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Meeting Report

Meeting report: 29th International Conference on Antiviral Research in La Jolla, CA, USA

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ABSTRACT

The 29th International Conference on Antiviral Research (ICAR) was held in La Jolla, CA, USA from April 17 to 21, 2016. This report opens with a tribute to the late Chris McGuigan, a Past-President of ISAR, then continues with summaries of the principal invited lectures. Doug Richman (Elion Award) investigated HIV resistance, Bob Vince (Holý Award) showed how carbocyclic nucleoside analogs led to abacavir and Jerome Deval (Prusoff Award) explained how his group chose to seek a nucleoside analog to treat RSV. ALS-8176 was active in a human RSV-challenge study and is being evaluated in children. The first keynote address, by Richard H. Scheuermann, reported on the remarkable progress made in viral genomics. The second keynote address, by Heinz Feldmann, gave an overview of Ebola virus disease. There were four mini-symposia, *Structural Biology*, *Diagnostic Technologies*, *DNA viruses* and *Zika virus*. Diagnostic assays are approaching an ideal aim, a compact instrument, simple to use with any type of sample, no sample preparation and a result within an hour. The diversity of HCMV is far greater than for other herpesviruses, typically, an individual having >20,000 single nucleotide polymorphisms (SNPs). During antiviral treatment, there is rapid CMV evolution which is presumed to be due to preferential selection of already present variants rather than by the creation of new variants. A selection of contributor presentations includes oral prodrugs for nucleoside triphosphate analogs, a new method for the synthesis of phosphoramidate prodrugs and the clinical evaluation of brincidofovir for treating transplant recipients with adenovirus infections.

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1. Introduction

This article provides an overview of the invited lectures at the 29th International Conference on Antiviral Research (ICAR), sponsored by the International Society for Antiviral Research (ISAR), which was held in La Jolla, CA, USA, from April 17–21, 2016. This year, the meeting opened with a heart-felt tribute by Andrea Brancale to his mentor and colleague, Chris McGuigan who died in March 2016. This meeting summary continues with reports of lectures by the recipients of ISAR's three major awards, held in memory of Gertrude (Trudy) Elion, Antonín (Tony) Holý and William (Bill) Prusoff. These are followed by summaries of the two keynote addresses, the main presentations within the four mini-symposia on *Structural Biology*, *Diagnostic Technologies*, *DNA viruses*, *Zika virus*, and four contributor presentations.

Because this review article simply provides short accounts of oral presentations, it is not generally accompanied by references to the scientific literature. Any descriptions of favorable treatment outcomes should not be taken as a recommendation for clinical use. Generally, I have added my personal comments on the meeting within the conclusion. In a few instances, I have added my own comment within the main text, indicated either by the wording or by the use of square brackets. Although this meeting report covers only a few of the presentations, it aims to illustrate the great diversity of topics discussed at ICAR, bringing together knowledge and expertise from a wide spectrum of antiviral research.

2. Chris McGuigan: a tribute to a remarkable innovator

Andrea Brancale, Cardiff University, Cardiff, UK

Much too young, Chris (Fig. 1) died from cancer on 11th March 2016.

Fig. 1. Christopher (Chris) McGuigan, 1958–2016.

Chris obtained his B.Sc., Chemistry, Hons (Class 1), at the University of Birmingham (1979) and his Ph.D. on Anticancer Drug Design (1982). He moved to the University of Edmonton, Alberta, Canada (1982–84) as a post-doctoral fellow, then to the University of Exeter as Demonstrator (1984–85), to University College London as Lecturer (1985–90), and to the University of Southampton as Lecturer (1990–94). His career at the Welsh School of Pharmacy began as a Reader (1994–95), as Professor (1995–2016) and becoming the Pro Vice Chancellor (2012–3).

As Professor, Chris was an inspirational leader, training >40 Ph.D.s and >50 postdocs, some of whom presented their work at various ICARs. Chris published >220 papers and obtained >100 patents. ISAR selected Chris to be the first person to receive the William Prusoff Young Investigator Award in 2001. Just 3 years later, Chris was elected as the ISAR President-Elect, becoming President two years later (2006–2008). Collaboration with Erik De Clercq and Jan Balzarini led to the discovery of Cf1743 (FV-100) (Fig. 2) which has highly potent activity against varicella virus (VZV). FV-100 is currently in Phase III clinical trials in patients with herpes zoster (shingles). Clearly, Chris was delighted to present Jan with the Elion Award in 2008 (Fig. 3).

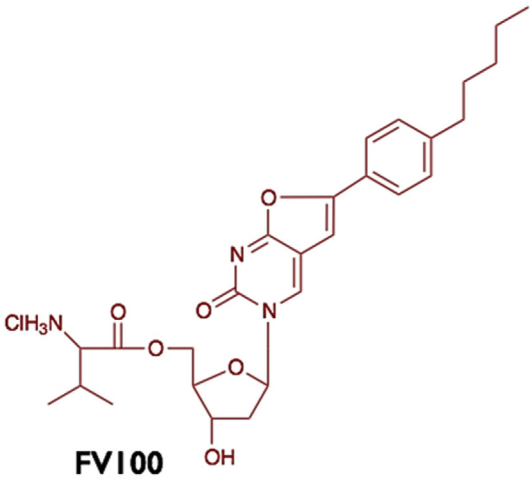

Fig. 2. Structure of CF1743 (FV-100).

Another compound, Cf2761 (INX-08189, often as INX-189) was synthesised and quickly shipped to Inhibitex on 11th Nov 2008. It was shown to have excellent activity against Hepatitis C virus (HCV) (EC_{50} 10 nM). The clinical milestones were quickly achieved, first into man - May 2010 and first efficacy in patients - Mar 2011.

Fig. 3. Chris McGuigan, as ISAR President, presents Jan Balzarini with the Elion award in 2008.

Unfortunately, the trials were stopped due to toxicity concerns. However, it did prove that the phosphoramidate prodrug approach was successful and it was adopted by others. Perhaps the most evident legacy of Chris' work is represented by sofosbuvir (Fig. 4) and tenofovir alafenamide, the "backbone" compounds in Gilead's single-tablet regimens (STR) for treating patients infected with HCV and HIV, respectively.

Moving the phosphoramidate (Protide) approach sideways from antivirals to anti-cancer, NUC-1031 (Acelarin®) (Fig. 5) has been licensed to Nucana. In a Phase I/II study in terminally ill patients with growing tumours, there was good disease control particularly with advanced gynaecological cancers. It is envisaged that Acelarin, used with chemotherapy, will reduce the development of resistance which often limits the efficacy of standard chemotherapy.

Andrea ended his tribute on a highly personal note. Chris had been a mentor, colleague and friend who died too soon. Our thoughts go to his family who have lost a husband and a father.

May I add my own comment? Andrea mentioned that Chris was the first recipient of the Prusoff award, in 2001. To my mind, his award lecture set a new standard. To this day, his mastery of slide creation, visually exciting but with clear information, has rarely been equalled. In my report of the 28th ICAR, I mentioned that the

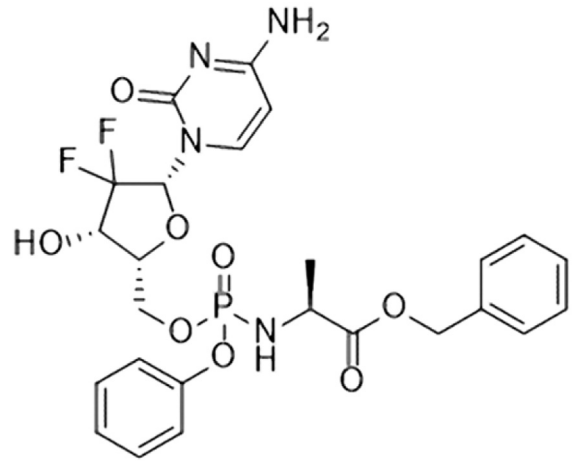

Fig. 5. Structure of NUC-1031 (Acelarin®).

Prusoff Award lecture, by Erica Ollmann Saphire, sailed straight into my top ten ICAR lectures. My top ten certainly includes the Prusoff Award lecture by Chris. His presentation style is uniquely memorable, even after 15 years. My other lasting memory is Chris excitedly showing us photos of his new-born daughter.

Maybe, his most important legacies are the many young scientists whom he had helped, including Andrea Brancale who gave such a heart-felt tribute to his mentor, colleague and friend. Andrea has been an active member of ICAR for many years, being the ISAR webmaster since about 2006.

3. Gertrude Elion memorial award lecture: antiretroviral drugs: history and future

Douglas (Doug) Richman, University of California San Diego, La Jolla, CA, USA

Doug (Fig. 6) first paid tribute to Trudy Elion for her pioneering work with nucleosides. In his Elion Award lecture, Doug briefly described the difficulties of the early days of the HIV epidemic but ends on the bright prospects for both treatment and prevention.

AIDS was first described in 1981 and HIV (human immune-deficiency virus) was discovered in 1983. In 1985, AZT (3′-azido-3′deoxythymidine) was the first drug to show antiretroviral activity, the first clinical data being reported in The Lancet (Yarchoan et al., 1986). In July 1987, the efficacy of AZT in the treatment of patients with AIDS or AIDS-related complex was reported in the New England Journal of Medicine (NEJM). For that time, this was a large-scale, long-term trial, 282 subjects for 24 weeks. At 24 weeks,

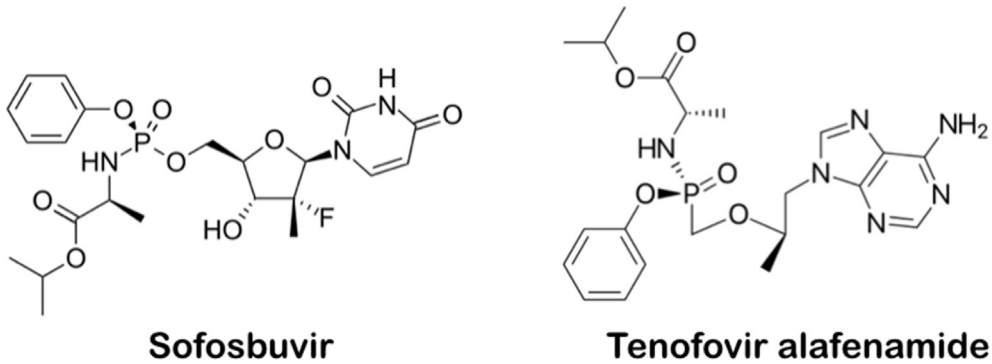

Fig. 4. Structures of sofosbuvir and tenofovir alafenamide.

Fig. 6. Bob Buckheit presents the Elion award to Doug Richman.

there was just 1 death in the AZT group vs 19 in the placebo group. This one study was sufficient for FDA approval.

Even in this first 24-week trial, there was a clear warning sign. In the group of less seriously ill patients (those with AIDS-related complex), the CD4 cell counts in the AZT group remained significantly ($p < 0.003$) higher than placebo group through to week 20. At week 24, there were too few patients to give a clear result. In the AIDS group treated with AZT, the mean CD4 cell counts remained higher than in placebo group through to week 20. However, after an initial rise above baseline at week 4, there was a steady decline.

Sequential HIV isolates (designated A036B, A036C and A036D) were taken at 2, 11 and 20 months from an individual patient being treated with AZT. There was about a 3 $\log_{10}$ reduction in AZT susceptibility (Larder et al., 1989). Doug commented that his grant application, to further investigate HIV resistance, was turned down because the reviewers asserted that essential enzymes for virus replication could not tolerate mutations. Doug recalled a comment by Arthur Schopenhauer (1788–1860) who noted that truth passes through three stages: first, it is ridiculed, second, it is violently opposed, third, it is accepted as self-evident.

During the next decade, it became clear that HIV resistance was a problem for each class of antiretroviral monotherapy, that sequential monotherapy led to multi-class resistance and that resistant HIV was being transmitted. Combination therapy with indinavir (IDV), AZT and lamivudine (3TC), vs IDV alone or AZT/3TC, gave the best efficacy (reduction of HIV RNA) throughout the two-year study (Merck 035, NEJM, 1997). This study showed the way forward, with several combinations able to reduce plasma HIV RNA levels to below the limit of detection, which was 400 copies/ml at the time. Most years, from the mid-1990s to the present, there has been a new drug or a new drug combination receiving FDA approval. Some notable advances were the approval of Truvada® (TDF, a prodrug of tenofovir) in 2001, the introduction of Atripla®, the first single-tablet regimen (STR) in 2006, and the approval of tenofovir alafenamide (TAF) in 2015.

In a combined analysis of two Phase III studies comparing Stribild (containing TDF) with the same combination but with TAF, the primary efficacy outcomes slightly favoured the TAF arm over the TDF arm. At 48 weeks, 92% and 90% patients had undetectable HIV RNA (<50 copies/ml), respectively, and at 96 weeks, 87% and 85%, respectively. One of the most important points is that, by 96 weeks with these current regimens, the virological failures with resistance were only 1.2% and 0.9%, respectively. All these resistant strains were genotypically susceptible to dolutegravir. Although the efficacy parameters for the TAF and TDF were similar, the TAF arm had an improved safety profile.

With the approval of improved therapies, and better understanding of how to use them, the proportion of patients, with newly detected drug resistance, has been decreasing with the year of antiretroviral (ART) initiation. There has been a marked improvement comparing therapy initiation prior to 1999 and after 2007. It is easier to avoid drug resistance than to limit it following suboptimal therapy. Unfortunately, transmitted drug resistance is still a problem, especially from patients who have had ART for more than 5 years.

From about 2005, there has been steady increase in the estimated proportion of HIV-infected patients receiving ART, from under 10% in 2005 to about 40% in 2014. The increase is largely due to patients in Africa receiving ART. In 2014, about 16 million patients world-wide were having ART, of these, about 12 million were in Africa. On the positive side, it has been estimated that over 8 million deaths have been averted. On the negative side, widely anticipated factors, contributing to HIV drug resistance in low and middle income countries (LMICs), have been confirmed: suboptimal regimens, limited resources to monitor viral load, drug distribution failures and perinatal ART rather than treating pregnant women. What does the future hold?

In the short term, the availability of TAF and dolutegravir (DTG) should provide marked improvements in ART in LMICs. Looking further ahead, there is the prospect of ART with prolonged intervals (several months) between doses. Cabotegravir (GSK-1265744, CAB) (Fig. 7) an integrase inhibitor, was investigated in a Phase I trial ($n = 10$ subjects/cohort), comparing monthly and quarterly repeat dosing. The 4 cohorts each had a loading dose (LD) of 800 mg intramuscular (im), then followed by a maintenance dose (MD) of CAB either 200 mg subcutaneous (sc) q 4 w 3 times, 200 mg im q 4 w 3 times, 400 mg im q 4 w 3 times or 800 mg im at 12 w, respectively. The target plasma concentration of CAB was 0.6 μg/ml. With the 12 w dosing interval, the CAB trough level was about 1 μg/ml. The 200 mg sc and im dosing were similar, both less than the 400 mg im dosing (trough levels about 2 and 3 μg/ml, respectively).

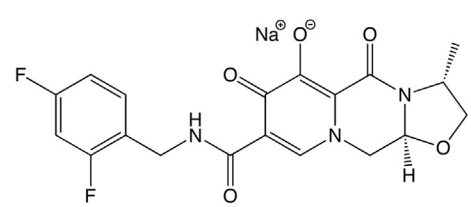

Fig. 7. Structure of cabotegravir (GSK-744), an HIV integrase inhibitor.

CAB and rilpivirine (RPV), an HIV NNRTI, are both under development as long-acting injectable nanosuspensions. In a Phase IIb trial, CAB + RPV (im) were compared to 3-drug oral ART (CAB + ABC/3TC). The patients were randomised after a 20-week induction period during which they were treated with daily oral CAB 30 mg + ABC/3TC, only those patients achieving HIV-1 RNA <50 c/ml were eligible to start the maintenance period (MP).

Enrolled patients were randomised 2:2:1 to CAB + RPV every 8 weeks (Q 8 W), every 4 weeks (Q 4 W), or remained on oral CAB + ABC/3TC (n = 115, 115 and 56, respectively). The primary endpoints were safety and virological failure (HIV RNA >50 c/ml) at 32 weeks into the MP (Intention to Treat-Maintenance Exposed (ITT-ME)). There was excellent virological success, 109 (95%), 108 (94%) and 51 (91%) patients, respectively, maintaining undetectable HIV RNA levels. The non-responders (HIV RNA >50 c/ml) were 3 (3%), 1 (~1%) and 1 (2%), respectively. There were only 2 patients who discontinued due to lack of efficacy, one each in the Q 8 w and oral groups. There seemed to be a good safety profile, with injection site reactions being common but acceptable.

MK-8591 (4′-ethynyl-2-fluoro-2′-deoxyadenosine, EFdA) (Fig. 8) is being developed by Merck, licensed from Yamasa. EFdA is a highly potent HIV RT inhibitor (PBMC, $EC_{50} = 0.2$ nM). It is a non-obligate chain terminator but it prevents translocation of the RT. Following a single oral dose (50 mg/kg) to rhesus macaques, the peak plasma concentration of EFdA is just over 10 μM, reducing to about 10 nM at nearly 180 h (7 days). In rhesus PBMCs, EFdA is taken up and converted to the diphosphate (DP) and triphosphate (TP) rapidly, > 100 μM at the first time point. At 7 days, the DP and TP are above 10 μM. This suggests the potential for once-a-week oral dosing. In a Phase Ib study, a single 10 mg oral dose in HIV-infected patients resulted in a 1.6 $\log_{10}$ decrease in viral load at day 7. The intracellular half-life ($t_{1/2}$) of EFdA-TP was 103 h. There was no evidence of resistance out to day 10 (1.8 $\log_{10}$ decrease in viral load). Parenteral formulations have given effective drug levels for 180 days. This suggests that twice-yearly dosing will be possible.

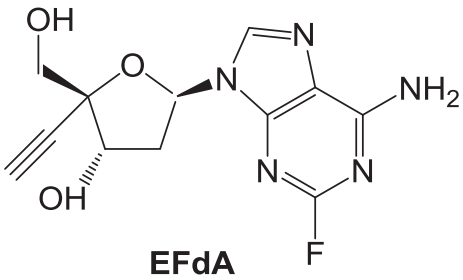

Fig. 8. Structure of EFdA (MK-8591), an HIV RT inhibitor.

Both CAB and EFdA look set to transform HIV therapy. It is likely that an induction period, using oral dosing will be necessary due to safety concerns in the event of a hypersensitivity reaction. Thereafter, an injection 2 or 4 times a year could give effective ART for patients with HIV infection or prevent transmission to uninfected subjects. The problems now associated with poor delivery of ART, and hence the potential for HIV resistance, in PMICs may be largely avoided. The prospect of interrupting HIV transmission holds out hope that the HIV epidemic may be gradually controlled.

4. The Antonín Holý memorial award lecture: acyclonucleosides to Ziagen

Robert (Bob) Vince, Center for Drug design, University of Minnesota, MN, USA

Bob (Fig. 9) started by acknowledging that the publications by Antonín (Tony) Holý had influenced his own work. When he joined the laboratory led by Howard Schaeffer, the team was focussed on nucleic acid chemistry. Bob was inspired by a molecular biology course on nucleic acids in his first year of graduate school. It was taught by Professor Roger Mantsavinos who had just arrived from

Fig. 9. Bob Buckheit presents the Holý award to Bob Vince.

Erwin Chargaff's lab at Columbia University. When Howard moved to Burroughs Wellcome to become head of the medicinal chemistry division, Bob moved to the University of Minnesota. Bob thanked Bill Shannon, who has attended many ICARs including this one, for their long-term collaboration.

Bob proposed the design and synthesis of acyclonucleosides as potential antitumor and antiviral agents based on the lectures presented by Dr Mantsavinos. The first of this series, acycloadenosine, was published in 1971 (J. Med. Chem. **1971**, *14*, 367). Although Howard Schaeffer had synthesised the compound and drafted this paper after his move to Burroughs Wellcome, he sent a letter to Bob suggesting that Bob should be an author as it had been his idea to synthesize these acyclonucleosides. This compound was shown to be a substrate for adenosine deaminase – so it was a biologically active molecule. It remained unknown if it could be recognised by a kinase to form the corresponding monophosphate (acycloadenosine-MP). With Howard's move to Burroughs Wellcome and Bob's move to Minnesota, this idea was shelved until acycloguanosine was later found active as an anti-herpes agent at Burroughs Wellcome. Subsequently in 1978 (Schaffer et al., 1978), the antiherpesvirus activity of acycloguanosine was published. [Over 3 decades later, acyclovir (ACV) and its oral prodrug, valacyclovir (VACV), are still in use. It is interesting to note that tenofovir, synthesised by Tony Holý, is also an acyclonucleotide – it is included, as its prodrug, in some of the most used single-tablet regimens (STR) for the therapy of HIV infected patients.]

Ara-adenosine (araA) was a known anti-herpesvirus compound. It is activated by kinases to give the mono-, di- and tri-phosphate which inhibited the viral polymerase. However, araA was also a substrate for adenosine deaminase to give ara-inosine which was inactive. Bob wondered if the corresponding carbocyclic araA

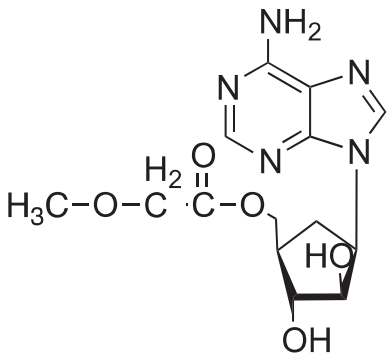

Fig. 10. Cyclaradine methoxyacetate.

would be stable. This compound, known as cyclaradine, was adenosine deaminase-resistant and had low systemic toxicity. Just before Bob was due to present his synthesis of cyclaradine at a meeting, he was told that Bill Shannon of Southern Research Institute had found that the compound had good activity against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and that he had suggested that Bob should consider withdrawing his presentation. Being unaware of the patent implications, Bob delivered his presentation as planned. An important lesson had been learnt the hard way!

The 5′-methoxyacetate prodrug, cyclaradine-MA (Fig. 10), was active in a topical model of genital infections in guinea pigs with HSV-2. Cyclaradine-MA (as 1% and 5% topical formulation) was compared to ACV (1% and 5%). Both compounds had good activity in this model, with cyclaradine apparently having slightly greater activity (Fig. 11).

Unexpectedly, there was a marked difference during the follow-up period. After ACV treatment, there were HSV-2 recurrences, whereas there were no recurrences following cyclaradine-MA treatment. Bob and Bill Shannon were co-authors of the publication (Vince et al., 1983). Although cyclaradine seemed to be an interesting compound, it was not developed as there could be no compound patent protection.

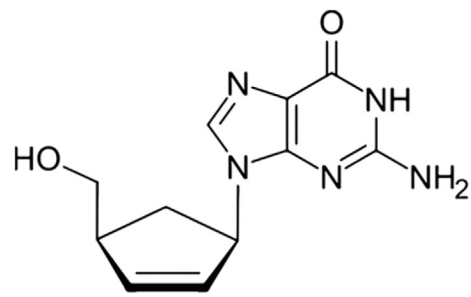

Fig. 12. Structure of (−)carbovir.

The next major advance came with the synthesis of a guanosine

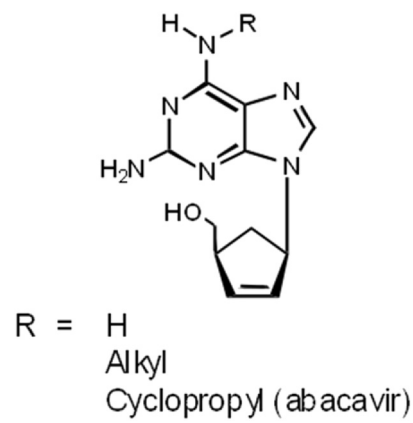

Fig. 13. Structures of some 6- substituted carbovirs covered by the Vince patent.

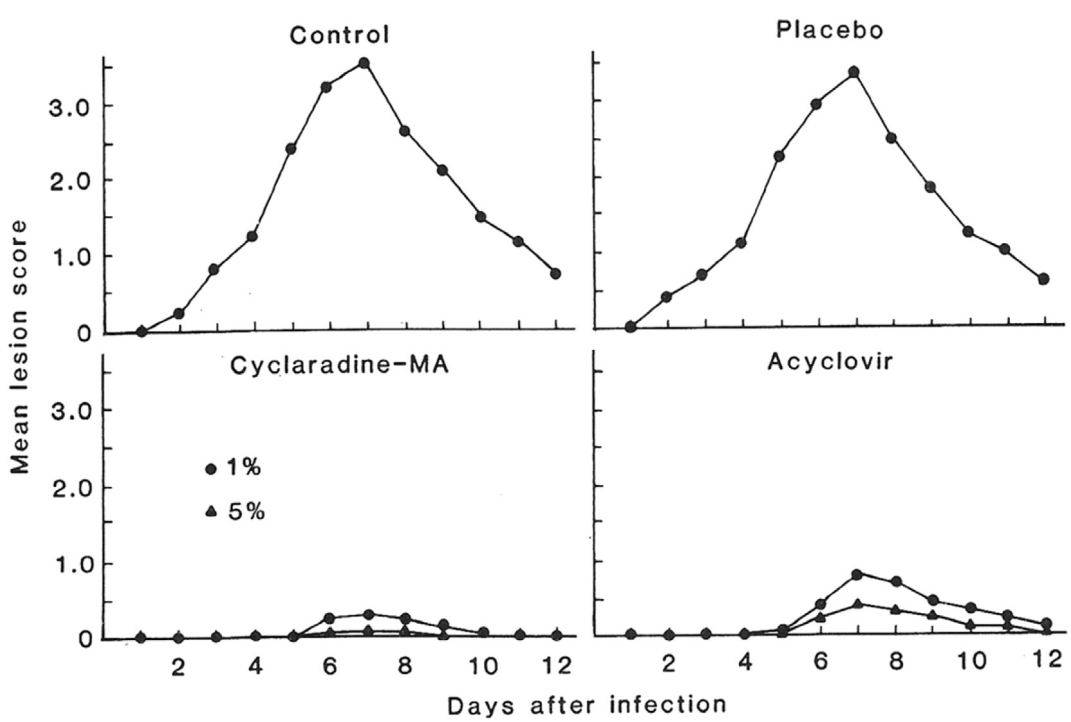

Fig. 11. Cyclaradine and acyclovir: *in vivo* anti-HSV-2 activity in guinea pig infection.

analog which became known as carbovir (Fig. 12). As in cyclaradine, the oxygen was replaced with a carbon but a double bond was used to give a ring with a similar shape to the ribofuranose. Mei Hua synthesised the first carbovir compound. To improve oral bioavailability, various alkyl analogs of carbovir were synthesised (Fig. 13). All these compounds were very active as HIV RT inhibitors, each analog being converted intracellularly to carbovir-MP which was then further phosphorylated to the active carbovir-TP. On 5th December 1988, a patent was filed with Bob and Mei as the inventors.

These carbovirs were licensed to GlaxoSmithKline(GSK). Cyclopropylcarbovir was synthesised by GSK which claimed that the cycloalkyl chains were not covered by the patent. However, as for all the other alkyl analogs of carbovir, cyclopropyl carbovir was converted to carbovir-MP and to the active form, carbovir-TP. Following a lawsuit, GSK had to pay University of Minnesota $600 million and royalties. Although the cyclopropyl group was not essential, GSK had done so much work with this compound that it was this carbovir analog that was developed to become abacavir. The royalties have enabled the university to start and fund the Center for Drug Design, with Bob as director.

5. The William Prusoff Young Investigator award lecture: new frontiers in antiviral drug development: inhibiting the polymerase of (−) strand RNA viruses

Jerome Deval, Alios BioPharma, San Francicsco, CA, USA

Jerome (Fig. 14) started by thanking Bob for his introduction: Jerome had obtained his Ph.D. in applied microbiology from the National Center for Scientific Research (Le Centre National de la Recherche Scientifique, CNRS), University of Provence, Marseille, France in 2004. Jerome's mentor was Bruno Canard who had been the Prusoff awardee in 2008.

Jerome noted that there are very few small-molecule drugs approved for negative strand (−) RNA viruses, none of these being virus replication inhibitors. In 2010, there was just a single (−)RNA virus replication inhibitor in clinical trials, T-705 which is now known as favipiravir. It has been approved in Japan for treating serious influenza infections and is in Phase III trials in the USA. In 2016, favipiravir and VX-787 were in Phase III and Phase II trials, respectively, for influenza infections, and five other compounds in Phase I trials. [BCX 4430 and GS-5734 are for Ebola virus infections and were included in ICAR lectures in 2014 and 2015 respectively.] Only one compound (ALS-8176), is being tested for respiratory syncytial virus (RSV) infections, this being the subject of this presentation. All these compounds are viral polymerase inhibitors but only four, favipiravir, ALS-8176, BCX3340 and GS-5734, are nucleoside analogs.

Fig. 14. Bob Buckheit presenting the Prusoff award to Jerome Deval.

There are no effective therapeutics for RSV infections which may be serious, even fatal, in children. Clearly there is an important unmet clinical need. Although the main RSV targets for potential drugs have been the fusion protein or any part of the polymerase complex, Jerome and his colleagues decided to screen only nucleoside analogs. Past experiences with herpes viruses, (HSV-1 and -2, VZV), HIV, HBV and HCV have shown that nucleoside analogs have provided the drugs with good broad-spectrum activity against many strains of the virus and have shown a high genetic barrier, limiting the problems due to virus resistance. Therefore, a small screening campaign was initiated in 2010. A selection of 100 structurally diverse nucleoside analogs was tested for activity against RSV vs lack of inhibition of cellular replication. This screen resulted in just one hit, 2′difluoro-4′azido-cytidine which was known to inhibit HCV but also known to have safety issues.

Structure-activity relationship (SAR) studies proved to be difficult. The aim was to discover a compound which had good selective activity for RSV, with the measures of selectivity being a lack of activity against HCV and cellular replication. There were many dead-ends but one compound, ALS-8112, emerged as a good candidate. It had good activity against RSV (EC_{50} = 0.1–1 μM against all tested A and B clinical isolates) and lacked cytotoxicity (CC_{50} > 100 μM). ALS-8112 lacked activity against HCV and rhinovirus, both (+)RNA viruses and against influenza virus, a segmented (−)RNA virus. However, it had activity not only against RSV but also against two other non-segmented (−)RNA viruses, parainfluenza virus type 3 (EC_{50} = 1.3 μM) and vesicular stomatitis virus (EC_{50} = 3.4 μM).

As expected for a nucleoside antiviral, ALS-8112 is activated to the ALS-8112-TP which inhibits the RSV RNA-dependent RNA polymerase (RdRp). ALS-8112 is efficiently phosphorylated to ALS-8112-MP by deoxycytidine kinase (dCK). In human primary lung cells, high levels of ALS-8112-TP (~800 pmol/10^6 cells) were formed. The half-life of ALS-8112-TP was about 29 h. ALS-8112-TP inhibited RSV polymerase (IC_{50} = 0.020 μM) and parainfluenza virus type 1 (IC_{50} = 2.3 μM) but not the polymerases of influenza virus or HCV. In collaboration with Rachel Fearns at Boston University, it was confirmed that ALS-8112-TP was incorporated efficiently into an RNA chain by RSV polymerase using a poly-G template, about 13-fold less than for the natural cytidine-TP. When 4 residues had been added, there was chain termination. This is the first example of RNA chain termination in RSV.

Upon prolonged incubation of RSV with ALS-8112, four resistance mutations (designated QUAD), were selected. Three of the four mutations (A789V, L795I, I796V) are within the conserved motif B of the polymerase domain of the L protein, the other being M628L. In collaboration with Marty Moore, Emory University, it was confirmed that these QUAD mutations, when introduced into the L gene, gave resistance to ALS-8112.

Although ALS-8112 seemed to be a promising candidate compound, it lacked sufficient oral bioavailability. As for various other antiviral drugs, possible prodrugs were made. The di-isobutyl ester (ALS-8176, Fig. 15) was selected. In a primate efficacy model, oral ALS-8176 reduced RSV in lung samples by > 4 $\log_{10}$ (to below the limit of detection) and by ~ 3 $\log_{10}$ in nasal samples.

In a Phase 1 study (n = 76), oral ALS-8176 dosing regimens were

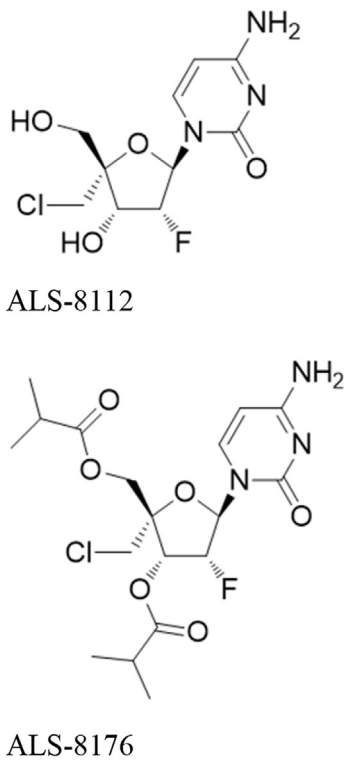

Fig. 15. ALS-8112 and its oral prodrug, ALS-8176 (4′-chloromethyl-2′-deoxy-3′,5′-di-O-isobutyryl-2′-fluorocytidine).

increased up to 2 loading doses (LD) of 750 mg followed by up to 26 maintenance doses (MD) of 500 mg twice daily for 14 days. No safety signals were identified. In a human RSV-challenge study, ALS-8176 was assessed in healthy volunteers. Of 62 participants inoculated with RSV (ITT population), 35 (56%) met the definition for RSV infection and were included in the ITT-infected population. There were three dosing regimens of ALS-8176, each twice daily for 5 days: 350 mg each dose, 750 mg LD and 150 mg MD for 9 doses, 750 mg LD and 500 mg MD for 9 doses. About 12 h after the first detection of RSV or on the morning of day 6, whichever occurred first, subjects were randomised and received the first dose of ALS-8176 or placebo. The primary efficacy end point was the area under the curve (AUC) for viral load in nasal washes, as determined by a RT-PCR assay for RSV RNA. There appeared to be an advantage when a loading dose was used, the reductions in AUC for viral loads

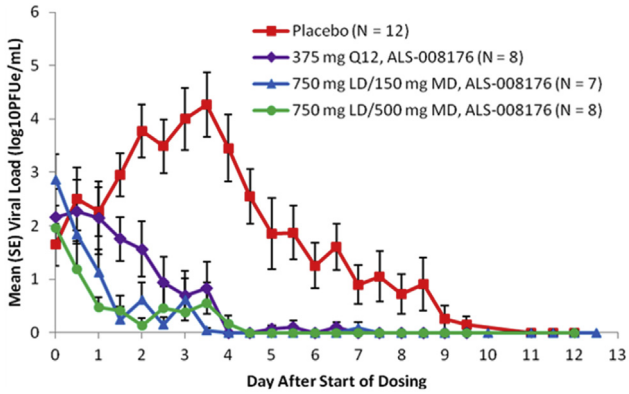

Fig. 16. ALS-8176 human RSV-challenge study in healthy volunteers (DeVincenzo et al., N. Engl. J. Med. 2015).

being 73.4%, 85.3% and 88.0% vs. placebo, respectively (Fig. 16). A secondary end point was the clearance rate of RSV RNA (the slope of the viral load change). It was most rapid in the two regimens with a loading dose, with reductions even at the first post-dosing sample at 12 h. In contrast, the viral load in the placebo group peaked about 3.5 days after randomisation. Importantly, this peak in RSV viral load was associated with a peak of symptoms. In the three treated groups, the mean symptom score and the mucus weights remained at about baseline throughout the observation period. This proof-of-concept study suggested that ALS-8176 was not only efficacious but that it appeared to be more active than a RSV-fusion inhibitor tested in a previous study of similar design.

ALS-8176 is currently being evaluated in young children. It is hoped that the results may be available by the end of the year (2016).

Jerome then switched focus, to influenza virus. Favipiravir has been approved in Japan for serious influenza infections and is still in Phase III trials in the USA. It is known that favipiravir-TP can be incorporated by influenza RNA polymerase, either base-pairing with cytidine (17-fold less than guanine) or uridine (30-fold less than adenine). Jerome and his colleagues were interested to discover what other polymerases could be inhibited by favipiravir-TP. They confirmed that norovirus RNA polymerase was inhibited but also that favipiravir-TP was a substrate for human mitochondrial RNA polymerase. This finding suggests that there is an opportunity to discover an analog of favipiravir which has better specificity for influenza RNA polymerase. Jerome's conclusion was that they are still far from having such a nucleoside analog. However, there are some non-nucleoside inhibitors of influenza polymerase in early clinical trials, for example, AL-794, S- 033188 and VX-787. Although a suitable nucleoside analog may be hard to find, it may potentially lead to the most effective drug.

6. Keynote addresses

6.1. Decoding viral genomics in the next-generation era

Richard H. Scheuermann, J. Craig Venter Institute, Rockville, MD, USA

Although Richard (Fig. 17) is based at the Rockville site, J. Craig Venter Institute (JCVI) has recently opened a new laboratory in La Jolla (Fig. 18), close to the site of this year's ICAR.

This presentation covered three topics (the third briefly):

a) How adaptive immunity drives influenza A virus evolution during a pandemic.
b) Identification of virulence determinants (causing paralysis) of enterovirus D68 during the outbreak in the USA (mid-August 2014 to mid-January 2015).
c) Identification of diagnostic regions of Zika virus and other human flaviviruses.

As an open resource for more information, two databases have been set up, for influenza (www.fludb.org) and the virus pathogen resource (www.viprbrc.org).

Prior to 2004, only 60 complete influenza A genome sequences were known. As of 6th April 2016, a total of 31,460 complete genome sequences were known, of these, 28,308 were for influenza A, nearly 20,000 of these being sequenced by JCVI. With such a large data set, one is able to seek much more detail about how antigenic drift allows seasonal influenza to thwart pre-existing immunity. Many immune epitopes in the hemagglutinin protein (HA) have been defined experimentally. Some B-cell epitopes are formed from continuous amino acid sequences but others from

Fig. 17. Richard Scheuermann presenting his keynote address.

discontinuous regions which come together in the folded HA. Three of the latter have been designated as Sa, Sb and Ca2. Is it possible that only a few of these B-cell epitopes are important for protective immunity? As a working hypothesis, sites, within which the epitopes of B cells and antibodies (Ab) overlap, may be the key targets of protective immunity. If a region of the HA protein is important for protective immunity, then non-synonymous substitutions will be selected (when a base change codes for a different amino acid). By comparing the rates of non-synonymous substitutions (e.g. CTA (Leu) => CAA (Pro), dN) and synonymous substitutions (e.g. CTA (Leu) => CTG (Leu), dS), it is possible to find amino acid positions which diversify more than expected (i.e. the ratio of dN/dS is large).

In a study of seasonal H1N1 clinical samples taken prior to the pandemic (pre-2009), 16 diversified sites were found in the HA. Of these, 11 were located in known B-cell/Ab epitopes, including Sa, Sb, and Ca2. All but one of these diversified B-cell/AB epitopes were in HA1 protein. Would these diversified epitopes be the targets of mutation as the 2009 H1N1 pandemic (pH1N1) lineage evolved in response to the development of population-level protective immunity in humans?

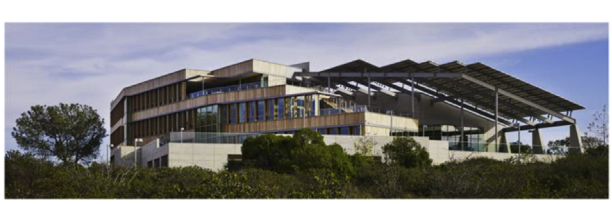

Fig. 18. The World's first Net-Zero Energy, Carbon-Neutral Biological Laboratory, JCVI at La Jolla, CA, USA.

In clinical samples of pandemic H_1N_1 influenza virus collected during the period 2009 to 2014, this data mining approach was used to identify regions of the HA protein that were diversifying and, therefore, may be targets of protective immunity in humans. Early in this pandemic, there was essentially no immunity in the infected population. After a while (2010–2011 and 2011–2012 seasons), community immunity became established. Late in the pandemic (2012–2013 and 2013–2014 seasons), the virus mutants, which escape existing immunity, would be favoured. The sequences in these three situations were compared to the pre-pandemic data.

There were 10 amino acid sites that differed (significantly) between the pre-pandemic H1N1 isolates and isolates from the recent 2012–2013 and 2013–2014 influenza seasons. Three of these sites (K180Q, D114N and S202T) were located in the same diversified B-cell/Ab epitope regions as identified previously, including Sa and Sb. However, there were additional diversified sites which are not known to be B-cell/Ab epitopes. It was noted that, when these substitutions happen, they seem to spread world-wide.

To test the hypothesis that mutations at the B-cell/Ab regions are important for protective immunity, a monovalent vaccine (inactivated A/California/07/2009) was used to raise antisera in a cohort of 10 volunteers. As expected, in a hemagglutination inhibition assay, the antisera had reduced reactivity to more recent isolates.

In summary, diversified epitopes appear to identify those B-cell/Ab epitopes that are key to protective immunity in humans. Although it is not possible to predict how these key epitopes will evolve, one can predict that it is these particular epitopes that will evolve selectively. When sequence variations in seasonal influenza includes changes to these key epitopes, it is time to change to a new vaccine.

Richard switched to his second topic – the 2014 enterovirus D68 (EV-D68) outbreak in the USA. Within the period mid-August 2014 to mid-January 2015, there were 1153 confirmed cases, including 14 deaths. As EV-D68 infections usually are mild, it is likely that there were many more unreported cases. Also within this period, there were 103 unexpected cases of paralysis of unknown etiology, some of which were associated with EV-D68 infection, for example 4/10 paralyzed children in CO, USA. Earlier (June 2012 to June 2014), there had been cases of acute flaccid paralysis (AFP) in CA, USA. Other cases of EV-D68 positive AFP cases were reported in Canada, France, Norway and Australia. Were genetic changes in EV-D68 linked to the increased disease severity and notable neurologic symptoms?

To answer this question, a phylogenetic tree was created and examined using a number of statistical tools. It was discovered that three distinct clades of EV-D68 were co-circulating during this outbreak. All the paralysis-causing isolates were within clade B1. There were various new nucleotide and amino acid substitutions – of particular interest, some of these sequences in EV-D68 are also found in the equivalent positions in paralysis-associated poliovirus, EV-D70 and/or EV-D71 isolates. It was noted that valine117 was 100% specific for clade B1 and associated with neuropathology in poliovirus infection.

An EV-D68 project has been set up with the aim to make targeted substitutions in various regions of the EV-D68 genome (5′UTR, polymerase gene and in the capsid binding genes,VP1, VP2 and VP3). These specifically mutated viruses will be tested in human neuronal cells (HTB-10, HTB-11 as used in poliovirus neurovirulence assay) and in non-neuronal cells (HeLa and A549) for comparison. It is hoped that further information will emerge linking sequence to disease.

The identification of diagnostic regions of Zika virus was covered briefly. A problem is that several flaviviruses are co-circulating in

Zika virus-endemic areas. The aim is to find viral peptide regions which are specific for each virus. The viruses are dengue virus (DENV) types 1,2,3 and 4, Ilheus virus (ILHV), Japanese encephalitis virus (JEV), West Nile virus (WNV), yellow fever virus (YFV) and Zika virus (ZIKV). It is hoped to develop an array screen for serum samples so that a result is obtained in hours rather than in days for a traditional ELISA screen.

6.2. Ebola virus: past, present, future

Heinz Feldmann, National Institute of Allergy and Infectious Diseases, Hamilton, MT, USA.

Fig. 19. Heinz Feldman giving his Keynote presentation.

Heinz (Fig. 19) stated that this presentation represented his own thoughts. Ebola virus was first recognised as a new virus in 1976 when there were two centers of infection, one near the Ebola river in Zaire (now the Democratic Republic of the Congo, DRC) and the other in Sudan near the border with the DRC. Ebola virus is closely related to Marburg virus, both being filoviruses. There have been sporadic outbreaks ever since 1976, but none nearly so large as the recent one in West Africa. In the worst-hit countries, Guinea, Liberia and Sierra Leone, there were 28,101 reported cases, of which 15,187 were laboratory confirmed, and 11,288 deaths. How did Ebola virus get to West Africa?

One suggestion has been via bat migration – but as yet, there has been no isolation of Ebola virus from bats. Was Ebola virus lying in wait for a long time? This strain of Ebola virus, designated Makona, does not seem to differ much from the Ebola virus in previous outbreaks in central Africa. It has similar virulence in primates, a comparable mutation rate and similar interferon antagonism. Could there have been higher transmissibility – no data but probably unlikely. This outbreak seems to have been driven by various local factors: poor health-care systems, mobility of the population across borders, resistance due to lack of understanding about virus infections, traditional customs (especially at burials).

One positive outcome from this outbreak is that there is a much larger cohort of survivors than in any previous outbreaks. Much data has been collected on the post-Ebola syndrome: musculoskeletal symptoms, blurred vision, weight loss, slowed-movement and pain, frequent headaches, depression, chronic pain, sexual dysfunction. Also, survivors have faced stigma which has hindered integration back into the community. A major concern has been the finding that Ebola virus RNA can be found in survivors many months after being discharged from an Ebola treatment center. Individual case studies on suspected sexual transmission have confirmed that not only can Ebola RNA be detected but transmission of virus remains possible. For example, a survivor was discharged on 7th October 2014; 5 months later (7th March 2015), a patient had unprotected sex with the survivor; a week later, this patient had symptoms and Ebola infection was confirmed. On 27th March, the survivor's semen was positive for Ebola virus RNA. The survivor and patient had the same distinctive Ebola virus sequence. It has been recommended that male survivors should either abstain or use condoms and that their semen be tested regularly until negative.

Since the first Ebola outbreak, it has been difficult to differentiate between malaria and Ebola virus infections. During the height of the Ebola outbreak in West Africa, many patients had Ebola virus, some had the malaria parasite (*Plasmodium*) and a few had both Ebola virus and plasmodium. More than one-third had neither Ebola nor malaria but were of unknown causes. One surprising finding was that the presence of *Plasmodium* seemed to increase the survival rate of an Ebola infection.

Throughout this Ebola outbreak in West Africa, there have been no approved drugs to treat Ebola infections. Favipiravir, a virus polymerase inhibitor approved in Japan for serious influenza infections, was tested in Guinea towards the end of this outbreak. There seemed to be a benefit to those patients who had low or moderate base line viral loads. Another viral polymerase inhibitor, GS-5734, was much more active than favipiravir against Ebola in a nonhuman primate model, but no clinical data are available. However, GS-5734 is a very promising drug for any future Ebola outbreak. One of the issues delaying the start of drug and vaccine trials was the ethical concerns over having a placebo control group for an infection with a high risk of mortality. For the favipiravir trial, it was agreed that the comparative group would be historical controls. For the Phase I vaccine trial, two, hopefully active, vaccines were compared for the induction of an immune response, there being no placebo group. In the ring vaccination trial, the vaccine was administered either immediately or after a short delay (see below). There has been a notable lack of published results.

Two vaccines, both expressing the Ebola virus glycoprotein (GP), were tested late in the Ebola outbreak, a chimpanzee adenovirus Type 3 (ChAd3-EBO-Z), developed by GlaxoSmithKline, and a recombinant vesicular stomatitis virus vector (rVSV-ZEBOV) developed by NewLink/Merck. Of these two, the rVSV-ZEBOV was further tested in a ring vaccination cluster trial in which clusters were randomised to have the vaccine either immediately or after a short delay (21 days) following index case identification. This vaccine seemed to be highly effective preventing Ebola disease; the vaccine seems to provide protection within 10 days.

This vaccine had shown good efficacy within a week in a primate model; rVSV-EBOV (5×10^7 PFU) was administered intramuscularly (im) either 28, 21, 14, 7 or 3 days prior to challenge (1000 PFU EBOV-Makona, im) ($n = 2$ or 3/group). The control group were administered an unrelated VSV-vectored vaccine. The day 28, 21 and 14 groups had all developed good antibody levels by the time of the challenge. The day 7 group did not give detectable antibody levels prior to challenge but the levels rose quickly in the next few days. There was 100% survival and essentially no symptoms in all

these groups. There was reduced survival in the day 3 group. There we no survivors in the control group.

There are ongoing efforts to improve the outcome of any future Ebola outbreak: strengthening public health, improving community awareness, negotiating cross-border co-operation and continuing international support. Although the rVSV-EBOV vaccine was deemed to be safe to use, there were unwelcome side effects – a safer vaccine is desirable. For clinical trials with potential drugs (e.g. GS-5734), clinical efficacy trials must be started immediately, with pre-approved trial protocols. There have been major advances in instruments (smaller and easier to use) for detecting and sequencing samples from Ebola patients. This will ease setting up the required infrastructure. In future, we need to have adequate stockpiles of drug and vaccine. This has been by far the worst Ebola outbreak but we now have some tools to limit any future Ebola outbreak.

7. Mini-symposium: structural biology symposium

This mini-symposium was organised by three past winners of the Prusoff Award, Andrea Brancale, Bruno Canard and Erica Ollmann Saphire.

7.1. Successful structure-based design of anti-AIDS drugs targeting HIV-1 reverse transcriptase: overcoming resistance through strategic flexibility

Eddy Arnold, Rutgers University, Piscataway, NJ, USA

HIV RT can exist in several forms, for example as a “closed fist”, changing to an “open hand” to grasp nucleic acid and nucleotide substrates. Structures of RT bound to non-nucleoside RT inhibitors (NNRTIs) and nucleic acid confirms that the drug prevents the incoming nucleotide reaching the active site.

Eddy mentioned his long-term collaboration with the late Paul Janssen (Johnson & Johnson). Together, they discovered and developed two HIV drugs, etravirine (approved in 2008) and rilpivirine (approved in 2011). To explain the efficacy of these drugs, even against mutant HIV strains, a “flexibility” hypothesis was proposed: a flexible molecule has more options to fit a pocket even after mutation. Recently, Arnold's group has started to use fragment screening with the aim of discovering new drug-binding sites.

7.2. RNA viral nucleases: from genome stability to innate immunity evasion

François Ferron, Centre National de la Recherche Scientifique, Aix-Marseille University, Marseille, France

Both single-stranded (ss) (−) RNA and ss (+) RNA viruses rarely encode a nuclease. Strikingly, *Arenaviridae* and *Coronaviridae* are the only two viral families that encode two viral nucleases, an endonuclease and a 3′-5′ exonuclease. What role do these nucleases play in virus replication and would that role be sufficiently critical to make these nucleases valid antiviral targets?

Arenaviridae are ss (−) RNA viruses with short bi-segmented genomes (~11kb) which encode just 3 proteins involved in replication/transcription. In contrast, *Coronaviridae* are ss (+) RNA viruses with a long genome (~27 kb) coding for 16 proteins for replication. Although *Arenaviridae* have only 3 proteins for replication, the large L protein is multi-functional with domains associated with endonuclease activity, polymerase activity and cap-binding properties. Cap-snatching (transferring the cap attached to host RNA to viral RNA) is an efficient strategy to limit viral RNA degradation by cellular 5′-3′ exonucleases. The three viral families, *Arenaviridae*, *Bunyaviridae* and *Orthomyxoviridae*, have an endonuclease, which is part of the polymerase protein, and use cap-snatching. Therefore an inhibitor of the endonuclease should enable the host exonuclease to degrade the viral RNA and so prevent viral transcription.

The *Arenaviridae* exonuclease is associated with a domain within the nucleoprotein. Mutations in this domain restores the interferon (IFN) response against the virus.

Initial screening has produced some potentially active compounds. One compound inhibits the endonuclease and is active (*in vitro* at 100 μM) against lymphocytic choriomeningitis virus (LCMV). The crystal structure, of the endonuclease domain complexed with this compound, has been obtained. A virtual screening approach against the exonuclease has led to the identification of one compound active *in vitro*.

In *Coronaviridae*, 16 non-structural proteins (nsp) are involved in replication; of these, nsp 14 is the exonuclease and nsp 15 is the endonuclease. Interestingly, the exonuclease requires the binding of nsp10 for activity. *In vitro*, SARS-CoV exonuclease mutant viruses are defective and have a 20-fold increase in mutation frequency. The exonuclease can remove one mismatched base pair. Modelling SARS-CoV exonuclease-dsRNA complex has given an insight as to how nucleoside analogs can be excised. A functional replication complex (nsp12 (RdRp) with nsp10/14) has been reconstituted and three compounds have inhibitory activity *in vitro*.

To summarise, the expected effect of inhibitors of the *Arenaviridae* endonuclease is to reduce transcription and of the exonuclease to restore the IFN response. Likewise, inhibition of the *Coronaviridae* exonuclease should restore the IFN response but also increase the mutation rate during viral replication.

7.3. Antibodies against Ebola virus: a global collaboration

Erica Ollmann Saphire, The Scripps Research Institute, San Diego, CA, USA

Hemorrhagic fever viruses include Ebola, Marburg and Lassa viruses. The surface of these viruses consists of glycoprotein (GP) which is highly glycosylated to form a protective coat of mucin. X-ray and light scattering has revealed the structure of the mucin, albeit at low resolution (10 Å). GP is produced in large quantities, is exported from cells and it dominates the immune response.

The Viral Hemorrhagic Fever Immunotherapeutic Consortium (VHFIC) is a global collaboration to evaluate all available monoclonal antibodies (mAbs) to Ebola, with the eventual aim to repeat this later with other viruses. In her Prusoff Award Lecture at the 2015 ICAR in Rome (Vere Hodge, 2015), Erica described the collaborative work leading to ZMapp, which emerged about February 2014. In August 2014, ZMapp was used on a compassionate basis to treat health care workers repatriated to the USA. ZMapp consists of three antibodies, 13C6 (binds to the top of the GP and recruits the immune system), 2G4 (binds to the base of GP) and 4G7 (also binds to base of GP).

This collaborative work continues with the investigation of antibodies obtained from survivors from the recent Ebola outbreak. The aim is to create a cocktail of antibodies with pan-Ebola efficacy. Also, work with Marburg virus is ongoing. So far, the majority of the antibodies investigated bind to the top of the GP.

8. Mini-symposium: Zika virus

This mini-symposium was organised by Pei-Yong Shi (University of Texas Medical Branch, Galveston, TX) and Heather Greenstone (Virology Branch, NIAID/NIH, Rockville, MD, USA).

Pei-Yong introduced this session which focused on the potential to treat or prevent Zika virus infections. One can start by learning

the lessons from past experience with dengue viruses. There have been no inhibitors, specifically designed for dengue, which have been taken to clinical trials. However, existing compounds have been tried. For example, balapiravir, a known HCV polymerase inhibitor, was tested clinically but found to be inactive because the dengue-induced cytokine production inhibited conversion of the prodrug to the active form. An adenosine analog, NITD008 was shown to have activity against dengue, yellow fever and West Nile viruses in cell culture assays and in animal models, but was abandoned due to toxicity concerns. It was also active against Zika virus in both cell culture assays and in a mouse model. Naturally, safety concerns are paramount as the greatest medical need is to treat pregnant women.

Another important area is to have point-of-care diagnostics. It is essential to distinguish infections with dengue, chikungunya (CHIK) or Zika viruses. Great progress is being made (see next section below).

The development of a mouse model was described by Justin Julander (Utah State University, Logan, UT, USA). Virus replication occurs in wild-type (wt) mice but typically the infection does not cause disease. In contrast, AG129 mice, lacking alpha, beta and gamma IFN receptors, proved to be a good choice as a model for Zika virus infections. Following subcutaneous (sc) inoculation of AG129 mice with Zika virus, various parameters were evaluated: disease signs (including conjunctivitis, hunching, lethargy, excitability, hind-limb paralysis), body weight and measurable viremia. To test potential antiviral compounds, the mice were infected with Zika Malaysia virus (sc, 10^2 pfu) and observed for at least 4 weeks. Viremia peaked at day 5 and reduced to baseline by day 9. The compounds were administered twice daily (bid) for 8 days starting 4 h before infection. The study parameters were weight change, survival and viremia. Several known compounds have been tested, including NITD008 but the most active compound so far was BCX4430 (viral RNA polymerase inhibitor). Previously, BCX4430 had been shown to be active in cell culture assays against a wide range of RNA viruses including filoviruses (Ebola and Marburg) and flaviviruses (e.g. yellow fever). BCX4430 was tested at two doses, 300 and 150 mg/kg. The higher dose gave good protection (90% survival) whereas the lower dose only delayed death (0% survival). On day 13, for both treated groups, there was little change in weight from baseline whereas the placebo group had lost weight. In this experiment, there were also uninfected untreated mice which gained weight throughout the 19-day period. In contrast, the mice, treated with BCX4430 (300 mg/kg), remained at their baseline weight. There was a dose-response reduction in viremia. The surviving mice (treated with the higher dose of BCX4430) were rechallenged but no more disease or mortality was observed.

BCX4430 was then tested in a therapeutic study in which treatment was started 1, 3, 5 or 7 days post-infection (dpi). There was good protection (80% survival) only with the group treated on day 1. With each further delay in the start of treatment, there was a reduction in the efficacy, although the mean day to death was delayed in mice treated beginning 3 and 5 dpi.

Although the yellow fever vaccine, using an attenuated strain of the virus, has been highly efficacious for many years (since 1937), the novel DNA YFVax technology may offer important advantages: there is no need for a cold chain for distribution, has high genetic stability (reduces production cost), can use needle-free administration (e.g. patches), no need for adjuvants and the technology can be adapted for other viral vaccines. Johan Neyts (Rega Institute, University of Leuven, Belgium), reported on pre-clinical research with DNA-YFVax. In hamsters, DNA-YFVax induces high levels of antibodies and a good cellular immune response, comparable to the standard yellow fever vaccine. Justin Julander showed that both the DNA-YFVax and the standard vaccines gave 100% protection in a hamster model of yellow fever.

It is planned to use this technology to create a DNA-Zika vaccine. Johan reported that he had just received the Zika virus transcript required to progress this work.

9. Mini-symposium: diagnostic technologies

This mini-symposium was organised by Mark Prichard (University of Alabama at Birmingham, Birmingham, AL, USA).

This session was introduced by Mark. For many years, there seemed to be little progress being made in the design of diagnostic kits for specific viruses. As there were no antiviral compounds to treat such viruses, there was no market for diagnostic assays – no assays, no way to develop a specific antiviral. Recently, technological developments in assay platforms that perform multiplexed PCR reactions has broken this negative cycle. Much recent progress has been made. Having used one of the platforms in his diagnostic laboratory for 16 months (Fig. 20), Mark commented that he had no idea that there were so many cases of adenovirus infections in the pediatric population in the hospital. He thought that this was a good time to set up a diagnostic mini-symposium. He recruited 6 firms to describe their assay platforms.

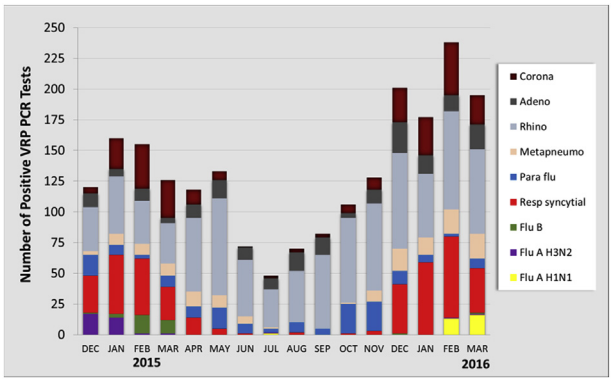

Fig. 20. Summary of viral respiratory infections in a pediatric hospital. The number of respiratory infections each month is shown and is stratified by the specific virus groups identified in the specimens. Waves of viral infections circulating in the community are readily apparent.

Company representatives were invited to describe their assay platforms.

- Introducing the Roche cobas® 6800/8800 virology assays. Pedro L Rodriguez, Roche Diagnostic Corporation, Indianapolis, IN, USA.
- Advancing multiplex molecular diagnostics. Scott O'Brien, GenMark Diagnostics, Carlsbad, CA, USA.
- FocusDx: flexible solutions for Molecular testing. Vivian Cintron, Focus Diagnostics, Cypress, CA, USA.
- Luminex applications in research and clinical settings. Aaron Benfield, Luminex Corporation, Austin, TX, USA.
- FilmArray – Point of impact rapid diagnostic improves patient care. Lou Banks, Biofire Diagnostics, Salt Lake City, UT, USA.
- High content imaging and analysis "The Cellomics Way". David Sweeney, Thermo Fisher Scientific, Waltham, MA, USA.

All the presenters gave an account of their diagnostic assays, not just the machinery but also the science and techniques used within the diagnostic platform. Some scientific approaches were unique to their firm but there were some common themes. Most

manufacturers have devised instruments which are small in size, moderately to easily portable, easy to set up and simple to operate. Sample preparation varies between simple and none required. Most steps are automated and the results, often in just over 1 h, are presented in an easily-understood format. This progression is ongoing with new systems in development or just being approved. Several firms offered panels which included both viral and non-viral agents selected because these cause a particular set of clinical symptoms. The aim is to make it easy for the physician to choose the correct test first time and avoid having several tests in sequence. Many of the assays and panels are approved by the USA FDA (Food and Drug Administration). To give an idea of the range of attributes available, I make a personal selection from each manufacturer (each attribute may be available from more than one manufacturer).

Roche Diagnostic Corporation: The Roche cobas® system has been used in many clinical trials for antivirals to treat HIV, HBV and HCV infections. These assays have a low detection limit and a wide linear range, an important factor for measuring the potentially high viral loads in untreated patients with these infections.

GenMark Diagnostics: GenMark system (total assay time 6 h) includes a respiratory virus panel, claimed to be the most sensitive available. They are replacing their GenMark machine with ePlex™ (coming later 2016) which does not require any sample preparation, fluid is moved with electricity, the assay time is 1.5 h and the output is a simple report (infectious agent in panel either detected/not detected).

Focus Diagnostics: Simplexa series have been developed since 2010. The machine's foot-print is small (1 sq ft) and easily portable (8 kg). The "universal disc" version can use untreated clinical samples (swabs, CSF, stool, urine, blood) or culture medium. The disc heats to 95 C to break open viruses (or bacteria) and then spins to separate the debris and the viral RNA. Result (viral RNA detection) is available within 75 min.

Luminex Corporation: The Magpix™ machine was launched in 2010. Their new ARIES™ machine simplifies the assay requirements. Several panels have been developed, for example their respiratory pathogen panel targets includes over a dozen viruses and some non-viral pathogens. Their gastrointestinal pathogen panel targets adenovirus, norovirus, rotavirus and various non-viral pathogens. Other panels in development include febrile disease agents (bacterial and viral), and vector-borne infections (those being transmitted by mosquitos, ticks and fleas). An expanded mosquito panel includes chikungunya, yellow fever and Zika viruses (also tests for malaria).

Biofire Diagnostics: Their "FilmArray" machine has a small footprint (6.5 × 10 × 15.5 in h,w,d) and is easy to locate near patients. Their assay requires 2 min on-hand time and about 1 h to result. They have 4 FDA-approved panels, three of which include viruses together with non-viral pathogens - respiratory, gastrointestinal and meningitis/encephalitis. A lower respiratory panel is in development.

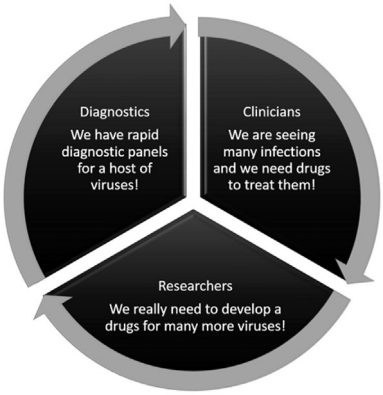

Fig. 21. The development of rapid diagnostic panels has initiated a self-progressing circle.

Thermo Fisher Scientific: The "Cellomics way" differed markedly from the others in this mini-symposium. Their system was the most complex to use but versatile and highly suitable for research and assay development. The cellomics system was claimed to be able to do "just about any cell based assay".

In his introduction, Mark summarised the recent progress and the resulting implications (Fig. 21). This was the first ICAR to include a mini-symposium on *Diagnostic Technologies,* a well-timed innovation.

10. Mini-symposium: DNA viruses

This mini-symposium was organised by Graciela Andrei (Rega Institute for Medical Research, Leuven, Belgium) and Rhonda Cardin (Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA)

10.1. Adenovirus monitoring and treatment in immunocompromised patients

Thomas Lion, Children's Cancer Research Institute (CCRI), Vienna, Austria

There are 7 species of human adenovirus (HAdV), designated A to G. Distributed among these species, 51 serotypes have been identified. In addition, most newly identified types (52–72) have resulted from homologous recombination events, either intraspecies or interspecies. In a cohort ($n = 138$) of pediatric transplant patients, 6% died from HAdV infection, and the contribution of these viruses to transplant-related mortality in this study exceeded 30%. Any species of HAdV can occur in peripheral blood, with predominance of species C, A, and B, and the onset of viremia may herald life-threatening disease. Nevertheless, screening methods must cover the entire HAdV spectrum in order to detect rarely occurring species, as exemplified by a patient displaying concomitant presence of HAdV species E, F, and B in peripheral blood. Moreover, the HAdV types or species causing viremia may switch during the post-transplant course and such change can be predicted by monitoring serial stool specimens.

There are no highly effective antivirals. Cidofovir has been the primary agent but is nephrotoxic. Its oral lipid-conjugate prodrug, brincidofovir (BCV, CMX001), appears to have a better safety profile but data in immunocompromised patients are limited. In patients after hematopoietic stem cell transplantation (HSCT), the incidence of HAdV viremia reportedly ranges from 3 to 15% in adults and from 6 to >40% in children. According to the current ECIL (European Conference on Infections in Leukemia) recommendations preemptive therapy should be started at first detection of HAdV in blood. However, in the presenter's experience, favorable outcome is most dependent on early onset of therapy and current data suggest that treatment decisions should be based on the monitoring of stool specimens to prevent invasive infection. The source of HAdV complications post-transplant may be *de novo* infection but much more commonly reactivation of the virus persisting in the gastrointestinal (GI) tract. HAdV replication in epithelial cells of the GI-tract can be conveniently measured by using stool samples. The risk of viremia is negligible in the presence of HAdV loads $\leq 1 \times 10^6$ copies/g, but increases to >70% above this critical threshold level ($p < 0.001$). According to studies in the pediatric HCST setting performed at the presenter's institution, presence of HAdV viremia is invariably preceded by detection of the virus in stool ($p < 0.01$). In their experience, the median time span between HAdV detection in

stool exceeding 10^6 copies/g and first appearance of the virus in blood was 11 days. Therefore, their center is exploring the possibility to change the basis for starting therapy, the new indicator being the HAdV load in stool $>10^6$ copies/g.

Although this change in protocol is expected to be beneficial, in the longer term, better treatment options are needed. Brincidofovir may be a promising option but development of additional anti-HAdV agents would be warranted. Another emerging approach with great potential is treatment with adoptive T-cell transfer.

10.2. Using preclinical models for papillomavirus-induced disease to define novel therapeutic and prevention strategies

Paul F. Lambert, University of Wisconsin, Madison, WI, USA

Several human viruses are well known to cause cancers, one of these being the human papilloma viruses (HPV), especially HPV16 and HPV18. The role of HPV16 oncogenes (E6 and E7) has been studied in HPV16-transgenic mice. It is also possible to study the effect of host factors, in particular, the potential of estrogen to act as a co-factor. Without an inserted estrogen pellet, no cancer was detected. It was discovered that estrogen contributed to the progression of cervical cancer. Furthermore, if the estrogen pellet is removed, the cancer regresses. Although the role of estrogen in human cervical cancer remains to be demonstrated, the known risk factors (HPV + oral contraceptives, HPV + many pregnancies, HPV + hormone replacement therapy (HRT)) all point towards estrogen being a factor.

In 2011, a murine papilloma virus (MmuPV1) was reported. Can this virus be used to provide a useful model? Infection of immunodeficient mice, either T-cell or B- and T-cell deficient, with MmuPV1 results in warts on the ears (the infection site). Although in normal mice, no warts are detected, is it possible that UVB radiation may have an impact? If UVB radiation is given either 24 h before of 24 h after infection, immunocompetent mice become susceptible to virus-induced warts. By giving the UVB radiation with or without shielding of the head and ears, it was shown that UVB acts systemically. It is known that UVB can induce systemic immunosuppression and this seems to correlate with the onset of MnuPV1-induced disease. As the disease can be followed for at least 6 months, there is a good opportunity to study the effect of potential anti-papilloma virus compounds.

10.3. Leveraging population genetics to reveal clinically relevant aspects of CMV evolution

Timothy Kowalik, University of Massachusetts Medical School, Worcester, MA, USA

Human cytomegalovirus (CMV) is one of the largest and most complex of human viruses. The viral DNA is about 235,000 base pairs (bp) with about 200 genes. Although infections are largely asymptomatic in healthy individuals, reinfection appears to be common. In contrast, serious disease is seen in immunocompromised patients and in new born children infected during pregnancy. Congenital CMV (cCMV) was a focus of this presentation.

In cCMV, an individual may have several genotypes. In the "CHIMES" study, which followed ~100,000 pregnancies, 40% of congenitally infected infants had $\geq$5 cCMV genotypes. In the report presented here, samples (48) from 18 patients were obtained from various sites (including urine, plasma, saliva) and deep sequenced. From 4.1×10^{10} CMV bases sequenced, 859,441 single nucleotide polymorphisms (SNPs) were detected, of these, 153,975 were polymorphic sites. This diversity is markedly more than for other herpes viruses, (Epstein-Barr virus (EBV), HHV-6A or HHV-6B) and also the RNA virus, West Nile Virus (WNV). The RNA viruses, Dengue virus (DENV) and hepatitis C virus (HCV), have slightly and much greater diversity, respectively. As may be expected, the diversity is not randomly distributed, with low diversity loci being enriched in regions encoding the DNA processing enzymes and capsid proteins. However, the prevalence of preexisting drug resistance alleles were around 1% for a panel of drugs, including those currently approved and three in development. In the viral DNA polymerase gene (UL 54), there were resistance alleles for ganciclovir (GCV), foscarnet (FOS) and cidofovir (CDV). It is similar for the genes encoding the viral kinase (pUL97) which phosphorylates (activates) GCV and is inhibited by maribavir (MBV), for the viral terminase (pUL56) which is inhibited by letermovir (AIC246), and for the target epitope (pUL 75) for the monoclonal antibody, MSL-109.

In untreated patients, the frequencies of the various SNPs remains largely stable. For example in one untreated cCMV patient there were several SNPs with frequencies almost 100%, a handful between 10 and 50%, and >20,000 SNP almost all less than 1%. Over a 700 day period, very few changes were seen. In contrast, in treated patients, there were a large number of SNPs changing frequencies, some from high to low and vice versa. The changes started immediately. Very many SNP had already switched frequencies by 10 days into therapy, with many further changes taking place during the next 20 days of therapy. This rapid CMV evolution is presumed to be due to preferential selection of already present variants rather than by the creation of new variants. Perhaps surprisingly, cCMV populations in urine and plasma samples can differ as much as between unrelated hosts.

In summary, CMV is as diverse as some RNA viruses, CMV population frequencies can change rapidly when colonizing new compartments and in response to antiviral therapies. Hence, variations within a host may not be represented by secreted virus. Lastly, reinfection (or superinfection) of "immune" individuals is common - this could explain how most individuals acquire such a vast number of SNPs.

10.4. Roseoloviruses: ships or icebergs in the sea of pathogens?

Philip E. Pellett, Wayne State University, Detroit, MI, USA

This presentation covered the topics, roseolovirus biology, spectrum of associated diseases and the use of antivirals. Like HCMV, the roseoloviruses (HHV-6A, HHV-6B, and HHV-7) are betaherpesviruses. Although HHV-6A was included in the presentation, this summary will focus on HHV-6B and HHV-7. Because assays to differentiate HHV-6A and HHV-6B were not widely used until recently, some of the findings for HHV-6B reported here may also apply to HHV-6A.

HHV-6B and HHV-7 infect $CD4^+$ T lymphocytes but use different receptors, CD134 and CD4, respectively. Both viruses are widely distributed worldwide, the seroprevalence being >90% by age 4 and > 80%, respectively. Both viruses are transmitted via saliva, modulate the immune system, persist for life with widely dispersed latent reservoirs from which they reactivate periodically. The HHV-6 (A or B) genomes contain telomeric repeats (TAACCC)n which are involved in recombinational integration of the complete viral genome into the telomeres of human chromosomes. Such integration has been hypothesized to be a mechanism of HHV-6 latency. Importantly, germ line integration of HHV-6 genomes enables Mendelian inheritance of the virus (inherited chromosomally integrated HHV-6, iciHHV-6). Following germ line transmission, essentially every cell throughout the body contains at least one virus genome. The iciHHV-6 prevalence is ~1% in the USA and the UK, but ~0.2% in Japan. Vertical transmission via iciHHV-6 should not be confused with the horizontal transmission that

occurs in >90% of the population. In whole blood of individuals with iciHHV-6, viral loads are typically ~10^6 copies/ml, but most, if not all, of the genomes detected are from cells that harbored quiescent integrated virus genomes. Thus far, iciHHV-6 has been associated with angina pectoris in large population-based studies, and with bacterial infections in solid organ transplant recipients. Importantly, high HHV-6 viral loads in iciHHV-6 individuals can lead to misdiagnoses and inappropriate therapeutic approaches in individuals with illnesses not related to the virus.

The known clinical spectrum of HHV-6 is more diverse than for HHV-7. HHV-6B is the major cause of roseola and associated febrile rash in young children, may cause febrile convulsions, and causes serious neurologic complications in transplant recipients. In contrast, in immunocompetent individuals, HHV-7 causes some febrile rash and perhaps 5% of roseola cases. Rarely, there may be neurologic disease. In immunocompromised patients, for example, after solid organ transplant (SOT) or hematopoietic stem cell transplant (HSCT), HHV-7 reactivation is common but causes little disease.

Good diagnostic assays, which discriminate HHV-6A and HHV-6B, have been developed. The established drugs employed for other herpesvirus infections (FOS, GCV, CDV) have limited activity against HHV-6B and their long-term use in immunocompromised patients is restricted due to toxicity. Philip Pellett mentioned that there was to be a presentation the next day by Jennifer Brooks on a new drug MBX-400 (filociclovir). It is a nucleoside analog (Fig. 22) with good activity against CMV, HHV-6A and B (EC_{50} 1.2, 1.3 and 2.5 μM, respectively). The compound has low solubility which may account for oral absorption being less than dose proportional – exposure following a 1350 mg dose was similar to a 350 mg dose. However, no safety issues were identified in a small Phase I dose escalation study (doses from 35 to 1350 mg).

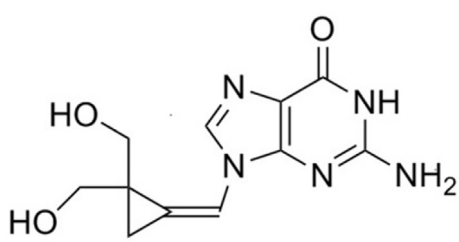

Fig. 22. Structure of filociclovir (MBX-400).

Adoptive T-cell immunotherapy is being evaluated for HHV-6 infections in transplant recipients. An excellent clinical response rate (94%, n = 11) has been obtained. In this approach, viral peptides are used to stimulate the generation of virus-specific T cells against 5 viruses (EBV, AdV, BKV, CMV, HHV-6) from HSCT donors. The process takes 10 days. Currently, this therapy is limited to treating just a few patients, but methods for establishing banks of such cells are being developed.

In summary, roseoloviruses are widely distributed and are common, but are usually mild pathogens in immunocompetent individuals. This has obscured their substantial contributions to consequential disease. It is now clear that HHV-6B plays a pathogenic role in serious and sometimes fatal cases of febrile status epilepticus and mesial temporal lobe epilepsy in immunocompetent individuals, as well as in CNS and other complications following hematopoietic stem cell transplantation. Therapeutic approaches are available for evaluation in controlled clinical trials. Successful multicenter trials for high priority disease targets will enable study of less well-established associations.

So, are roseoloviruses ships or icebergs? Certainly, these viruses are widely distributed throughout the world, but in immunocompetent individuals, infections often go unnoticed. Critically, there are still many unknowns but the application of new diagnostic assays will help define disease associations and aid clinical trials. It appears that we are transitioning from a state where many of the activities of these viruses were hidden from view, to one where their consequential clinical effects are becoming ever more visible.

10.5. Update on herpes simplex virus vaccines; are we getting any closer?

David Bernstein, University of Cincinnati, Cincinnati, OH, USA

In a clinical study of a GSK vaccine for HSV-2 (HSV-2 gD2 + adjuvant) conducted in 1995/96, the vaccine was given to HSV-1/HSV-2 seronegative subjects whose partners were HSV-2 seropositive. There was no protection for vaccinated men but there was an effect in women. In a similar study, but including subjects of any HSV serostatus, the vaccine gave no additional protection against HSV-2 to those who were HSV-1 seropositive initially. In a follow-up Phase III trial (NIH and GSK), the women were all dual seronegative. Unexpectedly, there was some protection against HSV-1 disease but not against HSV-2 disease. Also, the vaccine group had significantly more days of recurrent viral shedding than the control group.

More recently, Genocea has evaluated a novel therapeutic vaccine, GEN 003, containing a T-cell antigen, a B-cell antigen and a matrix-2 adjuvant. It had efficacy in a guinea pig model although recurrent shedding was reduced but not prevented. In a Phase1/2b trial, patients with moderate to severe HSV-2 infections (3–9 outbreaks/year) were evaluated. The vaccine was administered three times at 21 day intervals. Virus shedding was monitored for 4-week periods at baseline, just after the third vaccine dose, at 6 months and one year. Although there was not a clear dose response, the 30 μg dose of vaccine about halved both the days of virus shedding and of lesions for the periods just after vaccination and at 6 months but not at 12 months when both parameters were similar to placebo.

Another approach is to use replication-deficient viruses in which there are deletions in specific viral genes. One such vaccine (VC2) gave good efficacy in the guinea pig model, about comparable in protection as a gD2 vaccine. So, the search for a really effective HSV vaccine continues – a hard nut to crack!

11. Contributor presentations

At the Rome ICAR (2015), Chris Meier (University of Hamburg) described the synthesis of triphosphate prodrugs (Tri*PPP*ro's) of biologically active nucleoside analogs. Initially using diphosphate prodrugs, he reported that non-symmetrical prodrugs delivered nucleoside diphosphates more selectively as compared to the symmetrical prodrug. The results with symmetrical C_8H_{17}-Tri*PP*-*P*ro-ddBCNA-TP were shown and he reported that a non-symmetrical version of BCNA-TP had been synthesised and was being evaluated. At this meeting, two of his students gave presentations (sections 11.1 and 11.2 below).

11.1. Synthesis of nucleoside triphosphate prodrugs of abacavir and carbovir

Simon Weising, University of Hamburg. Hamburg, Germany

Simon described the synthesis of prodrugs for carbovir (CBV)

and abacavir (ABC), symmetrical $C_{11}H_{23}$-Tri*PPP*ro-CBV and $C_{11}H_{23}$-Tri*PPP*ro-ABC. Their anti-HIV activities were tested in CEM cells (a lymphoblastoid $CD4^+$ T-cell line). Compared with CBV, the CBV prodrug was 3.5- and 2.5-fold more active against HIV-1 and 2, respectively. Similarly, the ABC prodrug was 4.5- and 3.7-fold more active, respectively. The cytotoxicities of these prodrugs increased by about the same ratio, 3.9- and 2.3-fold, respectively.

These results confirm that this prodrug approach works successfully, even for ABC which is an approved drug for HIV therapy. ABC after initial monophosphorylation, acts as a prodrug of carbovir monophosphate which, after phosphorylation to its triphosphate form, inhibits HIV's reverse transcriptase. This opens the door to deliver the triphosphate of other nucleoside analogs which are not naturally phosphorylated *in vivo*.

11.2. Cell uptake of DiPPro- and TriPPPro-nucleotides by employing fluorescent nucleoside analogs

Inga Reimer, University of Hamburg. Hamburg, Germany

Inga described the syntheses of the symmetrical C_9H_{19}-Di*PP*ro, the non-symmetrical $C_4H_9/C_{14}H_{29}$-Di*PP*ro and $C_4H_9/C_{14}H_{29}$-Tri*PP*-*P*ro prodrugs of ddBCNA (Fig. 23). These prodrugs were incubated with CEM cells for either 15 or 60 min, the cells separated by centrifugation and lysed. The natural fluorescence of ddBCNA was used to detect the levels of ddBCNA phosphates.

With all these prodrugs, the desired di- or tri-phosphate was the major product after incubation for 15 min. Unexpectedly, after incubation of the non-symmetrical $C_4H_9/C_{14}H_{29}$-Tri*PPP*ro-ddBCNA for 15 min, the monophosphate was also present and it was the major product at 60 min.

The use of ddBCNA gave a convenient way to monitor the cellular entry of these prodrugs. By varying masking groups, the prodrug can be optimised for both cellular uptake and specific conversion to the triphosphate.

11.3. Microwave-assisted synthesis of nucleotide phosphoramidates

Cecilia M. Cima, Cardiff University, Cardiff, Wales, UK

As mentioned above, potentially active nucleosides have been converted into useful antiviral drugs by using the ProTide approach, first developed by Chris McGuigan at Cardiff. Notable examples are sofosbuvir (for HCV) and tenofovir alafenamide (for HIV). However, the standard synthesis has used a labile reagent at room temperature for a long time (ca 16–24 h) and has led to poor yields for highly modified nucleosides. The aim of this work was to develop an improved synthetic method using microwave irradiation (MWI).

The first step was to select a suitable stable phosphoramidating reagent which would retain reactivity towards the primary hydroxyl group under MWI conditions. For this initial evaluation using MWI, the temperature was 65 °C. With adenosine as a model compound (Fig. 24), the yields of the desired product were similar (42 and 40% with conventional and MWI heating, respectively) but the reaction time was much reduced (1 h and 2 min, respectively). Further optimization is ongoing.

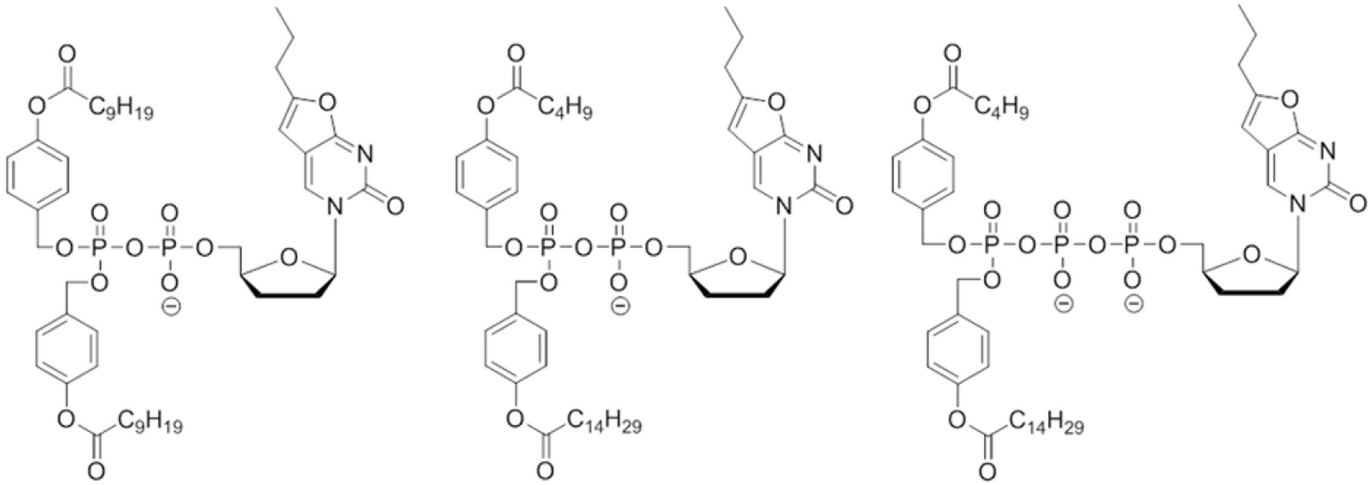

Fig. 23. Structures of the Di*PP*ro and Tri*PPP*ro prodrugs of ddBCNA.

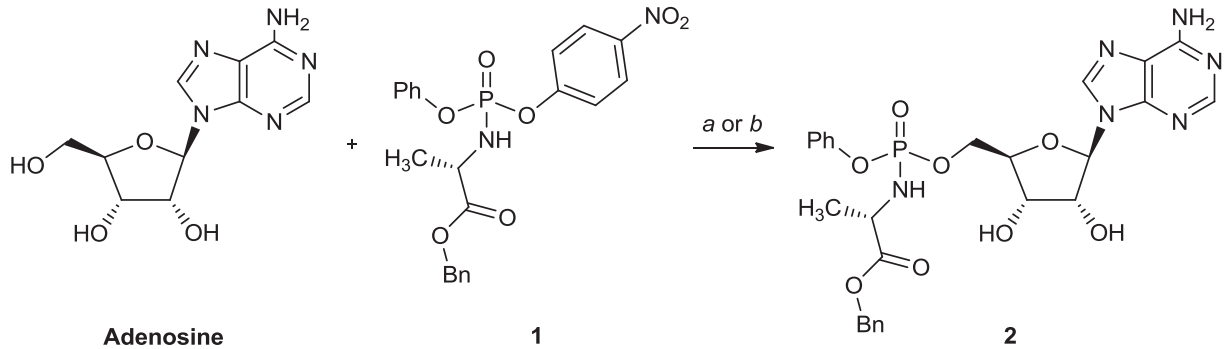

Fig. 24. Synthesis for comparing conventional heating (*a*: 1 h at 55 °C) and MWI heating (*b*: 2 min at 65 °C) using phosphoramidating reagent 1. Compound 2 is the desired product.

11.4. Role of adenovirus species and type on virological response to brincidofovir

Randall Lanier, Chimerix, Apex, NC, USA

Adenoviruses (7 major species, A-G) pose a serious risk of severe disease in transplant patients, particularly allogeneic hematopoietic cell transplant (allo HCT) recipients. Although brincidofovir (BCV, CMX001) is an oral prodrug of cidofocir (CDV), there has been no evidence of kidney or bone marrow toxicity detected in >1000 subjects receiving BCV. There have been two clinical trials evaluating BCV to treat AdV infections: HALT (CMX001-202) and AdVise (CMX001-304). There is also an ongoing expanded access study (CMX001-351).

In the AdVise study, there was a large reduction in viral load, especially for those patients whose baseline viral load was ≥1000 c/ml (Fig. 25).

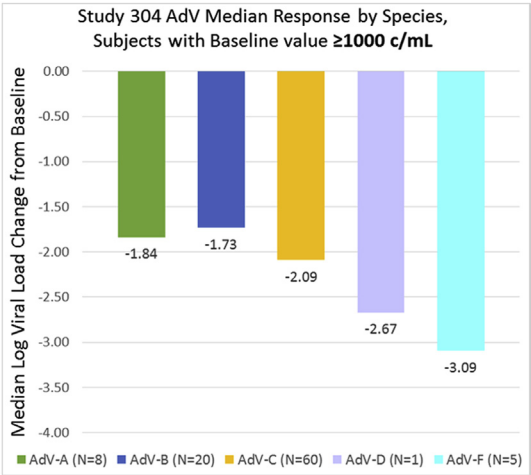

Fig. 25. Virologic response at last time on BCV therapy in patients with baseline viral load ≥1000 c/ml (AdVise, n = 94 AdV species in 89 patients, 2 patients had 2 species, 1 patient had 3 species).

AdV resistant mutants to BCV have been selected in cell culture: V303I and T87I/V303I. However, the changes in sensitivities to BCV have been modest, 2- and 6-fold, respectively. In the clinical trials, the AdV polymerase gene has been sequenced in cases where there was detectable plasma AdV at the end of therapy. One patient (HALT trial) developed mutation V303I on therapy and this was associated with virologic failure. Two patients (AdVise trial) had mutation T87I at baseline but these had complete virologic response by the end of therapy. This is a good result in such a challenging cohort of patients.

12. My personal comments and conclusions

Each year, ISAR presents three major awards, this year to Doug Richman (Elion award), Bob Vince (Holý award) and Jerome Deval (Prusoff award). Doug and Bob have each made important contributions to the HIV field. The path towards discovery of HIV therapies, which combine good safety with the ability to prevent the emergence of HIV resistance, is an amazing success story. Even now, starting a new chapter, cabotegravir and EFdA both have the potential not only to be used for therapy but also to prevent transmission. Atripla showed the way how two companies can work together to create a new, simple and effective single-tablet regimen. Likewise, if EFdA and cabotegravir were to be combined into one long-acting injection (e.g. once every three months), it would have the potential to limit HIV transmission even in the poorer countries of the world. These two compounds have complementary activities, first to reduce HIV DNA production, then to inhibit its incorporation into host DNA. We know that preventing transmission is an achievable aim. When trial subjects took Truvada (FTC/TDF) once daily prior to exposure (PrEP), transmission was essentially prevented, there being no seroconversions in subjects taking the drug daily. However, this trial also proved that once daily dosing was not an acceptable regimen for most of the trial participants. If women can have an injection of EFdA/cabotegravir with their monthly contraceptive injection, preventing HIV transmission may become a practical reality.

Whereas there are several excellent HIV therapies, Jerome Deval pointed out that there are very few small-molecule drugs approved for negative-strand (−) RNA viruses, none of these being virus replication inhibitors. In particular, RSV infections in young children can be serious, even fatal but there are no effective therapies. Jerome and his colleagues set out to discover a nucleoside analog to selectively inhibit RSV polymerase. They chose this aim because nucleoside analogs have the potential to be active against a broad range of virus strains and have a high genetic barrier to resistance. Indeed, nucleoside analogs have become the backbone of the therapies for herpesviruses, HBV, HCV and HIV infections.

Jerome described the work leading to the discovery of ALS 8112 and its oral prodrug, ALS-8176. The proof-of-concept trial in human subjects infected with RSV showed that ALS-8176 was highly effective and well tolerated. Currently, there is an ongoing clinical trial in infants (1–12 months old) hospitalized with an RSV infection (ClinicalTrials.gov, Identifier: NCT02202356). The primary outcome measures cover safety issues, the secondary aims covers the effects on the RSV infection, including viral RNA concentrations in nasal aspirates, emergence of resistance and changes in RSV polymerase. Also, pharmacokinetic parameters will be measured. The estimated date for completion is September 2016.

Although the efficacy of ALS-8176 in the adult trial was excellent, it is hard to predict how effective ALS-8176 will be in infants who have little or no natural immunity to RSV. However, one hopes that ALS 8176 has the potential to become a game-changer for patients with RSV infections.

There were two excellent keynote lectures, by Richard Scheuermann and Heinz Feldmann. Richard described how a large database of viral genome sequences has enabled the team to find answers to questions previously resistant to investigation. For example, which influenza epitopes will evolve selectively during a pandemic or which genetic changes in the genome of enterovirus D68 (EV-D68) were linked to the increased disease severity and notable neurologic symptoms? Heinz gave an interesting overview of Ebola virus outbreaks. He ended on an optimistic note that we now have some tools to help control any future outbreak, GS-5734, rVSV-EBOV, better instruments, for detecting and sequencing samples from Ebola patients, and we have learnt the need to improve community awareness. Hopefully, we shall never have such a long outbreak as this recent epidemic.

This year (2016), three important reports on Ebola trials have been published, on favipiravir in Guinea (JIKI trial, Sissoko et al., 2016) and in Sierra Leone (Bai et al., 2016) and ZMapp (The PREVAIL II Writing Group, 2016). Although none of these trials proved that the active treatment reduced mortality, the various trends in mortality, viral loads and symptoms all pointed towards a benefit sufficient to warrant further evaluation. The two favipiravir trials used historical controls – the reports justified this decision as

a pragmatic response to serious concerns raised by the local health care teams. The ZMapp trial was a randomised trial, although not blinded. It was not stated in the report, but I wonder if the number of available courses of ZMapp was so limited, there would not have been enough available ZMapp to treat all the expected patients, thus making a randomised trial more acceptable. However, in the ZMapp trial for patients recruited in Guinea, the standard of care included favipiravir, following the decision of the Ministry of Health. Surely, this points the way to a possible clinical trial design during a future Ebola outbreak - all arms of the trial should have potentially active treatments, for example, favipiravir, GS-5734, BCX4430 or ZMapp. Any highly active treatment would soon become apparent. Vaccination of health-care staff should reduce the risks associated with taking blood samples for viral load assays, an important parameter in any antiviral trial.

There were four mini-symposia, *Structural Biology*, *Diagnostic Technologies, DNA viruses* and *Zika virus*. My personal highlights include the presentation on the diversity of HCMV – far greater than for other herpesviruses, For example, one individual had >20,000 single nucleotide polymorphisms (SNPs) almost all at less than 1%. Typically, the prevalence of preexisting drug resistance alleles were around 1% for a panel of drugs, including those currently approved and three in development. During antiviral treatment, there is rapid CMV evolution which is presumed to be due to preferential selection of already present variants rather than by the creation of new variants.

Also, I like those presentations that open doors to new opportunities. For example, diagnostic assays are fast approaching an ideal aim, a compact instrument, simple to use with any type of sample, no sample preparation and a result within an hour. This has initiated a self-progressing circle, opening opportunities to develop drugs for more viruses.

Similarly, I chose to include in this report the prodrugs for nucleoside triphosphates because it widens the structural range of triphosphate analogs which may be specific inhibitors of viral polymerases. The phosphoramidate prodrug approach has been so successful, for example sofosbuvir and tenofovir alafenamide, but the standard synthetic method has led to poor yields for highly modified nucleosides – the use of microwave heating may have the potential to improve their synthesis. My report ends with brincidofovir which had good efficacy against adenovirus in hard-to-treat immunocompromised patients.

The three awardees gave excellent lectures which set the standard for the rest of the meeting. Together with the La Jolla sunshine, this was a memorable ICAR – I warmly thank the ISAR organisers for delivering another outstanding meeting.

Acknowledgements

I wish to thank all those authors who have kindly provided me with copies of their presentations and for giving me valuable comments. Also, I thank the President of ISAR for asking me to prepare this meeting report.

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