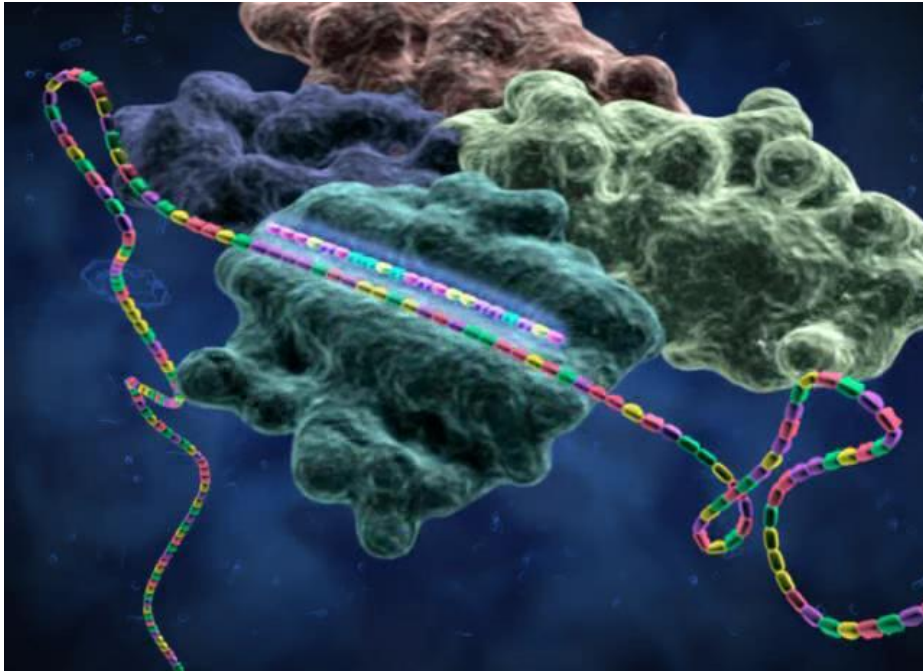


**UTILISING THE PRINCIPLES OF  
NANOTECHNOLOGY TO INVESTIGATE THE USE OF  
'PACKAGED' siRNA TO TREAT DISEASES SUCH AS  
CANCER**



[Image: <http://www.medgadget.com/archives/img/sirna.jpg>]

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## ABSTRACT

For some time, many cancer treatments have revolved around damaging tumour cells by changing or damaging the cells' DNA. Drawbacks of this include the obvious: damage inflicted to the cancer cells has the potential to affect other cells (an example being hair loss due to chemotherapy; the drugs used attack cells which divide quickly, including those responsible for hair growth).

Nanotechnology is a field that extends from the realm of supercomputers to that of the fundamental processes of the components of the body. This paper proposes the use of Small Interfering RNA (siRNA) as an alternative, or even supplant, to mainstream cancer treatments. siRNA is at the crest of a wave of new developments in nanotechnology and has a vast potential for use all across the field of medicine. This paper demonstrates the use of siRNA to change the way DNA is expressed in cancer cells, rather than damaging the genetic code directly.

Background reading into current research has also shown several other applications of nanotechnology, which could be used to augment this process. The issues set out in this discussion go to show the possibility of using siRNA as an effective worldwide treatment for one of the world's most prevalent conditions.

## INTRODUCTION

Throughout history, the progression of technology and understanding of the world around us has led to huge leaps in the field of medicine, particularly in the last three hundred years. Techniques such as vaccination and full-body scans have drastically reduced the impact of many diseases or conditions that otherwise have the potential to be crippling or even fatal. In some cases, threats have been completely eliminated; for example smallpox (caused by either the *Variola major* or *Variola minor* virus) has been completely eradicated from the natural environment, existing only in controlled conditions in laboratories.

However, current medical knowledge cannot resolve all ills. Cancer, in all its various forms, is the developed world's greatest health issue of the modern age, causing 27% of all deaths in the UK [1]. Nearly 300,000 people in the UK were diagnosed with cancer in 2007<sup>[2]</sup> (around 19 every hour), demonstrating just how prevalent a disease it is. Approximately one twentieth of NHS funding (around £4.5 billion a year) is spent on cancer treatment<sup>[3]</sup>, whilst the total economic burden for the UK is around £18 billion, set to rise to around £25 billion in the upcoming years<sup>[4]</sup>. The fight against cancer is described as "ongoing", but progress is being made all the time.

Nanotechnology is described as "engineering that deals with things smaller than 100 nanometres"<sup>[5]</sup>. In particular, nanotechnology deals with the manipulation of individual molecules. Due to the

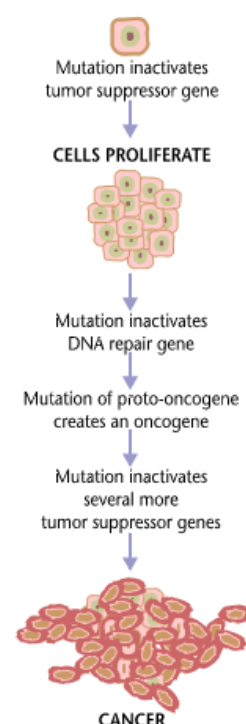


Figure 1:  
Simplified  
description of  
the  
mechanisms of  
carcinogenesis  
[i]

minute scale upon which this field is based, any toxicological effects of various atoms/molecules is vastly magnified in context (i.e. properties of molecules have a much greater impact when dealing with individual/very few molecules than they do when dealing with very large numbers of molecules). The use of nanotechnology also allows very small structures to be created and manipulated such as carbon nanotubes, which are deemed to be much stronger and more resilient than steel <sup>[6]</sup>. In terms of medicine, the development of nanotechnology creates the possibility of manipulation of cell membranes and even organelles within cells, such as altering channels in membranes to change the levels at which certain molecules can enter or leave the cell. A topic currently under great study is the use of nanostructures to create a targeted delivery system for drugs, wherein the nanostructure which contains the drug breaks down when it interacts with specific molecules.

Cancer is defined as “any malignant growth or tumour caused by abnormal and uncontrolled cell division” <sup>[7]</sup>. In essence it is the failure of the body to maintain normal cell growth. The uncontrolled division of cancer cells is linked to two main gene types: ‘oncogenes’ (genes linked to the promotion of cell growth and reproduction) and ‘tumour suppressor genes’ (also known as ‘anti-oncogenes’), which control any abnormal cell division <sup>[8]</sup>. The most common causes of extreme cell division are the disabling of anti-oncogenes (thereby removing any ‘failsafe mechanism’ in the case of abnormal cell division) or the over-prevalence existing or the creation of new oncogenes (thus greatly promoting cell division) due to genetic mutation. The reason that cancer emergence is not more common is that it usually requires many gene mutations to cause a cell to become cancerous, as well as the fact that most significant coding ‘errors’ can be resolved by the ‘self destruction’ of the cell (apoptosis) <sup>[9]</sup>. Factors linked to cancer incidence can either be genetic (i.e. a genetic predisposition to the unregulated growth caused by hereditary gene mutations) or environmental (i.e. external factors changing the DNA sequence in cells, causing a mutation).

In the past, the main two methods of combating cancerous cells have been radiotherapy and chemotherapy. Both of these treatments have their disadvantages; for example radiotherapy (the use of ionising radiation to damage cancer cells) poses a serious risk to tissues surrounding the target tumour. Chemotherapy involves the use of chemicals/cytotoxins to damage cells which divide rapidly (rapid reproduction being one of the fundamental properties of cancer cells). This issue with this treatment is that it attacks all cells which divide rapidly, including hair follicles and bone marrow, leading to such side effects as immunosuppression and hair loss. However, new chemical combinations have been tailored to respond to certain proteins found on the surface of cancer cells (rather than all cells that proliferate quickly), reducing the impact of side effects.

In the last few years a new technique has been developed: enzyme therapy. This largely involves the use of enzymes for two main functions, which could be referred to as 'direct' and 'indirect' therapy. 'Indirect' enzyme therapy focuses on using certain enzymes whose apparent functions seem to be reducing inflammation and 'cleansing' various parts of the body (such as the blood) <sup>[10]</sup>. Introduction of these enzymes (either directly to the area or into the pancreas) serves to clear away carcinogens, reducing the risk of any dangerous DNA mutations that lead to cancer. 'Direct' enzyme therapy involves the use of enzymes (usually proteases) to break down a protein layer which covers the surface of the cancerous cells, allowing the immune system to identify and combat them with greater efficacy <sup>[10]</sup>. The advantages of this are obvious; indirect therapy acts as a preventative which reduces the risk of cancer occurrence. Direct therapy appears to be an effective way of removing the protective coating of the malignant cells, thus increasing the effectiveness of the innate immune system (which uses 'tools' such as natural killer cells, which kill abnormal cells by stimulating apoptosis).

But what if you could combat the cancer on the most fundamental level, its genetic makeup? The transformation of a normal cell into a cancerous cell is dependent on a change in its DNA base code. What if it were possible to correct cancer-causing 'errors' in the DNA, or even completely disable a malignant cell? The addition, editing or removal of genetic sequences in abnormal cells creates an opportunity for the artificial stimulation of certain cell aspects, such as the deliberate over-expression of tumour-suppressant genes, the removal of oncogenes or even disrupting cell growth by disabling organelles within the cells. This method of combating dangerous cells will surely prove to be revolutionary in the field on cancer treatment in the very near future.

## DISCUSSION

In order to combat a specific disease with nanotechnology, two pieces of information are completely necessary: How to target and how to treat this disease.

### *Targeting:*

The easiest way to target any cell is to look at nature and copy the methods which work best in nature. We know that B-cells in our immune system target pathogens by identifying specific antigens on the pathogen's surface and then producing antibodies which attach to several of these specific antigens. This method, very simply described, can be copied with modern technology so as to target specific antigens with our own medicines. From there it is by no means difficult to insert particles, such as medicines, either directly into the cell or in such a way as to be diffused almost solely into the cell.

We know that using mass spectrometry and a sample of cancerous cells we can find disease biomarkers on many different cells. T-cell acute lymphoblastic leukaemia has PTK7 as a potential marker, for example (Shangguan et al) <sup>[11]</sup>. It is also not so difficult (Shangguan et al) to make proteins specific to binding sites, these proteins are called aptamers (a protein designed to target a specific molecule). This is one plausible method of targeting a specific disease with nanoparticles. We already know something about targeting cancers in this way. For example, we know that cancers express a protein known as Epidermal Growth Factor Receptor more strongly than do healthy cells <sup>[12]</sup>.

It has been shown to be plausible to treat breast cancers in such a way- those that are superficial, by binding gold nanorods to them and then using a laser to scatter light off the nanorods until all enzymes in the cancer are completely denatured <sup>[13]</sup>.

There are, of course, potential problems with this method of targeting, for example we know that two unrelated cells can have the same antigens. One example of this is some of the antigens on the bacteria that cause strep throat and the similarity between them and antigens on heart cells. In this case the immune system can, after the initial infection, try to attack the sufferer's heart. Though this example is not itself about nanotechnology, it does highlight the potential danger of using any targeting system to attack disease.

Another complication in some diseases is the similarities between cells. For example cancers are possibly some of the best targets for nanoparticle treatment, but due to their genetic makeup cancers are also some of the hardest to target without causing collateral damage.

Targeting is probably the most difficult piece of information to have at one hundred percent certainty about a given disease. It is difficult to be one hundred percent certain about the use of targeting information for a given disease but certainty is vital for the successful use of nanotechnology in medicine. The potential problems which would have to be treated if the targeting of a certain disease was in error can far outweigh the potential benefits.

Improving targeting could also involve developing nanoparticles with the ability to cross the blood-brain barrier, something which is also being researched at the moment. The blood brain barrier is a wall of endothelial cells tightly packed between capillaries and the brain [14]. There are few molecules which can cross this barrier, mostly small ions and molecules and lipid-soluble molecules. The vast majority of modern drugs are incapable of passing this barrier, which means that medics have historically been forced to inject drugs inside the Blood Brain Barrier, rather than going through it. Forming liposomes and loading them with nanoparticles as drugs is one possible of making a medicine capable of crossing the Blood Brain Barrier, and much less damaging than the one idea of simply making holes in the BBB to allow the passage of drug molecules through. This second method of treatment also allows pathogens through- the problem the BBB is there to prevent.

### ***Treating:***

Methods of disease treatment can be refreshingly simple; killing the cells which are causing the disease. This is often achieved through use of antibiotics- which cause problems because there is potential for bacteria to mutate and become antibiotic resistant, as with MRSA.

However, direct cell death is one of the best methods for nanoparticle treatment. Nanoparticles allow specific targeting of a particular cell and so can be used to very good effect. Delivery of poisons is possible with nanotechnology, but picking one's own poison for effect is perhaps better.

The well documented action of small interfering RNA [15] in the turning off of genes can most definitely be used to target pathogens. By turning off certain genes in any cell one can kill said cell. This is most definitely not "Star Trek Science" and has previously been used to treat macular degeneration (promising eye drug from S. F. Firm) [16].

The only potential stumbling block in its use is the method of delivery of RNA. If this can be achieved then this treatment will be possible. This may sound a little like Star Trek science, but this final piece of the puzzle is not implausible - we know, for example, that we can achieve RNA insertion by using viruses and it is plausible that we can copy this method by using nanoparticles similar to viruses. Insertion of siRNA into cancer can be targeted against several things. We can, prevent the creation of the Aurora B enzyme which helps cells divide, or any protein involved in the cell cycle, and you can prevent cancerous cells from dividing. Other proteins can also be stopped in a similar way. Aurora B seems to be one of the best targets because it is much more prevalent in cancerous cells and so turning off this gene in other cells would be less problematic, and therefore there would be fewer potential side-effects from using siRNA to treat cancer.

The uses of siRNA to treat disease are not just specific to cancer treatment too. Preventing bacteria from producing toxins can prevent symptoms of several diseases, and other

inhibitions can kill bacteria. Bacteria are also easily to specifically target, which is helpful when considering this treatment.

The problems with this RNA treatment method of treatment are also great. Turning off genes is a form of gene therapy and is still in its infancy as an idea. We do not fully comprehend what could happen to the body if when attempting to treat say, hepatocellular carcinoma, with siRNA via nanoparticles these nanoparticles might contaminate other cells in the body and cause random genetic variations in non cancer cells – even non liver cells. Whilst some of these changes might be innocuous, there is an obvious potential for disastrous side effects is which, due to the random nature of contamination would be difficult to predict and monitor.

Other than direct cell death, preventing cell division is another potential use of nanotechnology in disease management and treatment. Preventing them from dividing is an effective treatment of the disease, but there is still a drain on the patient's lifestyle involved in "feeding" the cancerous cells. Killing cancerous cells using nanoparticles to insert siRNA into them and turn off key enzymes involved in their continued existence is possible, but potentially even more problematic than conventional chemotherapy as the potential for affecting other cells is there and possible effects of this are less well known. Conventional chemotherapies use both cell death and prevention of division in treatment. The side effects on healthy cells (hair loss, diarrhoea) are well researched and understood. As nanotechnology therapy is in its infancy, one can only surmise that similar side effects will occur but it is difficult to predict if these will occur to a greater or lesser extent or if other unexpected side effects will result.

This form of treatment could also be used to treat several genetic disorders; this might be elegantly done in utero, but could be done (expensively, but definitely possibly) by targeting nanoparticles to the pertinent places once symptoms show.

For example, it is known that Huntington's disease is caused by the dominant allele. If it were known that the patient was homozygous or Huntington's disease, this particular allele can be turned off- the other chromosome will still code for Huntingtin (the protein which is not correctly produced in Huntington's disease) and as this second allele is different to the allele turned off this may now be the dominant allele- this means that if a nanoparticle were made to target nerve and brain cells, Huntington's disease and other genetic disorders caused by a dominant allele can theoretically be treated by the action of siRNA inserted via nanoparticles. The only reason the nanoparticles should not be made to target brain cells is the logistical difficulties involved in inserting them another way, injection would damage the brain and not targeting would require too many particles to be produced, but the research going into finding drugs able to pass the Blood Brain Barrier would also help with this problem.

## CONCLUSION

This paper not only shows the feasibility of using RNA to prevent the development (and even prevention) of tumours on its own, but demonstrates the ease at which it can be used in conjunction with most other types of treatments. For example, a combination of RNA-therapy and surgery may become a staple treatment for those suffering from cancer. In this scenario, RNA therapy would be used to arrest the growth of a large tumour and promote apoptosis (cell death) of the cancer cells by emphasising anti-oncogenes, until it is small enough to be wholly removed by surgery. Conversely, there is the issue of how well other treatments would interact with the use of the siRNA to edit genes in the cancer cells. For instance, chemotherapy and radiotherapy both kill cancer cells by damaging their genes, so could the interaction of RNA-therapy and chemo/radiotherapy cause a greater efficacy of the treatments, or render one or other of the treatments redundant? There has not been much indication of the latter as of yet, but the possibility remains.

The field of gene therapy is still very new, meaning little is fully understood in this area. Therefore, we cannot predict with complete accuracy the 'knock-on' effect of the changing various genes linked to cancer incidence. The removal of certain enzymes or the modification of the production of certain other proteins may cause the cell function to be radically changed, with the risk of those cells posing a threat to the surrounding tissue (such as causing the cells to produce chemicals which may be toxic to the surrounding cells). Thus, any proposed changes to cell DNA must be treated with caution.

Whilst this topic of study is still in its infancy, there have been definitive case studies of the successful use of siRNA to treat issues with cells. Whilst most of these have been tested on animals, there have been some examples involving humans, such as using siRNA to treat macular degeneration <sup>[16]</sup>. However, with the advancement of this method comes the familiar ethical arguments (particularly linked to religion). For example, do we (as humans) have the right to 'play God', per se, by changing the nature of cells? There are very few obvious secular ethical issues about this treatment (as few other life forms or potential lives are suffering for the benefit of others, such as in stem cell research) besides the ever-present argument that is linked to any scientific development in its early stages. This argument is, of course, whether the risks to early test subjects outweigh the potential benefit to others. However, it is not a major issue in this case, as early experiments have proven the use of siRNA to be fairly safe when used correctly.

Despite these problems, using siRNA in this way has a vast potential. It has been indicated that treatments along this vein show remarkably rapid responses in cancer patients. By targeting specific properties of the cancerous cells, this form of gene therapy can be equally effective in combating both solid tumours and non-solid (which cannot be removed by surgery, making them difficult to treat) tumours. All in all, it is patently obvious that RNA-

therapy will come to be a huge factor in the battle against cancer in the coming years, with the potential to save hundreds of thousands, if not millions, of lives.

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