Efficacy and effectiveness of oseltamivir unveiled

The Multiparty Group for Advice on Science (MUGAS) offered yesterday a timely satellite symposium on the review and statistical analysis of oseltamivir data. Nancy Cox (Centers for Disease and Prevention, USA) opened the session by welcoming the support provided by MUGAS for the urgently needed assessment of the efficacy and effectiveness of oseltamivir, one of the major neuraminidase inhibitors used to fight influenza.

Although vaccines are cornerstones of public health's arsenal for the prevention and control of influenza, antiviral drugs play an essential role in the fight. Their effectiveness has been the subject of debates for many years, as were early antivirals developed against influenza, such as the M2 channel blockers amantadine and rimantadine, in the 1980s and 1990s. The ongoing debate on the effectiveness of the newest class of antivirals, the neuraminidase inhibitors, has become a hurdle for public health response to seasonal and pandemic influenza.

An overview of the MUGAS-initiated statistical review of all available data was presented by Prof. Arnold Monto (University of Michigan, USA). The aim of the study was to offer a thorough, independent and transparent meta-analysis of published and unpublished data from clinical trials, carried during the procedure of licensure of the neuraminidase inhibitors. Despite different routes of administration, oseltamivir and zanamivir have a similar mechanism of action and data on both antivirals were evaluated. Prof. Monto outlined the overall approach used, with all data collected from randomized placebo-controlled clinical trials in adult and pediatric patients (therapeutic use only), and all analyses performed on an individual patient basis.

Preliminary results of the meta-analysis, soon to be published, were presented by Joanna Dobson (London School of Hygiene and Tropical Medicine, UK). Nine clinical trials on oseltamivir in adults were included, with similar inclusion criteria, intervention protocol and main outcome, defined as the time to alleviation of all symptoms. Two clinical trials were carried out in the elderly population and one trial in individuals with chronic cardiac or respiratory illness. Close to 70% of the patients proved to be infected with influenza A virus, mainly of subtype H3N2. Statistically significant reductions in the time to alleviation and in lower respiratory tract complications were observed, with no difference between age groups. Adverse events presented principally as gastrointestinal conditions, including vomiting and nausea. Three clinical trials in pediatric patients were included, with age and clinical heterogeneities calling for trial-specific analyses and secondary meta-analysis. Although no differences were found or results were inconclusive for two trials in asthmatic children, statistically significant reduction in the time to alleviation was detected in healthy children. Dr. Dobson concluded that although the use of neuraminidase inhibitors should be weighed against the risk, the meta-analysis provided strong evidence of their usefulness against seasonal and pandemic influenza.

The Post-pandemic Review of anti-Influenza Drug Effectiveness (PRIDE) study completed the MUGAS report and was presented by Puja Myles (University of Nottingham, UK). The observational study was performed to assess the usefulness of oseltamivir treatment during the pandemic of 2009, by conducting a systematic review and meta-analysis of published data. The review of 44 and 52 studies with mortality and severe disease as the outcome, respectively, demonstrated significant clinical effects of early treatment with oseltamivir. The standardization and meta-analysis of raw data obtained form 80 research groups in 38 countries of 6 WHO regions further supported the effectiveness of early oseltamivir treatment against mortality, as well as that of late treatment in critically ill patients.
Cross-protection against influenza: no longer a utopia?

Immunological basis for novel influenza vaccines
After hearing Marc Sprenger during the ESWI opening on Sunday and Ab Osterhaus at the plenary session on Monday, there was a clear take-home message: the efficiency of currently used influenza vaccines is subject to improvement, especially in rare occasions that the vaccine antigens poorly match the dominant circulating virus strains. Since this obviously indicates that novel generation influenza vaccines should be developed, the Monday immunology session was of a particular interest, since according to the abstracts all speakers would be touching upon important implications for the design of novel influenza vaccines.

Designing a universal vaccine: Are B- or T-cells important?
Even though the speakers did not focus on the design of novel influenza vaccines, it soon became apparent that all were interested in the defining the immunological correlates of cross-protective immunity. The factors that can actually confer cross-protective immunity were elaborately discussed, and the obvious next step would be taking this knowledge to the drawing board to design a universal influenza vaccine. The session was divided in two camps: those adhering to the importance of cross-reactive T cells and those who firmly believe in the importance of cross-reactive antibodies.

Stalk-specific antibodies: less potent but more broad
Presentations discussing the importance of cross-reactive antibodies boiled down to the same conclusion: due to antigenic drift usually only HA stalk-specific antibodies can be broadly reactive, in contrast to conventional HA globular head-specific antibodies that are highly strain specific. Apparently, these stalk-specific antibodies have a major disadvantage: they appear to be less potent in neutralizing virus than globular head-specific antibodies. Matthew Miller elegantly demonstrated that when tested in a polyclonal background, the neutralizing potency of HA-stalk binding antibodies was markedly increased relative to that of antibodies that bind to the HA-head domain. Taking it one step further and looking at antibody isotypes, Miller showed that IgA was capable of neutralizing virus with superior potency relative to IgG. Since Florian Kramer showed that stalk-specific cross-reactive antibodies are induced by natural influenza virus infection in animal models, it will be interesting in the future to further characterize the breadth of the response and the level of cross-protection in humans remains to be determined.

Cross-reactive T cells can protect from severe disease.
Focusing on the other arm of the adaptive immune system, natural infection with influenza virus leads to the production of influenza-specific T cells. Importantly, vaccination with an inactivated vaccine generally does not lead to the induction of an adequate CD8+ T cell response. In animal models it has been extensively shown that CD8+ T cells can confer heterosubtypic immunity, but evidence in humans is still largely lacking. Caroline van de Sandt showed that in blood from healthy donors, influenza B specific T-cells could be expanded and were capable of recognizing an influenza B virus from a completely different lineage. That cross-reactive T cells also have a protective effect was shown by Saranya Sridhar, taking advantage of the 2009 H1N1 pandemic. In an experiment of nature he used blood samples from 300 donors during the first wave of the pandemic, of which 180 were still negative for HI and VN antibodies against pH3N1, meaning that they had not yet been infected with the pandemic virus. He looked for cross-reactive T cells recognizing pH3N1 virus within these donors, and showed that the presence of these T cells correlated with less severe disease when these donors were naturally infected with pH1N1 virus. This shows that both B cells and T cells can contribute to cross-protection against severe disease caused by antigenically different strains.
**Influenza virions – Trojan exosomes?!**

During the session “Viral Structure and Replication” novel insights into the influenza virus particle were given. Besides the identification of a new functional site in the PB2 subunit and the crucial role of influenza RNA packaging signals during genetic reassortment, role of HA and M2 protein modification in assembly of influenza virus particles and fascinating new data about the virion architecture were presented.

The RNA-dependent RNA polymerase of influenza viruses is essential for viral transcription and replication. The PB2 polymerase subunit is responsible for the cap-‘snatching’-activity, whereby cap structures of the host cell’s premRNAs are transferred onto viral mRNAs. Nevertheless, the key functional sites of PB2 remain unknown. Dai Hatakeyama from Tokushima Bunri University showed nicely that the Val/Arg/Gly (VRG) site in PB2 is crucial for interaction with acetyl-CoA as well as RNA polymerase activity and viral replication in vitro.

The high variation of influenza viruses relies on the segmented RNA genome which allows genetic reassortment. Little is known about the packaging signals in vRNA complexes. Results presented by Catherine Isel from the University of Strasbourg clearly indicate that incompatibility between the vRNA packaging signals has negative effects on the genetic reassortment. Furthermore, she could show a crucial role for the M segment in packaging and reassortment.

Influenza viruses are known to build complex and pleomorphic virions which are difficult to analyze in detail. Here, Edward Hutchinson from the University of Oxford presented a new technique to allow a closer look to the content of these structures. By using quantitative mass spectrometry, he was able to demonstrate that the complement of viral proteins is conserved across different combinations of virus and host. Hutchinson’s data show that the examined non-structural protein NS1 is clearly associated with the virions. Additionally, Hutchinson revealed that a large number of host cell proteins are incorporated into virions. Even after an extensive, optimized purification protocol for the virions, a large number of associated host-cell proteins remain. Furthermore, Hutchinson showed that the incorporation of these proteins is also host specific, e.g. CD9 is only incorporated by mammalian hosts. He suggests that influenza viruses have co-opted parts of the exosome assembly pathway for their virion production, which leads to the host specific architecture of influenza viruses. This data raises the question of whether influenza virions are essentially Trojan exosomes.

Taken together, this session clearly underscores that the impact of the virus producing host is much greater than we suspected before. And in the future, we need to consider these differences when designing more effective vaccines.

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**THE ROLE OF HOST ORGANISM INCREASES IN INFLUENZA RESEARCH**

Data presented suggest that the choice of host organism for influenza virus research plays a previously underappreciated role in influenza virus biology, including genetic reassortment as well as vaccine production.
We need to work on collaboration

Yesterday morning’s SPI-track program kicked off with this simple key question. Indeed, in order to limit the burden, influenza should become a public health priority, and stakeholders should understand the severity and impact of flu, and be involved. The SPI-track generated a wide variety of ideas to tackle the hurdle. Here are some snapshots.

Better surveillance fuels earlier detection and more effective information. “Flu is a moving target and viruses are smart and simply unpredictable”, said Ab Osterhaus, Erasmus MC Rotterdam, The Netherlands. “If we want to conquer the beast, we need to use all available technologies and work on collaboration.” Surveillance systems are already very effective and make annual vaccine reformulation possible. On the basis of antibody presence in the population at large, it is possible to predict which viruses should be put in a vaccine.

But when it comes to inspiring changes in behavior, scientists need to empathize with their stakeholders. For example, the data proving vaccines are safe mainly originate from high-income countries. But in order to raise confidence in low income countries, we will need data local risk groups can identify with, based on studies in these countries, said Abdulla Brooks, from the Infectious Diseases Unit at Kamalapur Field Site in Bangladesh.

Raina MacIntyre from the University of New South Wales in Australia, demonstrated the role of influenza vaccination in preventing heart diseases. “There are many ways in which infectious diseases overlap with coronary diseases. Therefore, we have to engage cardiologists in our activities, and make them part of what we do. Not only talk to them, but partner with them.” Vaccination is perhaps not perceived as an art of heroic medicine, but in the end, it is preventing a large amount of heart diseases in people.

Ilaria Capua, both virologist and Member of the Italian Parliament, stressed not only the importance of communication with the public at large and with fellow scientists, but also with politicians, because in the end, they will decide on strategy and policy direction. “We need to understand why politicians behave the way they do. Politicians expect certainty. They are afraid of being criticized on their integrity. But our statements are full of ifs, or’s and when’s. And so politicians are completely confused on what they should do. As a result, we are selling them a hazard, a threat, and not something they can buy”, Capua said.

The Heinrich Pette Institute (HPI), located in Hamburg/Germany, is dedicated to basic research of the biology of most-relevant human pathogenic viruses and the pathogenesis underlying the respective virus-induced diseases. The institute’s long-established mission is to provide new technologies and solutions to improve therapeutic procedures for established and emerging viral diseases. These include AIDS, Influenza and Hepatitis as well as certain types of cancer linked to infections with Herpes-, Polyoma- and other DNA-Viruses.

The institute has created several advanced technology platforms that facilitate comprehensive investigation of the infection processes, including a High-Throughput Sequencing Facility, BSL2/BSL3 Small Animal Models and advanced Microscopy and Image Analysis. Research projects at the HPI comprise multi-disciplinary approaches and are closely linked with research groups in the Hamburg metropolitan region as well as other national and international collaboration partners.