



Slaying the Dragon

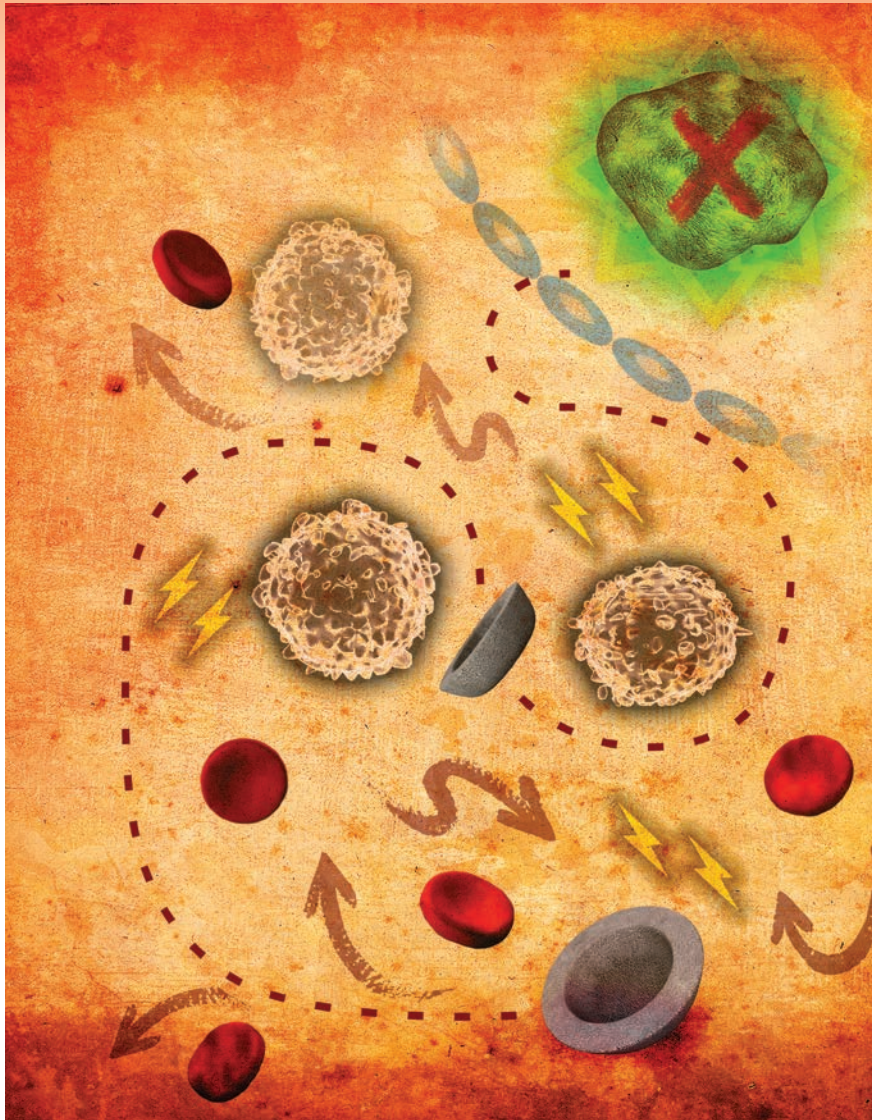
**Nanomechanics opens a new route
to the treatment of metastatic cancer.**

By Mauro Ferrari



nce cancers
metastasize,
they are
almost always

fatal. Researchers are looking at the nanoscale mechanics of tumors to discover means to attack—and kill—all kinds of cancer cells. If successful, this will be a cure for even metastatic cancer.



To reach and kill metastatic cancer cells, therapeutic vectors (gray disks) must make it past enzymes (yellow bolts), immune system cells (white globs), and the endothelial layer (blue-gray barrier).

adverse effect that the treatment gives to the patient. Simply put, the current treatments that are available to fight metastatic cancers have a therapeutic index so low that they provide only days or weeks of extended life.

And here is where nanotechnology and engineering mechanics come in. My colleagues and I are developing therapeutic drugs that can be tailored specifically to the weaknesses of these metastatic cancer cells and can be delivered to the cells directly, without poisoning the healthy tissues in the rest of the body.

For instance, my colleague Haifa Shen at the Houston Methodist Research Institute has developed a multi-functional particle system that takes advantage of the mechanics of blood flow through capillaries to preferentially attack lung cancer cells. In laboratory trials, Shen has had a 50 percent success rate in curing metastatic lung cancer in mice.

I will describe this approach in more detail, below, but first we need to take a few steps back to frame the problem in fuller detail, and set the stage for nanomechanics-based solution strategies. Perhaps the narrative can be eased by a metaphor—of the human body as a medieval fortress, protected by multiple defensive systems: Its high walls, encircled by a moat, with crocodiles to boot, archers on the walls, cauldrons of boiling oil to be used against attackers scaling the walls (including metaphorical cancer drugs, mistaken for enemies).

You get the idea, the human body comprises a sequence of built-in protections against attack (a.k.a., “bio-

We have been at war against cancer for more than a generation—officially since President Richard M. Nixon signed the National Cancer Act of 1971, and for years before that as well. And there have been many victories over the course of this war: Primary cancers are more and more often completely resolved, largely owing to advances in surgical techniques. But because cancer remains a leading cause of death in the U.S. and other parts of the developed world, many people view the effort against the disease as a failure.

As fearsome as cancers are, cancer cells are easy to kill. All cancer drugs are effective against cancer cells, as are many other substances—even tap water. The problem is that effective cancer drugs are also extremely damag-

ing to the healthy parts of the body, and therefore dosage cannot be increased at will without concern for major, potentially lethal adverse consequences for the patients.

The cancers which we cannot yet cure with any level of confidence are ones that have metastasized. That is, they have spread from the organ in which they originated to other organs. Indeed, the rate at which metastatic cancers, especially those that grow in the lungs, liver, and brain, are cured remains abysmally low and they are responsible for the vast majority of cancer deaths.

Medical researchers have a term, “therapeutic index,” which is a measure of how much therapeutic benefit we can achieve for a drug, per unit of

barriers”), which in biological reality comprise the surfaces lining the blood vessels (vascular endothelium), the trapping organs that selectively filter the content of the blood stream (e.g. liver and spleen, the reticulo-endothelial system), the membranes surrounding the cells of the body, and their inner organelles, and the safety pumps that cells use to expel noxious substances (multi-drug resistance efflux pumps).

In this medieval metaphor, let’s imagine the beautiful princess, symbolizing life itself, chained in a room—one among the thousands of rooms in the fortress—and a hideous monster, symbolizing a cancer metastasis, creeping up to her, with deadly intent.

The good news is, the monster is easy to kill: Just about any substance or weapon will kill it, as is true for cancer cells, which can easily be killed with tap water.

The bad news, however, is plentiful. First, we do not know in which room this tragedy is unfolding—and we need to get there quick. Time is ticking away.

Second, the monster has black magic powers, including the ability to modify the biobarriers around itself, so that it will be protected against whatever poisons and weapons we want to use

the cancer and kill it on the spot will do the princess no good, unless they come with a sequence of passwords. Drugs without the required complement of carrier passwords will end up in the toilet, or worse, will mostly kill innocent and helpful bystanders in other rooms.

Fourth, the very same poisons and weapons that kill the beast will also kill the princess—though some princesses are indeed a bit stronger than the beasts, by a tiny bit that correlates with the therapeutic index.

Fifth, actually there are many princesses, not only one, and they are all threatened by many different monsters, symbolizing the heterogeneity of metastases deriving from a single primary tumor. If any of the princesses dies, it’s game over for all. And, different monsters are vulnerable to different poisons and weapons, while all princesses are harmed by all drugs. And, different monsters can modify the biobarriers around them in different ways, each with new and more complex passwords.

Still surprised that metastatic disease is currently incurable?

So, what approaches have been used to address this horrifying scenario? We have four main lines of attack.

short: insufficient therapeutic index.

Second, in the last twenty years or so, all the rage has been on molecularly targeted, biological therapies, mostly monoclonal antibodies. These are really good at recognizing monsters from princesses, and delivering their deadly payload against the captors, rather than the hostages.

Great, but all of the biomolecular equipment they need to carry in order to achieve that bio-sniper capability makes them big and bulky, and generally incapable of making it through the defenses: They are picked up by the biological radars of the body, they are heard stumbling through the vaulted halls, and killed; they are too heavy to swim across the moat with rapidity, and succumb to the metaphorical crocodiles. Result: A modest survival advantage for metastatic patients, on the order of weeks, certainly no cure. Better therapeutic index, but still too low, still needing to flood a patient with drugs to get enough past the crocodiles, and still too much damage to innocent and productive citizens of the body.

Third—here enters nanotechnology! How about packing those chemo-drugs in little tiny nano-containers that will preferentially be collected in the rooms

where the princesses

meet the beasts? Great

idea, but you still need

the biological passwords to

get there. So, the first generation

nanodrugs (starting about

20 years ago) were approved to treat

cancers which were so stupid that they did not even lock the doors behind them. These cancers are very permeable to nano-sized agents through the so-called enhanced permeation and retention effect (EPR), which comes with a greater leakiness of their vascular walls.

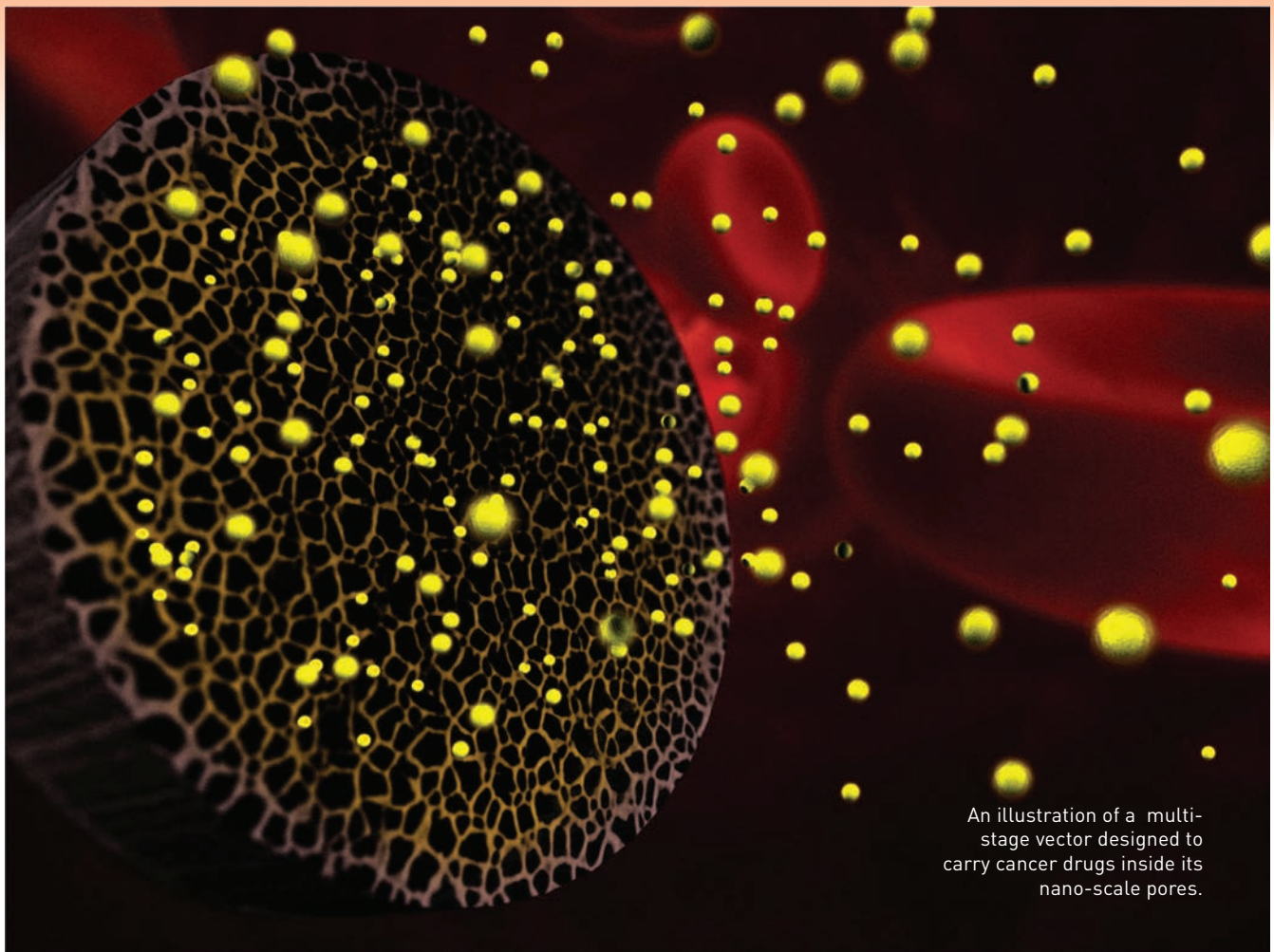
The nanodrugs use nanoparticle materials such as liposomes (imitation cell membrane) and albumin (a carrier

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against it. In reality, cancers have the ability to grow protective tissue around themselves (stroma), plus an adverse pressure gradient, and express molecular pumps that push poisons back to where they came from.

Third, the various barriers and protections in health and disease require special biological passwords to get through. Thus, the ability for our “prince charming drugs” to recognize

First, the poisons that can get into every room of the human fortress—the classical approach of IV infusion of small molecule chemotherapeutics. They will kill a lot of monsters indeed, but with this approach it is impossible to get them all without executing a princess or two, even though the poisons can be selected to act on processes that are more frequent in cancer than health—such as cell duplication. In



An illustration of a multi-stage vector designed to carry cancer drugs inside its nano-scale pores.

molecule of the body) as nano-scale drug containers and vectors, to be injected into the bloodstream. Nanodrugs in current clinical use have extended the lives of many metastatic patients on the order of weeks to months, which is good, but not enough.

Thus, fourth came the idea of adding to these nanoparticles a decoration of biomolecular recognition agents on the surfaces, such as antibodies that recognize cancer specifically, on the theory that this would keep the nanoparticle in the princess chambers longer, and away from the healthy parts of the body. Problem is, those metaphorical crocodiles that get the biologically targeted therapies (second approach, above) have a field day against these molecularly targeted nanoparticles, which are much fatter, slower, and juicier!

Then, no surprise that none of these

“actively targeted” nanoparticles has ever been approved for clinical use, though several are in clinical trials. Tell you a secret, I am not sure any ever will make to the clinic, and if they do, I doubt they will make much of a clinical impact.

Any impact is good, don’t get me wrong—but my only surprise here is that the vast majority of current nanomedicine projects deal with some variant of this ill-fated approach. Different materials, sizes, targeting agents, drugs—but probably the same crocodiles in their future.

Still, I am optimistic that the day is coming, soon, when deadly metastatic diseases can be definitively cured. And the approach that works will be based on the invaluable advances that were recorded in all of the prior approaches, chemo- to bio- to nano-.

Haifa Shen has recently demonstrat-

ed that a new, multifunctional therapeutic agent (MSV-pX) can completely cure about 50 percent of animals with breast cancers, metastatic to the lungs, in several different mouse models. For the same situation in the clinic there is no cure, no expectation of survival.

How did he do it? The key word is “multifunctional.” In turn, the key foundations of his successes are engineering mechanics and nanotechnology. Let’s explore how.

First, the vasculature feeding metastatic lesion is different; it has characteristic flow dynamics, which also reflect the organ in which they are located. Employing mathematical models of multiphase flow, which we have developed over the last 10 years, Shen was able to design particles that, upon reaching the lung cancer capillary bed, tend to accentuate their drift toward the vascular wall, and lodge there, or

Breast cancer cells are shown in dark pink in this micrograph. A nano-based, multi-stage therapy has shown good results.

even penetrate across a line of cancer endothelial cells into the cancer tissue.

This process of drifting (margin-ation), lodging (firm adhesion) and penetration (EPR, transcytosis, and paracytosis) is fundamentally enabled by the shape (disks, resembling platelets), size (3-micrometer diameter, 200 nm thickness), surface charge, and density. It is a physics-based choice of optimal design parameters, obtained in keeping with the principles of engineering mechanics that gives the preferential concentration at the tumor site, or: Mechanics begets therapeutic index. Different choices of physical design variables enable preferential concentration at other crucial metastatic sites, such as liver and bone marrow.

Over a hundred years ago, the Wright brothers tried a few designs to see if any of their contraptions would fly. Now, we design airplanes with the benefit of engineering mechanics and computer simulation for optimal design. Tell you what—I think the time has come that we do exactly the same thing with drugs, designing them for optimal concentration at target sites, based on the quantitative approaches of engineering mechanics.

Tell you more: If different cancers have different vascular and blood flow characteristics, how about we determine these first by radiological imaging (this is easy) and then we design the most suitable drug in a way that will optimize transport to the needed sites? In other words: Personalizing therapy by optimizing the engineering mechanics of its transport?

Alas, concentrate preferentially though they may at desired metastatic lesion sites, the Haifa Shen vectors do not penetrate through the cancer deep enough. They are simply too big. But then—ah-ha!—how about we load them

with smaller (second-stage) carriers and molecules?

As we saw, these by themselves if injected in the blood stream would never concentrate at the target site, but the idea here is they can be carried there by the “first-stage” Haifa Shen carrier. Sure, to accomplish this all you have to do is to make the first-stage particles suitably nanoporous (easy, done, patented) and load the “stage two” in the nanopores.

The solution that works against lung metastases is to load a second stage that is a molecule of a polymer (the “p” in MSV-pX), which is linked to a conventional “anti-duplication” chemotherapeutic drug (the “X”) by means of a chemical group which is cleaved in high-acidity environments (Don’t ask me why now; you will see later). This stage-two molecule is spewed out from the first-stage vector by diffusion, and during the chemical disintegration of the first stage carrier in the body—and by thermodynamic forces it forms nanoparticles, upon exiting the pores.

So, at this point you have a biodegradable microparticle for injection in the bloodstream, which can act as nanoparticle generator, can concentrate preferentially at the target cancer because of its physical characteristics, and upon getting there forms and spews out

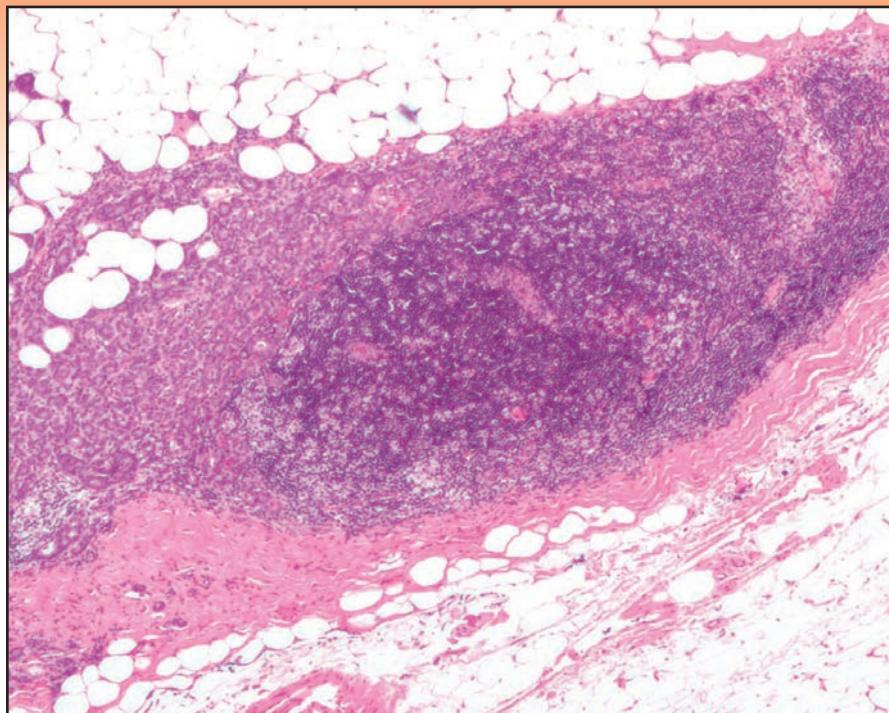
these drug-carrying nanoparticles – a Multi-Stage Vector (the “MSV”).

Why not just carry polymeric nanoparticles to start with, you ask? Too big for the nanopores of the first stage vector.

Why do you need nanoparticles, rather than just the polymer molecules with their linked drugs? Because the polymer with drug simply will not be taken in by the cancer cells.

The nanoparticle, on the other hand, has Trojan horse-like properties, and gets engulfed by the target cells by phagocytic processes. I suspect that the Trojans of old would not have taken into their city just any old, shapeless mass of wood. It had to look like a horse, right? So, we need nanoparticles, not polymeric strands—same idea.

Now, part three of this perilous journey through the cancer jungle swamps, plus crocodiles: Once the nanoparticles are picked up by the cancer cells, what happens to them is what happens to anything (nutrients, signaling molecules, etc.) that is picked up by receptor-mediated endocytosis in cells: They are enveloped in a lipid-bound container (vesicle, phagosome, then transforming into an endosome or lysosome during transport) and carried actively by transporter molecules along train-tracks (microtubules) that direct



them toward the cell nucleus.

As this happens, the interior of the vesicles becomes more and more acidic. It's part of the normal garbage disposal processes of our cells. As they get to the immediate vicinity of the nucleus (which is the ultimate target of the anti-duplication chemotherapy drug), the interior of the transporter biovesicle reaches a trigger point of acidity, which results in the pH-sensitive linker to be cleaved, and the therapeutic moiety to be freed from the polymer backbone.

In this free form it can diffuse out of the vesicle, and travel the very short distance to get into the nucleus, where it effectively kills the target cell. Victory. Haifa cured about 50 percent of the animals with lung metastases, which otherwise would have died in days to weeks.

Again, why don't we just inject the drug by itself? Can't reach enough concentration in the right place, resulting in bad therapeutic index. This is the classical chemotherapy approach.

Why not inject the chemo+polymer? Will not enter the cell as effectively.

Why not just find another way to marinate the cancer cells into a large dose of chemo, somehow delivered at the right site, rather than using the polymer backbone-turned nanoparticle to transport across the train tracks of the cells, toward the nucleus? Now, that's a good question to ask! Here goes the explanation—very, very clever of Dr. Shen: Cancer cells, especially those that are hardest to kill and that repopulate the cancer after most of it is wiped out by therapy (these are known as cancer stem cells), have this exceptionally powerful defense mechanism of multi-drug resistance enabled by a great many molecular pumps that sit at or by their outer cell membranes.

The pumps look for toxic agents that may have penetrated the cell, and when

they find them, they actively expel them from the inside of the cell, where they can hurt, to the outside, where they can be safely dealt with.

Free chemodrug diffusing into the cell will be spewed out most effectively. Chemodrug inside of polymer nanoparticle, on the other hand, is taken up by the vesicles and ferried far away

Cancer cells, especially those that are hardest to kill and that repopulate the cancer after most of it is wiped out by therapy, have this exceptionally powerful defense mechanism of multi-drug resistance enabled by a great many molecular pumps.

from these efflux pumps, right by the nucleus where it can carry out its mission impossible, past the defense of the dark-star cancer cell.

Again, all elements of this exquisitely designed multi-component drug—the MSV, the p, the X—are all necessary for its success, and they are all based on the mechanics of transport. And, please do not talk to me about mere “drug delivery systems.” What we have here is a new generation of multi-tasking drugs, and any component by itself is incomplete, exactly like those we use every day on thousands of cancer patients.

Other colleagues of mine are exploring further frontiers of multistage pharmaceuticals: Ennio Tasciotti strips some immune cells of their membranes, and uses them to cloak MSV carriers so that they will not be captured as readily by the filtering organs of the body. To use paratrooper analogies, if you are going to be dropped behind enemy lines, you might avoid capture longer to carry out your mission, if you wear the uniform of the enemy.

Elvin Blanco deploys second stages that carry more than one drug, with prescribed concentration ratios, to optimize their synergy. He can even get them to release at different, prescribed times.

Kenji Yokoi, Biana Godin, and Ennio

Tasciotti again hijack some cells in the body so that they will carry MSV therapeutics deepest inside of cancers. Carlotta Borsoi uses MSV systems to build a metaphorical road across the cancer jungle, so that conventional drugs can get there with greatest efficacy. Haifa Shen has developed multifunctional MSV cancer therapeutic vaccines. I

have been focusing primarily on using MSVs as components of a therapeutic strategy that suppresses the actions of the key driver genes of cancer.

All of these approaches could not succeed without the multi-disciplinary palette of clinical oncology, nanotechnology, cancer biology, mathematics, materials sciences, pharmaceutical methods, physics of transport, imaging technology, chemistry, biotechnology—and the necessary foundations of engineering mechanics!

So, I reckon it's time that engineering mechanics join forces with all of these disciplines, to finally win the fight against metastases. What say you? Haifa Shen and his colleagues have shown the way. There are prairies worth of space for other strategies and designs that will conquer different beasts in different fortresses.

Many lives to save, and we can do it, if we join forces and work together. Let's all get busy! **ME**

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