Plinian Eruptions

How Science and HIV Have Revolutionised Each Other

Speakers

Oliver Pybus
Daria Hazuda
Helen Rees

JUSTRI 2011 Science and Innovation Lectures

www.justri.org
Introduction
by Mike Youle

Welcome to the third Science and Innovation Lectures.
JUSTRI has now broadened out from just dealing with issues of HIV to start covering viral hepatitis and other infectious diseases. We continue to broach health inequalities and issues in populations where HIV is not very prevalent or not the easiest way to get people to think about those issues. Please see our website www.justri.org for updates on who we are and what we do.

This evening continues this annual series of lectures addressing areas prescient for HIV and associated infections, and tonight we have three fantastic speakers: Oliver Pybus from Oxford University, Daria Hazuda from Merck Research Laboratories and Helen Rees from the University of Witwatersrand in South Africa.

The title of the meeting, ‘Plinian Eruptions – How Science and HIV Have Revolutionised Each Other’, comes from the parallels between the eponymous description of a natural event and its subsequent understanding and how that has been mimicked by observation and subsequent understanding of many of the events of the HIV epidemic.

Pliny the Younger described the eruption of Vesuvius in 79 AD in which his uncle Pliny the Elder died. He recorded it in minute detail but not until almost 2000 years later, during the eruption of Mount St Helens in 1980, that technology was available to both record and begin to understand a Plinian eruption and the deadly pyroclastic flows it features.

With HIV we have been much faster to describe and treat it scientifically; it’s only 30 years since the first descriptions of AIDS in California in 1981. We now have 35 drugs and the ability to keep people with HIV alive and well. So it seemed apposite to discuss how economics, politics and social life have reacted to HIV and vice versa over this period.

JUSTRI is about to start another project – the AIDS archive UK. It seems to me that no-one is keeping the history of HIV and AIDS and it is important to document the evolution of this epidemic for future generations, to both inform and provide a body of knowledge that can grow as we face the next 30 years with this fascinating virus.

Our first speaker is Oliver Pybus.
How HIV and Darwin Changed Molecular Epidemiology

Oliver Pybus

Oliver Pybus is a lecturer at New College, Oxford, UK. He is also the UK Royal Society’s Research Fellow in Evolutionary Biology.

I’d like to talk today about how the discovery of HIV led to a revolution in the field of molecular epidemiology. Molecular epidemiology is the use of molecular information to study the spread, transmission and diversity of germs.

In terms of viral taxonomy, before DNA sequencing we could only classify viruses by their appearance under the electron microscope or by serology – the differing effects they had on the immune system.

In the last 20–30 years there has been a transformation in the amount of information we can get about viral taxonomy. We can now study and compare the genomes of different infectious organisms relatively easily and this has enabled the introduction of evolutionary ideas into epidemiology and public health.

Darwin first drew a phylogenetic tree – a diagram of how species develop – in 1839, about 30 years before The Origin of Species. We still draw similar phylogenies today.

There are three main reasons HIV changed the way we do molecular epidemiology.

Firstly, the discovery of HIV in 1983 coincided with the widespread adoption of DNA sequencing. From the start genome sequences of HIV were used to characterise the genetic diversity of the epidemic. The size of the epidemic and the scientific response to it led to an extraordinarily large set of genetic data to analyse and draw conclusions from.

Secondly, HIV has an exceedingly rapid evolution: its genome evolves one million times faster than the genomes of humans. We all know the clinical consequences of that in terms of drug resistance and the evolution of immune-escape mutations that allow the persistence of chronic infection. But there are positive aspects to this: it enables us to study and characterise evolutionary processes in our own lifetimes, and it also generates differences between infections so fast that we can use those differences to get high-resolution perspectives on HIV transmission. The transmission of HIV leaves a genetic footprint and we can use genetics to recover that.

Thirdly, this is the first disease to which the full power of evolutionary biology methods has been applied. There was a little bit of evolutionary research into influenza previously – but there is a genuine insertion of deep evolutionary concepts into molecular biology, which began with HIV providing a very strong linkage with evolutionary research.

I would like to talk about three areas to illustrate the use of molecular epidemiology. They are:

1. Phylogenetics – the study of evolutionary trees.
2. Molecular clock – this is a tool evolutionary biologists use to put a timescale on evolutionary trees. This has been particularly useful in HIV, enabling us to put a time scale on the pandemic and also on the age of individual outbreaks.
3. Coalescent theory – this is a mathematical theory derived from population genetics. It provides an explanation about how the phylogenetic tree we might observe helps us make inferences as to how fast the population has been growing or contracting, and therefore gives us information about past rates of transmission.
**Phylogenetics**

Here is a diagram of a typical modern phylogenetic tree. The black dots represent gene sequences for human, chimpanzee, gorilla, orang-utan – a related grouping – and a much less closely related ‘outlier’, the camel.

The red dots represent common ancestors. The length of the horizontal branches represents the number of mutational differences between the different sequences. In this particular way of drawing phylogenetic trees the vertical axis difference is not significant.
In terms of HIV’s family tree, this slide shows the time that different genetic sequences were discovered and entered into the database, and how through time, its family tree gets bigger and richer.

In 1987 we knew of subtype B and subtype D, as we would classify them today, with a handful of strains isolated for each one. A year later subtype A was discovered. By 1992 we had subtype G and in 1993 subtype H. Subtype C, which accounts for about 50% of infections worldwide, was not discovered until 1995. By 1997 we were getting quite a full picture of the different HIV subtypes, including circulating recombinant forms.
They look like distinct clusters that are really epidemiologically important, but this is only a consequence of the limited sampling, and it was only when we started doing studies of the huge genetic diversity of HIV in central Africa that we realised subtypes were not so distinct. Our view of HIV phylogeny is hugely biased by the money available to do such research and which patient samples get studied.

Using these methods to divide HIV into subtypes is useful for population epidemiology, but what other uses are there? One of the first ways phylogenetic trees were used was in forensic cases. However, the use of these phylogenetic trees to establish infection routes between individuals is much more controversial, especially if we try to use them to say who infected who.

One of the earliest cases was that of Dr David J Acer, a Florida dentist accused of infecting five of his patients between 1987 and 1989. Several of these patients turned out to have viruses that were very similar to the dentist’s – more similar to his than to the average local virus, while a couple of patients had virus that was no more similar than a random selection of local viruses, so were definitely not infected by him. There was a huge amount of controversy over this case as there were many opinions amongst evolutionary biologists, and the methodology of this analysis was later disputed.

Phylogenies can only supply circumstantial, not definitive, evidence as to the source of transmission, and shouldn’t be used as a tool in criminal prosecutions. Phylogenetics is not as certain as genetic fingerprinting. It can provide us with details of epidemiological linkage, and certainly establishes that the viruses of the dentist and several of his patients were closely linked. But it doesn’t tell us the direction of transmission, whether A infected B or B infected A, whether they were both infected by a third party, or whether a third party was infected by one and then transmitted to the other, or would only be able to under exceptional circumstances.

Also, people’s virus change within their bodies over time, so the virus in HIV recipients may look more like each other than they do the virus in the person who transmitted it, leading to false suppositions about recipients having infected each other.

We also have the problem of convergent evolution if there is evolutionary pressure, for instance the same drug-resistance mutations can evolve in parallel in two different patients who have, in fact, had no contact and are thus epidemiologically unlinked.
Molecular clock

Viruses evolve quickly. If we sample sequences at different times we can observe mutations accumulating between these time-points at a characteristic rate. In molecular clock trees, the branches represent time, not genetic difference. These trees can tell us how fast sequences evolve in an individual and therefore allow us to work backwards to an approximate time of infection.

In this slide, the vertical axis shows amount of genetic differences that have accumulated over time and the horizontal shows time. The slope therefore shows the rate of viral evolution. In general, this slope does not vary much between individuals; the molecular clock of HIV ticks regularly. This means that we can also use molecular clock technology to date the HIV pandemic in general. This date has been recalculated a number of times as we have refined our estimate of the rate of viral evolution and we can now say with a fair degree of certainty that the first transmission into humans of the common ancestor of HIV-1 type M – the pandemic virus – took place between about 1910 and 1935 and certainly during the first few decades of the century.
Molecular clock technology has also been used to correct an injustice. In March 1998, an outbreak of HIV was reported in 418 children in a hospital in Benghazi, Libya. Many of them also had hepatitis B and hepatitis C infection. The World Health Organization suggested the cause was nosocomial infection due to a lack of necessary equipment for safer injections in hospital. However, even at this time Benghazi was a hotbed of anti-Gaddafi sentiment so the following year the Colonel had five Bulgarian nurses and a Palestinian doctor arrested for deliberately infecting the children, hoping to use the subsequent outrage to improve his popularity in that region. Six years later, in 2004, the Benghazi six were condemned to death and a legal appeal began, with final judgement on sentence due in December 2006. In late October 2006, we finally got access to some virus sequences from the infected children. We performed ‘emergency phylogenetic analyses’ on the sequences and found that the HIV infections were all in one cluster, a very characteristic recombinant virus (CRF02AG) whose most closely related strains came from Ghana in West Africa. The hepatitis C viruses were in three clusters, two of genotype 4 and one of genotype 1, whose most likely origin was Egypt.
Benghazi Hospital Outbreaks

We also applied repeated molecular clock tests to date the likely date of origin of the viruses in the children (hepatitis C evolves even faster than HIV, so is also suitable for molecular clock technology). We dated the common ancestor of the HIV and hepatitis C clusters and found the latest date the strains could have infected the children. These dates always came out around the mid-1990s, at least a year before the nurses started working at the Benghazi hospital. After much legal wrangling the Benghazi six were finally released in July 2007, after spending eight years in jail.

**Coalescent theory**

Coalescent theory can be used not just to date the timing of epidemics but also their rate of growth. It relies on the fact that as you follow the evolutionary tree back in time, the rate of formation of nodes (common ancestors of diverging clusters) increases, because in the original small population of viruses each strain has given rise to a large number of descendants. In a shrinking epidemic, the opposite happens: the number of nodes increases as the viral population shrinks and the infection rate slows down.
We can use the shape of this phylogeny to calculate the rate of growth of epidemics and of viral strains within epidemics. When this is applied to the epidemic in Kinshasa in the Democratic Republic of Congo, the original urban centre of the HIV-1 epidemic, we can see that up until 1950 or so we do see exponential growth in the number of people with HIV, keeping pace with the exponential grow of the city population. Even so, we calculate that in 1950 there might have been only 1000–3000 infections in a city then populated by 200,000 people (1% prevalence). Between 1950 and about 1975–1980 we see super-exponential growth, to about 200,000 cases in a population of about 3 million (7% prevalence). After this the epidemic levelled off and there are now about 250,000 cases in a city that now has a population of 8.4 million (3% prevalence).

The spread of HIV-1 in Central Africa

We also used phylogenetics to isolate six large clusters of HIV subtype B that were largely restricted to the UK and then used coalescent theory to date the likely time of arrival and growth of these viruses. By analysing a sequence from the pol gene of 3500 viral isolates, 1500 from the UK and 2000 worldwide, we discovered six large independent transmission chains in this data set. There are probably more, but there are at least six. These are outlined in pink on the following page.
Coalescent analysis reveals that each chain entered into the UK in the first half of the 1980s, with two perhaps arriving earlier in the late 1970s. They then all went through a period of very rapid growth, which levelled off in the mid-1990s.

The cost of genome sequencing has plummeted in the last few years. This decline began as exponential and
comparable with “Moore’s Law” (costs halving every two years) and became super-exponential in 2007. For example, the cost of sequencing the entire genome of a species has gone from $60 million in 2003, when the human genome was announced, to $8 million in 2007, to only about $20,000 today. The most recent viral genome to be completed was that of the influenza virus causing swine flu, done on an automated platform for about $30,000, so maybe the next leap in molecular biology will come from another virus.
Advances in Science and Antiretroviral Drug Discovery: Past, Present and Future

Daria Hazuda

Daria Hazuda has been working at Merck Research Laboratories for many years and was instrumental in the development of integrase inhibitors as antiretrovirals, culminating with the bringing of raltegravir to the clinic. She continues to search for new targets and novel screening methods for new drugs for HIV and other disease areas.

Survival from age 25 years, N=3,990

I thought it appropriate on this, the thirtieth anniversary of the first notifications of AIDS, to start with a retrospective on advances in science and ARV drug discovery in the past and at present, and then really think about how we can get to the future.

HIV infection reduces life expectancy even in the highly active antiretroviral therapy (HAART) era. This figure shows the cumulative survival curves for HIV-1-infected persons (without hepatitis C co-infection) in the pre-HAART era (pink) and in the early (1997–1999, dark orange) and late (2000–2005, light orange) HAART era. Survival probability has improved considerably with the introduction of HAART and the subsequent expansion and improvement of the available treatment options. However, HIV-1-infected patients still have an approximately ten year shorter expected survival compared with uninfected matched controls (green). The authors of this study noted that “an ongoing effort is still needed to further reduce mortality rates for these persons compared with the general population”¹.

So we still need better therapies for HIV. And yet, I don’t really need to tell this audience what impact combination therapy has had on HIV. Anyone who remembers those first announcements in 1996 that a combination of three drugs had achieved viral undetectability for patients and elicited a durable and sustained antiviral response, knows that it was a turning point. It takes a long time to develop new chemical entities so it is a truly remarkable achievement that within twelve years we have enough drugs to enable us to give people the three or more drugs needed to inhibit HIV.

Part of this was of course due to the protease inhibitors (PIs). The introduction of PIs may be the first *bona fide* example of true structure-based drug development through x-ray crystallography of the HIV-1 protease. It enabled pharmaceutical companies to take small molecule inhibitors and convert them into highly potent and effective drugs.

**HIV Protease Structure Based Drug Design**

Structural design was essential not just to developing the first PIs, but, through understanding resistance, to the development of the whole PI class. There was an amazing number of second- and third-generation drugs synthesised to address issues of resistance, convenience and tolerability; we soon had a couple of dozen. Since it takes many years and over a billion dollars to develop one new chemical entity, it was an extraordinary achievement that we developed so many over 10-15 years.

The simple fact is that once we do something new in the pharmaceutical industry, we’re actually quite good at doing it again and again. Only one in 40 development candidates succeed, compared to one in five ‘me too’ compounds. It’s easy once you’ve got the tools to go back and do iterative improvements. It’s much more challenging to find something that’s entirely different.
Attrition occurs throughout the drug development process

- 1 in 40 dev candidates succeed for new targets
- 1 in 5 for “me too” compounds

Even with around 24 agents developed over the first decade, we still only targeted two of the HIV viral enzymes, despite the number of processes (and therefore drug targets) HIV needs in order to replicate. It took a further decade, from 1997-2007, to develop drugs of two entirely new classes, the integrase inhibitor raltegravir and the CCR5 inhibitor maraviroc. So, in its own way 2007 was as much a landmark year as 1996.

But why did new drug class development take so long?

I’ve talked about the discovery of raltegravir, in which I was involved, many times. It took a very long time to understand the cellular processes and develop the basic toolbox in order to test possible compounds for activity against HIV integrase and there were several hopeful classes of compound that fell by the wayside during the process. All this history has been well documented in a paper by Allison Johnson, Christophe Marchand and Yves Pommier, so I won’t discuss this class.

Instead I’ll use the CCR5 inhibitor class as an example of the kind of investment that is required.

- By 1986 we understood how HIV used the CD4 molecule as a cellular receptor and that this explained its tropism (preference) for cells that expressed this receptor.
- In 1994 the MIP-1α and RANTES chemokines were shown to inhibit HIV, indicating that HIV might use their cellular receptor as a co-receptor and that this receptor could be blocked by these proteins.
- In 1995 CXCR4 (called fusin at the time) was identified as the co-receptor for T-tropic (T-lymphocyte-tropic or syncytia-inducing) virus, and soon afterwards CCR5 was identified as the cellular receptor for M-tropic (macrophage-tropic or non-syncytia-inducing) virus.

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• In 1996 the Δ32 mutation was identified in CCR5 which, when homozygous (copies of the mutation in both chromosomes) conferred immunity to infection with M-tropic virus. This confirmed that CCR5 was an obligate co-receptor for this form of HIV and could therefore be a target for an inhibitor drug.

• In 1997 AOP-RANTES, an analogue of RANTES, was identified as the first synthesised CCR5 inhibitor candidate drug.

• However, it took another ten years for maraviroc to be licensed as the first CCR5 inhibitor.

As you can see, it can be a very long time from the first biological insights to the final development of a drug.

**With all the advances in genetic science could new putative agents be identified faster now?**

The answer, which is pretty remarkable, is yes, we can now do it within a month or two.

A few years ago several papers, the first by Mike Brass et al. and two others by Merck Research Laboratories and the US National Institute of Allergies and Infectious Diseases' Protein Interaction Project, used siRNA screening of a large library of the druggable human genome to identify host factors necessary for HIV replication. If you look at the three published papers, what’s clear is that they identified CXCR4 and CD4 as two of the essential factors. Nowadays, with enhanced technologies, a pharmaceutical company could do such a screen in less than a month.

**Genome scale siRNA screens >20,000 genes**

We can use tools such as knockdown screens, analysis of genome-wide associations and eSNP (single nucleotide polymorphism) associations to find as many associations as we can between genes, disease progress and possible drug targets. People thought this would be a revolution in our ability to identify targets. However, the difficulty is that there are very few targets that actually come up repeatedly in the different assays. Together, the three studies above only identified four essential factors that are necessary for HIV infection out of more than 20,000 genes. Although they actually identified approximately 1400 genes that had potential significance, there were merely four that all three screens picked out and only 19-22 selected by two of the three projects. In addition, those genes likely to be relevant generally only reside in the intersection of these very complex data sets.

**Why is it so difficult to pick out targets?**

When we pick out an association of host targets with common complex non-infectious disease genes with a well-defined phenotype (e.g. obesity) they tend to cluster in so-called modules. There appears to be a lot of genes that are associated with specific human diseases and they tend to be very highly co-regulated. They are highly conserved across species and this tells us that such an association is very relevant to the disease state. Infectious disease, however, may differ from complex diseases. We don’t see these very conserved associations. Viral pathogens such as HIV and HCV tend not to use co-regulated modules to regulate themselves. They tend to capitalise on a very diverse set of genes that are under different regulatory control. Thus, there is a challenge to understand the biological relevance of this although it suggests that there may be some evolutionary strategy by viruses to avoid using highly co-regulated genes. So if you want to identify novel targets, then directed approaches rather than random searches of the genome may be more fruitful. In our own research we found only 38 hits (positive associations) for genes associated with HIV replication through a genome-wide search of over 18,000 genes but 20 hits from 286 genes when the chromatin of cells targeted by HIV was examined.

**So how do we find new targets and where do we go from here in the next ten years?**

You have to understand what the target profile is that people are going to want and that you’re going to have to deliver.

We still need new drugs because the epidemic continues to spread, both in the developed and developing world. I believe there are still relatively few treatment options and limited drug classes with which to build effective combinations and distinct regimens. Many issues remain: women and girls, who are under-researched in trials, represent more than a quarter of all new HIV infections in the US; there is no vaccine on the horizon; reverse transcriptase inhibitors, the original class, still form the basis of most treatment regimens, and of current chemoprevention strategies including prevention of mother-to-child transmission, and we only have two or three of these drugs that are sufficiently tolerable for long term use; ideally, if we are going to use antiretroviral therapy for chemoprevention, we also need separate classes for this to avoid resistance.

So, all in all, we presently have a limited number of drug combinations we can actually put together successfully. Then there is also the question of finding a permanent cure for HIV infection, which currently persists despite suppressive therapy.

It is therefore encouraging that many people are still looking for new classes of antiretrovirals. One example is BMS’s entry-inhibitor that blocks the binding of gp120 to CD4, and small-molecule inhibitors of viral maturation/assembly. There are also novel ways of blocking reverse transcription and integration being investigated. See here a diagram of possible targets and identified drugs for each of them.
New mechanisms to attack HIV replication

Why can’t we cure HIV infection?

Now I want to spend the last few minutes looking at a different approach. We know that immune activation and pathogenesis, even in patients who have been on suppressive antiretroviral therapy for decades, has not been fully addressed, and that we do not have a cure for the disease. One of the main reasons is that we have a latent reservoir of HIV-infected cells, as identified by Robert Siliciano and colleagues in 2000-2003, which scarcely decays over time, although there has also been a lot of elegant science that suggests that this is not the entire story. Nonetheless, a lot of research is currently examining how epigenetic silencing of genes responsible for immune cell activation leads to latency. This phenomenon is caused by the enzyme histone deacetylase (HDAC), which causes chromatin (genetic material) in the cell to take a closed, compact form where gene transcription is repressed. HDAC inhibitor drugs—such as SAHA (vorinostat), an agent already licensed as cancer chemotherapy, could reverse this repression of transcription and allow the invisible latent cells to re-start transcribing HIV genes and thereby reveal themselves as HIV infected.

The problem with this approach is that it is potentially very toxic; drugs such as HDACs could over-stimulate the immune system and trigger a disastrous inflammatory reaction, similar to what happened in 2006 during trials of the T-lymphocyte stimulator TGN 1412 (CD28 monoclonal antibody) as a potential leukaemia and rheumatoid arthritis drug.

In our lab we have taken a very directive approach to see if we can map all the host factors that may be involved in maintaining HIV in a latent state. Instead of merely using genome-wide screens (which we have also done) we have also built libraries of siRNAs that are known to be involved in chromatin remodelling, and have identified a number of genes that could represent particularly interesting potential targets with respect to activation of latent HIV gene expression, as shown here¹.

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We now have a variety of basic compounds with which to see if we can affect HIV gene expression, at least in vitro. Recent studies have suggested that it is possible to get robust re-expression of HIV genes using these products in both cell lines and primary cells. However, one of the frustrating things is that not any single one of these can reactivate all of the latently infected cells. So if we are going to do this robustly we will need combination therapy that will target different pathways that contribute to HIV gene expression. By going back to our library and through further high-throughput screening we identified more novel HIV-1 latency activators and showed that they work synergistically with existing activators like vorinostat. Through these early studies we can start to work out a true drug development programme, using agents that target different pathways in HIV gene expression.

I said above that silencing HIV gene expression and thereby creating a stable latent reservoir is not the whole story. The latent reservoir is not stable but dynamic. HIV-infected individuals receiving effective ARVs for extended periods constantly replenish their viral reservoir. We need to understand the very specific processes that are required to maintain this reservoir if we are to deplete it successfully.

Another finding that has re-energised this field of latency is the concept that many of the underlying processes associated with the pathology we see in patients who are virologically well-controlled may also be responsible for the ongoing replenishment of the CD4 reservoir. To remind you, there is an increased incidence of cardiovascular disease, non-AIDS-defining cancers, osteopenia, cognitive impairment and frailty in people infected with HIV as they age, and one of the things common to these diseases is that they all have an important inflammatory component to them.

The size of the HIV reservoir (defined by RNA/DNA ratio) is associated with frequency of activated CD4+ T cells in rectal tissues.

![Graph showing correlation between HIV RNA/DNA ratio in rectal tissue and percentage of CD38+ HLA-DR+ CD4+ T cells in rectal tissue. Spearman's rho: 0.65, P=0.012.](image)

Hunt, Yukl and Wong

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Persistent low level viremia can be detected in nearly all patients on HAART

What could be driving this chronic immune activation?

‘Virologically well-controlled’ does not equate with a total absence of viral replication: Maldarelli and Palmer have found that in well-suppressed patients it is always possible to find viral antigens and the average plasma viral load is in fact 3.1 copies/ml. This may reflect ongoing replication of whole virus, but it could also represent production of viral antigens such as gp120 and tat that are themselves inflammatory. Either way, studies by Peter Hunt et al. have found that the size of the viral reservoir may correlate with immune activation in well-suppressed patients. So, the model is that ongoing viral replication drives immune over-activation, which in turn gives rise to ongoing pathogenesis, but I would argue that it is also a primary driver of persistent viral replenishment. This is why we can’t cure HIV with antiretroviral drugs. The science suggests that there are several non-mutually exclusive mechanisms that contribute to HIV persistence and ongoing pathogenesis, and we need a holistic model that explains how these work and how we are going to cure the disease. Each process suggests very specific ways one can think about intervening; eradication will require multiple approaches in combination.

Eradication will require multiple approaches in combination

We can induce HIV gene expression using HDACs and similar drugs and we can try to eliminate or regulate those cells using therapeutic vaccines and immune interventions such as anti-PD1 antibodies. PD1 stands for programmed cell death protein 1; this is an important regulator of apoptosis and has been found to be associated with certain types of cardiovascular disease. In conjunction with these approaches we can also intensify antiretroviral therapy to reduce residual viraemia and persistent viral antigen production. In addition, there is also the concept of CD4 cell modification as seen in the ‘Berlin patient’ who had a bone marrow transplant from an individual with defective CCR5 processing and in research by Jacob Lalezari, using zinc finger nucleases to attempt to bring about a state mimicking the Δ32 homozygous mutation and prevent ongoing viral infection of T cells. Finally, in an attempt to dis-inhibit HIV gene expression we are looking not only at HDAC inhibitors but at immunomodulatory cytokines such as IL-7.

So, I think we have a very exciting toolbox of potential options and some of these are currently being explored in small pilot studies. In the past, we made enormous advances in treatment, such as in 1996. I think 2011 will go down as the year we started to make really significant advances in using antiretrovirals for prevention. For the future, I think we need to think much more seriously about the ultimate goal of eradication. Thank you.

I’d just like to acknowledge the work done by researchers in this field like Steven Deeks, Alan Landay, David Margolis, Rafick Sékaly, Tae-Wook Chun, Douglas Richman and many others.

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The Sociopolitical Determinants of the Interaction Between the HIV Epidemic and Scientific Advances

Helen Rees

Helen Rees is the Executive Director of the Wits Reproductive Health and HIV Institute (WRHI); Ad Hominem Professor, Department of Obstetrics and Gynaecology, University of Witwatersrand and Honorary Professor and 2010 Heath Clark Lecturer, London School of Hygiene & Tropical Medicine.

I was a little intimidated when asked to do this talk, as I am not a politician; neither am I a social scientist or an historian. My interpretation of the title was, as a scientist, to try and look at how the synergy between science and politics has affected how we have responded to the HIV epidemic, both in HIV prevention and HIV treatment.

The HIV epidemic – a brief history

I am going to start with the history of the epidemic to see what lessons we have learned. As Oliver mentioned, HIV crossed over into humans in the Cameroon somewhere between 1884 and 1924, most likely from hunters butchering chimpanzees and gorillas. This phylogenetic tree depicts how HIV-1 crossed into humans several times from chimpanzees (types M and N) and gorillas (type O), as did HIV-2 from sooty mangabeys, with HIV-1 type M being the main source of the epidemic.

The epidemic moved along the Sangha river to what was then Leopoldville and is now Kinshasa, where the epidemic began. There was slow spread around that city in the 1950s to the 1970s and then the epidemic...
A silent epidemic became one of global concern. Anthony Fauci commented in 2010 in *Nature* that "HIV-1 flew below the radar for decades until social conditions such as the end of colonisation, migration to cities, increase in prostitution and promiscuous sexual activity made it easier for the disease to explode into a pandemic". I thought that was an interesting comment in the way that he talked about phenomena people were worried about, and stigmatised then, in the same way as people worry about, and stigmatise, them now.

If we break it down a bit, the first thing we can see is that he is talking about social conditions. Those of us who work in public health get nervous when social conditions are mentioned, as we feel there is very little we can do about them; we don't like things we can't easily intervene in. Take migration: a social phenomenon that made people nervous then as it does now, and one of the reasons that it puts fear into people's hearts is the possibility of migration as a vector of infectious diseases. Then there's an "increase in prostitution" and "promiscuous sexual activity": things we still stigmatise now. And "exploding into a pandemic": look at how anxious we continue to be about pandemics, for example, most recently about H1N1 flu. So, if you look at this language, it almost sets the scene for the problems we continue to have now in the way we respond to HIV.

In the 1980s, one of the things that happened early on in terms of people trying to explain what happened was the theory espoused by Edward Hooper in *The River* that experiments with early polio vaccines might have caused the jump of the virus from chimpanzees into humans. His theory was widely disputed and is now largely discredited, but it is an illustration of how difficult it has been to get people to understand AIDS and believe in the science of AIDS. Here he is making a link between AIDS and vaccines, another area that has, history tells us, also seen broad public questioning about scientific integrity.

Let's move on to the beginnings of the epidemic in the US, which – as Mike says – some of you may not remember. Here is a quote from Sandra Ford in *Newsweek* in 1981: "A doctor was treating a gay man in his 20s who had pneumonia. Two weeks later, he called to ask for a refill of a rare drug that I handled. This was unusual - nobody ever asked for a refill. Patients usually were cured in one 10-day treatment or they died."

Thus began the recognition that there was a new entity, a new disease syndrome, appearing. Retrospectively, many years later, we found the same virus in samples from Kinshasa, from a Norwegian sailor who had visited Africa in the 1960s, and from gay male patients in New York. At the time, though, none of this was known and there was massive fear of AIDS from 1982 onwards. At first called GRID – Gay-Related Immune Deficiency – it was then found in women, especially sex workers, in injecting drug users, and in Haitian immigrants. All groups that were stigmatised and ones that society already feared as a source of 'social infection': now they were seen as the source of physical infection too, and so the virus and the lifestyles got tangled up with each other. The stigma had begun. The book *And the Band Played On* may be familiar to some of you: it documents very well the fear and panic of those times.

There was a lot of speculation about what was causing this syndrome. Amyl nitrite ('poppers') and a semen-related immune reaction were suggested. The idea that it was a CIA-engineered plot was going around, as was the idea that it was an apartheid-regime plot a few years later in South Africa. And of course there was the idea that it was God's punishment on homosexuals too, though by 1983 Peter Piot had already published his observations of AIDS in the heterosexual population in what was then Zaire.

The idea that AIDS was caused by a virus – first put forward by hepatitis B experts, who noted that it appeared to be spread in a similar way – was quite strongly resisted in some quarters, partly because of the implications for blood banks, public health and health-care workers. This persisted even when HIV-1 was discovered in 1983, and we have seen similar resistance to new scientific findings later on. Finding out that AIDS was caused by a virus

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plinian eruptions

seemed to give rise to complacency as much as trepidation, as in the famous quote from the US Department of Health and Human Services secretary Margaret Heckler in April 1984 that “There will be a vaccine in a very few years and a cure for AIDS before 1990.”

By the end of 1983, AIDS had been documented in 33 countries with 1500 deaths noted – including the first case in South Africa – and the European epidemic had begun, with cases largely initially in men who had sex with men and injecting drug users, and later in African immigrants. By the end of 1987, AIDS was documented in 127 countries, with 63,000 cases.

The evolution of antiretroviral agents started in 1987 with the (controversial) licensing of AZT and in 1989 of ddi, and continues, as we have just heard. There were initial disappointments when the Concorde randomised controlled trial in 1992–94 found that there was little benefit to AZT monotherapy. However, in 1995 (1996 in Europe) the first protease inhibitor, saquinavir, was licensed, laying the foundations for today’s effective therapy combinations. However, 1996 also brought raised awareness of the long-term side effects of some of these drugs, with the first reports of lipodystrophy and other complications.

Treatment-as-prevention also began in these years: one landmark trial that sometimes goes unmentioned was the ACTG076 trial that took place in 1994, which found that giving AZT to pregnant women reduced the likelihood of their transmitting HIV to their babies by two-thirds. In a press release in 1996 the National Institutes of Health said: “researchers urge caregivers to offer AZT therapy to all pregnant HIV-infected women, regardless of their stage of disease.” In 1999 the HIVNET012 trial showed – again not without controversy – that single-dose nevirapine was also effective at reducing mother-to-child transmission, resulting for the first time in an ARV treatment being registered for use in all those who needed it globally. Not before time, as by this point AIDS was the fourth largest cause of death internationally, according to the World Health Organization (WHO).

Between 2000 and 2006 the numbers of people globally who were on antiretrovirals really started to increase (see figure), but I will return to the issue of global treatment access later when I come back to contemporary times.

Number of people on antiretroviral therapy in low- and middle-income countries, 2002–2006

![Graph showing number of people on antiretroviral therapy in low- and middle-income countries, 2002–2006](image-url)
Leadership and activism

So that was a quick thumbnail sketch of the early history of the epidemic up until the last decade. What I want to do now is talk about the importance of leadership and activism in this process.

The international agencies such as WHO and UNICEF have always had a role to play. Yet, Halfdan Mahler, who was then head of WHO, was quoted as saying in Zambia in 1985: “Do not make AIDS a front page issue” to the detriment of “malaria and other childhood diseases.” This debate has not gone away, as we will see later. In 1987 AIDS was first discussed in the UN General Assembly, with WHO declaring that it was committed to the “urgent, difficult, and complex task of global AIDS prevention.” But as late as 1991 Jim Grant, the well-respected head of UNICEF, said he didn’t want AIDS included in the UN Convention on the Rights of the Child. However, WHO has also been involved in campaigns like the ‘Three by Five’ initiative, which aimed to get three million people on antiretrovirals (ARVs) by the end of 2005 and to write clinical guidelines for HIV. Three by Five did not succeed in its primary aim but was part of the movement towards greater global access. So WHO and the other international agencies have always had a role to play – but are not always the prime movers in changing the course of the epidemic.

As early as 1984 we saw that political leadership could be crucial in affecting its course, when Yoweri Museveni, President of Uganda, declared that what was then called ‘Slim Disease’ was a catastrophe for the country. With his ‘zero grazing’ policy in support of monogamy he became the poster president for HIV prevention in Africa at that time, though it is important to remember that, in the mid-1980s, he also had a condom-promotion strategy. One way or another, with his leadership, he contributed to the fact that Uganda’s epidemic at this time was to some extent contained.
Museveni was not the only example of what forward-looking political leadership can do in HIV. President Fernando Henrique Cardoso of Brazil announced in the late 1980s that his country was going to treat every opportunistic infection in 1996, that it would manufacture its own ARVs and give free ARVs to all in need. He did that with no other country backing him – it was Brazil’s idea – and it had a major impact on the epidemic in that country.

Leadership can itself be misled, however. In 1995 Thabo Mbeki, then Deputy President of South Africa, held orthodox views on HIV, but after he became president in 1999, he became notorious for espousing HIV-denialist views on the cause of the epidemic, with his health minister Manto Tshabalala-Msimang adamantly refusing to allow the national rollout of ARVs, including nevirapine for pregnant women.
In 2000 there was a watershed international AIDS conference in Durban when, for the first time, activists in the developing world started to demand access to HIV treatment on the same basis as the developed world. There were protests on the streets and people from all walks of civil society joined hands.

This was an embarrassment for the big pharmaceutical companies and in parallel with this, 41 South African pharmaceutical companies summoned me, as the head of the Medicines Control Council, and Nelson Mandela to court in 1998. They disliked the legislation we had written that would allow parallel importing of drugs and an easier way to get round patents, so we could access generic drugs. The newspapers remarked that the court case looked like a replay of the conflict with the old apartheid regime. Ultimately, it became so embarrassing to the companies that their head offices made their South African subsidiaries withdraw the case.
One crucial development was that HIV was starting to be seen as a major security issue, and this played itself out in the United Nations. Here is US Vice-President Al Gore and the UN Secretary-General Kofi Annan debating AIDS as a security issue in the Security Council in 2000. A number of international declarations on AIDS were made, starting in the 1980s:

- The UN General Assembly 1987
- The Denver Declaration on Rights 1987
- The London Inter-ministerial Declaration 1987
- The Paris Declaration 1994
- The Millennium Declaration 2000
- The Abuja Declaration of the Organisation of African Unity 2001
- The Declaration of Commitment at the UN General Assembly Special Session (UNGASS) on AIDS 2001

The United Nations and the G8 then became involved in funding. In 2002 the Global Fund to Fight AIDS, Tuberculosis and Malaria was founded and in 2003 US President George W Bush launched the PEPFAR programme, the President’s Emergency Plan For AIDS Relief – probably the one thing he did right. PEPFAR truly transformed the landscape for HIV drug access, particularly in Africa. Bilateral donors became involved in many other discussions around how to fund HIV treatment access: one example was the Making the Money Work meeting in London, March 2005, co-chaired by US Global AIDS Coordinator Ambassador Randall L. Tobias, UNAIDS Executive Director Peter Piot, UK Secretary of State for International Development Hilary Benn and France’s Minister Delegate for Cooperation, Development and Francophony Xavier Darcos.
As a result of all these events the total annual resources available for AIDS went up enormously and continues to need to go up.

Another development happening in parallel was the importance of celebrity in profiling this disease. In 1985 Rock Hudson died of AIDS, and in 1987 Princess Diana made the important gesture of shaking the hand of someone with AIDS at a time when it was still not altogether clear what the transmission risks were. Equally important for Africa that year was the public announcement by Zambian President Kenneth Kaunda that his son had died of AIDS. In 1991 Magic Johnson announced he had HIV and retired from basketball, returning in 1996 after he had been put on antiretrovirals. In 1991 Freddie Mercury died of AIDS and Rudolf Nureyev died in 1993, as did the tennis player Arthur Ashe. An equally important event to South Africa was the stoning to death by fellow-villagers in 1998 of Gugu Dlamini, a young woman living openly with HIV. This reminds us of the courage needed to be open about living with HIV, and most of the celebrities who died were not open about their status, or not until shortly before their death. Many people have died of AIDS silently as have their families, so celebrity has been important but we have not always used it as well as we could.

There have also been a lot of good guys in the celebrity world who have been important in mobilising funding and awareness such as Elizabeth Taylor, Elton John, Bono, Annie Lennox – and here is a small picture of me talking to Richard Branson at our clinic, and...Brad Pitt. But will the attention of celebrities continue?

The media has been really important in the history of AIDS and in documenting its history has been used to promote awareness of the spread of the epidemic and has publicised controversies and injustices such as the Uganda witch-hunt against gay men. It has sometime raised uncomfortable issues. One is the debate around whether HIV gets a disproportionate amount of funding compared with other conditions that kill people in the developing world; this was raised in 2009 by the head of UNICEF, Mickey Chopra, who pointed out that diarrhoea kills 1.5 million young children a year in developing countries – more than AIDS, malaria and measles combined – but only four in ten of those who need the oral rehydration solution that can prevent death for a fraction of the cost of antiretrovirals get it. Another uncomfortable issue is how the fight against HIV can continue to be financed.
So, if leadership, celebrity, the media, culture, human rights, stigma, resources, access and ethics have influenced what we now have before us, how do all these interface with the issues facing us now?

Firstly, let us look again at stigma and human rights. AIDS has become ever more concentrated among the poor in Africa. In a survey from one South African workplace, HIV prevalence was 14.7% in semi-skilled workers, 5.8% in skilled workers, and 3.3% in managers. And of course it is concentrated by race too. At the same workplace prevalence in black people was 13.9%, whereas it was 3.2% in Indians and only 2.2% in white and coloured people. In Africa no less than elsewhere it is also a disease of men who have sex with men (MSM) and this reminds us how stigma continues to be immensely important in the way the epidemic develops. Prevalence amongst MSM in surveys from Cape Town and Mombasa in Kenya was found to be 40%, and in virtually all countries in Africa it is the same or higher than it is in the general population, as elsewhere. Yet legislation against homosexuality continues to be proposed and the prejudice against gay men has not changed much.

The most important consequence of this is that stigmatised, and highly at-risk, populations do not get access to help with prevention. Here, for instance, is a map of as much as we know about the availability of sterile injecting equipment to injecting drug users (IDUs) – and remember that the provision of sterile injecting equipment is one of the most effective methods of HIV prevention ever demonstrated.
Global estimates of the availability of sterile injecting equipment per person who uses drugs per year, 2010

What is striking is that availability is very different between neighbouring countries: for instance the UK provides more than 100 needles/syringes per IDU per year but Germany less than 20; Kazakhstan more than 100 yet Russia less than 20; Cambodia and Vietnam more than 100 yet Thailand less than 20. Decisions on the provision of injecting equipment are governed by prejudice against IDUs and how that is played out in the opinions of politicians, the public and the political will.

Below are the UNAIDS’ estimates for the proportions of selected population groups – sex workers, MSM and IDUs – who received HIV prevention assistance from programmes in 2008 and 2010. You can see for some of these higher-risk groups that the effort to provide prevention programmes has gone up. But if you look globally, you’ll see that after all these years we are just about hitting 50% coverage.

What about political leadership? I spoke about President Museveni of Uganda earlier on, and here we see an extraordinary reversal. Latterly under Museveni’s leadership there was an extraordinary stress on abstinence-only campaigns, partly guided by PEPFAR. Condoms had been widely promoted but enthusiasm for them decreased, homophobia was rampant and HIV prevalence has started increasing again. Here is a chart of the proportion of people who had multiple sex partners from 1989–1990 when the zero grazing policy was promoted. You can see a drop in the number of people who identified themselves as having multiple sex partners, but by 2000 – too early for any ‘treatment optimism’ to kick in there – it just went straight up again. The result of the reversal of political leadership is there for all to see.

*Multiple sexual partners in the past year, Uganda: percentage of population (age 15-49 years) that have multiple sex partners in past year in Uganda by sex and age*

But we still have good political leadership elsewhere and after a disastrous spell of leadership in South Africa we now have a very enthusiastic and committed Minister of Health, Aaron Motsoaledi. And of course we still have important leaders like Bill Clinton and Michel Sidibé carrying the fight forward; it’s important we don’t lose that kind of leadership and that it gets continually renewed.

What of the power of culture and cultural attitudes and how they affect the epidemic? One example is male circumcision, which – as we know from this Egyptian papyrus – has been taking place in Africa for thousands of years. I chair a programme implementation committee for our National AIDS Council and had to address the House
of Traditional Leaders and explain the science of circumcision to them and persuade them it was a good idea. I put the slide of the Egyptian papyrus up at the beginning of my talk to explain how male circumcision was a long-standing African tradition. There was one woman traditional leader in the room. When I put the slide up there was a sharp inhalation of breath and she got up and left the room. The male leaders came to me and explained that as a woman she was not allowed to talk about or witness anything to do with male circumcision and that I had offended her. Cultural attitudes are one of the things that have delayed the rollout of circumcision and we should not underestimate the power of that political debate. If you look at these figures for 2009 you can see by this point that apart from in Kenya very few men had been circumcised. Culture is sometimes used as an excuse to avoid implementing HIV initiatives that political leaders are afraid of.

<table>
<thead>
<tr>
<th>Country</th>
<th>Men already circumcised</th>
<th>MC campaign by 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenya</td>
<td>85%</td>
<td>90,000</td>
</tr>
<tr>
<td>Zambia</td>
<td>13%</td>
<td>16,000 in 11 sites</td>
</tr>
<tr>
<td>Swaziland</td>
<td>8%</td>
<td>5000</td>
</tr>
<tr>
<td>Botswana</td>
<td>25%</td>
<td>4300</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>25%</td>
<td>1800 in 4 sites</td>
</tr>
<tr>
<td>Rwanda</td>
<td>3%</td>
<td>Rollout to army</td>
</tr>
<tr>
<td>South Africa</td>
<td>30%</td>
<td>15,000 in 1 pilot site</td>
</tr>
</tbody>
</table>

What about funding? I’d like to mention here the female condom, a technology that has been around since 1993. It is available in 90 countries and 40 million are distributed annually but this only represents 0.28% of all condoms distributed worldwide. The reason given is cost; but new cheaper ones are available and I think the lack of push behind the technology is due to prejudices about gender. It will remain on the back burner as a technology, I think, unless women start to raise their voices and demand it.

The numbers of people on ARVs has gone up significantly in the last decade and now stands at over five million. If current spending trends continue, the annual cost of the AIDS response by 2031 will be about $30 billion and we will have to put in $30 billion a year for decades. This is unsustainable.

We have to bring an end to the epidemic as we cannot afford to keep treating it, but at the moment the total sum of all the money we have ever spent on the ultimate technology, an HIV vaccine, is $2 billion. Funding is going to be an ongoing problem.
One of the problems with funding is to do with poor health systems. Ninety per cent of the mother-to-baby transmission of HIV occurs within approximately 20 countries, those very same countries have a massive gap in providing mother-to-child-transmission prevention, and the main reason is poor health services. We cannot keep on pouring money into poor health systems.

Finally, there are ethical challenges that are coming our way. This lecture took place before the results of the HPTN 052 study, which found a 96% reduction in transmission from partners taking antiretrovirals, but the results were not a surprise; work done by Jared Baeten and colleagues had already predicted that the incidence of transmission from people with an undetectable viral load would fall by at least 90% compared with people with a viral load of 100,000 copies.1

In a well-known model, Timothy Hallett of Imperial College calculated the effect on HIV incidence if we started to treat people at different CD4 thresholds. Most developing countries cannot afford to treat at CD4 counts over 200 cells/mm³ but Hallett modelled for South Africa that if the median CD4 count at treatment initiation was 350 rather than its current level of 100 cells/mm³ then annual incidence would halve, from 2.5 to 1.25 per 100 persons per year, and decline by 50% to 1.75 per 100 persons per year if we achieved a median CD4 count of 200 cells/mm³ at treatment initiation. So we should be treating more comprehensively, but can we afford it?

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An increasing number of people are being treated worldwide. By the end of 2009, 52% of those with CD4 counts under 200 and 36% of those with CD4 counts under 350 cells/mm$^3$ were receiving ARV treatment, but that still means that nearly half of those who need treatment under 2006 WHO guidelines and two-thirds of those who needed treatment according to the 2010 guidelines were not receiving the treatment they needed. Can we ethically launch test-and-treat or treatment-as-prevention programmes – seeking out and diagnosing as many people as possible and putting them all on ARVs in order to reduce transmission – while half the world that needs treatment still does not get it? A similar but sharper ethical dilemma surrounds the question of providing pre-exposure prophylaxis. Also, constantly more people will be needing treatment and we should be treating them for reasons of tolerability with newer and more expensive ARVs. The policy space to negotiate with the pharmaceutical industry around generic production is going to shrink and funding shortfalls already exist.

To get around this we need to look at innovative ways to get medicines to everybody and innovative ways of sourcing funding. One idea that has generated a lot of publicity is the so-called Robin Hood tax on financial transactions. We need to fund research and development for products that are of use in resource-poor settings and we are going to have to look at reforms to patent laws, such as the UNITAID-supported patent pool.

These should not be forgotten…

- Human rights
- Equity
- Stigma
- Leadership
- Civil society
- Finance
- Access
- Drugs
- Research
- Media

Finally, this list should not be forgotten as we go ahead; we have learned from history that all these issues need to be considered if we are to continue to fight the HIV epidemic in the future. My thanks go to Francois Venter, Peter Piot and Seth Berkley.