Sweden is a privileged region in the HIV pandemic, but this was not at all clear when we opened Venhälsan, Södersjukhuset, Stockholm, in 1982. We saw those we diagnosed go on to become sick and die. However, we were fortunate to be able to take part in the worldwide quest for HIV drugs and rejoice in the breakthrough of highly active antiretroviral therapy (HAART) in 1996. In the early desperate days we explored the possibilities of various immune interventions, such as isoprinosine (which worked as well as zidovudine monotherapy (1)), and immunization with gp160 in collaboration with the Walter Reed Army Institute of Research (WRAIR) (which gave a transient survival benefit (2)). This led to the testing of the first DNA-based HIV vaccines with inserts coding for HIV early proteins, which were developed by Britta Wahren at Smittskyddsinstitutet (SMI) and Karolinska Institutet (KI), Stockholm (3). Earlier, in 1986, Gunnar Biberfeld from the same department had initiated a Sida/SAREC-supported programme in Tanzania, TANSWED. In 1992, I had the good fortune to join this programme. It focused on population-based epidemiology, natural "progression", laboratory capacity building, including HIV testing, mother to child transmission and HIV-related tumours, i.e. a very broad exercise to better understanding AIDS in Africa. Various cohorts were explored for their participation in trials of HIV vaccines, which at that point were only a dream (4).

At the end of 2010, an estimated 34 million people were living with HIV globally, including 3.4 million children under the age of 15 years. There were 2.7 million new HIV infections in 2010. Approximately 68% of all people living with HIV resided in sub-Saharan Africa, a region with only 12% of the global population. This region accounted for 70% of new HIV infections in 2010.

HIV prevalence peaked in 2003–2004 to 7.2% of the adult Tanzanian population, after which there has been a decline to an estimated 5.7%; higher among women (6.6%) than men (4.4%). The most important mode of transmission is unprotected heterosexual intercourse (80%), while mother to child transmission (MTCT) accounts for approximately 18% of infections. Provision of free HAART at Care and Treatment Centers (CTC) was rolled out countrywide in October 2004. By the end of 2010, the cumulative number of clients enrolled in HIV care was 740,040. A total of 244,148 patients were current on antiretroviral therapy at that time.

In 2001, Professor Fred Mhalu, our Tanzanian counterpart in TANSWED, called a meeting to explore whether Tanzania could play a role in the development of HIV vaccine. It so happened that the European Union (EU) had launched a call, due in one month, including prophylactic HIV vaccines. The University of Munich led the collaboration from Mbeya, and the research group from Muhimbili in Dar es Salaam teamed up with colleagues from WRAIR, University of Cape Town and the group at SMI/KI/Södersjukhuset, which had already come together around these issues to create a consortium to develop a vaccine designed for East Africa, i.e. a region were many HIV subtypes are circulating. Given the depressing news of the AIDSVax trial at that time, which showed no protection from anti-envelope protein antibodies, it was decided to focus on cell-mediated immunity that might not protect from transmission, but could potentially ameliorate disease if infected. The DNA plasmids vaccine developed at SMI/KI were to become the focus. This vaccine is unique, in that it contains Env and Gag coding plasmids against several subtypes; A, B and C.

HIVIS/TaMoVac study plan

<table>
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<th>Months</th>
<th>0</th>
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7 DNA Plasmids; KI/SMI
- Env A,B,C
- Gag A,B
- RT B
- Rev B

Recombinant MVA/NIH/WRAIR
- Env E
- Gag A
- Pol A

Attenuated vaccinia virus with HIV genes

Is priming with low dose intradermally equivalent to 'standard' dose intramuscularly?

Fig. 1. Study plan.
However, even back then it was evident that, although highly specific and safe, DNA vaccines are poor immunogens. To counter this we chose 2 approaches; the first was to prime a specific immunological response with DNA and then to boost these immune responses with analogous HIV genes of another subtype, E, in the potent vector modified vaccinia Ankara, (MVA) from WRAIR; and the second approach was optimization of the DNA vaccine delivery with a needleless injection device, Biojector, which since then has become the focus of our trials as it allowed reproducible intradermal injections. After a phase I study of these novel immunogens at Södersjukhuset and substantial clinical and laboratory capacity building in Dar es Salaam we launched the first phase I/II trial in 2007 (5). In this placebo-controlled trial we showed that there were no safety issues. All volunteers developed strong cell-mediated immunity. Intradermal injections with Biojector induced stronger and broader immune response with a quarter of the DNA dose used for intramuscular priming (6) (Table I). Furthermore, after the second MVA boost all immunized volunteers were shown, in routine diagnostic tests, to have seroconverted. The induced antibodies were principally directed against the envelope protein and were often neutralizing. It has been shown, in this neutralization is dependent on natural killer cells through antibody-dependent cell-mediated cytotoxicity (ADCC) (7). Thus, the vaccines and regimen could potentially protect from both infection and disease progression. We have recently extended this study in a subset of volunteers who have received a third MVA boost 3 years after the 2nd MVA in order to determine whether waning immune responses can be maintained in this way.

These promising results prompted a further trial in an effort to refine the intradermal DNA delivery. In the initial studies 5 needleless intradermal injections had been given at each immunization due to the small volumes that can be given intradermally. This is clearly not practical. A further complication was that the env and gag encoding plasmids had been given at different sites. Thus, the next step was to explore whether we could reduce the number of injections to 2 per immunization with a slightly higher concentration of DNA and whether all the plasmids could be mixed. This European and Developing Countries Clinical Trials Partnership (EDCTP)/Sida funded trial, which now included another site in Tanzania, Mbeya and 1 in Mozambique is now being analysed. A preliminary blinded analysis showed continued good tolerability and that there were only marginal differences between the groups with 2 or 5 injections or with mixed or separate plasmids. We recently gained access to an HIV envelope protein, gp140, in a novel adjuvant, GLA (Glucopyranosyl Lipid Adjuvant), which has been delivered to a subset of these volunteers in an effort to further boost the neutralizing anti-envelope antibodies.

This research has paved the way for a study to further augment DNA immunization using intradermal electroporation. In electroporation a series of electric pulses, over a period of 0.3 s, are given between 2 rows of 2-mm long electrodes inserted into the skin, placed on either side of the intradermally injected vaccine. In small animals this augments the immune response 10–100 times. Intramuscular electroporation has been demonstrated to augment the immune response to another HIV DNA plasmid vaccine; however this causes considerable discomfort, which is avoided by intradermal administration. Again, a phase I study at Södersjukhuset has demonstrated the tolerability and feasibility of the procedure, and a larger study in Tanzania and Mozambique is now planned, which will start at the end of 2013.

The network now consists of 3 collaborating centres. At the original unit at Muhimbili University of Health and Allied Sciences there is a dedicated clinic and laboratory with well-trained staff. Sida support is now almost terminated and the EDCTP is the main source of funding. Swedish regulations prohibited us from employing Swedish staff instead of local staff, and therefore local staff have been trained, both in Sweden and through frequent visits. The National Institute for Medical Research (NIMR) has now taken on a more prominent role in data management. The site in Mbeya, the Mbeya Medical Research Programme (MMRP), which is a collaboration between a very long-term broad German support effort and...
NIMR, has stepped in as a fully fledged partner. That site has also performed other HIV vaccine trials in collaboration with WRAIR. This effort is supported by EDCTP and University of Munich, and thus both clinical and laboratory German staff are on site. This is critical due to the remote location of Mbeya in the south-western corner of Tanzania. In Maputo a brand new enthusiastic team with a focus on the young population has been created on the basis of support from Sida. A whole new centre has been built and young researchers have been recruited to tackle all the aspects of the first HIV vaccine trial in the country. The effort is co-funded by EDCTP. All sites will engage in the electroporation trial referred to above with EDCTP funding, but funding after that is bleak. Given the excellent immunogenicity the next logical step is an efficacy trial, which would cost at least 50 million US$. There are very few funding sources for that kind of money and the competition to access them is fierce.

Considerable effort has been made to increase capacity in Tanzania and Mozambique. It has been most rewarding to follow a number of scientists in gaining their PhDs and now to find that 2 of them are Principal Investigators for the latest high-profile HIV vaccine trials. It is with special pride that we have shown it is possible to conduct all of the immunological studies at the highest international standards locally in collaboration with SMI/KI and University of Munich. It is indeed a privilege later in life to form new friendships in common efforts to address a cause we feel passionately about.

References

Fig. 2. Eric Sandström, Jonas Morian and Vera Dettmann together with some of the African volunteers.