

# RNA Interference and its effectiveness in treating the HIV Virus

BY  
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PASS WITH MERIT

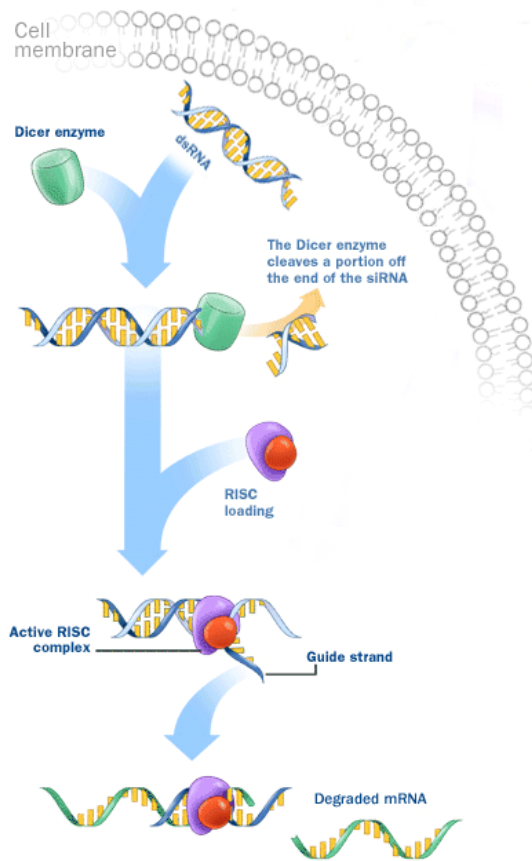
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## ABSTRACT

The HIV virus causes a gain of function mutation in the chromosomes of DNA, the product of which is the gene doing something positively abnormal. This mutant gene can cause errors in transcription, mRNA sequences and at the protein level. In this instance protein export or other steps crucial to the function of the protein can be affected. The method of siRNA interference has been tested through treatment in mice, and found that pre-treated mice were able to considerably reduce the expression of the virus. By evaluating the benefits and limitations concerning RNA interference, alternatives, and the question of future developments, it is concluded that it could be extremely effective in treating HIV, along with other mutation-caused disorders.

## INTRODUCTION

The HIV mutation is not amenable to simple addition of normal genes, which occurs in other types of gene therapy, as the presence of the normal allele does not prevent the mutant one from behaving abnormally. In these cases, the solution is to specifically target and inhibit the expression of the mutant gene, while maintaining the expression of the normal allele so the cell still functions normally.



**Figure 1**

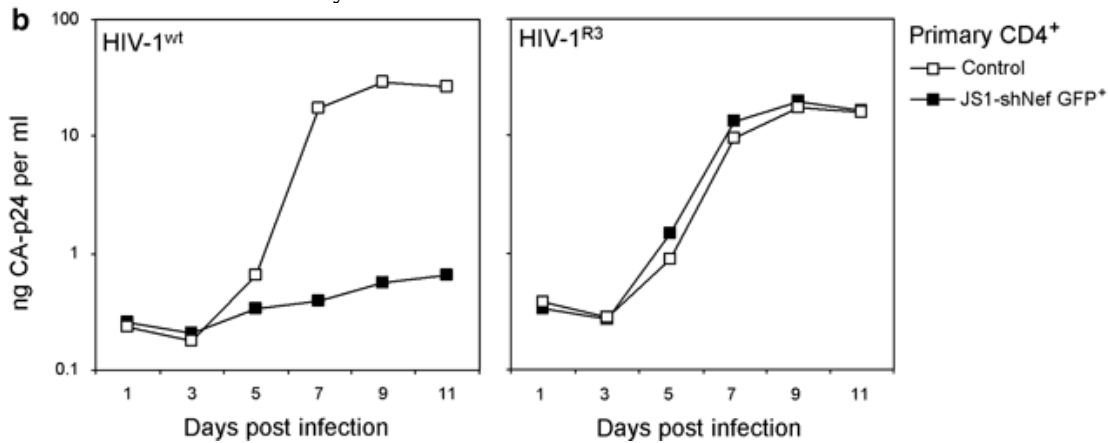
to which the HIV virus attaches. These mice were then infected with HIV, and observed for signs of the virus. Priti Kumar, PhD, Premlata Shankar, MD (see reference section) conducted this research, and he stated: "In mice pre-treated with the siRNA cocktail and then infected with HIV, we could not find any signs of virus for a long period of time, and then we treated mice whose immune systems had been reconstituted with cells from an HIV infected individuals, they were totally able to block expansion of the virus."

A new method has recently been tested, in which siRNA (short interfering RNA) are introduced into the cell. The sequence of this siRNA is complementary to the sequence of the mRNA being produced by the mutant virus-carrying gene. This means that the siRNA binds to the transcript of the mutant gene. The presence of these double-stranded RNA (dsRNA) triggers an interference response, called RISC (RNA -induced silencing complex) which means the siRNA is used as a therapeutic ribozyme, which cleaves the mutant mRNA upstream of the mutation site and before it gets to the protein. This means that as the siRNA is specific, it only binds to the mutant mRNA and the normal ones are still left to function. This therapy also targets the certain cells (T cells) that HIV infects, preventing HIV from entering them.

Figure 1 (left) shows the process of siRNA interference; how RISC is activated and the ribozyme (dicer) which cleaves the mRNA.

This method has been applied to the problem of HIV, first through treatment in mice. The mice were first pre-treated with a siRNA cocktail; which consisted of two siRNAs which "silence" different HIV genes, and one that prevents cells from expressing the molecule

This method was tested to see if it was applicable to humans by infecting mature human cells using the same method, and potent inhibition of the viral replication was observed, whilst leaving the siRNA resistant mRNA intact, confirming the sequence-specificity of the siRNA, and therefore its efficacy.



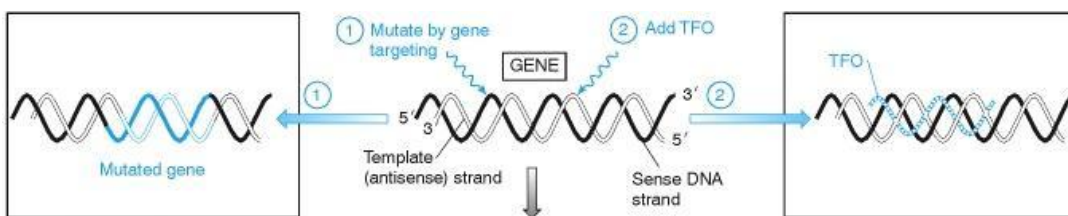
**Figure 2**

The left graph maps the expression of the HIV virus in a cell that has been given siRNA treatment, and the right diagram shows the expression of the HIV virus that is resistant to siRNA treatment. As is shown, the left graph shows a much reduced expression of the virus in the days following treatment. This gives credit to its effectiveness and shows promise for development into a long term cure for HIV.

## DISCUSSION

There are also alternative methods to siRNA interference; it is now found that gene silencing therapy is potentially possible at all three expression levels; the DNA level, the RNA level (siRNA) and the protein level.

At the DNA level, triple helix therapeutics inhibit the transcription of the mutant gene. It consists of short strand oligonucleotides which bind to the promoter region on a gene via Hoogsteen hydrogen bonds (variation of base pairing in nucleic acids) which disrupts the original hydrogen bonding. This means that the DNA can now form a triple helix formation (instead of a double helix), which can block the binding of transcription factors to the mutant gene. The oligonucleotides are delivered into the cytoplasm using cell permeabilization techniques or liposomes. Once delivered, the oligonucleotides can move into the nucleus by passive diffusion through the nuclear envelope, where, because they are gene-specific, they can bind to the mutant gene.



**Figure 3**

Figure 3 demonstrates how the method works, the gene-specific TFO (triplex-forming oligonucleotide) binds to the DNA to prevent transcription of the mutant gene.

The technology for this technique is improving rapidly; the oligonucleotides are now chemically modified to protect against nuclease attack once inside the cell, and cell permeabilization techniques have proved the most efficient methods of transferring them into the nucleus. However, this method of gene expression requires large amounts of oligonucleotides and in addition, there are difficulties caused by the limitations of relying on Hoogsteen hydrogen bonding. This type of bonding means that the target sequences have to carry all their purine bases on one strand, as the bonds use the purine bases as hydrogen bond acceptors, and the amine group as donors. *Human Molecular Genetics (Tom Strachan and Andrew P. Reed)* suggests that to solve this problem “replacement of the phosphate groups by different chemical groupings that allow triplex-forming oligonucleotides to “hop” from one strand of the bound DNA duplex to the other” needs to occur.

At the translational level, intracellular antibodies can be formulated to bind to a specific protein at a certain location within a cell, and destroy it. The antibody is designed to specifically bind to the protein which is infected with the mutant gene. The antibody is stably expressed and kept in the site of protein construction, where it either blocks this from occurring or inhibits the processing of the protein; in the case of the HIV protein for example, the antibody F105 is retained in the endoplasmic reticulum where it binds to the protein, thereby reducing its infectivity.

This method is shown to have advantages, as it is possible to design the intrabody to target molecules at various locations within the cell; mitochondria, golgi apparatus etc... by including an appropriate signal for localisation. These modulations would not be possible with the RNAi interference approach.

However, the screening and manufacturing of intrabodies is far more time-consuming and labour-intensive than the design and use of RNA. It is almost impossible to rationally design an antibody, and screening the antibody for suitability is a highly empirical process that is ultimately based on chance. Hence, it is unpredictable whether or not one may end up with a good antibody for a specific target protein.

The implications associated with gene silencing have to be considered when discussing its appropriateness for future use in medicine or veterinary science. Some religions object to the idea of manipulating the genes of our DNA; perceiving it as “playing God.” This objection is rooted in the principle that God has a plan for everyone’s lives, and by changing the very essence of our existence – our DNA – we are messing with God’s blueprint for life. This means that gene silencing could be rejected by groups in society as a treatment for HIV victims, as being against their religious beliefs; this could have serious implications for trying to introduce this development as a suitable treatment in the fight against AIDS.

Another implication of using siRNA to inhibit gene expression is the cost of producing the large amounts of siRNA needed to carry out the repeated procedures that are needed to continue silencing the HIV gene. It can be argued that the process may not be cost effective if the treatments have to be continued throughout the patient's lives.

On the other hand, there are several benefits to using siRNA as a method treating the HIV virus. The use of the artificial antisense strands can be synthesized simply and they can be chemically modified to have high intracellular stability, which protects them from being broken down by nucleases before they have had a chance to bind to the mutant mRNA. They are also sequence-specific, which means that they will only bind to the targeted gene, while the expression of the normal allele is not affected. When the siRNA is stably expressed in the cell it is shown to be efficient and durable in the reduction of expression of the HIV virus.

However, a large limitation of this method is that the siRNA will not pass through the cell membrane unaided, so the use of viral vectors and methods devised to insert siRNA as small molecule drugs are being explored. As the siRNAs are sequence-specific, they have to be altered to the particular mutation of the HIV virus present in the cell, which is expensive. Also, the siRNA have to be cleaved and designed to a particular length, as if long stranded foreign RNA enter the cell then it triggers an interferon response, which means the cell shuts everything down, causing negative side-effects on the immune system. This has serious safety implications for the patient and will be considered if the method is put forward for pre-clinical trials.

This technique would also require repeated insertions of the siRNA to continue to silence further mutations caused by the HIV virus. An improvement would be to find a way of silencing the HIV mutant transcript indefinitely, so the healthy allele can be expressed as normal. This could be found in a genetically engineered siRNA virus, that becomes part of a patient's stem cells, and eventually all T cells, so the cells then start producing siRNA themselves, making the virus "silencers" lifelong since they are now part of the patient's genome.

## CONCLUSION

It appears from the research and resources available that interference at the RNA level currently appears to be the most popular and promising development in the fight against HIV. As it is specific to the target mRNA carrying the mutated coding for the gene, it is efficient; only "silencing" the mutant part, while leaving the normal allele free to be expressed. However, as the RNA is so specific, this can cause problems, because the HIV virus has a high mutation rate, changing rapidly to different strains of the virus. If it mutates, then the specific siRNA loses its effectiveness, and therefore be regenerated to match the new mutation. The mutation rate of HIV is a huge factor in the development of this technique, because clearly it is neither cost-effective nor practical to be used as a long-term cure if the virus mutates too quickly for the siRNA to have an effect. Although, if the mutation of HIV can be mapped and predicted then there may be a economic and efficient way to overcome this problem.

The alternatives of therapy at the other two expression levels in the cell are still in the early stages, though techniques are improving rapidly. The unpredictability and labour-intensiveness of producing enough good specific antibodies for therapy at the protein level is a large limitation which does not apply to siRNA. The advantages associated at this level; the ability to target specific locations within the cell has great potential for gene therapeutics however, so the use of intracellular antibodies in gene therapies is by no means a closed door.

Therapy at the transcriptional level also shows promise; however it is difficult to see how this can be made gene specific, unless single gene transcription factors can be identified. Though if found, it could potentially be very effective in causing the rate of transcription of genes dependent on that particular factor to decrease.

Targeting mRNA using siRNA techniques still remains more attractive still, as mRNA remains much more accessible than the corresponding gene. DNA and RNA molecules can be modified to protect against nuclease attack, and for stability and can be transferred into the cell through a variety of effective means, a recent development being the use of lentiviral vectors.

RNA interference remains flawless in theory, the ethical, social and economic implications of introducing it still have to be considered. Applying the techniques to living cells, and overcoming limitations such as drug delivery and intracellular localisation of the siRNA remain obstacles to be overcome. Yet, David J. Looney, M.D., associate professor of medicine at UCSD and the VA San Diego Healthcare System [“said the new technique provides a new tool for research investigation aimed at elucidating the effects of different genes, and has the potential to modify gene expression in disease”] With the ongoing developments in cell and molecular biology worldwide, the successful application of this new method to numerous disorders, including cancer-causing mutations cannot be far away.

Kevin V. Morris, Ph.D, a post-doctoral fellow in Looney’s lab supports this supposition, with the positive view that [“theoretically, one could envision targeting virtually any gene at the level of the promoter and silencing that gene. This has implications in most biological processes in which one would want to down regulate the expression of a gene, such as those genes involved in virus infections such as HIV, as well as human cancers and certain genetic disorders.”]

So, the future looks promising for the application of this technique and with the ongoing advances in genomics this method becomes ever more viable. The possibility of effectively treating and curing a disease that over the past 27 years has killed over 25 million people, continues to drive interest in this field; so the day when RNA interference is used effectively as standard procedure is drawing ever closer.

Word Count: 2,304

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