in-house assays that are carried out in specialized research laboratories and can provide very relevant information on the cross-resistance profile of antivirals and help in providing treatment recommendations for patients who are in treatment failure. In view of clinical applications, there is a need for standardization regarding the minimal viral load required for the analysis, the type of cells used for antiviral activity assessment, the methods for measurement of viral replication, the cut-off for drug resistance etc...In any case, these assays should be part of the assessment of each new mutant arising during treatment with new drugs or during the course of new antiviral regimens (add-on or combination strategy).

Novel research assays have to be developed to study of the **fitness of drug resistant mutants**. This has been widely studied in the HIV field, but due to difficulties associated with the study models of HBV infection, little information is available for HBV mutants. These assays will be critical to gain more insight on the mechanism of selection of resistant mutants within the viral quasi-species. Indeed, the current phenotypic assays can only address the question of the replication capacity and drug susceptibility of these mutants. Their infectivity and capacity to spread in hepatocytes in the presence of antivirals (i.e. viral fitness) should be analyzed in detail to open new avenues in the development of treatment strategies to prevent the selection of the most fit mutants.

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Antiviral Resistance to New HCV Treatments

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The current standard of care for patients with chronic hepatitis C, combination therapy with pegylated interferon (IFN) alpha and ribavirin, is effective in approximately 80% of patients with hepatitis C virus (HCV) genotype 2 or 3 infection, but less than 50% of those with HCV genotype 1. As such, the majority of infected patients worldwide do not achieve the long term benefits associated with sustained, long term viral eradication. Additionally, therapy with pegylated IFN and ribavirin is prolonged, costly, and may be associated with adverse effects that are difficult for many patients to tolerate. Thus, more effective, more tolerable and/or more tailored therapies are required.

Basic molecular and virologic insights into the HCV have unraveled multiple targets for potential novel therapeutic agents. Unlike interferon and ribavirin, many of these classes of compounds are specifically targeted to HCV. Specifically Targeted Antiviral Therapy for Hepatitis C (STAT-C) has the theoretical potential to be effective in many more patients than current standard of care. Many drugs are in the preclinical developmental stage and several are in clinical developmental, a number of which are now also being tested in combination with pegylated IFN alpha with or without ribavirin.

Compounds targeting the HCV specific protease and polymerase enzymes, both essential for viral replication, are the most clinically relevant and furthest in the clinical trial development process to date. The high viral burden in patients with chronic HCV infection and the rapid turnover of the virus also promote the creation of a low fidelity system where a high likelihood of amino acid substitutions spontaneously occur frequently in any infected individual patient.

A number of protease and polymerase inhibitors have now advanced to Phase II clinical trials. To date, resistance profiles have been established for these drugs to varying degrees. In general terms, certain statements can be made: (1) short term monotherapy studies indicate the development of resistance with viral breakthrough after initial viral suppression (2) trough plasma drug concentrations correlate with the degree of viral suppression (3) resistance profiles tend to revert to the wild type after withdrawal of these drugs and over periods of weeks to months (4) there appears to be cross resistance between drugs acting on similar targets (5) resistance seems to be abrogated by the addition of peginterferon and ribavirin, due to the additive combined effects of greater viral load suppression by the addition of multiple drugs working through different mechanisms of action (6) the replicative fitness of these variants seems to be diminished, but more work is needed in this area (7) The sensitivity of each variant to a particular drug varies (8) double resistant variants are in general less sensitive to a specific anti-viral agent (9) the long term clinical effects of these variants are unknown at this time.

Further work characterizing the clinical phenotype associated with exposure to these new potent anti-viral agents, alone and in combination; along with determining the efficacy and side effect profiles and developing standardized nomenclature and testing strategies to describe and characterize resistance are now clearly important mandates. These strategies will be necessary if we are to develop these therapies effectively and to translate their use into the clinical practice setting most efficiently.

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Databases for Antiviral Resistance Monitoring

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Introduction

Antiviral resistance is emerging as the single most important factor in treatment failure using nucleos(t)ide analogues (NA). Since antiviral resistance mutations (both primary and secondary) selected under one agent may affect the efficacy of subsequent agents, database approaches allow the assimilation, correlation and integration of viral sequence data, *in vitro* antiviral drug sensitivity and relevant clinical information collected over time^{1,2}. Similar databases/search methods have been developed in HIV³⁻⁷.

Mutations associated with antiviral drug resistance to the NAs lamivudine, adefovir, telbivudine, tenofovir and entecavir have been identified for hepatitis B virus (HBV) using the SeqHepB system. SeqHepB is composed of a HBV genome sequence analysis program and a relational database which can then be used to correlate large numbers of patient clinical, routine pathology diagnostic data, viral mutational sequence information, and *in vitro* antiviral sensitivity and cross-resistance phenotypic data in an integrated and structured way for subsequent patient monitoring.

SeqHepB

The SeqHepB database currently contains routine pathology and specialised virology data for 1,921 patients, and is one of the largest database for HBV in the world. Associated with these patients, there are 340 clinical histories, 1,863 treatment histories, 189 biopsy results, and 23,812 specimen records. In terms of routine pathology tests performed on the samples, there are 25,596 records in the database, and these include HBV, hepatitis C virus (HCV), and hepatitis D virus (HDV) related pathology test results, as well as routine liver function and haematology test results. Samples within the database are also associated with 3,973 HBV genomic sequence information corresponding to 124,754 nucleotide or amino acid variation data points. The mutation data is correlated to an extensive data set of in-house as well as published *in vitro* phenotypic data on HBV antiviral drug sensitivity and resistance⁸⁻¹⁰.

Current Activities

The correlation of clinical, pathological and viral molecular biological data using different artificial intelligence techniques is facilitating the analysis of the pathogenesis and natural history of chronic hepatitis B in the era of antiviral drug resistance². The initial correlation of these multi-disciplinary data has identified novel mutations associated with antiviral drug resistance and cross-resistance to lamivudine, adefovir and entecavir. A linkage that exists between a 3-dimensional (3-D) structure viewing program and the database enables further analysis of potentially relevant mutations within the 3D model of the polymerase¹¹.

The overlap of the envelope gene with the HBV polymerase means that antiviral drug resistant HBV may have an altered envelope¹² and that this may have public health implications. Studies have shown that lamivudine resistant HBV (rtV173L+rtL180M+rtM204V) has a significantly reduced anti-HBs binding due to important changes in the overlapping hepatitis B surface antigen (sE164D+sI195M)¹².

Chronic hepatitis B is a disease with a complex natural history, and this complexity increases with the use of antiviral agents. The SeqHepB system is an important tool that will enable the physician to individualize patient management, to cope with the explosion of antiviral associated HBV mutations, and should prove to be a useful therapeutic guide in the clinical setting as new antiviral agents and combination thereof are trialled and implemented.

Future Directions

SeqHepB will enable virologists and physicians to individualise patient management, cope with the current explosion of antiviral drug associated HBV mutations, and to conduct cross-sectional retrospective or prospective studies on HBV-infected individuals undergoing antiviral therapy^{13,14}.

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NIH HCV and HIV Database

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The Los Alamos viral sequence databases

The Los Alamos HIV database project was started in 1986 by Dr Gerald Myers. It built on the Genbank database, which then also resided in Los Alamos. Over time the HIV sequence database added more background information, a website which provided data analysis tools, and databases of immunological epitopes, drug resistance mutations, and non-human vaccine trials. In 2002 funding was added to create a parallel hepatitis C database project, consisting of a sequence and an immunology database.

The HCV databases (1) were copies of the corresponding HIV databases, minus the data. The sequence database required some extra fields (e.g. ALT level and therapy outcome) and the parsing of the Genbank records was slightly different. The immunology database also needed modifications, but still used the same structure and many of the same fields and interfaces.

Over the past two years, the two projects have combined forces and created a new sequence database which can incorporate both viruses (and others) with only trivial modification. This new database is also structured to be much easier to annotate and maintain, and easier to search. New search interfaces are currently being developed and tested. Initially the change will be transparent to the users, the new interfaces will look almost identical to the old ones. Gradually more features will be added, and the final aim is to create one interface that can accommodate the needs of users of all levels.

At the same time, the HCV and HIV websites will also be unified. They will present a different look to avoid confusion, but the underlying functionality will be very similar, and the computer code will mostly be shared. This makes maintenance much less arduous, and also allows for fairly easy addition of other organisms.

One of the strengths of the HIV/HCV project compared to other pathogen databases is the virus-specific knowledge that is accumulated there, which is translated into sophisticated analysis tools, which are usually shared with the scientific community when they are robust enough. Virus-specific knowledge is incorporated into the database and the web tools in many different ways. One of the most basic is the use of a reference sequence, which can be used to retrieve sequences for a specific region from the database. In the case of HIV this involves in silico splicing of coding regions in the correct reading frame, to form a complete gene that can be translated if needed. For HCV, the process is a bit simpler since splicing is not needed. The Gene Cutter tool is able to locate all the genes that are present in a region, and also (within limits) to rearrange gaps in a submitted alignment in order to restore the reading frame.

Incorporated into the sequence database is a manually curated 'model sequence' which is used to align every sequence to a hidden Markov model (HMM) (2) as soon as the sequence is obtained from Genbank. The aligned sequence is stored along with the unaligned one. This

allows users to instantly align their retrieved sequences. The resulting alignment is usually quite decent, even though it often can be manually improved.

A third virus-specific feature offered by the HIV/HCV project is the ability to automatically align a user's sequence or alignment to any of the provided reference alignments. The location of the user's region relative to the complete genome is determined, the appropriate reference alignment is found, and using an existing alignment algorithm (Align0) (3) the two are aligned together. Finally, spurious gaps introduced by the alignment process are removed, and when appropriate the reading frame is restored.

This infrastructure serves to greatly facilitate the analysis of HIV and HCV sequences, because a lot of the time-consuming and mind-numbing work can be done automatically. We are currently looking for ways to expand these capabilities so they can also be used with other viruses.

The HIV/HCV project also offers numerous analysis tools, which were either developed for the site and therefore are unique to it, or that can be found elsewhere but are conveniently collected and provided with similar user interfaces. Examples of the former group are RIP (4), Glycosite (5), ELF, Peptgen, and Hypermut (6); of the latter, SNAP (synonymous/non-synonymous site analysis), PCOORD (7), TreeMaker, and FindModel. A lot of these tools are specific to HIV/HCV (mostly because they either use site-specific data or because they rely on organism-specific information, such as ORF and gene locations), but quite a few can be used for other viruses. And indeed, judging by references found in the literature, many people working on other viruses have found their way to the HIV/HCV website.

For the future, the HIV/HCV sequence database is planning several new tools and features. An automated classification system will be put in place to check submitted sequences and flag possible recombinants and dubious classifications. An easy-to-use interface will be added to view 3D protein structures and highlight specific amino acids and epitopes. A tool will be added to help users classify their own sequences and test them for potential recombination. And a tool that is currently only used in-house to generate high-quality alignments based on a hidden Markov model will be offered to users so they can generate their own alignments based on virus-specific knowledge; these alignments will be in-frame for all genes.

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