

Will RNAi provide a cure for lentiviruses such  
as Feline Immunodeficiency virus and HIV?

BY

Christine Viney

PASS WITH DISTINCTION

RESEARCH PAPER  
BASED ON  
PATHOLOGY LECTURES  
AT VET-MEDLINK 2009

Page 1 of 8

## ABSTRACT

HIV (Human Immunodeficiency virus) is a currently incurable disease and in this paper I propose a possible future treatment using RNA interference. This is a way of silencing gene expression by preventing proteins from being synthesised. I propose using FIV (Feline Immunodeficiency virus) in research on animals due to the similarities between the two diseases. I will discuss the method for getting RNA into host cells and also the ethical issues surrounding the research and the possible cure. These include the arguments for and against animal research and the potential for RNAi to damage an organism's immune system. In my conclusion I address potential problems of RNA interference treatment such as viral mutations.

## INTRODUCTION

It was discovered in the 1990s that when a gene was introduced to petunias with the intention of darkening their pigment some of the petals became half white and half purple (see figure 1). For several years this mystery remained unsolved until RNA interference (RNAi) was discovered by Craig Mello and Andrew Fire in 1998. They had found a way of silencing gene expression by using double-stranded RNA (dsRNA). They came about the discovery whilst investigating gene expression in the nematode worm *Caenorhabditis elegans*. Mello and



**Figure 1: Petunias' colour altered by RNAi**

<http://www.nature.com/nature/journal/v451/n7177/full/451414a.html>

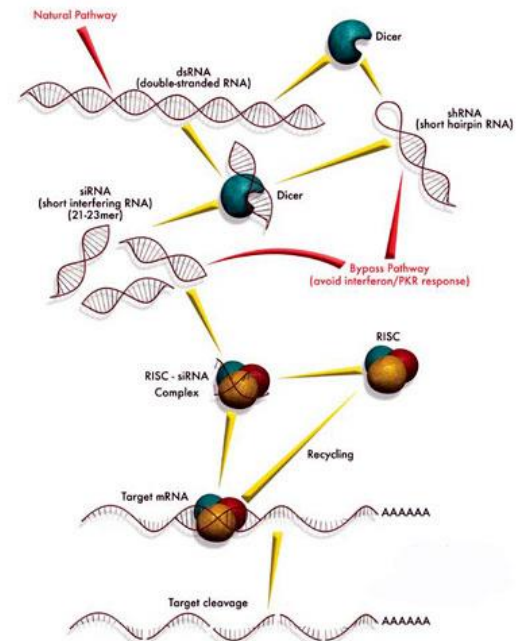
Fire found that when they injected sense RNA (messenger RNA) and antisense RNA (which can pair with the messenger RNA) into the worms the nematodes started displaying involuntary muscular movement usually associated with worms that lacked a specific gene. When a 'sense' strand pairs with an 'antisense' strand they form double-stranded RNA (dsRNA). The men then tested their theory (that they had silenced the gene that coded for the muscle protein) by injecting dsRNA into other worms. This dsRNA was specific to a different gene, but the same result happened: it appeared to stop functioning and the protein that it coded for wasn't synthesised. They went on to prove that gene silencing could be inherited and could pass from cell to cell [1].

RNA interference works by ensuring that during the process of protein synthesis the mRNA (messenger RNA) is destroyed before it can reach the ribosome. The mRNA transports genetic information from the DNA to ribosomes where protein can be synthesised. RNAi starts with double stranded RNA being cut by Dicer (a nuclease that cleaves dsRNA) into small interfering RNA (siRNA). The siRNA then couples with RISC (RNA induced silencing complex). The double stranded siRNA is unwound into single strands and, along with RISC, they cut mRNA into small pieces in specific places and hence destroy it (see figure 2).

Current research into RNAi includes improvement in "motor and neuropathological abnormalities" in a Huntington's disease mouse model [2]; providing a possible cure for cancer [3]; and "RNAi therapeutic and transgenic applications in bovine species" [4]. RNAi has the potential to cure genetic diseases such as Huntington's in both humans and other animals; prevent cancer from causing death in over 25% of the human population [5] and prevent viruses, from the common cold to HIV, from causing the disruption and death that they currently do. It is clear that the application of RNAi in both medicine and veterinary medicine may be of vital importance in future years. In this paper I intend to look at the use of RNAi in curing lentiviruses such as HIV and FIV.

Fire found that when they injected sense RNA (messenger RNA) and antisense RNA (which can pair with the messenger RNA) into the worms the nematodes started displaying involuntary muscular movement usually associated with worms that lacked a specific gene. When a 'sense' strand pairs with an 'antisense' strand they form double-stranded RNA (dsRNA). The men then tested their

theory (that they had silenced the gene that coded for the muscle protein) by injecting dsRNA into other worms. This dsRNA was specific to a different gene, but the same result happened: it appeared to stop functioning and the protein that it coded for wasn't synthesised. They went on to prove that gene silencing could be inherited and could pass from cell to cell [1].



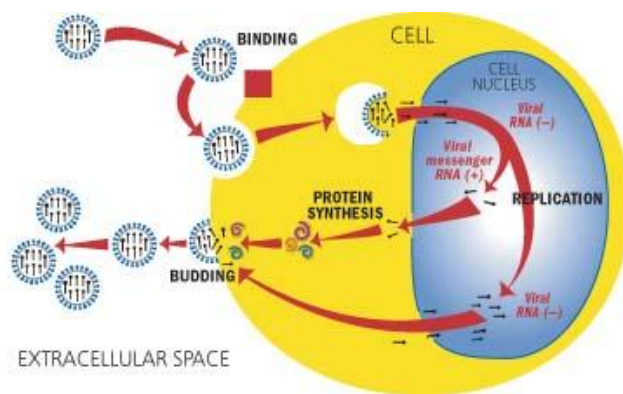
**Figure 2: How RNAi works**

<http://hedgehog.sfsu.edu/screens.html>

## DISCUSSION

I believe that with RNAi research it will be possible to eradicate all lentiviruses from both humans and animals in the future. A lentivirus is any member of the genus of retroviruses that has a long incubation period. This means that there is a long period of time, sometimes years, between the time of infection and the moment symptoms of the virus become apparent [6]. There are five groups of lentivirus species based on the hosts that they infect: Bovine, Equine, Feline, Ovine/Caprine, and Primate. Examples of lentivirus are Feline immunodeficiency virus (FIV) and Human immunodeficiency virus (HIV) amongst others [7]. None of the lentiviruses currently have a cure and so if RNAi research is successful it would be a major scientific breakthrough.

At the end of 2008 it was estimated that 33.4 million people were living with HIV/Aids in the world with 5.2% of adults (aged over 15) in Sub-Saharan Africa infected with the virus [8]. Consequently there has been much research done to try to find a cure. At present there is no treatment that will cure or vaccinate against all strains of the virus. I believe that one will be found for FIV using RNAi that could then be modified to cure HIV and other lentiviruses due to the similarities of all lentiviruses.



**Figure 3: How a virus replicates**

<http://www.news.cornell.edu/stories/Oct05/avianflu.the.virus.ws.html>

Viruses consist of genetic material inside a protein and lipid casing. Lentiviruses such as HIV and FIV replicate by first binding to a CD4 receptor (a protein) on the cell surface membrane of a T-Lymphocyte (a type of white blood cell that detects and fights foreign bodies in an organism) at lymph nodes. The virus fuses with the cell and releases its RNA into the cytoplasm. An enzyme called reverse transcriptase (which was inside the protein and lipid casing of the virus) is able to convert the single stranded HIV or FIV RNA into double-stranded DNA. Another enzyme, integrase, incorporates the new DNA into the host cell's DNA. When the cell receives a signal to become active, the lentivirus starts to replicate (this could occur after several years). Lentivirus proteins are created and "budding" occurs. This is when a part of the cell surface membrane

forms a new viral casing outside the cell containing the viral RNA. Subsequently, the virus moves to other lymph nodes in the organism so other white blood cells there are also infected [9] (see figure 3).

So far RNAi has been used to silence the HIV virus in cell cultures by Ying Poi Liu et al (2009). They showed that RNAi could "significantly inhibit the production of HIV-1 variants" *in vitro* [10]. HIV-1 is the more common type of HIV. HIV-2 (the other type) is usually only found in Western Africa. Both HIV-1 and HIV-2 cause a weakened immune system and clinically indistinguishable AIDS [11]. However, research now needs to start in animals.

I believe a cure for FIV will be found before there is one for HIV. Some people would say that there is no point looking for a cure to FIV when research time and money could be better spent searching for cures to human diseases. FIV is not a zoonotic disease: it cannot be passed to humans from cats therefore humans can never be infected by it. As people would rather find cures to treat humans, developments in human medicine are usually further advanced than veterinary medicine. I believe that a cure will be found for FIV before one is found for HIV because of the issues of clinical trials. However, finding the cure for FIV will lead to a leap forward in HIV research too.

It is easier to test cats in research labs rather than humans because it is possible to infect the animals and then keep them under constant monitoring; and they can also be dissected afterwards (which cannot happen in clinical trials on humans). In addition, it has been law in the UK since the Medicines Act of 1968 to test all new pharmaceutical products on "at least two different species of live mammal, one of which must be a large non-

rodent” [12]. HIV is a virus that is specific to humans and therefore other species cannot catch it naturally. Rhesus macaques (a species of monkey) can be artificially infected with the HIV virus in a laboratory, but the HIV has to be modified because rhesus macaques are only susceptible to certain strains of HIV-2. I believe cats will be used to find a final cure because cats catch all strains of FIV whereas rhesus macaques are immune to several strains of the HIV virus.

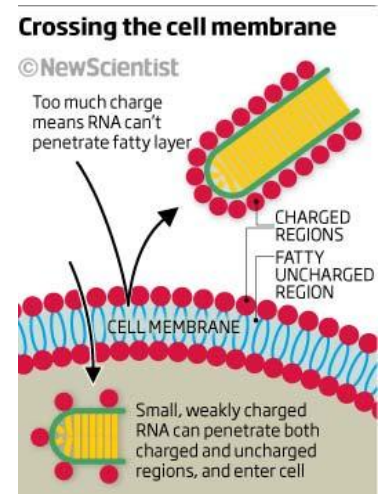
Feline Immunodeficiency virus attacks the immune system of cats by reducing the number of white blood cells which means the cat is less able to fight off infection. Consequently, it is often the case that a FIV positive cat dies or is severely ill because it contracts a secondary infection due to its weakened immune system rather than as a direct result of FIV. Between 1.5 and 3% of cats in the US have FIV; this value varies between countries. Often cats are not diagnosed with it because they may not display symptoms for many years and as a result an accurate percentage of the number of carriers is difficult to calculate. The virus is usually transmitted when an infected cat bites another cat. This is because the virus is contained in the saliva which then passes directly to the blood stream of the other cat. Currently there is no cure for FIV and the only preventative measure is a vaccine that has not been proven to work 100% of the time [13].

RNAi could prevent lentiviruses from replicating by silencing the viral DNA and thereby stopping a virus such as FIV from taking over the body. As described in the introduction, RNAi works by forming RISC (RNA induced silencing complex) from small interfering RNA. The RISC cuts the messenger RNA travelling from the nucleus to a ribosome and hence destroys it. FIV targets the white blood cells at the lymph nodes and therefore RNAi treatment would be injected directly into the blood stream. One way of getting the RNA molecules into the white blood cells is to chemically modify them so that they reduce their polar charge. Getting the RNA into the cells has always been an issue because they are polar (they have a charge) and therefore do not pass through the phospholipid bilayer. By reducing their polar charge the RNA can dissolve in both the water-containing plasma and the phospholipids (see figure 4). Anastasia Khvorova and her team have been researching this way of getting the RNA into the cell and, from what their research has shown, this appears to be a very effective method. So far they have managed to silence 17 genes and in this way it may be possible to cure FIV and other lentiviruses [14].

The main problem with this method of getting the RNA into the white blood cells is that the RNA has a very short half life (as little as a few minutes) because it is removed from the blood by the kidneys [15]. This means that a large proportion of the injected substance will be excreted before it has had the opportunity to silence viral genes and as a result a lot of the RNA is wasted. This will cause mounting costs. The potential cost of any RNAi treatment is a major drawback to finding a cure; however, the current cost of looking after someone with HIV or a cat with FIV may outweigh the costs of RNAi.

Another method of getting the RNAi into the cell that would overcome the issue of excretion of the RNA through the kidneys is also being developed. This involves the use of lipid nanoparticle capsules containing the RNAi which could be injected directly into the blood. They would prevent the RNA from being broken down by enzymes and excreted by the kidneys. It would also mean that the RNA would be able to get into the white blood cells easily because it would be possible for the lipid capsule to diffuse across the phospholipid bilayer [15]. Once within the white blood cells, the RNAi could prevent the virus from replicating by targeting and destroying the mRNA containing the genetic information for the virus. This would happen when the mRNA leaves the nucleus before the viral protein is synthesised on the ribosome.

A major problem with attempting to use RNAi to cure lentiviruses is that the current research would lead to a drug that only prevents the viral gene from expressing itself rather than completely removing the virus from an



**Figure 4: Chemically modified RNA molecules with reduced polar charges can enter a cell**  
<http://www.newscientist.com/article/mg20527494.200-disease-gene-blocker-sneaks-past-cell-defences.html?DCMP=OTC-rss&nsref=health>

organism. This would mean that a cat with FIV or a human with HIV would have to be injected with RNAi regularly for the rest of their life. According to John Maraganore, the CEO for Alnylam Pharmaceuticals (who are developing the lipid nanoparticle capsule), the drug his company are producing “is designed to be given via a 15-minute intravenous infusion, which could be given once every couple weeks”. A fortnightly injection would be very expensive especially if the patient is receiving treatment in a country that doesn’t have a government funded system like the NHS (i.e. they will have to pay for it themselves). If domestic cats start being treated with RNAi, it will also be very expensive for the owner. This also raises an ethical issue: is it fair to put anyone through this process twice a month for the rest of their life? People with HIV and cats with FIV can, for the most part, lead a normal life with their disease for several years before the effects of the lentivirus become severe. Therefore it could be argued that treating someone with RNAi isn’t justified.

If it is possible to cure lentiviruses, will there one day be a cure to all viruses? And should we cure them? Some would say that, if possible, viruses should all be treated with RNAi; however it may lead to a severely weakened immune system in the body. If all viruses have a cure then the immune system of an organism will never be needed to fight infection and will become superfluous (as all attacks on an organism from viruses will be dealt with by treatment from RNAi). After several generations this immune system might be so weakened that it couldn’t fight off such viruses as the normally harmless common cold. If a virus then mutated so that RNAi no longer worked, the effects would be devastating and could cause the extinction of a species. To ensure this doesn’t happen, RNAi should only ever be used to cure viruses that the immune system can’t fight (e.g. lentiviruses).

Another issue is the source for cats to test the effectiveness of RNA interference in treating FIV. When it comes to clinical trials in human diseases human volunteers can be used, but cats cannot volunteer themselves. Currently 0.08% of animal testing in the US is done on mammals (excluding rodents and primates) meaning a lot less than 0.08% of animal testing is done on cats [16]. Unlike rodents, people are less happy for cats to be tested on because 26% of households in the UK own a cat [17]. In contrast, EHI (Environmental Health Journal) estimates that professional pest control operators destroy 650,000 rats and mice in UK dwellings every year [18]. In other words, people don’t mind if humans are tested on because that is with the person’s consent; few people object to research on rats and mice (which would otherwise be killed by poison on the streets); however, there are more objections about the use of cats for research. One option for sourcing the cats is by using pets that already have FIV and using the owner’s permission as equivalent to the cat’s consent. Unfortunately, even that does not solve the problem entirely because, as with all research projects, a control is needed and this would take the form of a healthy cat. Therefore it is more likely that cats bred for research will be used. This raises such questions as: Is it right to torture another organism when that is illegal to do to humans in most countries? And are humans more important than other animals?

If RNAi becomes available for use in domestic cats issues may also arise when vets have to decide which cats should get treated with RNAi. Some may argue that as long as RNAi is proven to work, it should be used on any cat with FIV; however, this may not benefit all cats. For example, if the cat was elderly with the later stage of the disease it would not be appropriate to give it treatment because it may not have very long to live. In contrast, younger animals are often more able to fight diseases meaning RNAi is more likely to be effective in them. Owners may feel they are being unfairly treated if their elderly cat is not given a drug when their friend’s younger cat does receive treatment. How should a vet decide which cats should receive RNAi?

An even more contentious issue is who should get RNAi treatment in humans. Should it be only those who can afford it or anyone who needs it? 67% of cases of HIV are found in Sub-Saharan Africa [8], arguably the poorest region in the world. Most people in these areas will not be able to afford treatment meaning it will have to be given out for free if they are to receive it. Research companies are less likely to start projects that are unlikely to give them a profit and they will not like the idea of their treatment being given away. As a result, it may take longer than necessary to find a cure to HIV because drugs companies will be less willing to pay for research.

## CONCLUSION

Despite the obvious need for a cure to lentiviruses, especially HIV, there are some problems with the solution of using RNAi that I have proposed. One of the main problems with RNAi is that research is leading towards a cure that will need to be injected into the patient every fortnight. It could therefore be argued that RNAi isn't a cure, just a way of covering up the virus. Consequently, a new RNAi drug has to be developed which only needs to be injected a few times to be effective. It may be possible to develop a drug that can be taken every six months or once a year. However, this would mean the RNAi would need to stay in the body for all of that period. In addition, even if RNAi is used to cure HIV in one person, if that person has children then they will pass HIV on. Their children will then need to have RNAi and so the problem will not have been solved. Should it be made illegal for a person with HIV to have children? This seems morally wrong and it would be virtually impossible to implement.

It may be possible to incorporate a gene coding for RNAi into the DNA of cells so that when a cell replicates, the RNAi is also replicated. This would mean that a new gene would have to be inserted into the DNA to code for the RNAi. In this way the gene coding for the RNAi would effectively cancel out the gene for the virus. People may ask: why not just remove the viral gene rather than adding a new gene? The answer: it's easier to insert a gene into a host cell's DNA (lentiviruses do this already, of course) than it is to remove a gene from all the infected cells. Theoretically, this would mean that a person with HIV or a cat with FIV would only need to be injected once for the RNAi gene to be incorporated into the DNA and then again to ensure all the white blood cells have this altered DNA. This method would genetically modify the cell which raises more ethical issues such as: should humans alter an organism's natural DNA? However, if this method works and can also be incorporated into a person's gametes then their children will also have the RNAi gene. As a result, they would not show any symptoms of HIV. Approximately 9% of our DNA is already derived from viruses [19] so adding an RNAi gene wouldn't alter the human genome significantly.

Another problem with using RNAi is the likelihood of the virus mutating. Mutations occur regularly which creates a problem when trying to use a specific RNA molecule to target the virus. This is the main reason why no 100% effective vaccine or cure has been produced for any lentivirus. It would mean that a drug would need to be produced that could target several strains of the virus at the same time. It is likely that any drug produced would have to be modified annually to be effective against any new strains. Scientists may find a way for the RNAi to become non-specific (i.e. one form of RNAi could target several strains of a virus), but until then viral mutations remain an issue.

In conclusion, lentiviruses are a huge issue in both medicine and veterinary medicine because currently they have no cure. They cause a weakened immune system after, sometimes, many years of incubation (where the virus is within the body but causing no apparent problem) [6]. I believe that developments in RNAi research will lead to a cure for HIV through FIV. RNAi is a way of silencing genes by ensuring that during the process of protein synthesis the mRNA (messenger RNA) is destroyed before it can reach the ribosome. Viral DNA from lentiviruses such as HIV and FIV is incorporated into the host cell's DNA when the virus enters the cell. When the virus replicates it uses mRNA to transport its genetic information to other cells and hence spread the infection. RNAi that is specific to the viral gene can then be used to silence that gene and prevent the virus from replicating [9]. I propose one of two ways of getting the RNAi into the cell. RNA cannot pass through a cell surface membrane without help because it is polar and therefore doesn't dissolve through the phospholipid bilayer. The first method is by chemically modifying the RNA to reduce its polarity. The problem with this method is that the RNA can be easily removed from the blood by the kidneys [14]. The second method involves the use of lipid nanoparticle capsules containing the RNAi. This would be able to dissolve across the phospholipid bilayer and would prevent the RNA from being excreted [15].

Since RNA interference was first discovered by Craig Mello and Andrew Fire in 1998 [1] there has been much speculation as to its potential use in medicine from genetic diseases to a cure for cancer. I believe that in the future diseases such as HIV and FIV may be eradicated from society through the use of RNAi.

## BIBLIOGRAPHY

1. Craig Mello and Andrew Fire discovered the mechanism for RNAi in 1998 and in 2006 they won the Nobel Prize for their work:

[http://nobelprize.org/nobel\\_prizes/medicine/laureates/2006/press.html](http://nobelprize.org/nobel_prizes/medicine/laureates/2006/press.html)

2. Harper, S.Q. et al (2005) RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. Proceedings of the National Academy of Sciences of the United States of America, 2005, April 19

3. DeSano, J.T. et al (2009) MicroRNA regulation of cancer stem cells and therapeutic implications. The AAPS journal, 2009, December

4. Lambeth, L.S. et al (2005) Characterisation and application of a bovine U6 promoter for expression of short hairpin RNAs. BMC Biotechnology, 2005, May

5. Cancer statistics in the UK:

<http://www.statistics.gov.uk/cci/nugget.asp?id=915>

6. Definition of a lentivirus:

<http://medical-dictionary.thefreedictionary.com/lentivirus>

7. Index of viruses:

[http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs\\_retro.htm#Genus16](http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_retro.htm#Genus16)

8. HIV/Aids worldwide statistics:

<http://www.avert.org/worldstats.htm>

9. How a lentivirus replicates in an organism:

[http://www.aidsinfo.nih.gov/contentfiles/HIVLifeCycle\\_FS\\_en.pdf](http://www.aidsinfo.nih.gov/contentfiles/HIVLifeCycle_FS_en.pdf)

10. Liu, YP. (2009) RNAi-mediated inhibition of HIV-1 by targeting partially complementary viral sequences. Nucleic Acids Research, 2009, October

11. Information of the different types of HIV (HIV-1 and HIV-2):

<http://www.avert.org/hiv-types.htm>

12. HIV drugs, Vaccines and animal testing:

<http://www.avert.org/hiv-animal-testing.htm>

13. Information on Feline immunodeficiency virus:

[http://maxshouse.com/feline\\_immunodeficiency\\_virus.htm](http://maxshouse.com/feline_immunodeficiency_virus.htm)

14. Using chemically modified RNA molecules to block gene expression:

<http://www.newscientist.com/article/mg20527494.200-disease-gene-blocker-sneaks-past-cell-defences.html?DCMP=OTC-rss&nsref=health>

15. Using lipid nanoparticle capsules to allow RNA to enter cell:

[http://www.xconomy.com/boston/2008/12/23/alnylam-pushes-first-rnai-drug-that-circulates-through-body-into-human-test/?single\\_page=true](http://www.xconomy.com/boston/2008/12/23/alnylam-pushes-first-rnai-drug-that-circulates-through-body-into-human-test/?single_page=true)

16. Percentages of animals used in animal testing:  
<https://apbiostoga.wikispaces.com/Animal+Testing+Rights+Pd.+1>
17. Number of cat owning households in the UK  
<http://www.bris.ac.uk/news/2010/6826.html>
18. Statistics about animals in society:  
[http://www.understandinganimalresearch.org.uk/about\\_research/animals\\_society](http://www.understandinganimalresearch.org.uk/about_research/animals_society)
19. Ryan F. (2010) I, virus: Why you're only half human. New Scientist, 2010, January 29