Combating Viral Resistance to Treatment: VIRGIL after two successful years

2006 symposium report by Jenny Bryan, medical writer, UK

Two hundred and sixty people attended the Second VIRGIL International Symposium, Combating Viral Resistance to Treatment, held in Lyon, France on 23 May 2006, to hear about some of the important advances which are being made by the Vigilance Against Viral Resistance network.

Just two years after it was set up, the Vigilance Against Viral Resistance (VIRGIL) network has made enormous progress towards its key goals of establishing a comprehensive and integrated ultrastructure for the European fight against viral drug resistance.

'We have the basis on which to link basic research and clinical studies to improve the anti-viral treatment of our patients,' said VIRGIL's scientific coordinator, **Professor Fabien Zoulim**, in his introduction to the symposium.

He explained that, through its seven clinical, scientific, technological and societal platforms, VIRGIL has already taken major steps towards improving understanding, diagnosis, surveillance, and treatment of antiviral drug resistance, making the management of viral disease more cost effective, and integrating the research capacities of European centres of excellence.

Focusing mainly on hepatitis B, hepatitis C and influenza, research teams within the VIRGIL network are conducting ongoing clinical studies, and have established in vitro models for the study of the antiviral activity of a bank of antiviral agents, Professor Zoulim told delegates. As well as investigating the viral changes that confer drug resistance, extensive research is also providing important information on the host factors that contribute to the failure of hepatitis treatments and the virus-induced intracellular signaling pathways that are required for efficient influenza virus propagation.

Getting HBV treatment advice from the virtual doctor

The latest innovation in VIRGIL's portfolio is the new VirDoc online consultation system for clinicians in search of high quality advice about the management of hepatitis B patients who are resistant to treatment.

Dr Berlian Idris, from Erasmus University, Rotterdam, the Netherlands, described how physicians can submit information about their patient into the VirDoc online database. This is assessed through the Decision Support System and a preliminary report generated based on pre-installed decision trees, using therapeutic guidelines, published studies in high impact journals, Cochrane reviews and expert clinical opinion. Each report is then reviewed and revised by a consultant to provide a final report which goes out to the clinician.

'Patients benefit because they get maximally effective treatment, with minimal side effects, and physicians benefit because the treatment process is improved, so they can be more productive,' explained Dr Idris. 'Hospitals and health insurers also benefit from the improved efficiency and reduced cost of complications, and researchers will benefit from having a structured database of cases of anti-viral resistance,' he added.

Preventing resistance in HBV

Using drugs with maximal antiviral potency and low resistance over time, avoiding sequential monotherapy and treatment interruptions, and considering the use of combination treatment from the outset, were the three key strategies recommended by **Professor Rafael Esteban**, from HU Vale Hebron, Barcelona, for preventing HBV resistance.

He presented data showing that, while lamivudine resistance rises rapidly from 24% at 1 year to 70% at 4 years [Lai et al. Clin Infect Dis, 2003], adefovir resistance rises much more slowly from 0% at 1 year to 29% at 5 years [Colonno et al Hepatology, 2004]. Lower resistance rates have also been reported with the newer agents, entecavir (0% at 2 years in nucleoside naïve patients, 7% at 1 year in lamivudine resistant patients) and telbivudine (4.5% at 2 years in nucleoside naïve patients).

Professor Esteban also presented data to support the use of combination treatment in newly treated hepatitis B patients with, for example, only 2% resistance at 1 year with lamivudine and adefovir, compared to 20% with lamivudine alone [Sung et al. J Hepatol, 2003].

He recommended treating HBV according to the clinical efficacy profile, with lamivudine likely to be effective against N236T mutants, but with reduced activity against A181V mutants, and adefovir for all lamivudine resistant mutants. Entecavir also appears to be active against lamivudine resistant mutants, but at double the drug dosage.

‘If you know the profile of the drug, you can adjust the treatment at each point, and the earlier you address resistance the greater the benefit for the patient,’ concluded Professor Esteban.

Extra care with liver transplant patients

Liver transplant patients with HBV need to be on effective antiviral therapy before their operation and adhere carefully to their post-transplant protocol if they are to achieve a good outcome. This was the advice of **Dr David Mutimer**, from Queen Elizabeth Hospital, Birmingham, UK, who reviewed developments in HBV treatment for transplant patients over the last 15 years.

Before 1994, HBIg was used to prevent HBV recurrence in the transplanted liver. Development of resistant mutations resulted in increasing use of lamivudine between 1994 and 1997. But once again, mutations (YMDD mutants) have resulted in the need for a change in therapy for some patients – to combination treatment with HBIg and lamivudine, with or without adefovir.

Dr Mutimer warned delegates to take particular care when changing patients' immunosuppressive drugs as a small change in their immunity can be enough to trigger an HBV recurrence.

Transgenic mouse model opens door to novel anti-HBV therapies

A novel family of acylated HBV preS peptides has shown promising HBV blocking effects in a new transgenic mouse model, described by **Dr Joerg Petersen** from the University of Eppendorf, Hamburg, Germany. *In vivo* investigations were carried out using a urokinase-type plasminogen activator (UPA) mouse transplanted with hepatocytes from the common tree shrew, which is proving a more practical model for preclinical testing of potential HBV drugs than previously used chimpanzee, tree shrew, woodchuck and duck models. The UPA mouse can also be injected with human hepatocytes.

Non responders to HCV treatment: where next?

Future strategies for HCV management are likely to depend on tailoring therapy to how rapidly and how well patients respond to initial pegylated interferon alfa and ribavirin treatment. **Professor Heiner Wedemeyer** from Hannover Medical

School, Germany, predicted that extended therapy, retreatment and maintenance therapy will all play a role.

He reported results of a recent study showing that slow responder HCV genotype 1 patients who have detectable virus after 12 weeks treatment, but are HCV negative at 24 weeks, do better with 72 weeks of pegylated interferon alfa and ribavirin than standard 48 week regimens (relapse 46% vs. 87%) [Berg et al, Gastroenterology, 2006].

Doubling the dose of pegylated interferon alfa is a possible strategy for previous non responders – as shown in the RENEW trial, Professor Wedemeyer explained [Gross et al AASLD, 2005]. A mean SVR of 17% was achieved in patients treated with pegylated interferon alfa-2b 3μg/kg and ribavirin for 48 weeks, compared with 12% in those treated with the standard 1.5μg/kg dose (p=0.03).

EPIC^3 and HALT-C are currently investigating the potential of low-dose pegylated interferon alfa monotherapy as maintenance therapy for 3-5 years in non-responders to standard combination treatment. This follows promising results in COPILOT which showed the beneficial effects after two years of maintenance treatment with low-dose pegylated interferon alfa-2b on event free survival, compared to colchicine [Afdhal, AASLD, 2004].

'Our tasks for the near future are to identify non responders before week 12 of treatment, slow responders who need extended treatment, non responders who will benefit from retreatment, and those patients who will benefit from maintenance therapy,' concluded Professor Wedemeyer.

Viral kinetics and HCV treatment duration

Viral kinetics could be a useful tool in helping to tailor HCV treatment to individual patient needs, and for comparing the potential of new antiviral compounds. **Professor Stefan Zeuzem**, from Saarland University Hospital

Homburg/Saar, Germany, demonstrated how the two phases of HCV RNA decline correlate to different aspects of the treatment response. While the exponential phase 1 response which occurs during the initial 24-48 hours of treatment is indicative of viral decay, the more prolonged phase 2 response is indicative of the rate of loss of infected cells, and correlates well with SVR to interferon-based therapies. Plateauing or rebound effects seen during phase 2 are likely to indicate development of treatment resistant mutations.

Professor Zeuzem presented phase 1/2 data on a series of new HCV protease inhibitors, including SCH 503034. He explained that the typical pharmacodynamic and pharmacokinetic profile of pegylated interferon alfa-2b continued to be seen when SCH 503034 was added, but with reduced viral load with the combination treatment.

'Viral kinetics allow for direct assessment of efficacy and, in the long term, we hope that the initial decline that is seen can be used to predict the duration of treatment which is needed for each patient,' concluded Professor Zeuzem.

Update on HCV culture systems

The lack of a culture system for HCV has seriously hampered treatment research. But the recent demonstration, by **Dr Takaji Wakita**, from the Tokyo Metropolitan Institute for Neuroscience that subgenomic replicons of the JFH1 genotype 2a strain cloned from an individual with fulminant hepatitis replicate efficiently in human hepatoma cell lines (Huh7), and secrete viral particles, has been met with great interest [Wakita T el al. Nature Med, 2005].

At the symposium, Dr Wakita reported that infection efficiency has now been improved using Huh7 permissive cell lines, and that CD81 expression appears important for permissiveness, though other factors are also likely to be involved.

‘We now have a system to analyse the HCV lifecycle which should help us devise new drugs that target the virus at different stages,’ concluded Dr Wakita.

Vaccine therapy for HCV

A therapeutic vaccine for HCV which uses Modified Virus Ankara (MVA) as vector for NS3-NS4 & NS5B is expected to enter phase 1/2 clinical trials this year, following promising preclinical results. **Dr Genevieve Inchauspe**, from Transgene, France, which is developing the vaccine, brought delegates up to date with the problems that have been associated with previous vaccines. For example, the addition of core and envelope proteins to a pervious MVA NS3 vaccine significantly reduced its efficacy in chimpanzees. So increasing the number of antigens in an HCV vaccine may not be the answer.

New treatments for H5N1

Additional neuraminidase inhibitors and novel neuraminidase inhibitors could soon be added to the limited range of antiviral agents currently available to treat the H5N1 strain of avian flu.

In a wide ranging review of current and future influenza treatments, **Professor Erik De Clercq**, from the Rega Institute Leuven, Belgium, explained that H5N1 owes much of its virulence to the fact that it binds to receptors in the lower respiratory tract and therefore has greater potential to cause pneumonia than H3N2 strains which bind in the upper respiratory tract.

The influenza neuraminidase is essential for release of new viral particles from host cell receptors, and the neuraminidase inhibitors, zanamivir and oseltamivir, block this process. Professor De Clercq pointed out that both drugs are effective if given early in the infection, though the oral formulation of oseltamivir has proved more practical than the inhaler route used for zanamavir.

Resistant mutations have been identified against both drugs. The recent identification of H5N1 virus with a H274Y substitution in neuraminidase, conferring high-level resistance to oseltamivir in two of eight Vietnamese patients, both of whom died, resulted in suggestions to change the current strategy for the treatment of H5N1 infection to include additional antiviral agents [de Jong et al. New England Journal of Medicine, 2005].

Professor De Clercq described two new neuraminidase inhibitors, peramivir and (RWJ-270201) and A-315675, which have been shown to be active against both zanamivir- and oseltamivir-resistant A and B influenza viruses (Mishin et al. Antimicrob Agents Chemother, 2005.

In addition, the substituted pyrazine compound, T-705, and the fungal extract, flutimide, have shown promising anti-influenza activity via their inhibitory effects on viral polymerase.

H5N1 mutants: update

In vitro, H5N1 isolates from poultry and humans are susceptible to both zanamivir and oseltamavir, but different clades may have different susceptibility patterns, **Professor Bruno Lina**, from the University C Bernard, Lyon, France, told delegates. Clade 1 viruses seem to be less susceptible to neuraminidase inhibitors than clade 2 viruses. They are also resistant to amantadine while clade 2 viruses are susceptible.

Professor Lina warned that, in highly replicating viruses such as H5N1, fitness reduction may not impair transmissibility. Amantadine resistant isolates of H5N1 are highly transmissible, he said, and 119 mutants have been shown to be transmissible in ferrets, while a 274 mutant seems to have been transmitted from a child infected with H5N1 [de Jong et al. New England Journal of Medicine, 2005].

How ferrets can overcome influenza resistance

Ferrets remain the best animal model for studying influenza, according to **Dr Rob Lambkin**, from Retroscreen Virology Ltd., at Queen Mary's School of Medicine and Dentistry, London University. He explained that influenza in ferrets closely resembles infection in humans in terms of symptoms, viral distribution and immunity.

Recent ferret experiments have shown that neuraminidase resistant mutants (H3N2 Arg292Lys) are significantly less pathogenic than wild type virus, and have markedly reduced fitness (H1N1 His274Tyr). They also have reduced transmissibility (H3N2 Arg292Lys).

How the influenza virus uses host cell signalling systems

A growing understanding of the way that the influenza virus uses host cell signalling systems in the infection cycle may yield new targets for antiviral drugs. **Dr Stephan Pleschka**, Assistant Professor of Virology at the Institut für Med. Virologie at the Justus-Liebig-Universität Gießen, in Germany, described recent research showing the importance of viral activation of the cellular Raf/MEK/ERK (mitogen-activated protein kinase (MAPK)) signaling cascade for export of ribonucleoprotein (RNP) complexes late in the infectious cycle. Accumulation of haemagglutinin in the membrane, and its tight association with lipid-raft domains, has been shown to trigger activation of the MAPK cascade via protein kinase C alpha activation and to induce RNP export [Marjuki et al. J Biol Chem, 2006]. In contrast, the non structural NS1 protein of the influenza virus is essential to counteract virus induced signaling pathways for antiviral defence mechanisms, and hence to promote efficient viral replication.

Drug targets for SARS

Future antiviral drug developments against severe acute respiratory syndrome coronavirus (SARS-CoV) will depend on improved understanding of the viral replication complex, predicted **Professor Willy Spaan**, from Leiden University

Medical Centre, the Netherlands. He explained that nidoviruses, such as SARS-CoV have unusually large RNA genomes and complex lifecycles, making their replication systems difficult to elucidate. But a growing number of potential targets within the replicase region of the viral genome are being identified.

Understanding HIV drug resistance

Though not currently one of the three key viral infections on which VIRGIL is focusing, HIV is providing important information about mechanisms of drug resistance. **Professor François Clavel**, from Inserm, Paris, France, reviewed research into mechanisms of resistance against protease and fusion inhibitors used in HIV treatment.

He explained that the HIV protease is a key enzyme for replication and has been a popular target for inhibitory drugs owing to its small size, simple structure, and ease of synthesis. The active site is highly conserved and most resistance sites are outside the binding site of the enzyme, so cumulative mutations are required to achieve significant levels of resistance.

‘The result of the mutations is to change the shape of the enzyme, so that inhibitors cannot make good contact points, but this has a negative effect for the virus because it makes it more difficult for the enzyme to bind to its substrate,’ said Dr Clavel.

Two mutations in the Gag region of the HIV genome, at L449F or A431V, are most commonly found in resistant strains, and it appears that these enable the virus to overcome the adverse effects of changes to the shape of the protease enzyme. But, as Dr Clavel pointed out, there is a price to pay in terms of reduced viral fitness. Whether a measurable reduction in viral fitness could be used, alongside CD4 count and viral load, to monitor response to treatment remains uncertain, and there appears to be a component of fitness, as measured in recombinant assays, that is unrelated to resistance.

Fusion inhibitors are a newer group of anti-HIV drugs which block fusion of the gp41 envelope protein to the cell membrane prior to formation of the fusion pore which facilitates viral entry. Dr Clavel explained that enfuvirtide is a fusion inhibitor which is useful for salvage therapy. But if the response is insufficient resistant mutations occur. Two complementary mechanisms appear to be involved:

- selection of mutations in the HR1 region of gp41
- selection of HIV quasi-species that offer the most favorable environment for expression of these mutations

Although not prevalent before escape, these *env* species display an apparent increase in fitness *in vitro*.

Dr Clavel explained that viral recombination is another key mechanism that HIV uses to retain diversity and optimise infection in individual patients. He described recent studies demonstrating extensive viral recombination in HIV patients, including those failing antiretroviral therapy [Charpentier C. J Virology, 2006].

Managing HIV drug resistance

Viral mutations are so common in HIV patients that drug resistance testing has a role to play, even in treatment naïve patients. **Professor Juergen Rockstroh**, from the University of Bonn, Germany, presented data from extensive clinical trials of resistance in ART naïve patients, those undergoing treatment for mother to child (MTC) prophylaxis, following first treatment failure, following multiple treatment failures and after treatment interruption.

Tailoring HIV treatment to viral genotype helps patients with resistant strains to achieve comparable immune responses to those without resistance [Oette M et al. J AIDS, 2006]. Treating pregnant women in developing countries with triple therapy to prevent MTC transmission may be more expensive than single drug

therapy, but carries a significantly lower risk of resistance. Development of resistance in the first year of treatment is declining, and has been linked to lower viral load at baseline and less advanced disease.

Three quarters of long term users of anti-HIV drugs are likely to have developed resistance to at least one agent [Richman DD et al, AIDS, 2004], and multiple treatment failure remains a significant problem. Even so, it is still a reasonable expectation to achieve low viral load levels in multiply resistant patients, concluded Professor Rockstroh.

VIZIER: new targets for new antivirals

Thirty potential antiviral compounds have been identified under the VIZIER programme, 13 have been purchased and 1 is being actively tested on infected cells, reported project coordinator, **Dr Bruno Canard**, from the University of Marseille, France.

Started in November 2004, VIZIER has been set up to identify new drug targets against RNA viruses through comprehensive structural characterisation of their replicative machinery. Implementation is through interaction of five scientific sections:

- Bioinformatics for genome annotation, target selection and date integration
- Virus production and genome sequencing: >70 complete genomes have been sequenced including all of the flavivirus family, and >1000 targets (PCR products) have been sent for protein expression and purification
- HTP protein production
- HTC crystallisation and structural determination: 28 crystal structures have been elucidated from a variety of viral families
- Target validation

‘With the problem of emerging viruses, we wanted to bring research together and design new anti-viral agents,’ concluded Dr Canard. ‘By bringing all this information on viruses together, we hope that, when a new virus, like SARS, emerges, we will be able quickly to identify it and work out its structure, so that we can save years in the development of effective new treatments.’

COPY ENDS

For further information about VIRGIL please contact:

Dr Jerome Weinbach
Senior International Project Manager
Inserm Transfert
101 rue de Tolbiac
75 013 Paris
FRANCE
Mob: +33 6 76 63 05 50
Tel: +33 1 44 23 67 33
Fax: +33 1 44 23 67 45