ratio of total drug concentration would appear to be of limited value unless, for the receptor in question, total drug levels have been shown to be relevant. In the absence of this link, literature evidence suggests: (i) the importance of considering free drug concentrations in the brain; (ii) that the equilibrium theory of free drug applies to the 'CNS compartment'; and (iii) the potential use of CSF drug concentrations as a surrogate for free drug concentration in the brain.

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Stacking up the armory against viruses

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The recent *Future Antivirals: Latest Developments in HIV, Hepatitis, Herpes, Influenza, RSV & SARS* conference (12–13 November 2003, London, UK) focussed on the major advances and novel solutions in the field of antiviral drug development. At present, antiviral R&D programs are concentrating on the development of drugs designed to inhibit the action of viral proteins, but there is increasing evidence that host cells will also afford important targets for therapeutic agents [1,2]. Antivirals that exploit the host response already exist, for example, interferon-α (IFN-α). Although there are chemotherapies available for the treatment of influenza, herpes simplex, varicella–zooster, cytomegalovirus, respiratory syncytial virus (RSV), papilloma, hepatitis B, hepatitis C and HIV infections, there is still a significant unmet medical need for novel drugs to combat these diseases. Perhaps of greater concern is the need for drugs to treat newly emerging infections, for example Severe Acute Respiratory Syndrome (SARS) and West Nile Virus (WNV).

Emerging viral infections

In terms of emerging diseases, an interesting outlook was presented by Paul Kellam (University College of London; http://www.ucl.ac.uk). As a result of the continuing improvement in molecular and biochemical technologies, the number of viruses and associated infections that can be detected is constantly increasing, as illustrated by the recent SARS outbreak. SARS-CoV was unambiguously identified in November 2002. In April 2003, the link between SARS-CoV and the disease was established, and in May 2003 the full genome was sequenced. Meanwhile, Pfizer (http://www.pfizer.com/main.html) launched a drug development program on the SARS-CoV 3CL protease. Wade Blair (Pfizer) reported on anti-SARS-CoV compounds that show activity in an *in vitro* retroviral assay. Pfizer pursued homology modeling of the SARS-CoV 3CL protease, based on the human rhinovirus 3C protease (HRV-2 3C protease) crystal structure, and their current lead out of this program is

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AG7122, which shows efficacy in a cellular replication model.

Patrick Iversen from AVI Biopharma (http://www.avibio.com/home.html) reported on a different development strategy for treating SARS that exploits antisense therapy. AVI Biopharma uses antisense phosphorodiamidate morpholino oligomers (PMOs), which are uncharged and stable, to target c-myc (AVI4126) and CYP3A4 (AVI4557) in cancer. The company currently has nine ongoing clinical studies researching the efficacy of using PMOs in the treatment of cancer. To date, they have not observed any serious adverse side effects in any of the 250 patients participating in the clinical studies. AVI-Biopharma decided to apply PMO technology to the field of antivirals and translated it into an extremely promising research program. Furthermore, Iversen reported a record time of nine months for the progression of the PMO AVI4020 (used in the treatment of WNV) from concept to Phase Ib clinical trials. Similarly, the activity of various PMOs against several other flavivirus models (e.g. Dengue-2 and hepatitis C) was observed. In summary, PMOs are a safe, effective and rapid novel antisense technology that show promise for antiviral therapy. However, one potential problem with PMO therapeutic agents is the method of administration; the stable state of PMOs is uncharged, which renders them unsuitable for oral administration.

Although RSV affects significant numbers of infants, elderly and immunocompromised people, the pathology of the virus has not been extensively researched. Malcolm Carter (Arrow Therapeutics; http://www.arrowt.co.uk) gave a comprehensive overview of compounds in development and already in use for the treatment of RSV. For obvious reasons, the fusion inhibitor programs of Wyeth (http://www.wyeth.com), Viropharma (http://www.viropharma.com), and Bristol–Myers Squibb (http://www.bms.com) are on hold or discontinued. Arrow Therapeutics has identified a potent and promising lead series of compounds, which is represented by the clinical candidate A60444. A60444 entered preclinical development in 2003 and clinical trials are planned for early 2004. A60444 has a completely novel mode of action.

Nucleoside analogs – panel discussion

The panel discussion tackled the question: 'Nucleoside Analogs – have they been exhausted?' J.P. Somadossi (Idenix; http://www.idenix.com) and R. Schinazi (Pharmasset; http://www.pharmasset.com), emphasized that there will be continued research into the development of novel nucleoside inhibitors against viral polymerases. The development of novel nucleoside analogs remains an attractive prospect for the future of antiviral drug discovery for the following reasons: administered orally; 50 years of experience in nucleoside chemistry; a well defined pharmacology and mechanism of action; ease of production; low protein binding; potentially low drug–drug interaction; a high probability of regulatory approval; and low development costs. However, K. Powell (Arrow Therapeutics) raised several clear counterarguments: a need for highly trained nucleoside chemists; a clearly defined toxicology [3]; and the potential of viruses to develop resistance.

Undoubtedly, nucleoside inhibitors have become efficient front-line therapies against life-threatening viral infections (e.g. HIV), but the time has come to generate new drugs that not only lower the viral load in patients, but also eradicate the virus from the host. The application of this novel intervention strategy to HIV and hepatitis C was particularly well covered at the conference.

Novel intervention strategies in HIV

David Stammers (University of Oxford; http://www.ox.ac.uk) reported on the successful application of structure-based design for non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the treatment of HIV. NNRTIs act by inhibiting HIV reverse transcriptase. The binding of NNRTIs to HIV reverse transcriptase induces a conformational change in the enzyme, which subsequently introduces steric effects that inhibit the mode of action of HIV reverse transcriptase. On this basis, the third generation of NNRTIs (e.g. TMC125 and GW8248) has been successfully developed.

Akhter Molla (Abbott Laboratories; http://www.abbott.com) summarized the clinical results for Kaletra, which is a combination of two HIV protease inhibitors (PIs), Ritonavir and Lopinavir, and is currently the bestselling HIV drug. Kaletra is well tolerated and exhibits significant antiviral effects in single and multiple PI-experienced patients. Molla reported that although Kaletra has a greater activity than the PI Atazanavir, studies indicate that Atazanavir is likely to become the largest competitor of Kaletra in the HIV drug market because of the once-daily administration of Atazanavir (which is unique amongst the PIs) and the low level of lipid-related side effects (as compared with the other five marketed PIs [4]) associated with the drug.

Barney Koszalka (Trimeris; http://www.trimeris.com) summarized the new field of HIV-entry inhibitors and, beginning with a host cell approach, presented some insights into the development of CXCR4/CCR5 ligands. The most promising compound appears to be a CCR5 antagonist, the spirodiketopiperazine derivative, GW873140 (also known as AK602 and

ONO4128), which commenced Phase I clinical trials in July 2003. In addition, Koszalka summarized research performed on inhibitors of the glycoproteins gp120 and gp41. Roche recently launched (March 2002) Fuzeon (T20), a peptide drug that acts by blocking the HR1–HR2 domain interaction of gp41. Fuzeon returns the CD4 T-cell counts in patients to almost 100 cells/mm^3, which is a 'magic number' for clinicians. However, Koszalka advised that Fuzeon should be employed as early in therapy as patients are able to tolerate because of the side effect of adverse reactions occurring at the injection site, which is exacerbated by the administration of twice-daily injections, and leads to a discontinuation rate in 4% of the treated patients. A second-generation fusion inhibitor currently under development (T1249) shows *in vitro* activity against Fuzeon-resistant viruses.

Novel intervention strategies in hepatitis C

Worldwide, ~170 million individuals are infected with the hepatitis C virus (HCV). Current treatment for hepatitis C is restricted to interferon-α (IFN-α) ± ribavirin; however, ~50% of patients infected with this life-threatening virus do not respond to this therapy, which underlines the seriousness of the disease and the urgent (and unmet) medical need for an alternative treatment. The lack of a cellular replication model for HCV complicates research into novel therapeutic agents for HCV. The HCV replicon cell system is recognized as the current 'gold standard' in HCV research [5].

Nathaniel Brown (Idenix) described Idenix's NM283-program, which was designed to develop a novel treatment for hepatitis C rapidly. NM283 is a nucleoside inhibitor of the HCV polymerase and showed efficacy in a chimpanzee model. Furthermore, the FDA accepted NM283 for Phase I/II clinical trials, which started in 2003.

Michael Cordingley (Boehringer Ingelheim Limited; http://www.boehringer-ingelheim.com) explained the development of BILN2061, which is an inhibitor of the NS3 protease. In early 2003, BILN2061 was the most promising candidate for the next generation of hepatitis C drugs. The final structure of BILN2061 was optimized from co-crystallization studies of a peptide-like lead structure and is the result of a challenging peptidomimetics approach. BILN2061 has potent activity *in vitro* (in the replicon system), but it is not bioavailable in chimpanzees. In a Phase I trial, BILN2061 demonstrated potent activity and a viral drop of three logs after a two-day treatment. However, routine chronic safety testing of high, supra-therapeutic doses in animals identified adverse reactions to the drug. Boehringer Ingelheim is currently studying the available preclinical data to determine their impact on the clinical development of this compound.

Bert Klebl (Axxima Pharmaceuticals; http://www.axxima.com) presented an approach based on the identification and validation of a host-cell target in hepatitis C. The gastrointestinal glutathione peroxidase (GI-GPx) is specifically downregulated in HCV replicon cells. Validation experiments using the replicon system revealed that ectopic overexpression of GI-GPx leads to the inhibition of RNA replication. The GI-GPx promoter contains retinoid acid response elements, and addition of retinoid acid and its derivatives to the replicon system results in inhibition of the GI-GPx promoter. Therefore, retinoid acid (a market drug for acne) could provide an alternative medical therapy for treating hepatitis C, particularly for IFN-α non-responders.

Although the panel discussion addressed the future of nucleoside inhibitors, which are attractive from an economical perspective, current R&D efforts are moving in a different direction with alternative viral targets becoming the key targets for research. Host cells are also evolving as potential targets for the treatment of viral infections. The future of antiviral drug development holds great interest. We look forward to the next conference for an update on the progress of this exciting field of research.

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