

The JOURNAL of GEOETHICAL NANOTECHNOLOGY



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Challa Kumar, Ph.D.

This article was adapted from a lecture given by Challa S. S. R. Kumar, Ph.D. during Terasem Movement, Inc.'s 3rd Annual Workshop Webinar on Geoethical Nanotechnology, July 20th, 2007.

I am going to touch briefly on technologies that are currently under development and then move a step ahead, into more of a philosophical regime rather than a scientific regime, and see what should be done, the main goal of us scientists, in moving ahead to these technologies.

Many of us are already familiar with nanotechnology. We are inundated on a daily basis with plenty of information via newspapers, magazines, or scientific research publications. There is also plenty of money available to conduct the nano research, both from the governmental agencies, as well as from private agencies. We are all also familiar with how nanotechnology is going to achieve varying things, scientifically and otherwise.

"Nanotechnology is no longer a myth, it is science based on real life successes."

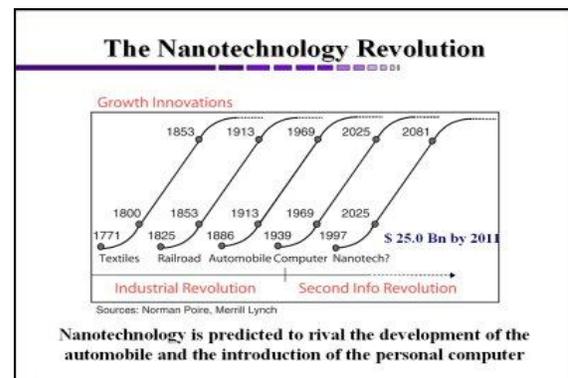


Image 1

Nanotechnology is no longer a myth, it is science based on real life successes. It is going to bring in a market value of about \$25 billion by the year 2011, which is not very far away; it is just four years more. It is truly a revolution, competing with some of the great

revolutions there have been so far such as textiles, railroad, automobile, computer, and so on.

Having really seen the impact of the nanotechnology revolution, I want to give you a heads-up on exactly what the nano scale is. It takes about one nanometer to reach one billionth of a meter; the depiction that you see is equal to one nanometer.

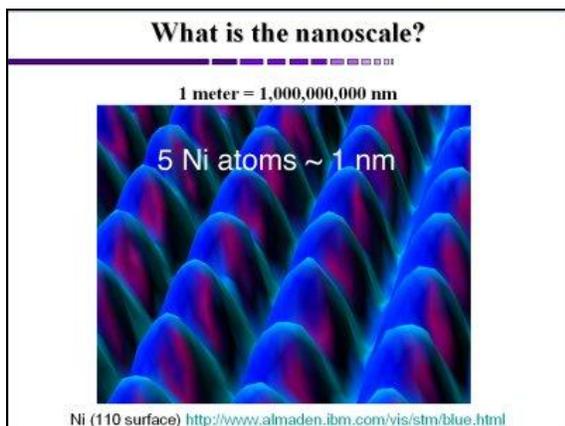


Image 2

There are several examples that we could use to define nanoscience [1] and nanotechnology, both natural as well as manmade.

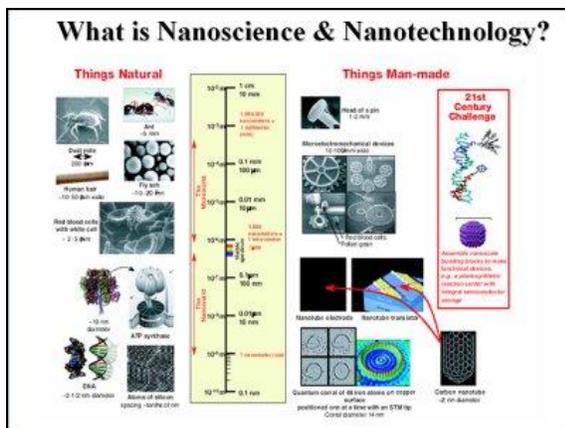


Image 3

Nanoscience is all about understanding the scientific aspects of materials and technologies of the nano scale. Present nanotechnologies are about converting this basic science into

applied benefits. I am going to outline why these nanomaterials are important, and why we feel the importance of the nanomaterial is not only within the manmade things, but also in the natural environment. The nanomaterials are really important because the materials possess very, very unique properties.

Take gold for example; we are all familiar with gold. You can see these ornaments are owned by many people in different countries. Solid gold is bright yellow in color, whereas the nano-sized gold is bright red in color. The optical and mechanical properties of the gold change with size.

Think of any property, the nano scale has very unique properties. The idea is to convert, take advantage of these nano scale properties, and build items by utilizing those unique advantages.

Nanotechnology is not really new; it's been there in nature, we now have the opportunity to understand the presence of nanoscience and nanotechnology in nature. There are several, very exciting examples. For example, abalone pearls. I am sure we are all familiar with abalone pearls, which are extremely expensive and are made up of nanostructured calcium carbonate with protein and carbohydrate mortar. That's what makes these abalone pearls worth millions of dollars.

Whereas the familiar chalk that we use for writing on the board is also made of calcium carbonate. Chalk is worth pennies when compared to the millions of dollars of value of associated with abalone pearls. That is the kind of value that nanotechnology and nanoscience possesses within the natural environment.



Image 4

There are a few more examples, the gecko for one. The gecko has the ability to provide an extreme and aggressive competence in climbing mountains. The ability of the gecko comes from the structures of the gecko's foot, which is all made of nanostructured material.

Such nanostructure provides unique properties and unique advantages.

Similarly, there are power plants, literally power plants which are chloroplasts in various plants that we've seen, and that is what is responsible for energy, and this is again a nanostructured material.

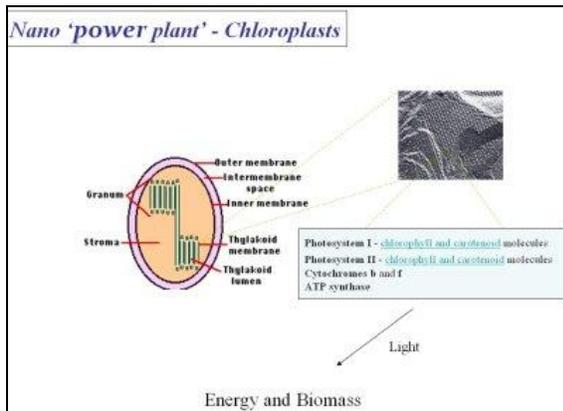


Image 5

Another example is the the Manuka beetle cuticle, which has a nanostructured liquid crystals that have very interesting optical properties that makes the cuticle iridescent.

While there are innumerable number of examples in nature, it is exciting to know that we are now, slowly unraveling these mysteries.

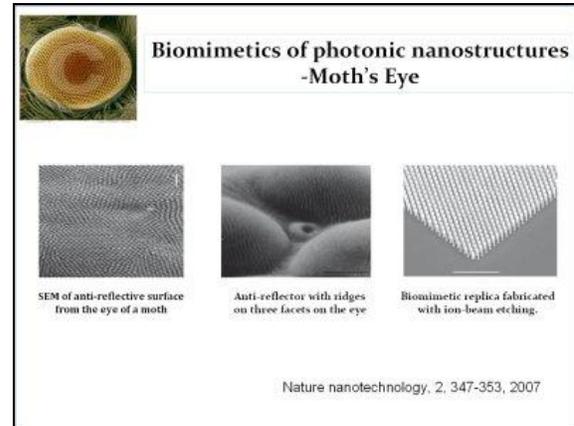


Image 6

When we examine the moth's eye using the traditional electron microscope technique, we would realize it has some very interesting nanostructures, and that is what is responsible for the behavior of the eye.

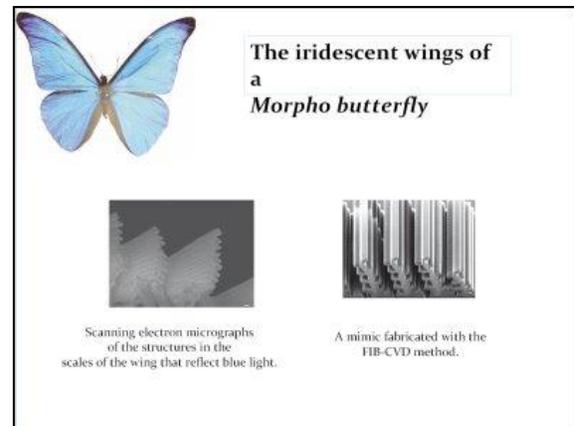


Image 7

When you look at this beautiful Monarch Butterfly, you see beautiful colors; different colors of the butterflies which are shown in nature. What you see here is the wing of the Morpho butterfly, and those colors are due to the nanostructure, which is responsible for the tint in the blue line.

Having learned all these lessons from nature, we are slowly trying to mimic nature. Trying to see how best we can incorporate the nanostructure process of the materials in nature, and how to convert them to our advantage. A recent publication of Nature and Nanotechnology [2] describes how a tobacco mosaic virus conjugated with nanoparticles is being developed as a digital memory device (*Nature Nanotechnology* 72(1), 72-77, 2006).

What good example is greater than the human body itself? The human body is an array of various nanotechnological materials and nanotechnological principles, so the human body is really an epitome of the advantages that nanoscience and nanotechnology can offer.

Having really looked at what the life sciences offer in nature, man is trying to translate those into the laboratory. When you look at the manmade nanotechnologies of what we have today, one could literally make a variety of different sizes and shapes of these nanomaterials.



Image 8

You have rods, nanocubes, nanotetrapods, carbon nanotubes, nanowires, nanofiber, nanoplates, and so on. These are all being made on a commercial scale in laboratories.

The commercial potential of the advantage is very obvious. Look at the way the carbon nanotubes are being developed for a variety of products for their unique and excellent properties being the lightest and yet the hardest material ever known to humankind.

The technologies that are being developed in terms of biomedical applications are multifunctional polymeric nanoparticle platforms; nanosensors for in vitro bio-analysis, drug delivery, and diagnosis, and so forth.

There are also technologies that are close to commercialization or already commercialized. For example, the technology that has been recently commercialized in Japan is a drug delivery system for topical applications. This is a nano-based technology that really changes fine wrinkles of an aged hairless mouse. The treatment helped in ridding the wrinkles (Pharm Tech Japan (2005), 21 (12, Rinji Zokang), 2000-2004).

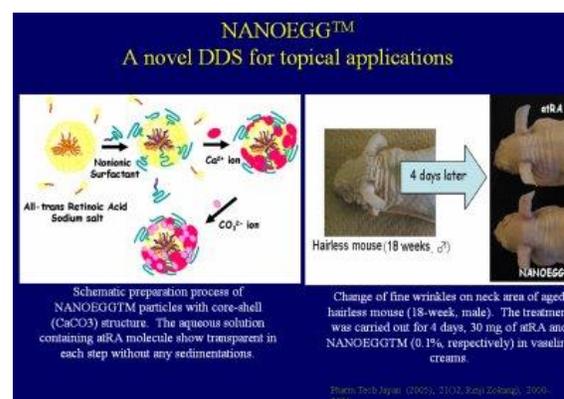


Image 9

Another very interesting nanostructured material that is being developed for integrated cancer imaging and therapy is silica core gold shell nanoparticles.

The beauty of these materials, the way they are being designed, is that by just tuning the optical properties of near-infrared rays, one

image the tumor, as well as subject it to thermal therapy. These technologies are, again, under commercial development (C.Loo et al 2005).

One of the papers that I am very fond of and citing all the time was published in 2005 -- it's "An Elegant Design of Polymeric core-shell Nanoparticles for the Treatment of Angiogenesis." [5] The design is so elegant that it has shown tremendous promise in the mice studies, and is currently under development for human trials. Once injected into the bloodstream, the core-shell particle is selectively taken up into tumor tissues, where the lipid layer rapidly releases a drug that kills endothelial cells and disrupts blood vessels. The inner core gradually releases a chemotherapeutic drug to destroy the cancer cells. (Sengupta et al 2005).

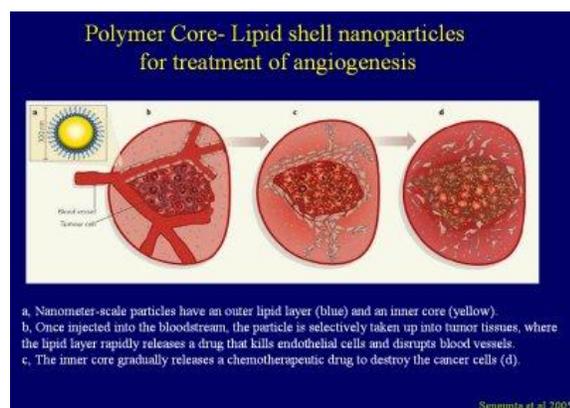


Image 10

This brings us back to my own laboratory where we are trying to engineer new site specific, controlled released drug delivery systems what my colleague, Professor Leuschner [3], described as integrating the LHRH Ligand Based Therapy [4] for controlled release of anticancer drugs.

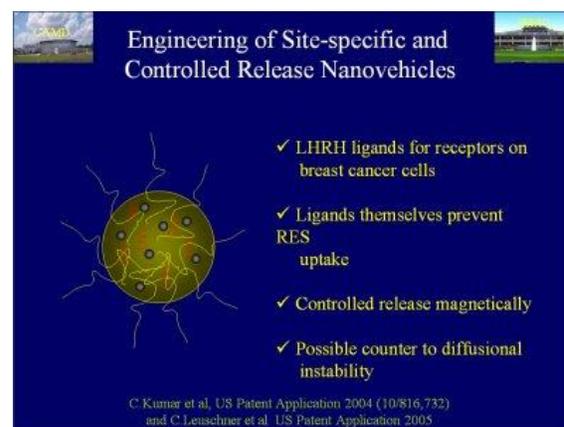


Image 11

This release is unique in the sense that we are trying to have a magnetically modulated controlled release. Wherein one could, in principle, have a three-pronged approach targeted and controlled release delivery of anticancer drugs.

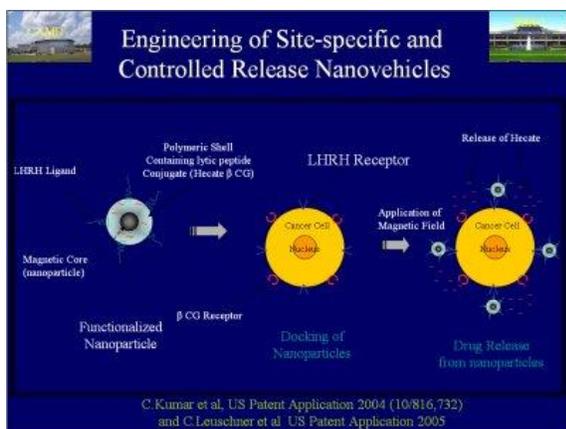


Image 12

This is currently under development in our laboratory. We are looking at various specific aspects. For example, the first thing we demonstrated is to see if the oscillating magnetic field can control the release of drugs in this material. We have shown by looking at magnetic polymer composite materials, and then we have demonstrated it is indeed possible when you apply oscillating magnetic fields (*Langmuir*, 21(5), 2042 -2050, 2005).



Image 13

I think I can properly address the question: Why are you looking at commercialization aspects and technology for LHRH based

"This is no longer science fiction, it is science based on real life examples."

materials or imaging as to the treatment?

The first thing being developed is LHRH-SPION contrast agents for magnetic resonance imaging of cancer and the preliminary results that we got, both in animal studies, as well as intra studies, are very, very encouraging

"I think it all leads to universal consciousness." (Breast Cancer Research and Treatment, 99(2), 163-176, 2006).

This is where nanotechnology is so far and there are several more exciting opportunities that nanotechnology provides, and those nanotechnologies have to be screened, but are not far away.

This is no longer science fiction, it is science based on real life examples. For example, there are military suits that are being developed which can monitor health (which can ease injury), and communication, enhancing a soldier's performance.

In addition, such military suits are so lightweight that one could literally leap into tall buildings in a single bound.

Similarly, imagine going to a doctor and having the cancer detected as well as treated at the same time on a single visit. It is a possibility; it is no longer a myth. One could talk about space elevators [6] - these are all technologies that are currently being developed.

While such technologies are being developed, one may be worrying about the concepts on nano wars. There's much discussion going on in trying to understand what fine control and influence one could have utilizing these nanomaterials for biomedical applications. I think such dialogue is very, very crucial and important while the technologies are being developed.

I recommend a very interesting book by Michael Crichton, *Prey* [7]. I think this also helps one to really understand the various other aspects of these new technologies that are being developed.

Having given you a brief idea about how these nanotechnologies are revolutionizing different disciplines and different phases of life sciences, I think one may ask the question as to where this is all leading. I think that is the most important question in my view.

The purpose of moving into higher end technologies, day in and day out, is to enhance our quality of life, or is there something else? I think that's the crucial question which one needs to answer.

I think the most important lessons that nanotechnologies provides is for our lives. That crucial understanding is what I would call nano-thinking. Nano-thinking is the ability to think small while thinking big. It is the difference between a commercial thinking and an integrated thinking. When you have such thinking, you cannot only enhance your ability to do wonderful science, but also increase your ability to look inward.

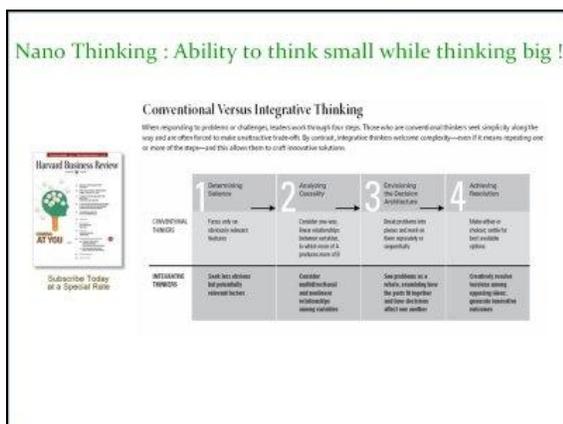


Image 14

Coming back to my question of what all of these technologies and continuous

enhancements in our level of thinking - level of implementing our minds leads to? I think it all leads to universal consciousness.

Our mind is an extraordinary source of highest potential. The mind is really responsible for bringing out these new technologies and new approaches, and helping us to understand nature and the world better. The mind is what is really sharpening us, and then the sharpening is what is going to bring us towards universal consciousness.

There is a very interesting statement from ancient Scriptures, VEDAS, from the Hindu religion, The statement is "Anoraniyam Mahatmayam". What it means is that the universe, or the universal consciousness, is just one, which is the smallest of the smallest, and biggest of the biggest. This statement truly conceptualizes nanoscience.

Therefore, there is only one thing which carries both the small and the big, and that is the universal consciousness. I think that all research and technologies are moving in that direction.

I would like to end with two very important statements from Einstein that really summarize where all of these technologies are leading to. The first one is:

"The most beautiful and most profound emotion we can experience is the sensation of the mystical. It is the source of all true art and science. He to whom this emotion is a stranger, who can no longer wonder and stand rapt in awe, is as good as dead. To know what is impenetrable to us does really exist, manifesting itself as the highest wisdom and the most radiant beauty which our dull faculties can comprehend only in their primitive forms. This knowledge, this feeling is the center of true religiousness."

Another rather very profound statement from Einstein is:

"Our time is distinguished by wonderful achievements in the fields of scientific understanding and the technical applications of those insights. Who would not be cheered by this? But let us not forget that knowledge and skills alone cannot lead humanity to a happy and dignified life. Humanity has every reason to place proclaimers of high moral standards and values above the discoverers of objective truth. What humanity owes to personalities like Buddha, Moses, Mohammed, and Jesus ranks for me higher than all the achievements of the enquiring and constructive mind."

Endnotes

1. Nanoscience – "refers to the ability to manipulate individual atoms and molecules, making it possible to build machines on the scale of human cells or create materials and structures from the bottom up with novel properties. Nanoscience could change the way almost everything is designed and made, from automobile tires to vaccines to objects not yet imagined."

<http://www.nsf.gov/news/overviews/nano/index.jsp> January 22, 2008 3:26PM EST

2. Nature Nanotechnology Magazine –

"Digital memory device based on tobacco mosaic virus conjugated with nanoparticles."

www.nature.com/nnano/journal/v1/n1/pdf/nnano.2006.55.pdf January 22, 2008 3:32PM EST

3. "An Elegant Design of Polymer Prolepsis Nanoparticles for the Treatment of Angiogenesis."

<http://circ.ahajournals.org/cgi/content/full/110/10/1219> January 22, 2008 4:53PM EST

4. Carola Leuschner, Ph.D. - Assistant Professor, William Hansel Cancer Prevention, Pennington Biomedical Research Center at the

University of Louisiana.

[/www.pbrc.edu/...](http://www.pbrc.edu/) January 23, 2008 11:32AM EST

5. LHRH Ligand Based Therapy – "[B]reast cancers and their metastases can be targeted through their LHRH (luteinizing hormone releasing hormone) receptors."

Further information available at:

<http://www.nsti.org/procs/Nanotech2005v1/1/M57.03> January 23, 2008 11:38AM EST

6. Space Elevator - In a 1978 science-fiction novel called *Fountains of Paradise* Arthur Clarke described a strong filament or cable being lowered from a geosynchronous satellite and used by the engineers of the future to move things up and down from earth—a space elevator. Let's ignore for a moment the tremendous problems involved—atmospheric turbulence, the Coriolis forces, the ravages of ozone and radiation up there—and think about how strong such a cable should be. It takes freshman college physics to figure that the tension in a cable is proportional to its specific gravity $\rho = 1.3$, a square of the earth radius R , and a simple integral: $\int (1/r^2 - r/R^3) dr$. The integral spans 22,300 miles all the way from the ground to the synchronous orbit, accumulates a lot and produces a strength requirement of 63 gigapascals. As speculative as it is, the story benchmarks this number. None of the materials now known to humankind get close to such strength. Fullerene cables someday may.

[http://www.americanscientist.org/...](http://www.americanscientist.org/) January 23, 2008 11:48AM EST

7. Michael Crichton's, Prey – "In the Nevada desert, an experiment has gone horribly wrong. A cloud of nanoparticles—micro-robots—has escaped from the laboratory. This cloud is self-sustaining and self-reproducing. It is intelligent and learns from experience. For all practical purposes, it is alive. It has been programmed as a predator. It is evolving

swiftly, becoming more deadly with each passing hour. Every attempt to destroy it has failed. And we are the prey.”

<http://books.google.com/...> January 23, 2008
12:26PM EST

Bio



Professor Challa S.S.R. Kumar, Ph.D.

Is with the Group Leader of Nanofabrication at the Center for Advanced Microstructures and Devices with the Louisiana State University in Baton Rouge, LA. Dr. Kumar is a member of the Scientific Advisory Board of the Maximum Life Foundation and one of the recipients of the Nano 50 Technology Award, 2006 for work in Magnetic-Based Nanoparticles for Cancer Imaging & Treatment.



Imaging and Treatment of Cancer through Combinations of Nanoparticles and Hormones.

Carola Leuschner, Ph.D.

This article was adapted from a lecture given by Professor Carola Leuschner, Ph.D. during Terasem Movement, Inc.'s 3rd Annual Workshop Webinar on Geoethical Nanotechnology, July 20th, 2007.

Dr. Leuschner discusses in detail, the work of her research team at the Pennington Biomedical Research Center, Baton Rouge Campus of the Louisiana State University, which employs nanotechnology aimed at targeting and eradicating breast cancer cells while leaving normal cells unaffected.

I will give a brief background about nanotechnology, cancer, targeting cancer, and then talk about preclinical development of a contrast agent for magnetic resonance imaging (MRI) [1], which includes synthesis, studies in vitro and in vivo [2] testing; magnetic resonance imaging; and then preclinical development of a new treatment for cancer, specifically in metastatic disease. Again, synthesis and in vitro and in vivo testing are involved.

Nanotechnology involves particles that are

"Nanoparticles can be used to infiltrate sub cellular compartments."

between one and two hundred

nanometers. To have a comparison, about a million nanometers is the size of a dust mite, so you can imagine how small nanoparticles are.

The small size has many advantages because nanoparticles are smaller than cells, and smaller than a subpopulation of cells, the so-

called organelles in cells. Therefore, nanoparticles can be used to infiltrate sub cellular compartments.

They are too small to obey classical physics, however, too large to apply to quantum physics. They have a large surface, and due their small size and modification change properties are certainly abundant. They are small enough to enter tumor cells and also normal cells, and they can be used for biomedical applications.

Applications in nanotechnology and medicine would be, for example: building sensors for biomolecules. It can be used for imaging, for example, if the contrast agent is linked to a delivery system. They are for treatments through hyperthermia, and also for building biomedical devices.

Cancer as a disease is still untreatable. If you look at development and changes in death rates in the U.S., heart disease death rates between 1950 and 2001 definitely dropped way down.

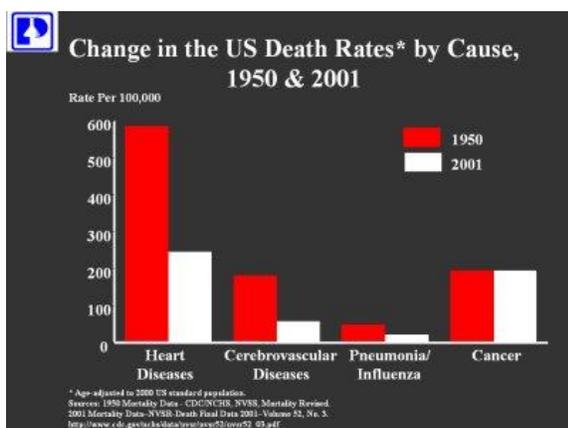


Image 1

However, cancer death rates still stay stagnant, and there was no improvement in cancer treatments regarding survival. The main reason for that is that there is something which is called metastatic disease [3]. We

have primary tumors and metastatic disease to distinguish.

Primary tumors can be treated if early recognized and diagnosed; if the patient contracts metastatic disease (a disease from the primary tumor building up metastases in

"Nanotechnology can certainly build a bridge in the gap to detect early stage metastatic disease."

lymph nodes, bone marrow, and other

parts of the the body), that stage of the disease is causing the five year survival rate to drop drastically in lung cancer, and less than 10 percent and 20 percent in colorectal cancer. Therefore, we have less than 50 percent survival rate in patients with metastatic disease.

The big problem in that case is also metastatic disease is not readily detectable.

Nanotechnology can certainly build a bridge in the gap to detect early stage metastatic disease. Nanoparticles can be designed for implant and hormone treatment modalities to increase treatment efficacy.

Currently applied methods for detecting metastatic disease, we can distinguish between invasive methods, which require anesthesia and, for example, among those are tissue biopsies; the tumor needs to be at least greater than one centimeter in diameter; bone marrow aspiration is very invasive and it is also painful.

In the case of noninvasive methods for example mammography or ultrasound, the tumor needs to be about one centimeter and has a 36 percent detection rate by ultrasound. More sensitive methods of detection are X-ray, PET and CT [4], that can detect 60 percent of the lesions which are already missed in a mammogram, and they offer higher sensitivity,

and can detect tumor sizes between 6 millimeter and one centimeter.

Magnetic resonance imaging (MRI) can certainly be improved dramatically by using a contrast agent. Looking at contrast the agents, I will further elaborate on that method to develop better agents. There are already clinically approved contrast agents for MRI on the market. They accumulate mainly in the liver and, therefore, can certainly improve the resolution for magnetic resonance imaging in this organ. Another contrast agent is the iron oxide nanoparticle of 25 nm size, for example, that would be applicable for the detection of lymph node metastases.

Both of these magnetic resonance imaging contrast agents have the disadvantage that they do not necessarily accumulate within the cancer cells. We want to specifically detect the cancer cells, not the surrounding tissue, and also reduce the background to have a higher sensitivity.

Here, the next graphic shows nanoparticles for detection of lymph node metastases in prostate cancer patients. These nanoparticles are carried into the lymph nodes by lymphocytes, and you get basically an indirect image by having the surrounding of the tumor itself labeled.

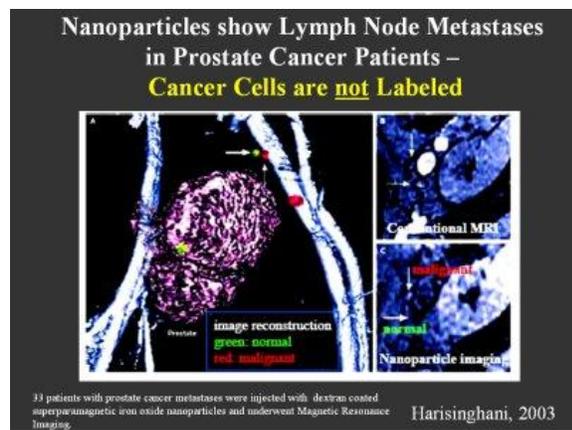


Image 2

Whatever area is not occupied by the tumor cells in the lymph node, as found in that approach, is taken up by the lymphocyte containing nanoparticles. You have a differential image acquisition; it is an indirect acquisition. You have to make two magnetic resonance images (at least two), to get a contrast image in order to make a diagnosis.

In our approach, we seek to directly label cancer cells in vivo. In order to do that we have been looking at surface receptor expressions of cancer cells and we have found (others have also published) that hormone receptors, luteinizing [5] hormone-releasing hormone receptors, are over-expressed in most of the ten leading cancer cases. For example, prostate cancer, colon cancer, even melanoma and cancer of the kidney, breast cancer, etc.

There are a lot of cancers which have specifically over-expressed these receptors, and these cancers could be targeted with our approach, which I will explain. The luteinizing hormone-releasing hormone receptor is a receptor that binds to LHRH, which is a decapeptide, ten amino acids long, and naturally released from the brain and regulates reproductive function. However, cancer cells also over-express LHRH receptors, therefore, can be targeted.

We have synthesized and characterized superparamagnetic iron oxide nanoparticles. These nanoparticles are the basis for contrast agents for the magnetic resonance imaging. Superparamagnetic iron oxide nanoparticles are produced through adding chemicals to iron chloride. These nanoparticles have been characterized and it has been shown that they are not toxic, super-paramagnetic, and through physical and chemical measurements, these particles have been thoroughly analyzed in Dr. Challa Kumar's lab [6].

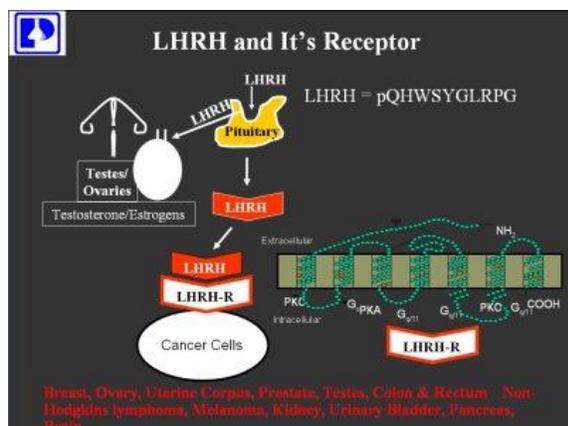


Image 3

In summary, it is important that these particles are super-paramagnetic and, therefore, they increase the contrast in magnetic resonance imaging.

These particles, in order to use them as specific targeting molecules or entities for magnetic resonance imaging, have to be connected with a targeting agent which is, in this case, the luteinizing hormone-releasing hormone. That is binding directly to the cancer cells, that is called the LHRH.

Here you see in this panel, the LHRH iron oxide nanoparticle that has (in the peptide-bound stage), a small reduction in electronic saturation, and it changes its features. The iron oxide nanoparticle is positively charged, whereas the LHRH-SPION is almost neutral, which is a very important finding.

If we are looking at the accumulation of iron oxide nanoparticles, there are LHRH conjugated nanoparticles in breast cancer cells, which over express LHRH receptors.



Image 4

You can already see by looking at the different shades of blue, which in this case is an iron detection, there's free iron oxide nanoparticles. It's a fairly light blue. There is much darker blue, which means much more iron from the nanoparticles are inside the cells in the case when we have specifically targeted the LHRH-SPIONs to the cancer cells.

That is a direct receptor-mediated endocytosis [7] has been proven by co-incubating these cancer cells with the ligand LHRH, blocking the receptor for LHRH and basically preventing the LHRH-SPIONs to enter into the cell, which shows in the lighter blue stain at the lower row of the panel.

Another important part is that we do not want nanoparticles to be taken up by macrophages [8]. Depending on charge and surface, and also size, macrophages take up injected nanoparticles, which would basically be removed from the circulation and therefore, would be unable to reach the target organ or the target cells.

If you look here at the free SPIONs, the SPIONs are very easily taken up by macrophages. Looking at the conjugated LHRH-SPIONs, we have very poor uptake, meaning we have a very extended circulation time and increase the chance to deliver the

nanoparticles to the cancer cells, to their target.

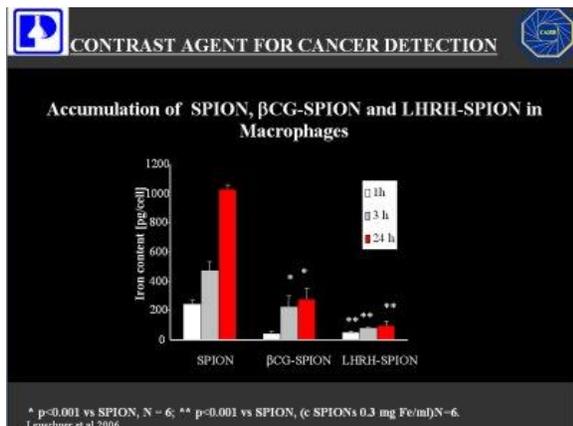


Image 5

The next part of preclinical development involves in vivo experiments. In most in vivo experiments we use a cancer xenograft mouse model in which we have implanted the human breast cancer cell line. These cells also proliferate well, so we can always find the metastases in each organ of the mouse. The mice were intravenously injected with the iron oxide nanoparticles.

After twenty hours, the mice are necropsied; each individual organ is investigated. In histology procedures iron is detected by a prussian blue staining. We also homogenized each individual organ in order to determine how many tumor cell are in each organ and how much iron is detectable in those tumor cells.

Looking at the relative iron distribution in mice after injection of free iron oxide nanoparticles (SPIONs) and conjugated iron oxide nanoparticles (LHRH-SPIONs), if you look at the tumor, we have a very high accumulation of LHRH-SPIONs in the tumor itself, and fairly low accumulation of iron oxide nanoparticles (SPIONs).

Compared to injection of just the free iron oxide particle, we can show that specific targeting through LHRH-SPIONs is certainly improving the accumulation of the contrast agent.

Now if we have a look at the lungs, we have a fairly high amount of LHRH-SPIONs accumulating in the lung tissue. Looking at free iron oxide nanoparticles, there is a very small amount of iron detectable in the lung tissue.

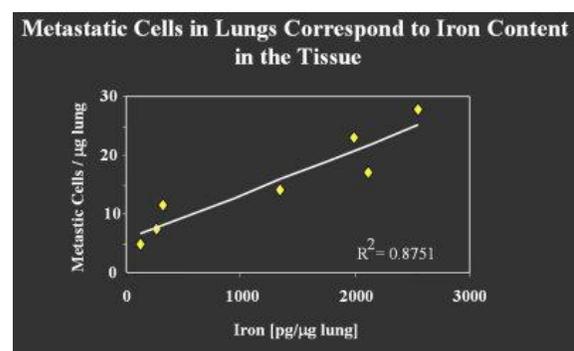


Image 6

Both of these animal groups had lung metastases. Looking at the lung tissue in animals without any tumor, there is absolutely no accumulation of LHRH-SPIONs. Therefore, we have again, a very specific accumulation of nanoparticles conjugated to LHRH, which are directly related to the extent of metastatic disease.

In transmission electron microscopy images, you can see clusters of iron in lung cells, which are the metastatic cells in this case. Looking at and comparing the two images, i.e. normal SPIONs, we hardly see any accumulation of iron in the tumor cells.

We can directly correspond the iron content in the lung tissue to the number of metastatic cells in these tissues. We receive a linear relationship proving that we can very specifically label the individual metastatic cells

in the lung tissue. That was our first successful step in developing the contrast agent.

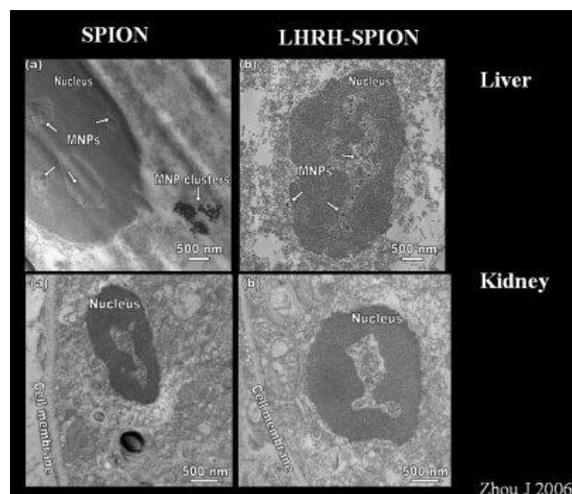


Image 7

We also needed to know if we had a large accumulation of nanoparticles in other organs like the liver and kidney. Sure enough, we find that there's very little of the conjugated nanoparticles in the liver and kidney, most of the free particles obviously accumulate in the liver.

The application of our study is in magnetic resonance imaging. In this case, we see magnetic resonance images from tumors of mice that have been injected with iron oxide nanoparticles from the experiment before. We can see already an increase in contrast, when we are looking at tumors from mice which have been injected with LHRH-SPIONS. In different MRI acquisitions and calculations, an increase in contrast compared to a normal contrast agent and a specifically targeted contrast agent.

Another important finding is, that these two images, which are Anisotropy Map images from mice which bear a tumor. The tumor is here from the same section.

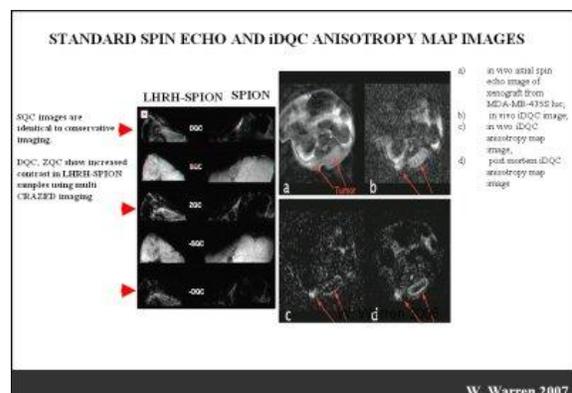


Image 8

If you look at the part marked by the red arrow, you see a lighting up of a small lump, and a lighting up of a circle, which is much sharper because this is a post mortem acquisition; we have a very sharp circle, and a very sharp lump to the right, both are areas of live tumor cells.

When we dissect the tumor, the center of the tumor always contains dead material, therefore unlikely to be labeled however; we can successfully label live tumor cells which are represented in the outer circle. This lump is very small, but it still contains exclusively live tumor cells. So the whole lump has accumulated the iron of the nanoparticles which can be imaged in magnetic resonance imaging.

This part of the article will demonstrate the preclinical development of any treatment, which is very similar to the previous approach, except that a drug is added to the iron oxide nanoparticles.

In order to cure cancers, we have to certainly

"The LHRH-lyticpeptide construct is killing cancer cells from the outside by destroying the cell membrane."

reduce the systemic toxicity. Also it's important

to specifically target the diseased tissue, which has been shown in previous studies already

that we have been able to target specifically the cancer cells by putting LHRH on the surface of the nanoparticles.

That also spares healthy tissue. Previously, we had shown a very small accumulation of free iron oxide nanoparticles in the peripheral healthy tissue. We have also shown that we can retain the targeted nanoparticles in the tumors and metastases.

And here we have conjugated the iron oxide nanoparticles with a drug, that is chemically a lytic peptide (Hecate), which is the same compound group as the LHRH. The LHRH-lytic peptide construct is killing cancer cells from the outside by destroying the cell membrane. Here we have the same membrane and expose it to a lytic peptide. It's a peeling of the outer membrane of a cell, showing how a cell dies with that particular approach.

Obviously we had to ask ourselves the question if we construct a nanoparticle with a drug, is it still toxic or does it lose its toxicity by being processed and bound to the nanoparticle?

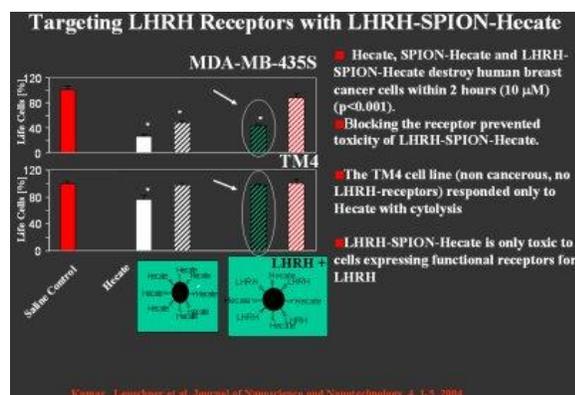


Image 9

In the in vitro experiment, we could show that we still have a very nice reduction of live cells; meaning this construct retains the toxicity of the cancer drug. It is very specific because the

second cell line, the TM4 cell line, does not express the receptors. The cell line, therefore is not targeted, therefore it is not harmed.

It retains its viability, even under incubation of the cancer drug with the nanoparticle in comparison with the breast cancer cells, which has more than 60 percent cell reduction by incubation.

In an in vivo experiment using the above introduced cancer xenograft mouse model we intend to test if we can destroy cancer cells, in particular metastatic disease with this approach. We have a saline control group and groups of xenografted mice for the different treatment groups. These mice are bearing breast cancer xenograft, a very large tumor.

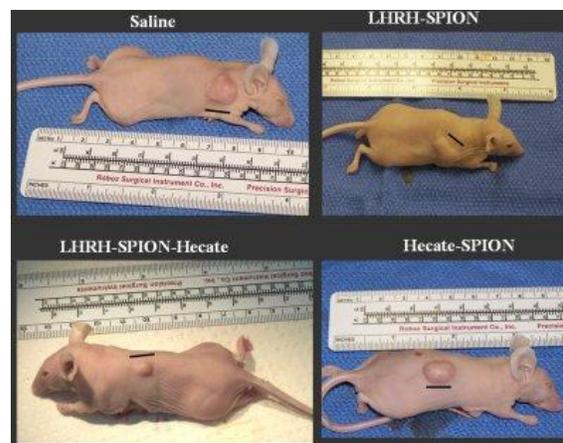


Image 10

In the groups injected with LHRH SPIONs, just the contrast agent, the large tumor is retained. There is no cell death of tumor cells observed. However, if we have injected conjugated the SPIONs with LHRH in combination with a drug, we have a reduction of the primary tumor.

The important point is that if we leave out the targeting moiety, we cannot destroy the tumor cells. It is a very important that the tumor cells are accumulating the SPIONs bound together with the drug. They can only do this

by being targeted with the ligand, which is specifically binding on the tumor cells. Without that we have no binding and no tumor cell reduction.

This is a summary image of what happened and how many metastases and live tumor cells we have after the treatment. The saline controls are in red. We have a fairly high number of tumor cells. We have a fairly high number of metastases from the primary tumor, and a fairly high number of lung metastases.

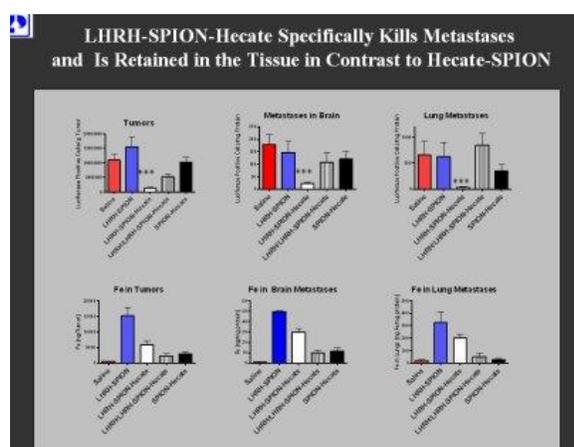


Image 11

If we treat with a LHRH-SPION-Hecate construct, we drastically reduce the primary tumor, we reduce brain metastases, and also lung metastases. We can specifically destroy these cells with that construct.

We also have (depending on the injection), an increased accumulation of iron. This is the iron accumulation in the tumors. We have increased iron accumulation in the brain, and also in the lungs, all of which have been treated with LHRH-SPION-Hecate.

If we just look at SPION-Hecate, the untargeted compound, we have very little, accumulation on primary tumors, no destruction of brain metastases, no destruction of lung metastases, and very little accumulation of iron in tumors, brain

metastases, or lung metastases suggesting that this is a very specific compound we are testing here.

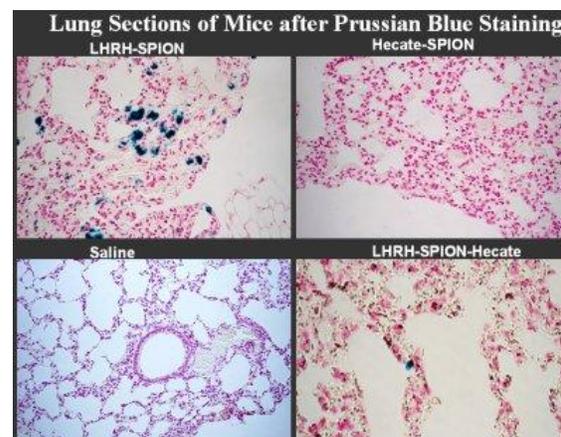


Image 12

This section shows lung sections which have been excised out of treated mice with LHRH-SPION-Hecate. This is iron, what you see here, iron particles directly accumulating in the lungs. LHRH-SPION injected animals really have not accumulated iron in the metastases of the lung, which we cannot even see in Hecate-SPION injected mice. No labeling because of no accumulation of SPIONs in the lungs.

To summarize our results, we specifically are

“...we really do not see any side effects and it is a very safe approach.”

able to target and destroy breast

cancer xenograft, and the metastases from lymph nodes, bones, brain, and lung. Unconjugated construct of SPION with the drug is not effective in destroying tumor and metastases.

The nanoparticles are retained in treated tissue even after destruction of metastases, which would open up a new avenue to simultaneously monitor and treat at the same time, so that would certainly have future impact on our imaging and treatment modalities.

We have a very specific mechanism of action, it is a concept that is dependent on expression of surface receptors on the target cells. We do not see any side effects like changing body weight. Liver and other weights are unaffected by treatment, so are the hematological parameters.

Liver and kidney functions are absolutely unchanged in treated animals. Platelets, erythrocytes, leukocytes [9] are normal in treated or untreated animals, so we really do not see any side effects and it's a very safe approach.

In conclusion, this treatment approach may have promising application for simultaneous treatment imaging, and may be useful for directly monitoring treatment response non invasively in cancer patients.

Our Research Group	
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Support: Nutrition and Chronic Disease, Pilot award, Pennington Center, DARPA, NSF	

Image 13: Research Group

I would like to thank our research group from the Pennington Biomedical Research Center, the Center for Advanced Microstructure and Devices; Doctor Kumar, Josef Hormes. Doctor Warren and Doctor Branca from Duke University and Louisiana Tech's, Doctor Lvov and his new chemistry department.

Endnotes

1. Magnetic Resonance Imaging (MRI) – n. The use of a nuclear magnetic resonance

spectrometer to produce electronic images of specific atoms and molecular structures in solids, especially human cells, tissues, and organs.

The American Heritage STEDMAN'S Medical Dictionary. Boston, New York: Houghton Mifflin Company, 2004: 480.

2. In vitro – adj. In an artificial environment outside a living organism. **In vivo** – adj. Within a living organism.

The American Heritage STEDMAN'S Medical Dictionary. Boston, New York: Houghton Mifflin Company, 2004: 424.

3. Metastatic – adj. 1. Transmission of pathogenic microorganisms or cancerous cells from an original site to one or more sites elsewhere in the body, usually by way of the blood vessels or lymphatics. 2. A secondary cancerous growth formed by transmission of cancerous cells from a primary growth located elsewhere in the body.

The American Heritage STEDMAN'S Medical Dictionary. Boston, New York: Houghton Mifflin Company, 2004: 506.

4. PET - Positron emission tomography - a nuclear medicine medical imaging technique which produces a three-dimensional image or map of functional processes in the body.

http://en.wikipedia.org/wiki/Positron_emission_tomography January 2, 2008 4:43PM EST

5. Luteinizing - v. lu·te·in·ized, lu·te·in·iz·ing, lu·te·in·iz·es - v.tr. To cause the production of a corpus luteum in. v.intr. To develop into or become part of the corpus luteum.

<http://www.thefreedictionary.com/luteinize> January 2, 2008 4:51PM EST

6. Challa S. S. R. Kumar, Ph.D. - 2002 - Present Group Leader, Nanofabrication, Center for Advanced Microstructures and Devices, Louisiana State University, Baton Rouge, LA.

<http://www.camd.lsu.edu/staff/ckumar.html>

January 3, 2008 11:06AM EST

7. Apoptosis – n. A natural process of self-destruction in certain cells that is determined by the genes and can be initiated by a stimulus or by removal of a repressor agent. *Also called programmed cell death.*

The American Heritage STEDMAN'S Medical Dictionary. Boston, New York: Houghton Mifflin Company, 2004: 58.

8. Macrophage – n. Any of the large phagocytic cells found in the reticuloendothelial system.

The American Heritage STEDMAN'S Medical Dictionary. Boston, New York: Houghton Mifflin Company, 2004: 478.

9. Platelets – n. a minute, irregularly shaped, disk-like cytoplasmic body found in blood plasma that promotes blood clotting and has no definite nucleus, no DNA, and no hemoglobin. *Also called blood platelet, thrombocyte.*

The American Heritage STEDMAN'S Medical Dictionary. Boston, New York: Houghton Mifflin Company, 2004: 640.

10. Erythrocyte – n. see red blood cells. Red blood cell – n. Abbr. RBC, rbc. A disk-shaped, biconcave cell in the blood that contains hemoglobin, lacks a nucleus, and transports oxygen and carbon dioxide to and from the tissues. *Also called erythrocyte, red cell, and corpuscle.*

The American Heritage STEDMAN'S Medical Dictionary. Boston, New York: Houghton Mifflin Company, 2004: 276, 700.

11. Leukocyte – or leucocyte – n. See white blood cell. White blood cell – n. Abbr. WBC Any of the colorless or white cells in the blood that have nucleus and cytoplasm and help protect the body from infection and disease through specialized neutrophils, lymphocytes, and

monocytes. *Also called leukocyte, white corpuscle.*

The American Heritage STEDMAN'S Medical Dictionary. Boston, New York: Houghton Mifflin Company, 2004: 458, 886.

Bio



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Dr. Leuschner's areas of interest are development of new methods for treating prostate, breast, testes, and ovarian cancers based on targeting lytic peptides to specific receptors on the cancer cell membranes. In addition, the detection and treatment of spreading tumor cells, which later can lead to metastases, are her main areas of interest. She is also very much interested in collaborating with colleagues of the nutrition and cancer groups to study whether nutrition and lytic peptide conjugate treatments can benefit the patients.

"The take home message is that you need to have a targeted entity to kill the cancer cells," Dr. Leuschner says. "Without [an] LHRH targeting moiety, the nanoparticle-drug construct doesn't kill the cancer cells and it's like a generally systemic chemotherapy drug."