Can you set the scene for your research, and explain the thinking behind your new method of targeting HIV infections?

According to the World Health Organization (WHO) there are more than 35 million people living with HIV and, in 2013, 1.5 million have died of AIDS-related illnesses.

An HIV infection is currently incurable because the virus inserts its genetic information into the DNA of host cells (this stage is known as a provirus). By doing this, it essentially becomes part of the cell, and is therefore not susceptible to standard therapies.

However, the inserted viral DNA is flanked by sequences called long-terminal repeats (LTRs), and these are crucial to our work.

Site-specific recombinases are enzymes that bind to specific DNA sequences. If these sequences present twice, the enzymes work as pairs of molecular scissors and cut out the sequences that lay between the two recognition sites. Because the integrated virus is flanked by two sequence-identical LTRs, we predicted that if we reprogrammed the enzyme to recognise a sequence present in the LTR, the enzyme would be able to excise the provirus.

How did you go about doing this?

The reprogramming was done by directed molecular evolution. Basically, this is the same technique that has been used by humans for centuries during plant or animal breeding. The only difference is that in our case we are breeding a very small molecule. Using the forces of evolution, we were able to slowly adapt the enzyme to recognise sequences that are present in the LTR of HIV-1. Expression of these enzymes in HIV-1 infected cells deletes the provirus and cures the cell.

What were the main findings of your in vivo analyses?

HIV-1 does not infect mouse cells. To test whether our approach works in an organism, we made use of mice that can be transplanted with human blood cells. The human cells can then be infected with HIV, and we tested our engineered recombinases in this system. Strikingly, humanised mice that were treated with our recombinase showed a constant drop in viral loads, and in most animals the infection was undetectable at the end of the experiment in comparison with control animals where the viral loads increased over time. Importantly, we did not observe any side effects in the animals or in the human cells, indicating that the anti-HIV recombinase is well tolerated.

You have moved from testing these enzymes in cell cultures to using HIV-infected human cells in mice. Can you explain how you use the tailored site-specific Tre-recombinase LTRs in HIV-1 to fight the infection in mice?

The anti-HIV recombinase is delivered to the human cells within the mice via a gene transfer vector. The expression of the recombinase is under the control of a HIV-specific promoter, so only cells that are HIV positive actually produce the enzyme.

Once produced, the enzyme binds to the LTR sequences of the provirus and snips it out of the genome. This way, the cell is cured and can do its normal job again. Because T cells, which are normally fighting infections, are the prime target of HIV, this reconstitution of the immune cells also helps in fighting the infection by boosting the immune system.

Looking ahead, how close are you to human testing? If successful, what do you anticipate to be impact of this antiviral strategy for eradicating the HIV virus?

With the production of the second generation anti-HIV-1 recombinase Brec1 we are very close to conducting our first clinical tests. In fact the German regulatory authorities, namely the Paul Ehrlich Institute, have positively reviewed our application to perform a clinical trial. We are currently trying to raise the money to start such a trial.

It is difficult to anticipate how well this antiviral strategy will work in humans. However, we expect that our approach will at least reduce the viral load in the treated patients such that they can refrain from or reduce their intake of antiretroviral drugs.

The best case scenario would of course be full eradication of the infection, thereby curing the patient.
The point of return: reversing the effects of HIV infection

IN 2008, ALL eyes were on Germany as the epicentre of global HIV/AIDS innovation. This was all down to one man – not a researcher, but an anonymous patient known only as ‘The Berlin Patient’. After 13 years of taking antiretroviral drugs to fight his HIV infection, this man was then diagnosed with acute myeloid leukaemia and subsequently given a stem cell transplant, which – owing to the donor cells possessing a mutation that conferred HIV-resistance – had the fortunate and unexpected side effect of curing him from the HIV infection.

The patient was later revealed to be Timothy Ray Brown, a native of Seattle, USA. This case not only rocked the media worldwide, but also sent HIV/AIDS research into overdrive. A cure seemed more in reach than ever before, and many further trials were planned.

Fast forward another five years, however, and the initial wave of excitement had abated. Sadly, neither the so-called ‘Boston Patients’, nor the ‘Mississippi Baby’ lived up to the media hype, and despite initial promise following these similar treatments, none of the patients remained HIV-free.

As is so often the case with medical research, it seems there are no easy answers when it comes to combating the complexities of the HIV virus. However, there are still plenty of resourceful and pioneering scientists around the world dedicating their lives to finding a cure.

A NEW HOPE?

Now, it seems, Germany could be right back where it was in 2008, as a team from the Dresden University of Technology has been working on a new method that overcomes one of the biggest challenges facing HIV/AIDS researchers everywhere.

The HI virus is a true master of disguise. When it has entered the human body, it will invade macrophages and T cells – cells belonging to the host’s own immune system – and spew out its own insidious mixture of RNA and enzymes. The viral single stranded genome is then transcribed into double-stranded DNA, which it integrates into the host’s chromosomes, from which it can wreak terrible damage while remaining hidden and untouched by the immune system.

The virus’ apparent invulnerability to attack has been a seemingly insurmountable issue for HIV/AIDS researchers, until now. Professor Dr Frank Buchholz has spent 15 years working on site-specific recombinases that can remove HIV genetic material from infected human cells, essentially reversing the effects of the infection. Finally, he feels his team has reached the point where they can translate their findings into the clinic.

THE HUMAN FACTOR

The fruit of the Buchholz Lab’s labours is an engineered HIV-1 long terminal repeat (LTR) site-specific recombinase, or to put it in layman’s terms, a pair of molecular scissors. Using genetic modification, cells can be coaxed into expressing this enzyme, which then cuts away HIV genetic material from the host immune cells. Once the HIV material has been excised from the host cells, it cannot survive in the host and dies. In addition, the immune cells are returned to full function, allowing them to return to their job of protecting the body from pathogen attack.

Already in 2007 the group together with colleagues from the HPI in Hamburg could show that the approach works to eradicate the virus from cells grown in culture. Very recently, the same groups have revealed a second generation, broad-range anti-HIV-1 recombinase (Brec1) applicable to the majority of HIV-1 strains and subtypes. Importantly, Brec1 has demonstrated its utility in an animal model without measurable side effects. Following successful trials in the lab using humanised mouse models of HIV, the team now wants to take its work to human trials. Only then will the researchers be able to see if their method, which has been so successful thus far, can make a real difference to HIV patients around the world.

To successfully administer the recombinase to patients, an effective gene therapy approach...
is required that uses the patient’s own CD34+ hematopoietic stem cells. On paper this is simple enough; the team extracts blood from the patient, introduces the recombinase blood into the body. The use of the patient’s own stem cells should mean that the body will not reject the modified blood, and then over time the molecular exciser will do its job and the rejuvenated immune system – with all of its macrophages and T cells back in working order – will be able to polish off any remaining pathogens.

A REASON FOR OPTIMISM
While the encouraging results of the humanised mouse trials are certainly cause for celebration, and serve as a wonderful proof of principle, it is important to remain realistic. The Buchholz Lab is currently looking for the funding it needs to carry out its desperately needed human trials that can take this method into the realm of practical applications. Buchholz himself predicts that, given the right set of circumstances, treatments based on his team’s current work could be contributing to the fight against HIV/AIDS within a decade.

It would not stand alone, of course; combining their findings with some of the other work being done to fight the disease could potentially make for even more effective treatments. One suggestion the Dresden group has floated is their method in combination with immune-boosting medication, to ensure the virus is decisively driven out of the body.

And HIV may not even be the only end goal. Buchholz envisages a future in which the team’s method is used to treat other human retroviral infections, such as human T-lymphotropic virus (HTLV), as well as diseases caused by genetic inversions, such as haemophilia A.

The fight against HIV/AIDS has seen enormous progress in its relatively short history, especially given the intangible nature of the virus itself. Ultimately, like so many other ideas, experts have had to consign bone marrow transplants to the scrapheap – declaring them overly expensive and risky. Despite this, the joint UN Programme on HIV/AIDS has declared that they anticipate an end to the HIV/AIDS epidemic by 2030 – an encouraging goal, but undoubtedly an ambitious one, too.

With fresh ideas such as the ones being proposed by Buchholz and his team providing renewed hope for the future, perhaps this target is achievable after all.

TURNING POINTS IN THE FIGHT AGAINST HIV/AIDS

While the epidemic has been undoubtedly the most horrifying of the modern era, the concerted efforts of myriad dedicated researchers and healthcare professionals has enabled enormous strides to be taken in a relatively short space of time

1981 – First clinical observation of AIDS
1983 – HIV virus identified
1984 – CD4 identified as main HIV receptor
1996 – Combined antiretroviral therapy introduced
2001 – First generic antiretrovirals
2009 – The Berlin Patient is declared functionally cured of HIV infection

BUCHHOLZ LAB

OBJECTIVE
• To use different strategies to dissect and manipulate gene function in mammalian cells

KEY COLLABORATORS
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DR FRANK BUCHHOLZ conducted his thesis work at EMBL Heidelberg. He then performed postdoctoral work at UCSF before starting his own laboratory at the MPI for Molecular Cell Biology and Genetics in Dresden. In 2010, he was appointed full Professor at the Medical Faculty of the Dresden University of Technology. His research interests are in genome engineering and functional genomics.