Discovery and Evaluation of Therapeutics against Dengue
Workshop Summary

Chromos building, Biopolis, Singapore
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Organized by the Novartis Institute for Tropical Diseases (NITD) and the National Institute of Allergy and Infectious Diseases (NIAID)

NIAID Participants

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NIH Collaborators

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Stanley Watowich (Associate Prof.), Department of Biochemistry and Molecular Biology and Sealy Center for Structural Biology and Molecular Biophysics, The University of Texas Medical Branch, Galveston, Texas

NITD Participants

Gu Feng (PI, Leader 'host response and target' project)
Paul Herrling (Head of Novartis Corporate Research, Chairman of NITD board)
Chen Yen Liang (PI, Leader 'NS5 RNA-dependent RNA polymerase' project)
Alex Matter (Director NITD)
Christian Noble (PI)
Lim Siew Pheng (PI, Leader 'NS5 Methyl Transferase' project)
Wouter Schul (PI, Interim Unit Head, Leader 'Dengue Therapeutic Antibody' project)
Wang Qing Yin (PI, Leader 'cell-based dengue inhibitors' project)

NITD Collaborators
Martin Hibberd (Group Leader Infectious Diseases, Genome Institute of Singapore (GIS))
Julien Lescar (Assistant Prof., Nanyang Technical University (NTU))
Paul Young (Assistant Prof. / Reader, University of Queensland, Australia)

Goal of Workshop

Dengue virus is one of the most significant arthropod-borne viral diseases in the world, causing globally 50-100 million cases of dengue fever per year and tens of thousands of deaths, primarily in children. The four types of dengue virus, which historically have affected selected tropical and subtropical regions, have been spreading to new geographical areas and thus have become a serious public health threat worldwide. No vaccines or drugs are currently available to treat or prevent dengue infection. The goal of this workshop was to provide a forum for scientists from NITD, NIH and their respective collaborators to review the dengue therapeutics under development, to identify the most promising compounds in the pipeline and to identify areas of possible collaboration between different groups in order to expedite the licensing of one or more dengue antiviral drugs.

Summary of Discussion

Treatment of dengue fever (DF), and dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS)

Workshop participants seemed to agree that an antiviral approach for a dengue treatment is viable and promising, but that the time of start of treatment may have a considerable influence on the efficacy of an antiviral drug. Particularly, patients who already have high cytokine levels or entered DHF or DSS may not benefit from reduction of viral replication. Therefore, the greatest impact of an antiviral is likely to be on the treatment of DF. The importance of rapid diagnosis to the initiation of appropriate patient management and therapy was emphasized.

The possibility for treating with anti-inflammatory or immunomodulating therapeutics was briefly discussed, with TNF α as one of the most prominent targets, but with the caveat that suppressing pro-inflammatory cytokines may enhance disease. Alternatively, it was proposed that treatments that increase vascular integrity may counteract the vascular leakage, although great care has to be taken when this is combined with rehydration therapy, to avoid over-hydration.

The benefit for the use of drugs in combination has been clearly demonstrated for treatment of infectious and other diseases. Dengue is an acute infection and the risk of emergence of resistance would not be expected to be high. Nonetheless, the emergence of resistance to one class of influenza drugs was apparent within three days of treatment and resistance to other classes was also observed. Consequently, the development of at least two antivirals which attack different viral targets should be a goal. In addition, an effective therapy might involve the combination of one or more antivirals with immunomodulatory or other activity.

Clearly, a validated animal model that faithfully reflects DHF and DSS observed in patients would be of great value for the evaluation of therapies.

Cell-based screening approached vs. target-based screening approaches
There was a consensus that both approaches have merit and should be pursued in parallel. There was also the realization that cell-based screens based on whole virus replication (CPE assay) and cell-based replicon screens in which only a limited set of viral genes are involved are both useful approaches. The importance of target identification of drugs found in cell-based screens ("target deconvolution") to allow a compound to progress into lead optimization or clinical selection was recognized. For target-based approaches in silico docking can provide a useful starting point and there are improvements in this approach under development that may greatly reduce the number of false-positives that are generated.

Evaluation of targets
1. NS5 RNA-dependent RNA polymerase is a promising target with its fundamental role in viral replication and the known successes for antiviral drugs targeting viral polymerases encoded by other viruses.
2. NS5 methyl-transferase activity has been studied less than the polymerase but is becoming increasingly well understood and plays a key role in viral replication. With two distinct substrate binding pockets, it makes a promising target for inhibitor development. It was reported that purified NS5 methyl-transferase may have some specific RNA methylation activity which should be taken into consideration when developing a screening assay.
3. NS3 protease remains a well validated target considering its crucial role in dengue protein processing and the promising results observed for hepatitis C virus (HCV) and HIV protease inhibitors. However, efforts at NITD for the last three years have not yielded any potent leads. Dr. Watowich's lab and Dr. Young's lab are both working on new approaches to find NS3 protease inhibitors and reported promising results.
4. NS3 helicase is an essential enzyme for viral replication, but may be hard to target as it has a large active site that binds dsRNA and an ATP pocket with low specificity, with no known allosteric pockets. Efforts at NITD for the last three years have not resulted in any leads. Moreover, there are no known helicase inhibitors on the market, although there are inhibitors of the herpesvirus helicase-primase that are in mid- to late stage development. However, Dr. Lescar presented the full NS3 crystal structure with an RNA molecule bound, opening new possibilities for targeting sites in the RNA binding region of the protein.
5. Envelope (E) protein is a viral structural protein and potential non-enzymatic drug target. The presence of natural antibodies in recovered patients that neutralize the E protein could be considered as validation of this target. The therapeutic potential of anti-E monoclonal antibodies was considered to be promising based on data obtained with a related flavivirus, West Nile virus. Recent insights into the dengue E protein's structure, spatial organization on the viral surface, conformational changes during the membrane fusion process, and the ability to inhibit fusion by disrupting protein-protein interactions has encouraged new efforts to find small molecule fusion inhibitors. Although

disrupting protein-protein interactions by small molecules is notoriously difficult, a focused screening combined with structural and functional biology studies could yield interesting drug opportunities. A better assay to measure inhibition of membrane fusion would greatly help the development of a drug against the E protein.
6. siRNA approaches that target the viral genomic RNA and/or mRNAs may have great potential if successful strategies for their effective delivery, sustainability, and safety can be demonstrated.
7. Other viral targets, e.g. NS1, prM, and capsid, have functions or activities that are not understood well enough to assess their suitability as targets for inhibition although ongoing research may change this situation.
8. Human host targets essential for viral infection and proliferation are not well understood. More efforts need to be spent on finding the human proteins necessary for virus propagation. Alpha-glucosidase was suggested as a potential cellular target since it is known to play an important role in the proper glycosylation of the virus, has some known inhibitors, and has been well studied for treating HCV. Indeed, one of these inhibitors was shown to have protective activity in a mouse model in which DV-2 is injected intracranially, and two others were shown to significantly reduce viremia and inflammation when dosed orally in a mouse model in which DV-2 is injected intraperitoneally.

Clinical and epidemiological studies

The possibility of linking genomic sequences data with clinical outcomes and data was discussed. A large NIAID genomic program is currently supporting the collection and full genomic sequencing of hundreds of dengue viral isolates from around the world. The genomic data is deposited in a public database (GenBank) within a month of its generation. Everybody agreed that adding clinical data linked to the genomic information would allow researchers around the world to identify genetic traits of the virus and/or the host that might cause or contribute to disease severity and would be highly beneficial. NIAID and the National Center for Biotechnology Information (NCBI) will explore the possibility of upgrading the current system to allow more comprehensive information to be shared with the public.

A goal of many studies has been the identification of biomarker(s) that could reliably predict progression to more serious disease. To this end, the possible comparison and coordination of clinical studies of biomarker correlates of disease severity and disease progression in different populations was considered. Several presentations discussed efforts ongoing in several parts of the world to find biomarkers in individuals with dengue fever that would identify patients likely to progress to DHF/ DSS. This would allow public health authorities in dengue endemic areas to concentrate their limited hospital resources on patients at highest risk of severe morbidity. This should improve overall disease outcome and drastically reduce the healthcare costs. It was suggested during the workshop that it would be beneficial to compare the data on biomarkers collected by different groups in different populations. It was further recommended that the various groups conducting studies of natural history and pathogenesis meet and, to the extent possible, agree on the collection of data on a core of disease-associated

parameters. These parameters will facilitate comparisons among studies of patient populations differing in factors such as locale, nutritional status and economic status. There was general consensus on the importance in collecting as much cytokine data as possible in the course of natural history studies.

Resistance

Resistance is an inevitable risk but there was a consensus that several factors would reduce the risk of rapid emergence of resistance. The expected short duration of treatment; self limiting nature of the infection; the need for vector transmission; and somewhat lower mutation rate of the dengue polymerase (compared to HIV polymerase) are factors that reduces the risk of resistance, even with monotherapy. However, to be able to treat resistant dengue should it arise and to reduce the risk of resistance emergence by use of combination therapy, the search for new anti-dengue therapeutics has to continue beyond the first drug becoming available.

Other issues

Efforts were described to generate algorithms for the diagnosis, evaluation and triage of patients with suspected dengue illness. As similar efforts are ongoing in Thailand and Vietnam by different groups, it was suggested that the data that is generated in one population is compared and validated with data from patients in other dengue endemic areas. Investigators responsible for the different studies have been put in touch to discuss the possibility of a comparative study.