Professor Igor Lukashevich provides an insight into his research, which aims to curb rising infection rates of yellow and Lassa fever with an innovative new vaccine

YF17D vector for Lassa fever vaccine

Could you offer an insight into the background of the project?

Recently the yellow fever vaccine YF17D has been successfully used as a vector for live vaccines against flaviviruses (Japanese Encephalitis, West Nile, and Dengue), and as a vaccine vector for flavivirus-unrelated B and T cell epitopes. We use a full-length infectious cDNA clone of the YF17D as a vector for LAS virus genes, GPC and NP, encoding major antigens, glycoproteins and nucleoprotein, respectively. We are testing the hypothesis that YF17D/LAS recombinants will effectively express LAS virus major antigens and induce protective immune responses in experimental animals. Our goal is to assess the safety, immunogenicity and efficacy of YF17D/LAS recombinants in small animal models (mice, guinea pigs) before pre-clinical trials in non-human primates.

What could YF17D offer compared to its predecessors? What are the main advantages and disadvantages of this viral strain in vaccine production?

The YF17D vaccine is cheap, used as a single dose, and has a highly favourable benefit-risk profile. More than 500 million have been vaccinated, with rare adverse events.

Sequencing of the YF17D genome and recovery of YF17D from full-length complementary cDNA allows production of a homogeneous cDNA-based vaccine *in vitro*. This technology also introduces tissue culture cells as a vaccine substrate and facilitates the manufacturing process. In cooperation with our partners we further developed a cDNA-based approach and proposed infectious DNA (i-DNA) immunisation based on recovery of infectious YF17D from cDNA *in vivo*. The i-DNA combines the benefits of conventional DNA immunisation with high-efficacy of live viral vaccines.

The full-length cDNA is generated and introduced into Medigen's plasmid vector containing optimised eukaryotic promoter and regulatory elements necessary for efficient transcription of genomic YF17D RNA in eukaryotic cells. The full-length genomic RNA transcribed from i-DNA is expected to be capable of launching attenuated 17D vaccine in mammalian cells *in vivo*.

Could you elaborate on the genetic stability issues that you have discovered thus far?

In our project we used YF17D vaccine as a vector to express the major immunogen of LAS virus, intracellular glycoprotein precursor, GPC. The vector, YF17D, is genetically very stable. However, molecular basis of YF17D attenuation is still unknown. So, dealing with a genetically-homogeneous population of YF17D derived from cDNA provides a kind of peace of mind. The major problem we faced was stability of the LAS virus GPC insert into the YF17D. After several passages *in vitro* or *in vivo* the GPC gene was spontaneously deleted; it appears the YF17D vector has size limitations for foreign insertion. We decided to clone the LAS virus GPC gene as two antigenic sub-units, GP1 and GP2, producing two recombinant viruses, YF17D/LAS-GP1 and –GP2. These new vaccine stocks had much more stable profiles and were used for vaccination-challenge experiments.

Why are there no plans to verify that the recombinant vital vectors still function adequately as yellow fever vaccines?

We already tested that immunisation with recombinant YF/LAS vaccine-induced protection against fatal LAS virus infection and induced antibody responses against vector, YF virus. It must be mentioned that protection in the case of LAS is based on CD8+ CTL responses and protective immunity against YF is associated mostly with induction of neutralising antibodies. Initially, the project plan focused on induction of anti-LAS virus protective immunity. We are now extending our research into anti-YF immune responses and we are working with mice lacking the type I interferon (IFN-α/ß) receptor (A129). Wild-type YF virus induces a fatal infection in A129 mice resembling human disease. In contrast, YF17D is non-pathogenic in these mice. Preliminary results indicate that YF17D/LAS recombinant vaccine induces neutralising antibodies against YFV in titers comparable to those induced by the parental YF17D vaccine. The next step will be evaluation of protective efficacy of the recombinant vaccine in A129 mice challenged with wt YFV.

Do you believe that the introduction of a vaccine solves the whole problem; is there also an issue of public image which stops people seeking assistance?

I believe that introduction of a safe, efficacious, and affordable vaccine will effectively control the infection. The previous success of massive YF17D vaccination programmes in 1940-1953 demonstrates this. The WHO is trying to fix the shortage of YF17D and motivating countries to extend YF17D vaccination. This project is dealing with improvement of YF17D-based technology and development of a bi-valent vaccine to combat YF and LF for public health benefits in West Africa.

How close are you to conducting pre-clinical non-human primate trials?

For pre-clinical studies we have already designed a programme with our potential biotechnology partner (SAFC Pharma, Carlsbad, CA, USA) for pilot manufacturing this vaccine for phase I safety evaluation and efficacy trials in non-human primates. This program includes four milestones with a 48-month timeline, with an approximate cost of $4 million.

What are the plans for the future of this project?

We are looking to make YF17D-based vaccines against VHFs circulated in South America, because endemic areas of these diseases overlap with YF-endemic areas, and have been collaborating with colleagues from University of Buenos Aires, Dr E Damonte and Dr C Garcia. We are also focusing on further development of i-DNA vaccine technology in collaboration with Dr P Pushko from Medigen, Inc.

A vaccine for Lassa fever

By re-engineering the yellow fever vaccine YF17D, researchers led by **Professor Igor Lukashevich** are hoping to produce a new, recombinant vaccine which will provide immunisation against both yellow fever and Lassa fever.

YELLOW FEVER (YF) and Lassa fever (LF) are two viral hemorrhagic fevers (VHFs) endemic to west and central Africa. There is currently no vaccine for Lassa fever, and although a widely-used and successful vaccine for yellow fever, attenuated YF17D, has existed since the mid-20th Century, ecological and social changes, ineffective public health policy and insufficient vaccine coverage have meant that the last 15 years have seen an increase in the number of cases.

Lassa fever is a preventable infection that touches the lives of hundreds of thousands per year in West Africa. In recent times, the wars in Liberia and Sierra Leone, and unrest in Guinea and Nigeria, have added to the problem by displacing many in rural areas, increasing exposure to virus-carrying rodents. Furthermore, the increase in poor living conditions in West Africa has brought the disease to the slum areas of larger cities. In the late 1980s and early 1990s there were conservatively estimated to be as many as 300,000 infections from Lassa virus per year, with perhaps as many as 20,000 deaths; a more recent estimate based on current populations in rural areas suggests these figures are now even higher. In endemic areas, the 'at risk' LAS virus sero-negative population may now be as high as 59 million, with an annual incidence of illness of 3 million, fatalities of up to 67 thousand, and up to 3 million re-infections. A similar level of risk in any developed country would warrant a major research effort such as that into HIV in the U.S. and Europe, but to date, few resources have been allocated, despite the existence of a candidate vaccine that could prevent illness and death. Such a sizeable disease burden makes the development of an effective and safe vaccine against these two diseases a high priority for public health.

TEACHING AN OLD VACCINE NEW TRICKS

Professor Igor Lukashevich is Associate Professor of Medicine at the Institute of Human Virology, University of Maryland School of Medicine. In collaboration with partners in the U.S. and the Netherlands, he is driving an innovative study which seeks to develop a recombinant vaccine, based on YF17D, which protects against both Lassa fever and yellow fever, with the long-term goal of controlling both diseases in Africa.

As Lukashevich explains, the need to address these diseases has never been more pertinent: "Because of the failure to continue mass vaccination campaigns and a shortage of YF17D," he explains, "a resurgence of deadly yellow fever in many African countries began in the early 1980s. Now, it is a re-emerging illness in tropical and sub-tropical areas of Africa and remains a major health threat in South-America. Worldwide, it is estimated to affect 200,000 individuals annually, of whom approximately 30,000 will die mostly in Africa".

Theoretically, more effective vector control could significantly reduce the risk of infection for these diseases in Africa. However, Mastomys natalensis, a natural rodent host for LAS virus, is widespread in sub-Saharan Africa and traditionally used as a protein source in poor rural areas, which makes rodent control impractical. Moreover, climatic and environmental factors pose significant difficulties to vector management, as Lukashevich outlines: "Aedes mosquitoes are responsible for transmission of YF virus between monkeys, from monkeys to humans, and between humans, and in underdeveloped rainforest areas during the rainy season, vector control is very difficult to achieve. The vaccination is the only effective measure to control these infections in Africa".

PREVENTION RATHER THAN CURE

The study has three specific aims: firstly, to generate and validate a set of vaccine candidates from recombinant YF17D/LAS replication-competent viruses; secondly to assess their ability to induce antigen-specific antibody and cellular immune responses in vaccinated animals; and thirdly to test their efficacy in protecting guinea pigs against LAS virus challenge.

Lukashevich is keen to point out that, rather than treatment, the project is about the development of a prevention strategy to control LF and YF. "The LF treatment strategy is limited to ribavirin," he says; "however, this drug is effective at a very early stage of the infection. It is also toxic, expensive, and impractical for widespread use in West Africa. There are no antiviral drugs to treat YF patients".

The project is driving innovation and the team believes there is a mandatory need to improve current YF17D technology. One new approach they have taken is the proposal of infectious DNA, or i-DNA. The i-DNA turns a small number of cells in the tissues of the vaccine recipients into cell-scale 'factories' which

manufacture vaccine inside the tissues of the recipient. If successful, this could revolutionise the development, manufacture, and application of live attenuated viral vaccines.

"The i-DNA technology targets the major current problems," explains Lukashevich. "Genetic stability and homogeneity of the initial master and working viral stocks, their production and controls, i-DNA master stock which is much easier to control and manipulate than live virus stocks, and bacterial-based production of recombinant DNA, is well-established technology. DNA-based vaccines are significantly cheaper in terms of storage and transportation and do not require a 'cold-chain'."

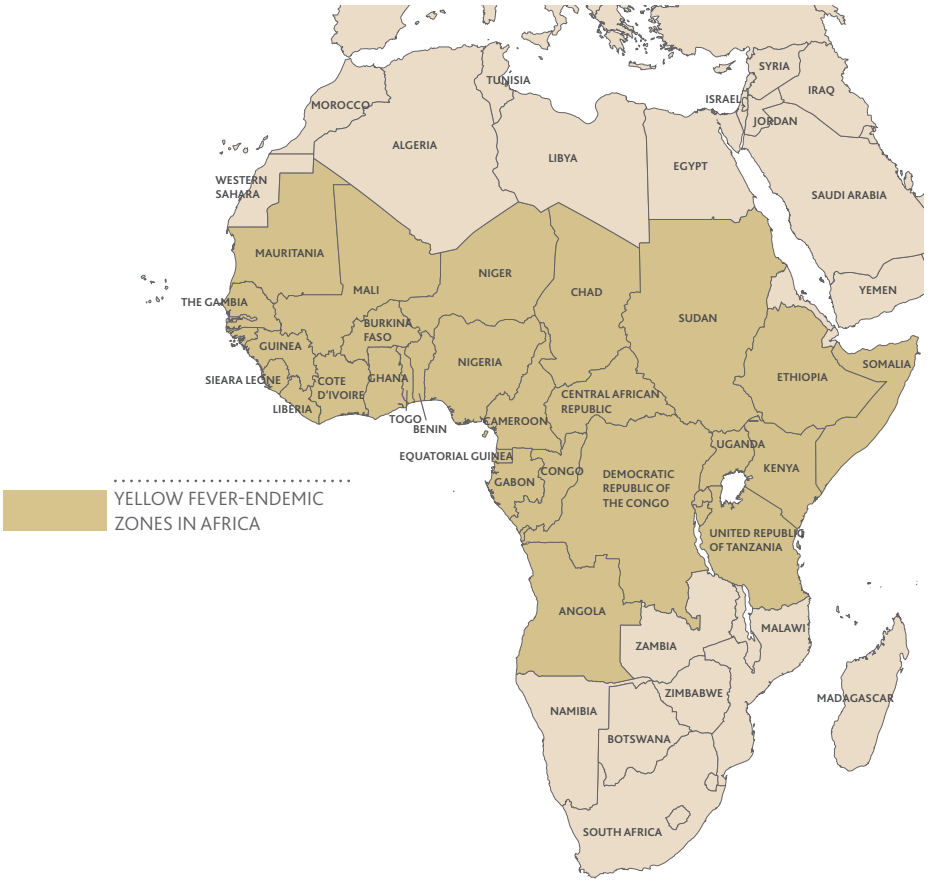
AVAILABLE WITHIN 10 YEARS

The cost-effectiveness rate for YF17D is also favourable: a vaccinated child is fully protected over a 50-year life time for an investment of a few cents cent per year. Lukashevich believes that the introduction of new generations of YF17D and YF17D-based recombinant vaccines will continue this trend, and that under favourable conditions, including appropriate financial support, the involvement of a key industry player in the project, and the availability of clinical trial infrastructure, the new vaccine will be available for use in Africa and other badly-affected areas within 10 years.

This of course raises questions about whether those people already infected, or at risk of being infected, can be helped by the new vaccine.

Many cases of LAS infection result in sub-clinical or flu-like infections, providing natural immunisation against the second round of the infection. The overall case-fatality rate for rural LF is about three per cent, rising to 16 per cent among hospitalised patients. However, in certain groups of patients – notably pregnant women, new-borns and children younger than five years old – the case-fatality rate can be more than 50 per cent. "In addition," remarks Lukashevich, "in West Africa, as many as a tenth of the potential vaccines have an altered immune status. Indeed, we have shown that LAS virus sero-prevalence rate in HIV-1 positive individuals was almost twofold that of HIV-1 negative persons. So, immuno-compromised HIV-infected individuals can represent an additional vulnerable group for LAS virus infection".

The team is making progress in expressing LAS virus nucleoprotein (NP) in the YF17D vector. The role of NP in protection against fatal LAS virus infection is controversial. There is an evidence that in experimental rodents this protein is contributed to viral control at early stage of the infection. A single inoculation of a plasmid encoding full-length LAS NP induces effective CD8+ T cells responses against close-related and distantly-related arenaviruses. "The LAS virus is a heterogeneous species and an effective vaccine must protect against all virus genotypes," notes Lukashevich. "Unfortunately, LAS NP-based vaccine was found not to be effective in non-human primates. So, we are working to better understand the role of NP in pathogenesis and immune responses."



INTELLIGENCE

RECOMBINANT YELLOW FEVER 17D-LASSA VACCINE

OBJECTIVES

The priority of this study is to identify viral and host genes that control virulence, innate immunity, and to elucidate how the Lassa virus escapes adaptive immune control. The second goal is to design safe and efficacious vaccine.

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