ISAR News

Newsletter of the International Society for Antiviral Research

Women in Science *Current research* *ICAR Rome update*
ISAR member profiles *Election candidates* *Antivirals on the horizon*

Editor, Anthony Vere Hodge Guest editor, Mike Bray

WELCOME TO ISAR NEWS

Dear ISAR members,

Welcome to the autumn, 2014 issue of ISAR News! As Bob Buckheit has mentioned in his president's letters, the Publications Committee and Board of Directors decided at the Raleigh ICAR to expand our newsletter from two to four issues a year, and to include articles about research by ISAR members and other scientific content. This issue inaugurates these changes, and we hope you'll find it interesting and rewarding to read.

The first two articles provide a profile of ISAR Women in Science chair Amy Patick and information about the WIS Career Development Award. These are followed by interviews with two leading experts on chikungunya and hepatitis C, Scott Weaver and Heiner Wedemeyer, then by discussions of antiviral drugs and vaccines and the Ebola epidemic by Mike Bray and Anthony Vere Hodge. Next you'll find profiles of two ISAR members doing interesting work, Nesya Goris in Belgium and Ashoke Sharon in India. In the following article, Luis Schang discusses a novel approach to herpesvirus therapy, through control of epigenetic modification of viral chromatin. The final sections cover three important topics for ISAR members: the election of two members of the Board of Directors, plans for the 28th ICAR in Rome, and a list of future conferences of interest that will be posted on the ISAR website.

As this issue of ISAR News goes to press, the Ebola epidemic in West Africa is taking an increasing number of victims and spreading fear throughout the world. This unexpected outbreak is a painful reminder of the worldwide burden of viral diseases and the continuing need for effective drugs and vaccines. On behalf of society members, we wish to express our deep concern about the Ebola outbreak and send our condolences to all those families who have suffered losses.

In the mid-20th century, some scientists confidently predicted that humanity would soon control and eventually eliminate infectious diseases, but subsequent decades have removed any cause for optimism. Through the emergence of novel viruses, the continual evolution of drug-resistant variants and the intractable problem of chronic viral diseases, the challenge of infectious diseases and the importance of antiviral research are as great as ever. For those of us who cannot respond directly to the Ebola epidemic, rededication to our work may be the best contribution.

For the ISAR Publications Committee
Mike Bray
Anthony Vere Hodge

IN THIS ISSUE

WOMEN IN SCIENCE

An interview with WIS founder Amy Patick

Amy Patick, chair of the ISAR Women in Science committee, has had an interesting and varied career. She first became interested in virology while working on a research project on Rio Bravo virus as an undergraduate at Wellesley College and went on to earn her PhD in Medical Microbiology from the University of Wisconsin, Madison, with a research project on a herpesvirus. She did her postdoctoral training in the Department of Immunology at the Mayo Clinic, focusing on picornaviruses. She then joined Bristol-Myers Squibb as an investigator in HIV research.

In 1994, Amy moved to Agouron Pharmaceuticals, which was acquired by Pfizer in 2000. At Pfizer, she held various leadership positions, eventually becoming Head of the Antiviral Biology Therapeutic Area. While still at Agouron, she led the preclinical effort in the successful approval of the HIV drug nelfinavir. Most recently, she was Vice President for Biological Sciences at Genelabs Technologies and Vice President for Research at Adamas Pharmaceuticals.

Amy has been an ISAR member since 1992, and was the second woman president, serving from 2008–10. She now chairs the Women in Science committee.

How has the situation of women scientists improved during your career, and what hasn't changed?

I've spent much of my career in the pharmaceutical industry, with experience ranging from small start-ups to large companies. Looking back over the past 25 years, I think women are slowly being recognized for their achievements, and more women now occupy significant leadership positions.

Some issues worthy of continued attention include the ability of women to be compensated fairly, to advance in their careers without gender bias, and to be recognized for their scientific achievements without discrimination. Many women also juggle career development with raising families, and achieving an optimal balance of work and personal life can be challenging. We should also strive to recognize successful female scientists and cultivate them as role models.

Who has been your own role model during your career?

I have many people to thank, but I'm especially grateful to Dr. Karen Biron. As a young graduate student, I became very familiar with her research, as she was a prominent and published scientist. I "got my courage up" to introduce myself at a conference, and when I saw Karen at a later meeting, she remembered my name! Through the years, she's been very kind and supportive of me and other young scientists.

Karen also served as an important role model as the first woman president of ISAR, and she encouraged me to run for the position. I was grateful to be elected and to have the chance to give back to a Society that has provided me with so many opportunities for so many years.

When did the idea for a Women in Science committee occur to you?

The idea came during a 2012 ISAR Board of Directors meeting, when we were brainstorming about new educational or career development initiatives. I pointed out that we were lacking a forum that focused on the challenges and opportunities encountered by women scientists while managing their career progression in today's environment. I suggested that we create a group for this purpose, and said I would be happy to take the lead. At the next ICAR, I organized the first WIS activity, a "speed dating" event, in which participants moved from table to table to sample several topics, similar to one I had attended earlier at an Association for Women in Science workshop.

Who have been your key supporting players?

The WIS committee is comprised of a number of prominent women scientists, including Graciela Andrei, Rhonda Cardin, Kara Carter, Heather Greenstone, Ann Kwong, Jennifer Moffat, Anneke Raney, Katherine-Seley Radtke and Karen Watson-Buckheit. They have embraced the WIS initiative with strong enthusiasm and a collective desire to help women scientists. The ISAR President-Elect, President, Past

President and Board members have all been very supportive.

Do some WIS committee members have defined roles?

Anneke Raney has taken the lead in fund-raising activities for the WIS scholarship program, with support from Roger Ptak. Rhonda Cardin is coordinating a pilot mentorship program for young women in ICAR, and Jennifer Moffat helped create a questionnaire for the Raleigh meeting to measure success and understand what our attendees would like to do in future efforts. All WIS committee members play active roles.

What are your goals for the WIS program?

I would like to continue to provide a forum and create initiatives that focus on the challenges women face in their career development. Since most current members of the WIS committee are from North America, we would like to expand our leadership to include women from Asia, Africa and Europe. I would also like to see ISAR include more women in leadership positions and have more female officers and board members. I encourage ISAR to recognize women for prominent awards. To date, only one woman has won the Gertrude B. Elion Award, and none has received a William Prusoff Award.

WIS Career Development Award

The ISAR WIS Committee is now accepting applications for its Career Development Award. Up to three awards will be given annually to advance the careers of women with potential for significant contribution in the field of antiviral research, by providing funds to attend a conference, visit another laboratory, take a course or acquire specialized training. Each award will consist of a $1500 stipend, a 2-year ISAR membership and a commemorative certificate.

To be eligible to apply for this program, a woman scientist must:

- be a current undergraduate or graduate student or hold a doctoral degree and have no more than five years of cumulative postdoctoral experience;
- be performing undergraduate, graduate or postdoctoral work in antiviral research and/or related areas.

More information about the award and additional guidelines can be found on the ISAR website, http://www.isar-icar.com. The application deadline is December 31, 2014.

CURRENT RESEARCH

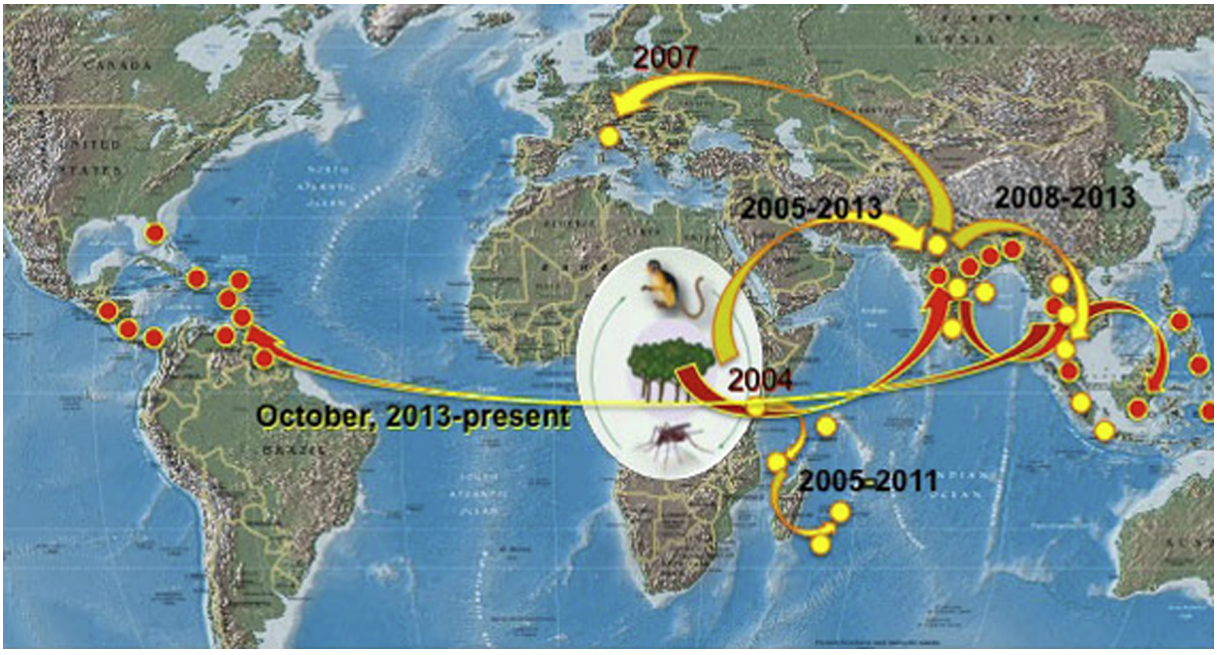
Chikungunya in the Western Hemisphere: How will it spread? Are new drugs and vaccines needed?

An interview with Scott Weaver, Director of the Institute for Human Infections and Immunity at the University of Texas Medical Branch, Galveston.

Chikungunya fever (CHIK), caused by a mosquito-transmitted alphavirus, was first described in Africa in the 1950s, and is now widespread in Africa and Asia. The name comes from a local African word for "to become contorted", describing the severe joint pains that occur in the acute illness, and which may persist for months to years in some patients. In 2013, an outbreak of CHIK was detected on the island of St. Martin in the Caribbean, and it has since spread widely (see map).

Is it known how CHIKV "jumped" to the Western Hemisphere?

It's almost certain that the Caribbean outbreak was initiated when an infected traveler arrived from Southeast Asia or the Oceania region. Because CHIK has an asymptomatic incubation period of 1–3 days, air travelers can board flights unknowingly infected. Once they reach their destination, if they are exposed to *Aedes aegypti* or *A. albopictus* mosquitoes during the viremic phase of the acute illness, local transmission can ensue.



Spread of chikungunya virus to the Caribbean, 2013. Red dots: spread of Asian lineage virus, 1958–2014. Yellow dots: spread of Indian Ocean lineage virus, 2004–2013.

Was the entry of CHIK into the Western Hemisphere inevitable?

Yes, I believe it was inevitable, and in fact the recent introduction was probably not the first. There is evidence that outbreaks of acute febrile illness in the Americas following the establishment of trade from Africa in the 16th century probably included CHIK (Carey, 1971). As has been well established for yellow fever (YF), arboviruses transmitted among humans by the mosquito *A. aegypti* could travel on ships, even with onboard circulation, then initiate epidemics at their destination by introducing both the virus and vector.

In the case of YF, a permanent nonhuman primate-sylvatic mosquito cycle was also established in South America forests, increasing the risk of frequent emergence into urban transmission cycles. CHIKV may also have enzootic potential, because its ancestral enzootic cycle in Africa is nearly identical to that of YFV.

Is the epidemic spreading faster than anticipated?

The spread has actually been a bit slower than I expected. Colleagues in the Caribbean and Central America have told me that their rainy season, which normally occurs from June-November (coincident with the North Atlantic hurricane season) has been dryer than usual, which may explain the relatively slow spread seen so far on the mainland of Latin America.

How does the virus now circulating in the Americas differ from that which emerged in Indian Ocean countries a few years ago? What are the implications?

There are two lineages of CHIKV, Asian and Indian Ocean (IOL), both of which originated from enzootic strains in the eastern half of sub-Saharan Africa (Volk et al., 2010). The Asian lineage spread to India and Southeast Asia some 60–70 years ago, while the IOL emerged in coastal Kenya in 2004. The virus now circulating in the Western Hemisphere is from the Asian lineage.

Importantly, the two lineages differ in their ability to be transmitted by the mosquito species *A. aegypti* and *A. albopictus*. *A. aegypti* tends to be found in cities, and can't survive cold winters, while *A. albopictus* thrives in both urban and rural locations, and can survive in temperate climates.

While the Asian lineage of CHIKV is confined to *A. aegypti*, the IOL can adapt to utilize *A. albopictus* as a principal epidemic vector through mutations in the E1 and E2 envelope glycoprotein genes that enhance infection of the mosquito midgut (Vazeille et al., 2007; Tsetsarkin et al., 2007, 2014). We can therefore predict that the Asian virus now circulating in the Americas will be transmitted more efficiently by *A. aegypti*, and may be limited in the near-term mainly to tropical and subtropical regions.

In Africa, CHIKV is maintained by mosquito transmission among wild primates. Could a similar cycle become established in the Americas?

Interestingly, in a few Caribbean Islands there are large populations of imported African green monkeys, which are known CHIKV enzootic hosts in Africa. They may represent an opportunity to initiate enzootic circulation if appropriate mosquito vectors are present. However, I doubt that these populations are sufficient to maintain continuous transmission.

In South and Central America, many other species of nonhuman primates occur in much larger populations, and some serve as enzootic hosts for YFV. If these develop sufficient viremia after infection with CHIKV, they could support continuous circulation of the virus, but this would again depend on the vector competence of local sylvatic mosquitoes.

Will it be possible to eradicate the disease?

The prospects for eradication of the urban CHIKV cycle are poor in the short term, but better in the long term. Eliminating urban transmission through mosquito control is highly unlikely, considering the lack of success with dengue, which has an identical epidemic cycle. However, some new strategies for the control of *A. aegypti*, as well as for reducing its competence to transmit dengue and CHIK offer greater promise in the long term.

The best chance of eradicating CHIK lies with vaccination. Unlike dengue virus, CHIKV is antigenically conserved, and reinfection does not play a role in pathogenesis, so it's a relatively straightforward vaccine target, and several promising products are in preclinical or early clinical evaluation (Weaver et al., 2012).

The major challenges to vaccine development are the unpredictability of the disease, the lack of continuous surveillance and the difficulty of conducting clinical efficacy studies. The FDA "Animal Rule" is a possible solution to the latter problem, but it is unclear if the hundreds of millions of dollars typically required to bring a vaccine to licensure in the U.S. will be forthcoming.

Where would vaccination play the greatest role in the Western Hemisphere?

U.S. populations are at risk, particularly in the southeast where *A. aegypti* is well established, as demonstrated by the occurrence of CHIK in some residents of Florida who had not traveled to outbreak areas. However, epidemics will probably be limited in scope compared to Asia, Africa or Latin America, where there is much greater contact between mosquitoes and people.

The greatest impact of a vaccine, especially if it could be deployed rapidly, will be in large Latin America cities where tens of millions of people are at high risk of CHIK, and experience with dengue indicates that major epidemics are inevitable. Once CHIKV has swept through Latin America, a vaccine will still be very useful to reduce endemic disease and potentially eradicate the urban cycle, if coverage is adequate.

How could a vaccine be tested for efficacy?

Ideally, a vaccine could be tested in a major Latin American city as CHIK arrives, because attack rates are typically around 50%, and there are few inapparent infections. This means that vaccination of a relatively small number of volunteers could demonstrate efficacy. Unfortunately, this opportunity will quickly slip away, and experience from Asia has shown that, after major epidemics have come to an end, data on the continuing incidence of endemic disease are scarce. Planning a test of vaccine efficacy thus becomes extremely difficult without supplementary surveillance.

The most feasible route to vaccine licensure in the U.S. is probably a Phase I safety trial in 50–100 healthy volunteers, followed by efficacy demonstration in an animal model. Rodents are not very useful for this purpose, but rhesus and cynomolgus macaques are excellent models of the human disease

Is there a need for antiviral drugs?

Antiviral drugs could be extremely valuable for CHIK, particularly if they are effective during the late stages of acute infection as well as for controlling the chronic arthralgia that often affects victims for months or even years (Schilte, 2013). A major need for basic research is the development of animal models of chronic arthralgia, so that pathogenesis can be better understood and antiviral products can be evaluated preclinically.

Unfortunately, the pathogenesis of persistent CHIK-related arthralgia is still poorly understood. Virus and, to a greater extent, viral RNA have been detected in some patients a few months after the resolution of the acute illness (Hoarau et al., 2010), suggesting that persistent viral replication may play a role in chronic disease. However, the identification of chronic activation of macrophages in joints also suggests that targeting the host response will be a fertile approach for drug development. Both direct-acting antivirals and immunomodulatory approaches will probably be needed.

Scott Weaver co-chairs the Global Virus Network Chikungunya Task Force. For up-to-date information on CHIK and the epidemic in the Western Hemisphere, go to http://gvn.org/chikungunya-task-force.

Carey DE. Chikungunya and dengue: a case of mistaken identity? J. Hist. Med. Allied Sci. 1971;26(3):243–62.

Hoarau JJ, et al. Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. J Immunol. 2010;184(10):5914–27.

Schilte C, et al. Chikungunya virus-associated long-term arthralgia: a 36-month prospective longitudinal study. PLoS Negl Trop Dis. 2013;7(3):e2137.

Tsetsarkin KA, et al. A single mutation in chikungunya virus affects vector specificity and epidemic potential. PLoS Pathog. 2007;3(12):e201. Epub 2007/12/12.

Tsetsarkin KA, et al. Multi-peaked adaptive landscape for chikungunya virus evolution predicts continued fitness optimization in *Aedes albopictus* mosquitoes. Nat Commun. 2014;5:4084.

Vazeille M, et al. Two Chikungunya isolates from the outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito, Aedes albopictus. PLoS One. 2007;2(11):e1168.
Volk SM, et al. Genome-scale phylogenetic analyses of chikungunya virus reveal independent emergences of recent epidemics and various evolutionary rates. J Virol. 2010;84(13):6497–504.
Weaver SC, et al. Chikungunya virus and prospects for a vaccine. Expert Rev Vaccines. 2012;11(9):1087–101. Epub 2012/11/16.
Weaver SC. Arrival of Chikungunya Virus in the New World: Prospects for Spread and Impact on Public Health. PLoS Negl Trop Dis. 2014;8(6):e2921.

Antiviral therapy of chronic hepatitis C: a time of rapid progress

An interview with Heiner Wedemeyer, senior physician in the Department of Gastroenterology, Hepatology and Endocrinology, Medical School of Hannover, Germany.

During the nearly two decades you've been involved with the treatment of hepatitis C, how have the course of therapy and the prospects for a cure changed?

I have experienced two major paradigm shifts in the treatment of hepatitis C. Fifteen-20 years ago, we could treat very few patients, and liver transplantation was the only hope for patients with liver cirrhosis. The introduction of PEG-IFN/ribavirin therapy was a major breakthrough. Many patients could be cured and we recently gained evidence that this cure translated also into improved overall survival rates. However, IFNá and RBV were sometimes associated with severe side effects and more than 50% of patients could not be treated at all (Manns et al., 2006).

For about 10 years, we optimized PEG-IFN-based therapies and tried to ''individualize" treatment duration based on host factors, on-treatment response kinetics and side-effect profiles. With the introduction of novel direct-acting antivirals we are experiencing a new era of HCV therapy. DAAs with excellent side-effect profiles have been introduced and more drugs are expected during the next two years. By then, a treatment option should be available for almost every patient, leading to HCV elimination. Most importantly, this response seems to improve liver function even in patients with advanced liver cirrhosis (Lee and Friedman, 2014).

Recent progress has been especially rapid. What changes have occurred in antiviral therapy during the past year?

In December, 2013 the FDA approved the first-in-class HCV polymerase inhibitor sofosbuvir, which was the key step on the path to interferon-free therapies for hepatitis C. Sofosbuvir is very potent across all genotypes and has a very high barrier to resistance. At the same time, the second-generation protease inhibitor simeprevir was approved, allowing the (off-label) combination of two DAAs, which has been widely applied in the USA (Kohli et al., 2014). Sofosbuvir was approved by the European Medical Agency in January and simeprevir in May. Since August, European clinicians have had an additional option, as the first-in-class NS5A inhibitor daclatasvir became available. Thus, we now have different options to ''mix and match'' anti-HCV drugs in Europe, with sofosbuvir being the backbone of current therapy.

What changes will we see in the coming year?

We can expect the next major steps forward as early as November. The combination of the NS5A inhibitor ledipasvir with sofosbuvir has been tested in large Phase 3 trials as a ''single-tablet regimen'', leading to cure rates in genotype 1 patients of 95–100% with 8–12 weeks of therapy. Under the trade name Harvoni, it was approved by the FDA on October 10th, 2014. In addition, a 3-drug combination therapy has been developed with a ritonavir-boosted protease inhibitor (ABT-450), an NS5A inhibitor (ombitasvir) and a non-nucleoside NS5B inhibitor (dasabuvir), which has also cured almost every genotype 1 patient, even those presenting with cirrhosis (Liang and Ghany, 2014).

Overall, there will be various outstanding, highly effective and very safe therapies available for patients infected with HCV genotype 1. Therapies are not ideal yet for genotype 3 and only limited data are available for genotypes 4–6.

What are the remaining challenges for eliminating hepatitis C in Europe, the US and other high-income countries?

Even though recent data are absolutely exciting, there are still several clinical challenges. First, we do not know to what extent the natural history of chronic hepatitis C can be improved in patients with advanced and decompensated liver disease. For some HCV genotypes (e.g. genotype 3), we still do not have a perfect treatment which works in every patient. More data on drug-drug interactions are needed, as well as safety data in individuals with renal impairment.

We also do not know if HCV resistance will become a challenge for some patients – in particular if resistance against the HCV NS5A protein has been selected (Schneider and Sarrazin, 2014). More drugs are expected for 2016 which should be able to cover some of the remaining challenges. New treatment will also aim to cure HCV with even shorter therapies (e.g. 4–6 weeks).

Finally, we must make sure that patients with HCV infection are identified (Edlin and Winkelstein, 2014). I can only cure a patient if I know that the patient is HCV-infected. General practitioners and other doctors need to become aware of these great achievements and that it is worthwhile testing now!

Most people with HCV infection live in countries where they have little access to treatment of any sort. What is the best approach to reducing the disease burden?

During the coming years, treatment must be prioritized based on urgency of therapy. This means that individuals with advanced fibrosis and cirrhosis should receive therapy first. If fibrosis has been excluded by reliable methods, it can be justified that these patients may wait some more years before therapy is initiated. However, it must be emphasized that HCV-infected patients also have an increased risk for extrahepatic morbidity and mortality.

Thus, it will be absolutely crucial to make sure that therapies will become cheaper and affordable. One has to keep in mind that early treatment of HCV is cost-effective. If we treat now we will save costs 10–20 years from now.

The availability of new DAA therapies means that people frequently exposed to HCV, such as injecting drug users, may be treated, then re-infected. What is the likely outcome in such cases, and how should these patients be managed?

We need to re-think how to address injecting drug users with HCV. The idea of therapy could be ''treatment as prevention''. Many patients could be cured with just 8 weeks of treatment with 1 pill a day. Thus, e.g., hepatitis C could be treated in methadone clinics and together with substitution we may simply cure HCV infection. These are unique opportunities. However, this only makes sense if all patients are treated at the same time. Re-infection will be possible, and thus if the goal is to eliminate HCV from a patient population, then treatment should be aggressive and broad (Grebely and Dore, 2014).

Edlin BR, Winkelstein ER. Can hepatitis C be eradicated in the United States? Antiviral Res. 2014;110:79–93.
Grebely J, Dore GJ. Can hepatitis C virus infection be eradicated in people who inject drugs? Antiviral Res. 2014 104:62–72.
Kohli A, Shaffer A, Sherman A, Kottilil S. Treatment of hepatitis C: a systematic review. JAMA. 2014 Aug 13;312:631–40.
Lee YA, Friedman SL. Reversal, maintenance or progression: what happens to the liver after a virologic cure of hepatitis C? Antiviral Res. 2014;107: 23–30.
Liang TJ, Ghany MG. Therapy of hepatitis C – back to the future. N Engl J Med. 2014 370:2043–7.
Manns MP, Wedemeyer H, Cornberg M. Gut 2006 Sep;55(9):1350–9.
Schneider MD, Sarrazin C. Antiviral Res. 2014; 105:64–71.

Antivirals for Ebola
Mike Bray, Bethesda, MD, USA

The Ebola epidemic in West Africa is the most challenging problem facing antiviral research today. Far more people have died in the current outbreak than in all previous Ebola epidemics combined, and the disease is also affecting many countries outside of Africa, through the travel of potentially infected people and the need to treat doctors and nurses who have been accidentally exposed to the virus.

More than 20 Ebola outbreaks have occurred in Africa since the virus was first identified in 1976. All were contained and eliminated within a period of weeks to a few months, probably because they took place in relatively remote areas with low population density. The much greater size of the West African epidemic does not appear to reflect any change in the causative virus or its mode of transmission; instead, its size has resulted from the spread of the disease to urban areas and the extensive movement of infected people. Halting the epidemic will largely require the massive implementation of infection control measures, in which a role for antiviral therapy has not been defined. However, effective treatments are important for the management of Ebola patients in settings where such drugs can be administered.

Antivirals currently under evaluation can be divided into two categories. The first consists of products that are already licensed for other indications, or have undergone safety testing in Phase 2 and 3 trials, and are available in adequate quantity to be given to patients under compassionate use protocols. At present, there are two drugs in this category. A compound familiar to readers from past ICAR presentations is favipiravir (T-705), a drug being developed by the Toyama Chemical Company for the treatment of influenza, which is now licensed in Japan and undergoing extensive Phase 3 trials in the USA and elsewhere. Favipiravir was reported to be active in mouse models of lethal Ebola virus infection in two papers in *Antiviral Research* earlier this year, providing a basis for its clinical use (Oestereich et al., Smither et al., 2014). Evaluation in nonhuman primates is under way. Favipiravir has been given to a number of Ebola patients in Europe, but case reports have not yet been published.

Most surprisingly for antiviral researchers, the compound brincidofovir (CMX001), which was previously considered to be specific for DNA viral diseases, and is being developed by the Chimerix company for the treatment of poxvirus, herpesvirus, adenovirus and other infections, has been reported to have anti-Ebola activity. Even though this discovery has only been posted on the company website (http://www.chimerix.com), the established safety record and dosage experience have made it possible to administer brincidofovir to several hospitalized Ebola patients under compassionate use protocols.

The second category of antivirals that have been administered to patients in the current epidemic, or are being considered for use, are compounds that have been developed specifically for the treatment of filovirus infections, but for which human safety data are lacking. Because these drugs were still in preclinical evaluation at the time the West African epidemic became a major concern, large-scale production has not taken place, and only limited quantities are available.

The experimental therapy that has received the greatest attention is ZMapp, a ''cocktail'' of three monoclonal antibodies targeting the Ebola surface glycoprotein (Qiu et al., 2014). Treatment with ZMapp prevented the death of Ebola-infected macaques when initiated after the animals had already developed fever and viremia, a later time point than has been achieved with any other product. Because of extensive experience with monoclonal antibody therapies in humans, compassionate use of ZMapp was approved for two American healthcare workers infected while treating Ebola patients in Sierra Leone. Both survived, but whether therapy contributed to the outcome is not known. To date, seven Ebola patients have received ZMapp, and two have died. Further treatment cannot proceed until more drug has been produced.

A second approach is a small interfering RNA product, TKM-Ebola, developed by the Tekmira company. A 2010 study found that treatment with siRNA targeting mRNA encoding the Ebola virus polymerase, begun soon after virus challenge and given in multiple daily doses, prevented the death of infected nonhuman primates, and equivalent molecules have also proven effective in Marburg virus-infected macaques (Geisbert et al., 2010; Thi et al., 2014). The observation of fever in some subjects in a Phase I trial caused TKM-Ebola to be placed on clinical hold, but it was still considered sufficiently safe and potentially effective to be administered to an American doctor infected in West Africa.

Antisense molecules (phosphorodiamidate morpho-lino oligomers, or PMOs) targeting filovirus mRNA sequences have also proven protective in macaques infected with Ebola or Marburg virus (Iversen et al., 2012). Sarepta Therapeutics recently reported the encouraging results of single-ascending-dose studies of two combinations of PMOs (Heald et al., 2014).

Another promising Ebola therapy is the nucleoside analogue BCX-4430, which was described in an invited lecture at ICAR earlier this year. The compound was highly effective in rescuing macaques infected with an otherwise lethal dose of Marburg virus, a related filovirus (Warren et al., 2014). The results of testing against Ebola have not been published, but it seems reasonable to expect that the compound will be active. BCX-4430 has not yet been evaluated for safety in humans, but Phase I trials are planned.

The other area in which the West African epidemic is driving rapid progress is the evaluation of Ebola

vaccines. Research over the past two decades has resulted in the development of at least half a dozen candidate vaccines, all of which have protected nonhuman primates against the disease. However, only two have elicited good protection after a single dose, which would be highly desirable in an emergency setting such as the current outbreak. These two products, a replication-defective chimpanzee adenovirus encoding the Ebola surface glycoprotein and a recombinant vesicular stomatitis virus with a similar gene insert, are now in expanded safety trials. These are discussed in more detail in the following article by Anthony Vere Hodge.

The Ebola epidemic will clearly be with us for months to come, and it will be a major focus of discussion at the upcoming ICAR in Rome. I hope that by that time, reports from the field will show the number of cases to be declining, if not reduced to zero. I also hope that this tragic event will result in the development and widespread use of effective drugs against Ebola virus and other highly pathogenic agents, and that it will lead to more effective preventive measures against other viral threats.

Thoughts and opinions are those of the author, and not of the US National Institutes of Health.

Geisbert TW, et al., 2010. Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study. Lancet 375:1896–905.

Heald, A, et al., 2014. Safety and pharmacokinetic profiles of phosphorodiamidate morpholino oligomers with activity against Ebola virus and Marburg virus. Antimicrobial Agents Chemother 58:6639–6647.

Iversen PL, et al., 2012. Discovery and early development of AVI-7537 and AVI-7288 for the treatment of Ebola virus and Marburg virus infections. Viruses 4:2806–30.

Oestereich, L, et al., 2014. Successful treatment of advanced Ebola virus infection with T-705 (favipiravir) in a small animal model. Antiviral Res. 105:17–21.

Qiu, X, et al., 2014. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature 514:47–53.

Smither, S, et al., 2014. Post-exposure efficacy of oral T-705 (Favipiravir) against inhalational Ebola virus infection in a mouse model. Antiviral Res. 104:153–5.

Thi, EP, et al., 2014. Marburg virus infection in nonhuman primates: Therapeutic treatment by lipid-encapsulated siRNA. Sci Transl Med. 6:250ra116.

Warren, TK, et al., 2014. Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. Nature 508:402–5.

A personal view: From cozy research to the harsh realities of Ebola

Anthony Vere Hodge, Reigate, UK

Mike Bray has given a good summary of those compounds and vaccines which have shown promising efficacy against Ebola virus. My aim is to provide ISAR members with an account of the difficulties in progressing these potential therapies and the progress which may be expected in the next 3 months (to the next issue of ISAR News). I have been fortunate to attend many ICARs and, for several years, the ISAR President has asked me to write the scientific report. The report on the 2014 ICAR in Raleigh, NC can be downloaded at http://dx.doi.org/10.1016/j.antiviral.2014.08.009. For more information on presentations, please visit the ISAR web site (http://www. isar-icar.com). Over the years, there have been many presentations which, either directly or indirectly, help me to assess the information currently available on the internet. Necessarily, as facts are in short supply, I shall be expressing my personal opinions.

The 10th ICAR (1997) was held in Atlanta, GA, USA. At the end of the conference dinner, two ISAR members, both talented amateur musicians, wrote and performed a song about antiviral research, including research on Ebola virus. It was very well received by us attendees. The song looked forward to the day when there would be good therapies for all viruses. Since 1997, what has been done to fulfil that aspiration?

During those intervening years, huge progress has been achieved in many areas of antiviral research, for example in the treatment of HIV, HBV and HCV. In contrast, progress with Ebola has been

limited. I think that it is easy to be critical of this lack of progress. However, one should take into consideration that, since the first known outbreak in 1976, there have been many years without any known human cases. All previous outbreaks have been controlled by careful isolation of Ebola patients. At the 24th ICAR, 2011, in Sofia, Bulgaria (Vere Hodge, 2011), Mike emphasised the difficulty in evaluating potential antivirals for Ebola. First discovered in 1976, there had been <3,000 cases identified before 2014, with only six outbreaks having >100 cases. All were brought to an end within weeks to a few months, making it impossible to plan and carry out antiviral drug trials.

Despite all the difficulties and competing priorities, various organisations, including the NIH, USA, have continued to support research on Ebola. For example, specialised testing facilities have enabled compounds to be tested in cell culture and in animal models. In the article above, Mike has summarized some lead compounds which have been shown to have anti-Ebola activity. This work has been continuing, albeit without an apparent sense of urgency. As the 2014 epidemic increased, there was the ethical question about sending untested drugs to treat people who would be in no position to give informed consent. In August this year (2014), The World Health Organisation (WHO) issued a statement: ''In the particular circumstances of this outbreak, and provided certain conditions are met, the panel reached consensus that it is ethical to offer unproven interventions with as yet unknown efficacy and adverse effects, as potential treatment or prevention.''

This guidance seems to have been well received by the antiviral research community. It stimulated the move from a cozy research timetable to the harsh realities of this Ebola outbreak in which the number of new cases is doubling every 4 weeks, as estimated by WHO. I draw two conclusions. Firstly, to have a real impact on the current outbreak, any vaccine or drug must be made available in large quantities. Secondly, this is the first outbreak that continues to have, unfortunately, sufficient new cases to enable the running of clinical trials. It is my hope that it is also the last such outbreak – any future outbreak, hopefully, will be quickly controlled by a combination of containment and stockpiles of at least one vaccine and of at least one drug.

Prior to 2014, the sporadic and unpredictable nature of Ebola outbreaks made it virtually impossible to evaluate the efficacy of any potential vaccine in humans. Fortunately, some candidate vaccines have shown promising results in pre-clinical (non-human) studies. Mike identifies two products, a recombinant chimpanzee adenovirus encoding the Ebola surface glycoprotein and a recombinant vesicular stomatitis virus (VSV) with a similar gene insert, which have elicited good protection after a single dose. In collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), these vaccines are now in expanded safety trials. To my knowledge, only the former, an investigational vaccine being developed by GlaxoSmithKline (GSK), (http://www.gsk.com/en-gb/our-stories/health-forall/our-contribution-to-the-fight-against-ebola/) is expected to have the initial data on safety and immunogenicity from these expanded Phase I trials by the end of this year (2014).

The experimental VSV-Ebola vaccine (VSV-ZEBOV), being developed by NewLink Genetics Co., is also expected to have initial safety and immune response data, from two small dose-ranging Phase I trials, by the end of 2014 (http://www.nih.gov/news/health/oct2014/niaid-22.htm). While NIAID tests the VSV-ZEBOV vaccine candidate as a prime-boost strategy, the Walter Reed Army Institute of Research (WRAIR) is evaluating the vaccine as a single injection. On the NewLink Genetics company web site (http://newlinkgenetics.com/development-pipeline), there is no mention of plans to start large scale production. However, in an interview with Science, NewLink CEO Charles Link mentioned the possibility of having many doses available by April, 2015, but admitted there were still unresolved concerns about vaccinees transmitting VSV to livestock (Link and Cohen, 2014).

Rather than wait for their Phase I trial results, GSK has started manufacturing 10,000 doses, to be completed within the next few months. If the Phase I trials are successful, Phase II clinical trials can be started early in 2015 and will involve thousands of front-line workers in Sierra Leone, Guinea and Liberia. The next stage will be to give the vaccine to people at high risk of infection with the aim to limit further spread of the epidemic.

To my knowledge, there have been no completed Phase II trials of any anti-Ebola drugs. The design of clinical trials needs urgent consideration. It would be helpful if all trials followed a similar format so that reasonable estimates of relative efficacies will be possible. I wonder what will be the design of a clinical trial in Ebola virus-infected patients. Conventionally, double-blind, placebo-controlled trials have been used to assess the efficacy of a new drug treatment against the current standard of care. As the current standard of care results in 50 to 80% mortality, such an approach seems to be ethically unacceptable. A different approach is needed.

At the recent 27th ICAR (Raleigh, NC, USA), Myron Cohen described the clinical trial organised by the HIV Prevention Trials Network (HPTN) in 2005. The couples were randomised to have either immediate or delayed antiretroviral therapy (ART). Both groups received the same care. Such an approach

could be considered for patients with Ebola - perhaps patients presenting early with low grade fever could be randomised to receive active drug either immediately or after a short delay. Certainly the fever could be monitored and it would be useful, if it could be arranged, to have measurements of serum viral titers. Discussions may be ongoing but I have not seen any news of different research groups and regulatory agencies getting together to reach consensus on a clinical trial design. Perhaps the WHO are in a good position to co-ordinate such discussions.

Brincidofovir (CMX001) is being evaluated in clinical trials for efficacy against herpesvirus, CMV and adenovirus infections. It seems that the production of brincidofovir is well able to supply hundreds of doses needed for these clinical trials. Perhaps, there may be sufficient brincidofovir to treat health care workers who become infected with Ebola but I doubt that production capacity is sufficient to treat many patients.

To my knowledge, favipiravir (formerly known as T-705, now also know by the brand name Avigan), is the only drug with both evidence of activity against Ebola virus and which has the production capacity to impact the current outbreak. Toyama Chemical Co., a subsidiary of Fujifilm Holdings Corp., is progressing favipiravir for influenza. In March this year, favipiravir was approved in Japan for influenza. Given the possible efficacy against Ebola, WHO have given their approval for its use. Toyama Chemical Co. announced on 10th October 2014 (http://www.fujifilm. com/news/n141020_02.html) that there are stocks of Avigan Tablets for 20,000 courses as well as an additional stock of active pharmaceutical ingredient which will be used to produce sufficient favipiravir to treat 300,000 patients. It is hoped to start clinical tests on Ebola patients in Guinea by mid-November. It is intended to provide favipiravir for free to treat Ebola patients in Africa.

The plan for favipiravir is most welcome news but will it greatly impact this current outbreak? Unfortunately, research is rarely that simple. A recent press conference announcement from NIAID (http://www.c-span.org/video/?c4511940/favipiravir-doesntwork) reported that a test of favipiravir in monkeys gave some delay in time to death but no increase in survivors. It was suggested that favipiravir was ineffective since there was no increase in survival rates. To date, the full details on these studies have not been reported. Nevertheless, I suggest that it is important to consider various factors before dismissing as insignificant the finding that there was a delay in time to death.

At the 24th ICAR in 2011 in Sofia, Bulgaria, the Prusoff Awardee, Brian Gowen, described research on arenaviruses (Vere Hodge, 2011). Work on highly pathogenic arenaviruses must be performed in BSL-3 or -4 containment but Pichinde virus (PICV) infections of hamsters and guinea pigs may be studied in BSL-2, making them convenient models for initial research. In the hamster PICV model, favipiravir (T-705) gave 100% protection when treatment was initiated 5 days after infection. However, there was less efficacy when treatment was delayed until day 6 or 7, just prior to when the animals began to succumb to infection. This decreased efficacy may be explained by pharmacokinetic studies in uninfected and infected hamsters. At day 7 after infection, the time (Tmax) of peak plasma concentrations of favipiravir was much delayed in infected hamsters (from a few minutes to approximately 1 h) and the plasma concentrations were reduced. This finding illustrates how important it is to evaluate a drug not just in healthy human subjects but also in patients with the target infection.

There are numerous challenges with conducting studies in monkeys within BSL-4 containment, which could have negatively affected the outcome of the study with favipiravir. For example, evaluation of oral favipiravir is less than ideal as the monkeys would require repeated anesthesia for oral dosing, which would be very hard on the animals and certainly impact their ability to survive Ebola virus challenge. In reality, patients suffering from Ebola hemorrhagic fever would not likely be able to keep down oral medications, and therefore intravenous, intramuscular or subcutaneous delivery formulations would be the most relevant to treating such patients. To this end, monkey studies should be designed to evaluate favipiravir and other test therapies considering the most likely human treatment scenario and, if possible, also include some supportive therapy as would be given to patients (Brian Gowen, personal communication).

In 1997, the ''antiviral song'' was just an aspiration. Today, we are faced with the harsh realities of an Ebola outbreak in which the number of new cases is doubling every 4 weeks, as estimated by WHO. The GSK vaccine and favipiravir are being produced with an urgency and on a sufficiently large scale to have a marked effect on this outbreak. Let us hope that there will be good news to report at the 28th ICAR in Rome.

Link C Jr., Cohen J, 2014. Infectious diseases. The Ebola vaccine underdog. Science 346:534.

Vere Hodge A, 2011. Scientific report: highlights of 24th ICAR, 8–11 May, 2011, Sofia, Bulgaria. Antiviral Chem Chemother 22:75–85.

Vere Hodge R.A, 2014. Meeting report: 27th International Conference on Antiviral Research, in Raleigh, NC, USA. Antiviral Res. 111:143–153.

ISAR MEMBER PROFILES

Nesya Goris

Most of us like cats, but not many of us have the chance to develop new treatments for cat diseases. Nesya Goris is one of the lucky few who does both. As senior director for antivirals at Aratana Therapeutics, she guides programs aimed at developing therapies for feline herpesvirus, feline immunodeficiency virus and feline calicivirus infections, together with parallel efforts targeting diseases of dogs.

Looking back at her education and work history, Nesya notes that she arrived at her present position by a very indirect route. In high school in Heverlee, Belgium, she followed an intensive course of Greek and mathematics, and after entering university, she first pursued acting, then engineering. Trying to settle on a career, she eventually realized that one of her strongest and most constant passions was a love of animals (she's always had pets, and now owns two cats, a dog and a horse). This led her to dream of becoming a professional dolphin trainer, and on being told that such work required a thorough grounding in biology, she promptly switched to a science curriculum, eventually receiving a master's degree in biology in 2002.

Unfortunately, the Belgian dolphinarium had no openings for trainers at the time she graduated, so she discussed her situation with her veterinarian, who directed her toward the Veterinary and Agrochemical Research Centre (CODA-CERVA), which acts as the Belgian National Reference Laboratory for animal diseases.

This proved to be the turning point. The opportunity to study foot-and-mouth disease (FMD) and other infections of livestock was irresistible, and after applying to CODA-CERVA for any sort of position, Nesya was hired as a researcher in 2002. Her first project focused on antigenic profiling and molecular characterization of FMDV proteins. She also received funding to work on a unique antiviral approach to the control of FMD, through the development of potent and selective inhibitors of picornavirus replication, in collaboration with the team of Johan Neyts at the Rega Institute in Leuven. Her research led to the award of a Ph.D. in Veterinary Sciences at the University of Gent in 2008.

The timing was right – her graduation coincided with the founding by Leuven University of a spin-off company, Okapi Sciences, with the unique slogan "Antivirals for Animals", and Nesya was hired as chief scientist. Encouraged by the expanding use of antiviral drugs for human diseases, Okapi Sciences had the goal of introducing antiviral therapy into veterinary medicine. Even though millions of cats and dogs are infected by viruses each year, there are no approved species-specific antivirals. Based on the well established treatment of human herpesvirus infections, Nesya and her colleagues perceived an opportunity to develop similar therapies for ocular herpesvirus disease in cats and for the lethal herpesvirus infections of ornamental carp (koi), prized by collectors in many countries.

In addition to treating these and other conditions of companion animals, there was also the intriguing possibility that antiviral drugs could be used to intervene swiftly in outbreaks of livestock diseases. FMD and classical swine fever (CSF) pose continuous threats to livestock populations, especially in areas of high farm density such as the Netherlands. When a virus introduction is detected, the minimal control strategy imposed by the European Union is often insufficient to halt the epidemic. Additional measures such as preemptive culling encounter ethical objections, and, because the antibody response induced by emergency vaccination may make it impossible to rule out recent infection, vaccination may result in prolonged export restrictions. In contrast, immediate antiviral prophylaxis could potentially give instantaneous protection, without inducing an antibody response.

Experimental validation of this approach has been obtained in CSFV-infected pigs treated with an oral antiviral (Vrancken et al., 2009) The molecule significantly reduced the level and duration of viremia in treated pigs, and also blocked transmission to uninfected sentinels. These results served as input parameters in a stochastic model describing within- and between-herd transmission, which simulated epizootics in an area in The Netherlands with farms of varying sizes and pig types (Backer et al., 2013). Modeling showed that, once an introduction of CSFV was

detected, antiviral treatment of all herds within a 2-km radius was as effective as vaccination in halting an outbreak, and was more effective than preemptive culling in a 1-km radius, both from an epidemiological and economic perspective. Similar results were obtained for the Belgian setting (Ribbens et al., 2012).

During its first five years, Okapi Sciences grew from a nucleus of five people to a company of 19, most of whom were involved in research and development. In 2013, Okapi signed an agreement with Novartis Animal Health for the joint development and commercialisation of a drug for feline herpesvirus infections, the first time a small-molecule antiviral was developed specifically for veterinary use. Okapi also had another feline therapeutic under clinical evaluation, with several other candidate drugs in the pipeline to treat cat and dog diseases. At the same time, the concept of using antivirals to contain viral diseases in livestock continued to make progress through the laboratory evaluation of candidate drugs (Osiceanu et al., 2014) and the theoretical modeling of outbreaks, leading to several publications.

Earlier this year, Aratana Therapeutics saw the commercial potential of antiviral therapies for cats, dogs and other pets and acquired Okapi Sciences. Aratana has a broad portfolio aimed at developing innovative therapies for osteoarthritis, pain, cancer and other conditions. In addition to her continuing work on antivirals for animal diseases in the Leuven laboratory, Nesya serves as Aratana's vice-president for discovery research. She welcomes inquiries regarding possible collaborations, and can be reached at ngoris@aratana.com.

Backer JA, Vrancken R, Neyts J, Goris N, 2013. The potential of antiviral agents to control classical swine fever: a modelling study. Antiviral Res. 99:245–50.

Osiceanu AM, Murao LE, Kollanur D, Swinnen J, De Vleeschauwer AR, Lefebvre DJ, De Clercq K, Neyts J, Goris N, 2014. In vitro surrogate models to aid in the development of antivirals for the containment of foot-and-mouth disease outbreaks. Antiviral Res. 105:59–63.

Ribbens S, Goris N, Neyts J, Dewulf J, 2012. Classical swine fever outbreak containment using antiviral supplementation: a potential alternative to emergency vaccination and stamping-out. Prev Vet Med. 106:34–41.

Vrancken R, Haegeman A, Dewulf J, Paeshuyse J, Puerstinger G, Tignon M, Le Potier MF, Neyts J, Koenen F, 2009. The reduction of CSFV transmission to untreated pigs by the pestivirus inhibitor BPIP: a proof of concept. Vet Microbiol. 139:365–8.

Ashoke Sharon

Dr. Ashoke Sharon directs the program of Drug Discovery Education and Research (http://www.dder.in) in the Department of Chemistry at the Birla Institute of Technology (BIT), Ranchi, Jharkhand, India. His laboratory is the first at the Institute to be dedicated to antiviral drug discovery, and one of few such labs in India.

Ashoke performed his doctoral research in medicinal chemistry at the CSIR-Central Drug Research Institute in Lucknow, India. After receiving his Ph.D. in chemistry, he began postdoctoral work in David Chu's Drug Discovery Group at the University of Georgia, focusing on the design and synthesis of new chemical entities as antiviral candidates. He attended his first ICAR in 2007, in Palm Springs, where he won first prize for a postdoc in the poster competition.

Ashoke notes that attending ICAR gave him a unique opportunity to talk with a number of eminent antiviral researchers, including Eric De Clercq, Masanori Baba, Chris Meier, Dale Barnard and Chris McGuigan. That experience, and the ''extraordinary'' support from his advisor, David Chu, helped to propel him towards a career in antiviral research.

In 2009, Ashoke returned to India to become an assistant professor in the Department of Chemistry at BIT, Mesra. This renowned academic and research center, located in Ranchi, the capital of Jharkhand State. It was established in 1955 by the industrialist B. M. Birla as a focus for mobilizing the enormous intellectual capital of India. The Institute has become a premier destination for engineering and scientific research, and has made significant forays into international academics, by entering into international ventures and collaborations with world-renowned universities.

Once at the BIT, Ashoke initially found it challenging to establish a new section for antiviral drug development in the Chemistry Department. However, with encouragement from the department and institute administration, together with financial support from Indian government research grant agencies such as the Department of Science and Technology (DST), the Department of Biotechnology (DBT) and the University Grants Commission (UGC), he was able to establish a new section for antiviral medicinal chemistry. The DDER program was initiated through the mentorships of Drs. Debashis Mitra in the area of HIV biology, Debprasad Chattopadhyay for herpes simplex virus and Masanori Baba in hepatitis B and C.

The DDER team. Row 1, left to right: Mr. Rahul K. Jha, Ms. Monkia Yadav, Dr. A. Sharon, Dr. Chandralata Bal, Ms. Afsana Parveen. Row 2: Ms. Farhana Rozy, Mr. Kaustav Sahoo, Mr. Tanmoya A. Pradhan, Mr. Rajan Kumar.

Ashoke's research team focuses primarily on understanding the 3-dimensional structure of biological macromolecules and recognition of the chemical scaffold as the essential first step in antiviral drug discovery. Their overall objective is to conduct synergistic use of structural chemistry and biochemistry information involving computer-aided approach for the synthesis of novel antiviral molecules, directed mainly against HIV, herpes simplex virus and hepatitis B and C viruses.

The DDER group recently revealed the molecular balancing phenomenon in HIV integrase by raltegravir, using triple aromatic stacking recognition mode (Figure 1), and also highlighted the molecular basis of resistance to raltegravir and the role of the flexible loop in HIV-integrase (Balaraju et al., 2013). Ashoke's team has also employed a conformational mimetic approach for the design of new carbocyclic nucleosides, based on the key role of conformation (sugar ring and base disposition) to have better binding with the viral polymerase as a competitive inhibitor (Kasula et al., 2013). Because the correct conformation depends on viral polymerase selectivity, the HCV RdRp and the HBV polymerase will have different conformational requirements.

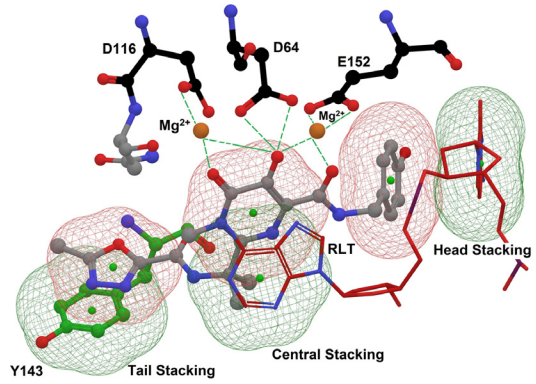


Figure 1. The triple aromatic stacking system (head, central, and tail) involves the $\pi\ldots\pi$ interactions mediated by raltegravir (RLT) to yield a stable WT HIV IN–RLT complex (Balaraju et al., 2013).

In addition to nucleosides, the DDER group is also working to synthesize non-nucleoside antiviral molecules using viral target protein and ligand structure analysis. They have recently identified a novel pyranone carboxamide as a potential anti-HCV scaffold (Konreddy et al., 2014). Further, they have used a structure-based approach to optimize the molecules as promising anti-HSV agents. (Karampuri et al., 2014) Thus, the discovered scaffold by DDER group has potential against viral infections and should be exploited for related RNA viruses such as chikungunya and dengue. This should make it possible for the DDER team to extend their development of new antivirals by collaborating with biologists in the area of these emerging RNA viruses.

Ashoke notes that medicinal chemistry is very strong in India, especially at the Birla Institute. Looking to the future, he says that "the increased involvement of Indian industry and government grant agencies suggests the possibility of substantial growth in antiviral research." He notes that the silent burden of viral hepatitis is a growing concern in India, while outbreaks of dengue and chikungunya present an urgent need for antivirals. Ashoke would be especially glad to hear from ISAR members who could help with preclinical evaluation of new drugs against these diseases. He can be reached at asharon@bitmesra.ac.in.

Balaraju T., et al., 2013. Aromatic interaction profile to understand the molecular basis of raltegravir resistance. Structural Chem. 24:1499–1512.

Karampuri, S., et al., 2014. Anti-HSV activity and mode of action study of á-pyrone carboxamides. RSC Advances 2014, 4, 17354–17363.

Kasula A., et al., 2013. A conformational mimetic approach for the synthesis of carbocyclic nucleosides as anti-HCV leads. ChemMedChem 8:1673–1680.

Konreddy A., et al., 2014. Synthesis and anti-HCV activity of 4-hydroxyamino á-pyranone carboxamide analogues. ACS Med. Chem. Lett., 5:259–263.

ANTIVIRALS ON THE HORIZON

Latent herpesvirus infections: could epigenetic modulation prevent reactivation?

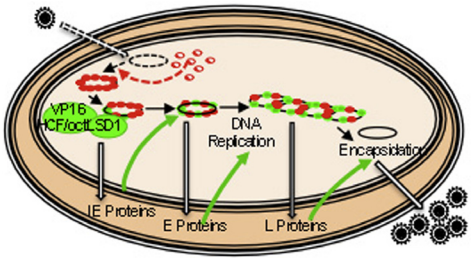
Luis Schang, Professor, Department of Biochemistry, U. of Alberta, Canada

Most antivirals have been developed to target specific viral proteins, an approach that has resulted in 57 approved drugs and many under development. However, these antivirals have proven to be ill suited against latent viral infections, such as HIV or herpes simplex viruses 1 and 2 (HSV-1/2). When latent, these viruses express no proteins to be targeted. No drug therefore inhibits their reactivation, resulting in the need for life-long or recurrent treatment. A great deal of research has focused on viral latency and reactivation, with a recent emphasis on epigenetic regulation. This has led to a new paradigm, in which drugs are used to prevent reactivation from latency.

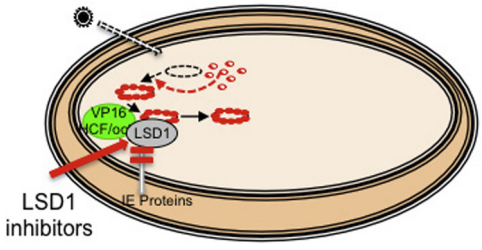
My lab was among the first to consider epigenetic regulation in lytic HSV-1 infections, when we discovered that the same promoters are differentially regulated if they are located in the viral genome or in the genome of the infected cell. We noticed that a small-molecule protein kinase inhibitor which inhibited expression of HSV-1 IE genes did not inhibit expression of a reporter GFP driven by the ICP0 promoter recombined in the genome of the same cell (Diwan et al, 2004).

More recently, we found that HSV-1 chromatin is highly dynamic (Lacasse and Schang, 2012, Conn et al., 2013), which brought together the apparently contradictory results of chromatin immunoprecipitation and nuclease protection assays from many other groups. The finding of the dynamic nature of HSV-1 chromatin was critical to the now general acceptance that chromatin plays a major role in the regulation of HSV-1 gene expression during lytic infections.

"Epigenetics" broadly describes the mechanisms that regulate gene expression by modulating chromatin structure. Three types of proteins participate in epigenetic regulation: the "writers", which add covalent modifications to the histones or DNA; the "erasers", which remove them; and the "readers", which interact with the modified histones or DNA to exert the action.



Above: cells attempt to silence infecting HSV-1 genomes by assembling silenced chromatin onto them. However, the virus recruits LSD1 and other chromatin remodelling factors to the IE promoters via VP16/oct1/HCF, allowing expression of the IE proteins, which then induce further remodelling of viral chromatin to activate transcription of E and L genes. Reactivation of transcription of latent HSV-1 genomes also requires LSD1-dependent chromatin remodelling. (Red: silenced nucleosomes. Green: transcriptionally active nucleosomes. Dashed lines: events that occur during lytic infection, but not reactivation).



Above: LSD1 inhibitors block LSD1-dependent chromatin remodelling, preventing HSV-1 gene expression and reactivation.

Some histone modifications are generally repressive for transcription, while others are generally activating. For example, methylation of histone H3 lysine 9 (H3K9) is usually associated with transcription repression, whereas methylation of H3K4 is usually associated with activation. H3K4me2 and H3K9me2 can

both be demethylated by LSD1, the first identified histone demethylase (Kooistra et al., 2012). LSD1 uses a FAD-mediated amine oxidase reaction, which requires a free electron pair, to demethylate its substrates. The chemistry of the reaction is well understood, as is the inhibition by mono-amino oxidase (MAO) inhibitors that make covalent adducts with the FAD co-factor (Suzuki et al., 2011).

The structure of LSD1 has been solved in multiple complexes (Andreoli et al., 2013). LSD1 is overexpressed in prostate cancer, undifferentiated neuroblastoma, ER^- breast cancer, bladder cancer, lung cancer and colorectal cancers, and is required for acute myeloid leukemia (AML) (Kooistra et al., 2012; Helin et al., 2013). There are good *in vitro* and cell-based assays for LSD1 inhibitors. LSD1 has thus become the target of several drug development programs. Two of the most advanced inhibitors, GSK2879552 and ORY-1001, have entered Phase I/IIA clinical trials in the USA and Spain.

Lytic and reactivating HSV-1/2 infections are regulated in part by epigenetic modifications. A body of biochemical, genetic and cell biology evidence strongly supports a model in which cells attempt to inhibit the expression of HSV-1/2 virus genes by assembling the infecting viral genomes in silenced chromatin (Conn et al., 2013; Oh et al., 2014). However, viral transcription activators disrupt these silencing mechanisms to activate viral gene expression and replication. For example, the VP16 recruits the cellular cofactor HCF, which brings a complex containing the histone demethylase LSD1 and the histone methyl transferase Set1 or MLL1. LSD1 removes the inactivating H3K9me2, allowing Set1 or MLL to then add the activating H3K4me3.

Other HSV-1 transcription activators, ICP0 and ICP4, further disrupt viral chromatin. HSV1/2 chromatin is consequently highly dynamic This dynamic chromatin cannot silence viral gene expression any longer, and the virus replicates (Conn et al., 2013; Lacasse et al., 2012). In contrast, the chromatin of latent genomes is stable and harbors silencing markers (Bloom et al., 2010; Cliffe et al., 2013). The mechanisms resulting in the formation of silenced chromatin during the establishment of latency are still only poorly understood, but those governing remodeling during reactivation appear similar to those during lytic infections. The major difference is that VP16 is not present early during reactivation. However, HCF is still recruited to the latent viral genomes, bringing LSD1 to remove the di-methyl group in H3K9.

The importance of HSV-1/2 epigenetics has recently started to be probed with LSD1 inhibitors. Early experiments used mostly non-specific inhibitors, such as (±)-*trans*-2-phenylcyclopropan-1-amine (trancyl promine, TCP) (Liang et al., 2009), which were originally developed as MAO inhibitors for the treatment of depression, but were later found to also inhibit LSD1. TCP inhibited HSV-1/2 replication and gene expression in culture, with the expected increases in the association of the HSV-1/2 genomes with the silencing H3K9me2, and stabilization of the viral chromatin.

More recently, the effects of specific, albeit not too potent, LSD1 inhibitors have begun to be tested. OG-L002 inhibited HSV-1/2 replication at micromolar concentrations, whereas concentrations up to 50 μM of clorgyline inhibited MAO, but not LSD1 (Liang et al., 2013). In a mouse model, 40 mg/kg of OG-L002 ip daily starting 7 days before infection and continuing after infection resulted in 5- to 10-fold lower levels of HSV-2 genomes in the trigeminal ganglia (no clinical or virological data was reported). Moreover, *ex vivo* HSV-1 reactivation in neurons explanted from latently infected mice was also inhibited at 10–50 μM OG-L002. These results invited more extensive pre-clinical evaluation of LSD1 inhibitors in animal models.

After several years of basic discovery science, the potential clinical applications of viral epigenetic modulation are starting to become visible. This emergence has been fostered by a series of bi-annual conferences on ''Chromatin Control of Viral Infection'' (CCVI) hosted by the NIH since 2010. At the recent meeting on September 18–19, a collaboration of several of the most prominent groups testing anti-herpetic antivirals presented the results of the first evaluation of LSD1 inhibitors in a variety of pre-clinical models representing all stages of clinical HSV infections, from acute infection to reactivation. The results appear most encouraging. Considering that clinical trials of highly specific LSD1 inhibitors are on-going, one would expect the clinical effects of epigenetic inhibitors as antiviral drugs to be tested soon.

The importance of epigenetic regulation is not limited to HSV-1/2. LSD1 itself is also required for the lytic infection of other nuclear DNA viruses that establish latent infections, such as HCMV (Liang et al., 2013). It is tempting to speculate that an epigenetic drug could be used to target an oncogenic virus and its oncogenic mechanism at the same time. At the very least, the testing of epigenetic drugs against viral tumors would allow the prompt clinical exploration of their antiviral activities. Encouragingly, recent results (Liang et al., 2013 and CCVI 2014) also support an important role for epigenetics in the regulation of EBV and KSHV lytic replication, latency or reactivation.

In summary, a novel paradigm is emerging in the antiviral therapy of latent infections. It may be possible to use epigenetic modulators to maintain

latent viral genomes in a repressed state, preventing the periodic recurrence of disease. Current clinical trials of LSD1 inhibitors and data accumulating on the effects of LSD1 inhibitors on HSV-1/2 reactivation in animal models hint that the potential of epigenetic regulators of viral latency may soon become clear.

Andreoli F, et al.: Modulation of Epigenetic Targets for Anticancer Therapy: Clinicopathological Relevance, Structural Data and Drug Discovery Perspectives. *Current Pharmaceutical Design* 2013, 19:578–613.

Bloom DC, et al.: Epigenetic regulation of latent HSV-1 gene expression. *Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms* 2010, 1799:246–256.

Cliffe AR, Coen DM, Knipe DM: Kinetics of Facultative Heterochromatin and Polycomb Group Protein Association with the Herpes Simplex Viral Genome during Establishment of Latent Infection. *Mbio* 2013, 4.

Conn KL, Hendzel MJ, Schang LM: The Differential Mobilization of Histones H3.1 and H3.3 by Herpes Simplex Virus 1 Relates Histone Dynamics to the Assembly of Viral Chromatin. *Plos Pathogens* 2013, 9.

Conn KL, Schang LM: Chromatin Dynamics during Lytic Infection with Herpes Simplex Virus 1. *Viruses-Basel* 2013, 5:1758–1786.

Diwan, P. et al. Roscovitine inhibits activation of promoters in the HSV-1 genome independently of promoter-specific factors. J Virol 78:9352–9365.

Helin K, Dhanak D: Chromatin proteins and modifications as drug targets. *Nature* 2013, 502:480–488.

Kooistra SM, Helin K: Molecular mechanisms and potential functions of histone demethylases. *Nature Reviews Molecular Cell Biology* 2012, 13:297–311.

Lacasse JJ, Schang LM: Herpes Simplex Virus 1 DNA Is in Unstable Nucleosomes throughout the Lytic Infection Cycle, and the Instability of the Nucleosomes Is Independent of DNA Replication. *Journal of Virology* 2012, 86:11287–11300.

Liang Y, et al.: Inhibition of the histone demethylase LSD1 blocks alpha-herpesvirus lytic replication and reactivation from latency. *Nature Medicine* 2009, 15:1312-U1112.

Liang Y, et al.: A novel selective LSD1/KDM1A inhibitor epigenetically blocks herpes simplex virus lytic replication and reactivation from latency. *MBio* 2013, 4:e00558–00512.

Oh HS, et al.: A Targeted RNA Interference Screen Reveals Novel Epigenetic Factors That Regulate Herpesviral Gene Expression. *Mbio* 2014, 5.

Suzuki T, Miyata N: Lysine Demethylases Inhibitors. *Journal of Medicinal Chemistry* 2011, 54:8236–8250.

Tsai K, et al.: Viral Reprogramming of the Daxx-Histone H3.3 Chaperone During EBV Early Infection. *Journal of Virology* 2014, *in press*.

ISAR ELECTIONS

Candidates for the ISAR Board of Directors

This year the Nominations Committee was charged with finding four candidates to fill two Board seats currently held by Roger Ptak and Rhonda Cardin, whose terms expire at the end of the 2015 ICAR. Roger and Rhonda have both agreed to run for a second term, and Anneke Raney and Simon Tucker have also agreed to be candidates. All of them has given of their time and talents to the Society and would make excellent Board members.

The election is being held through the ISAR website beginning on November 20th and continuing for one month. We strongly encourage all members to vote, and wish all of these excellent candidates the best of luck.

Rhonda Cardin

Rhonda is an associate professor at Cincinnati Children's Hospital Medical Center. She received her A.B. from Washington University in St. Louis in 1983, then began her PhD studies at Tulane University in New Orleans. After the lab moved to Louisiana State University in Baton Rouge, she received her PhD in microbiology in 1989. She received her postdoctoral training in Ed Mocarski's lab at Stanford University, on cytomegalovirus pathogenesis and latency.

In 1994, Rhonda joined the lab of Dr. Peter Doherty, 1996 Nobel Laureate in Medicine, at St. Jude Children's Research Hospital, to study gammaherpesvirus immunology. In 1998, she joined Park-Davis Pharmaceuticals as a senior scientist to oversee the in vivo herpesvirus antiviral program.

After the Pfizer, Inc, merger with Parke-Davis and a short period at ChemoCentryx, Inc., a chemokine therapeutics company, she returned to academia in 2003. Her lab in Cincinnati studies CMV pathogenesis and latency.

Since 2003, Rhonda has been a co-PI for NIH contract evaluation of antivirals and vaccines in CMV and HSV animal models. In 2009, she was president of the Women's Faculty Association at CCHMC. She serves as a grant reviewer for the NIH and AHA, and is a reviewer for multiple journals, including *Antiviral Research*.

Rhonda joined ISAR in 2003 and has presented at ICAR each year, co-chaired herpesvirus plenary sessions, and currently serves on the finance, membership, publications, and Women in Science committees. For the past three years, she has served on the ISAR Executive Board.

Roger Ptak

Roger Ptak is an accomplished scientist with over 20 years of research and project management experience in antiviral drug discovery and development. He received his BS degree in biology from the University of Notre Dame in 1992. From 1993–99 his work supported the discovery of novel herpesvirus and HIV-1 inhibitors at the University of Michigan.

In 1999 he joined the Southern Research Institute Department of Infectious Disease Research in Frederick, Maryland where he is currently Program Leader for the In Vitro Antiviral Drug Development Program. In this role he manages an extensive staff of scientists and research technicians responsible for execution of in vitro assays and assay development for the discovery and development of antiviral drugs and topical microbicides on multiple large government programs and a wide range of contract research projects for the biotechnology and pharmaceutical industry.

In addition to his work at Southern Research, Roger is affiliated with the Frederick County Hepatitis Clinic, where he serves as Board President and Treasurer. He has co-authored 68 peer-reviewed publications and review articles related to antiviral research, and he is an Ad hoc reviewer for multiple journals related to antiviral drug discovery and development.

As a member of ISAR since 1995, he has actively served the Society through participation on a number of committees including the Web Site and Conference Committees. Roger is currently a member of the Society's Board of Directors, and is the Chairman of the Finance Committee, for which he has helped raise over $1M to support ISAR/ICAR

Anneke Raney

Anneke's career in virology began in hepatitis B virus (HBV) research at The Scripps Research Institute, and she earned a PhD from the University of Warwick studying HBV transcriptional regulation. Anneke initiated the discovery efforts to develop antiviral therapies for the treatment of chronic HBV at ICN/Valeant Pharmaceuticals. As Section Leader at Valeant, Anneke led multidisciplinary teams to drive HBV and HCV programs towards the clinic. As Director of Virology at Ardea Biosciences, Anneke led HIV discovery and pre-clinical programs and supported HIV clinical development. At Gilead, she led projects to explore new approaches to cure chronic HBV infection.

Anneke is currently Associate Director of Alliance and Project Management at Avidity NanoMedicines,

providing strategic input and R&D coordination towards the development of antibody-siRNA complexes (ARCs™). She has co-authored more than 30 peer-reviewed articles, is a patent inventor and has delivered numerous presentations at international antiviral research conferences.

Anneke has been fortunate enough to attend ICAR several times since 2007. She has been an active member of the ISAR Women in Science committee since its inception. She was a moderator of the inaugural 2013 ICAR/WIS Roundtable, helped organize the 2014 Roundtable, and is an ISAR Mentor to two, early-career women scientists. She has also been raising support for the newly established WIS Scholarship Fund.

Anneke chaired the Corporate Sponsorship Committee and was on the organizing committee for the Women in Science and Technology Conference for two years in the San Diego Chapter of the Association for Women in Science.

Simon Tucker

Simon P. Tucker is vice-president for research at Biota Pharmaceuticals, Inc. Biota is an anti-infective R & D company focused on the discovery and development of new drugs. Biota was the originator of the first drug in the neuraminidase class of influenza antivirals and has a particularly strong background in respiratory antivirals.

Before joining Biota, Simon held the position of senior lecturer and head of the Gene Therapy Laboratory at the University of Glasgow, UK and before then Senior Research Investigator, Infectious Disease Research, G.D. Searle Research and Development, St Louis, MO, USA.

Simon received his BSc (Hons) degree in biochemistry from the University of Sussex, UK. He completed his PhD in 1988 through the University of Reading, UK while studying avian influenza in the Director's Group, Institute for Animal Health, Pirbright, UK. His postdoctoral work was undertaken at the University of Alabama, Birmingham, AL, USA where he also served as a Senior Research Fellow before joining Searle.

His background is in virology with a particular interest in the discovery and development of new therapies. He has led multidisciplinary discovery teams and has been associated with numerous potential drugs that reached clinical trials, together with some that achieved registration and marketing. He has also overseen the development and launch of one the first influenza point-of-care diagnostic tests for influenza A and B.

Simon has taken an active in role in ISAR and, together with presenting on numerous occasions and participating in a variety of society initiatives, has served on the Publications Committee for many years.

ICAR 2014 SCIENTIFIC REPORT

The official report on the 2014 meeting in Raleigh, NC, written by Anthony Vere Hodge and reviewed and approved by the ISAR Publications Committee, is available as an Open Access article in *Antiviral Research*:

Vere Hodge R.A., 2014. Meeting report: 27th International Conference on Antiviral Research, in Raleigh, NC, USA. Antiviral Res. 111:143–153.

It can be downloaded for free at http://dx.doi.org/10.1016/j.antiviral.2014.08.009.

ICAR 2015

Update: 28th ICAR in Rome, Italy

The 28th International Conference on Antiviral Research (ICAR) will be held in Rome on May 11-15th, and will commence with a session on Drug Discovery and Development 101. This will be followed by a keynote address by Dr. Rafael De Francesco, head of the virology program at the Fondazione Istituto Nazionale Genetica Molecolare. A keynote presentation on the Ebola epidemic is also planned.

There will be a free afternoon on Wednesday, May 13, to allow participants to network and to take in the sights of one of the most beautiful and historic cities in the world.

ISAR is committed to conducting a meeting of the highest scientific standards for oral and poster sessions. We welcome abstracts of original research in any area of antiviral drug discovery and development. Abstracts will be evaluated for scientific merit by a panel of reviewers. Authors must agree to disclose the structure of any compound for which biological data are presented in a poster or talk.

Online abstract submission

Abstracts for oral presentations and posters must be submitted no later than February 6th. Abstracts are submitted online via the SPLtrak system, following the instructions below. Abstracts will not be accepted in any other manner.

Abstracts are limited to 2500 characters and spaces, including the title, authors/affiliations, and body text. The character limit is 2000 for abstracts that include a figure. The system will automatically impose these restrictions when you enter your abstract.

Instructions for abstract submission

- Create a new abstract submission account or sign in to your existing account.
- On the linked page, click on the "Go to Abstract Submission" link and follow subsequent instructions.
- Note that the title, authors, text, etc. are entered separately.
- After entering the abstract title, click on the "Save" button which takes you to the next task, "Authors". Fill in this section and click "Save".
- This will take you to the section to enter or upload the body of your abstract.
- On the "Abstract Submission" page you may edit or delete your abstract at any time until the abstract is officially submitted. Select "Submit Complete Abstract" under "Status" to submit the final version of your abstract.

Publication of abstracts

The program for the 28th ICAR, including titles of presentations and names of authors, will be posted on the Society's web site on or shortly after March 1, 2015. All attendees are encouraged to submit their posters online, and they will be made available on the website prior to the meeting.

Poster awards competition

ISAR will again sponsor a poster awards competition for graduate students, postdoctoral fellows and young investigators. Further information on awards is available on the ISAR website (http://isar.phrm.cf. ac.uk/node/6) or from Mark Prichard, chair of the program committee, at mprichard@peds.uab.edu.

Any investigator whose abstract is approved for an oral presentation, but would like to compete for a poster award, should prepare a poster and upload it to the website at least 7 days before the meeting. Candidates will present their posters at the meeting, and should have slides available if they are selected for the "shotgun" poster presentation.

Shotgun poster presentations

In our continuing effort to increase the involvement of society members in the annual meeting, ICAR will once again include an oral poster presentation session which will be open to graduate students, postdoctoral fellows, and other young investigators. Poster presenters should bring 3–4 slides summarizing their presentation, as the selection of presenters will be made at the meeting after review of the posters. Two presenters will be selected as co-chairs for this session.

Important ICAR Dates

Abstract submission deadline February 6, 2015
Abstract acceptance notification March 16
Travel grant notification March 20
Late-breaker abstract deadline March 18
Advance rate registration deadline April 17
Registration cancellation deadline April 17

Travel Grants

Applications must be submitted to Graciela Andrei, ISAR secretary (graciela.andrei@rega.kuleuven.be) by February 6th. You must first submit an electronic copy of the abstract you intend to present prior to seeking a travel award. Please submit your abstract early and ask for an early response because you are

seeking a travel award. Travel grants will be awarded only to participants whose abstracts are selected as oral or poster presentations and who attend the meeting and present their work.

CALENDAR OF FUTURE CONFERENCES

Simon Tucker has prepared a calendar of future conferences on antiviral therapy, medicinal chemistry and other topics of interest. ISAR members can access the calendar by logging in to the society website.

ISAR NEWS

ISAR News is an official publication of the International Society for Antiviral Research. It is published four times a year as a free article in *Antiviral Research* (http://www.journals.elsevier.com/antiviral-research) and is posted on the ISAR website (http://www. isaricar.com).

ISAR News is prepared by the ISAR Publications Committee: Anthony Vere Hodge (chair), Masanori Baba, Andrea Brancale, Mike Bray, Rhonda Cardin, José Esté, Joy Feng, Brian Gowen, Justin Julander, Aruna Sampath, Luis Schang, Ashoke Sharon, Bart Tarbet and Simon Tucker.