Modeling Cancer Biology:

BY KRISTIN COBB, PhD

How mathematical models are transforming the fight against cancer

The most common test for prostate cancer (known as PSA screening) misses aggressively growing prostate tumors—the type typically seen in young patients. It's a fact that was accepted by the medical establishment in 2004 only after a seven-year study of 9000 men appeared in the *New England Journal of Medicine*. But **Kristin Swanson, PhD,** predicted the test's inadequacy in 2001 using a single differential equation—a "back of the napkin calculation" that "a high school student could answer." This is the type of powerful insight that mathematics can offer cancer biology, says Swanson, who is an assistant professor of pathology and applied mathematics at the University of Washington.

Unfortunately, mathematics has remained largely untapped and under-appreciated in cancer biology. Though mathematicians have been deriving formulas about cancer for decades, their work has been confined to mathematical and theoretical biology journals—a set of dense journals that the average biologist doesn't read. Biologists are also skeptical: How can cancer, which is so complex and unpredictable, be reduced to a set of neat equations?

But cancer biology may be at a turning point. Never before has there been a greater need for the field to embrace mathematics and computation. As biological data pile up at an astonishing rate, there is growing recognition that only quantitative approaches can pull it all together. As a result, quantitative cancer models are slowly making their way out of the theoretical and math journals and creeping into mainstream cancer biology. Leading biology journals like *Cell* and *Cancer Research* now contain theory sections. And, in 2003, the NIH established the Integrative Cancer Biology Program—which now funds nine inter-disciplinary centers that are applying quantitative modeling and systems biology approaches to cancer (the ICBPs).

These efforts promise enormous pay off. Modeling can streamline wet-lab experiments; give scientists deeper insight into how tumors develop, grow, and spread; and even predict a patient's prognosis and optimal treatment regimen.

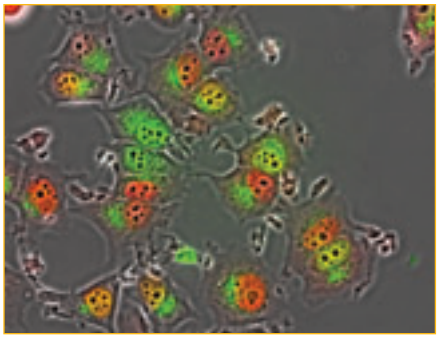
"Biologists tend to think of modeling as some sort of magical thing or black art," says professor **Philip K. Maini, PhD**, director of the Center for Mathematical Biology at Oxford University. "But we haven't done anything extra; we haven't done any jiggery-pokery or put any voodoo in there."

Mathematicians simply translate biologist's hypotheses into a formal set of testable equations, he says.

"Biologists are the first people to tell us that biology is very complicated; it's highly non-linear. Yet biologists use verbal reasoning, which is linear reasoning, which is the wrong model," he says. Mathematical models are needed to reach beyond where human intuition and linear thinking can take us, he says.

ple, **Natalia Komarova, PhD**, associate professor of mathematics at the University of California, Irvine, models the initiating event in colon cancer—the inactivation of the APC tumor suppressor gene. Normally, APC causes cells to enter apoptosis at the end of their "term" in the colon tissue, which helps prevent cancer.

Cells in the colon are constantly exposed to the elements, and thus have a high risk of mutation. Thus, it is imperative that colon cells turn over quickly. The bottom of each microscopic pit of colon tissue (called a crypt) contains adult stem cells whose job is to produce daughter cells to continually replenish the colon. These daughter cells climb up the crypt, differentiate into colon cells, and die off in about a week. It is a delicate balance, however: the quick turnover helps prevent cancer in the daughter cells, but the frequently dividing stem cells are vulnerable to accumulating mutations.



DNA-Damage Control. When cells are exposed to DNA-damaging radiation, they produce p53, an anti-cancer protein that causes damaged cells to undergo apoptosis (programmed cell death). Here, cells express fluorescently tagged p53 (green) and Mdm2 (red) following gamma irradiation. Time-lapse microscopy shows that, following DNA damage, p53 and Mdm2 levels undergo a series of pulses that vary in number from cell to cell. Courtesy of Galit Lahav's lab, department of Systems Biology, Harvard Medical School.

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What follows are some examples of how modeling is adding insight to intuition—from cancer initiation to metastasis and from the molecular to the patient level.

A Cancer Cell is Born: The Subcellular Level

Cancer arises through a series of genetic changes. Mutations in proto-oncogenes allow cells to grow and divide without the need for normal growth signals, and mutations in tumor suppressor genes allow cells to evade normal checks and balances—such as anti-growth signals and programmed cell death (apoptosis). Mutations in genes that detect and repair DNA damage facilitate the process by upping a cell's mutation rate.

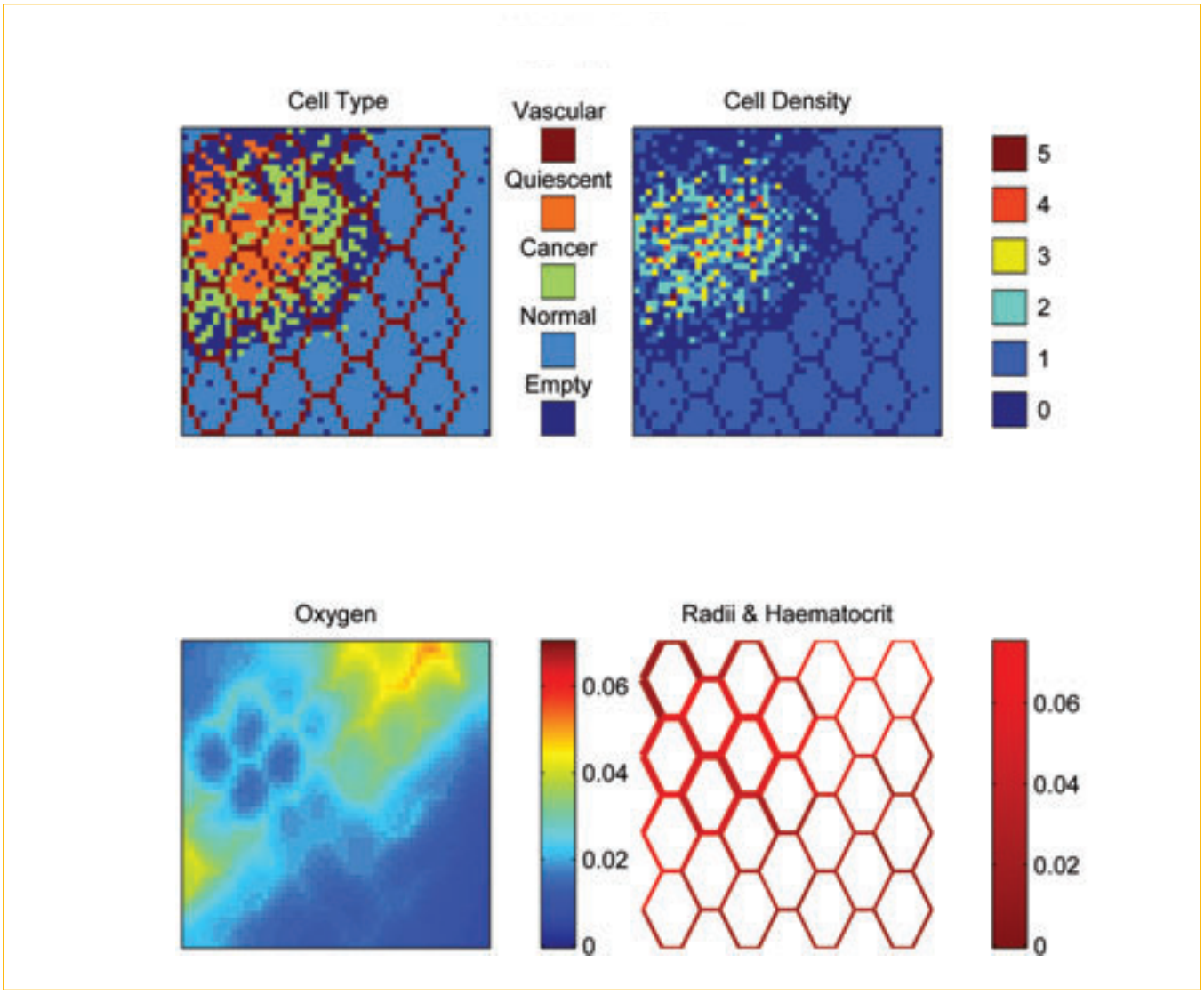
Stochastic mathematical models help investigators test hypotheses about how cancer mutations accumulate. For example, Komarova tries to answer this question mathematically—by calculating the optimal number to minimize a person's chance of getting mutations in the APC gene.

One question that cannot be reliably answered experimentally is how many stem cells are in each crypt. Komarova

"A situation like this is perfect for the application of modeling because in the model we can assume that there is one stem cell or that 50 percent of them are stem cells and we can see what happens," Komarova says. It turns out that, for young people, having many stem cells minimizes the chance of cancer. But for older individuals, having a few stem cells is the best strategy. Likely, evolution favored the optimal strategy for young people, since evolution acts on those of reproductive age, she says.

Besides probabilistic models of mutation "hits," other researchers model the signaling pathways involved in growth, anti-growth, and cell death. Typically, these models consist of systems of ordinary differential equations. Each equation describes the rate of change in the concentration of a particular enzyme, substrate, receptor, or signaling molecule as a function of its production, degradation, and reaction with other network players.

For example, **Galit Lahav, PhD**, assistant professor of systems biology at Harvard Medical School, models the p53 signaling network. p53 is a tumor suppressor gene that plays a crucial role in apoptosis, among other important anti-cancer functions. If specialized sensors in the cell detect DNA damage (or other dangers, such as oncogene over-



Virtual Angiogenesis. In these snapshots from a computer simulation of tumor growth and angiogenesis, the top panels show the presence and density of tumor cells at time=5; cells in the core of the tumor become quiescent because oxygen cannot reach them. As the tumor grows, tumor cells secrete angiogenesis factors that cause new blood vessels to grow and supply extra oxygen and red blood cells to the tumor (bottom panels). Courtesy of Philip K. Maini.

expression), they trigger p53 to initiate a cascade of events leading to the cell's death. More than half of all human cancers contain a mutation in p53, making it the most common cancer mutation.

Lahav studies the p53 network both experimentally and theoretically. "We go back and forth from the bench to the computer," she says.

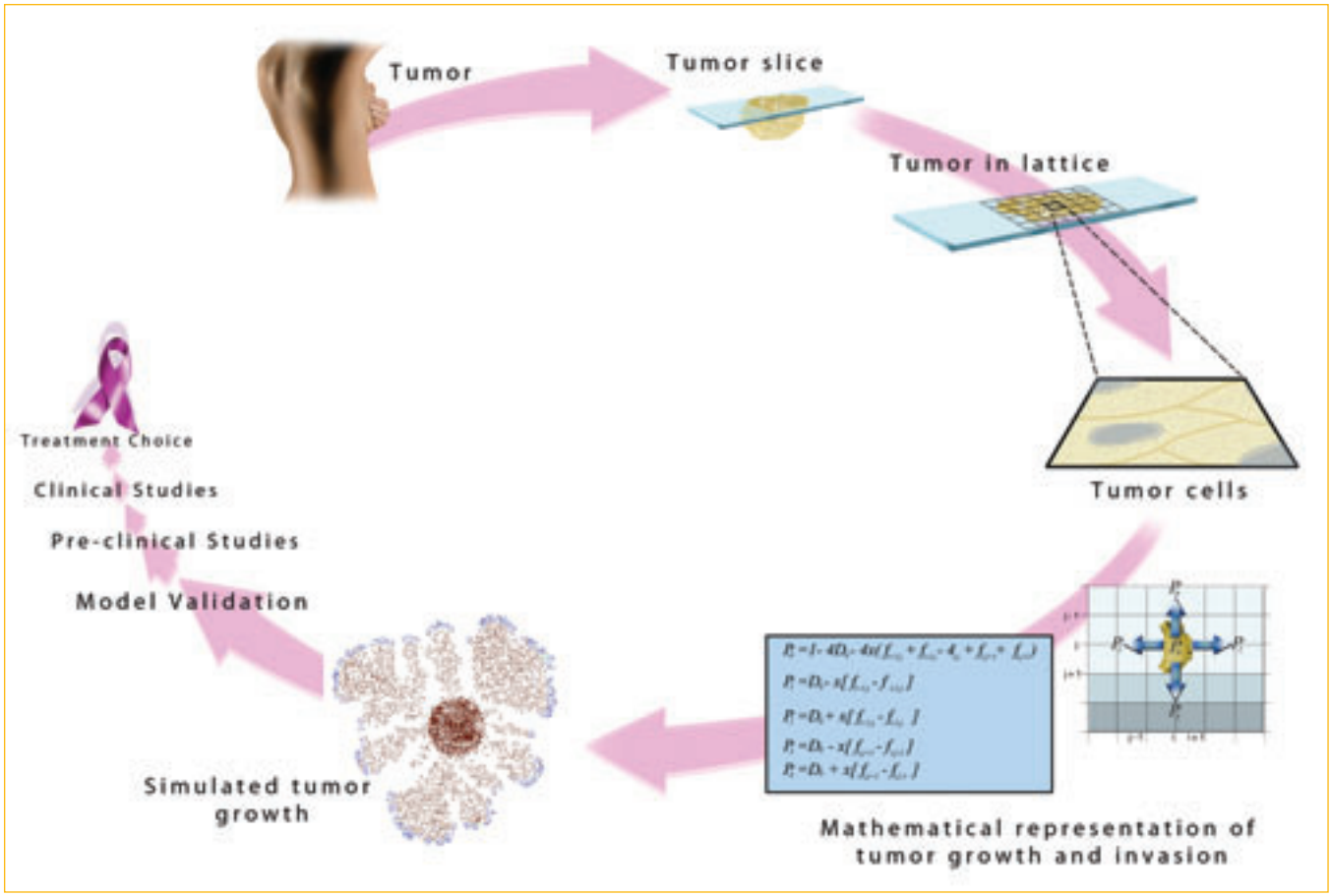
In the lab, Lahav uses fluorescence microscopy to measure the changing levels of p53 and other proteins of interest (all tagged with fluorescent markers) after a cell is exposed to DNA-damaging gamma radiation. On the theoretical side, she uses a series of ordinary differential equations to predict the changing levels of p53 and related proteins, such as Mdm2, which is involved in a negative feedback loop that regulates p53.

"The idea of the models is to help us predict how the network will behave in response to different treatments and to suggest new experiments," she says.

For example, she discovered that levels of p53 oscillate following gamma irradiation, and she is using modeling to help understand these oscillations. If they are important for apoptosis, then some cancer drugs may work better if delivered in pulses rather than continuously, she says.

The Growing Tumor: The Cellular Level

Once tumor cells have acquired the ability to propagate unchecked, they grow into a small ball of cells—which mathematicians model as a growing spheroid. Initially, the tumor feeds on oxygen and nutrients that diffuse to its surface. But these supplies cannot penetrate deep into the tumor, so cells in the core become dormant or die of starvation. The limited nutrient supply curbs the tumor's growth to about half a millimeter in diameter—and if the story ended here, the tumor would be harmless.



Forecasting Invasion. This graphic depiction of a mathematical model developed by Vito Quaranta and Alexander Anderson predicts whether a tumor will become invasive. The tumor is represented on a two-dimensional grid. Each virtual cell is accounted for on the grid and its behavior (e.g., growth, movement, death) is tracked based on mathematical functions and partial differential equations. Solving these equations in sequential time-steps generates a computer simulation of tumor growth and invasion. This approach has the potential to predict disease outcome based on precise quantities measured in the tumor of a specific patient. The model was described in: Anderson et al.* Cell. *2006 Dec 1;127(5):905-15. Courtesy of the journal* Cell. *Graphic by Dominic Doyle.

Unfortunately, as cells in the center become starved of oxygen (hypoxic), they release chemicals that stimulate angiogenesis—the growth of new blood vessels. These chemicals encourage blood vessel cells (endothelial cells) to migrate toward the core of the tumor and supply it with blood. Now the hungry tumor can feed unhindered. At the same time, the tumor gains a connection to vessels throughout the body, giving it an escape route for metastasis.

One strategy for modeling angiogenesis is to set up systems of partial differential equations that describe how the tumor and vasculature are changing in both time and space (how their shapes are changing). For example, **Zvia Agur, PhD**, President of the Institute for Medical BioMathematics in Israel, has modeled angiogenesis using three interconnected modules of partial differential equations. Her equations describe: the changing volume of tumor cells (which depends on factors such as oxygen concentration); the changing volume of immature blood vessels (which depends on how quickly tumor cells release VEGF, a potent angiogenesis factor); and the changing volume of mature blood vessels (which depends on molecular signals that promote maturation). "The simplest model we could make was quite complex," Agur says.

She also set up an experimental system to validate her model. Her team implanted small balls of ovary cancer cells into mice and measured changes in the size and shape of the tumors and the blood vessels using MRI. For each individual tumor, Agur simulated its expected growth in the computer and then compared the simulation results to the actual results from the lab—and the prediction was quite good, she says.

She then simulated what would happen if tumors were treated with anti-angiogenesis drugs, and got a surprising result: The model showed that treatment with a single anti-angiogenesis drug is not sufficient to eliminate a tumor; rather, combinations of anti-angiogenesis drugs are needed.

"At the time, the anti-angiogenesis drug Avastin was very much in the news, and people thought that it could be used on its own," Agur says. "Genentech was doing extensive clinical trials using Avastin monotherapy, and it took them another year or so to realize that we were right."

Invasion and Metastasis: The Tissue Level

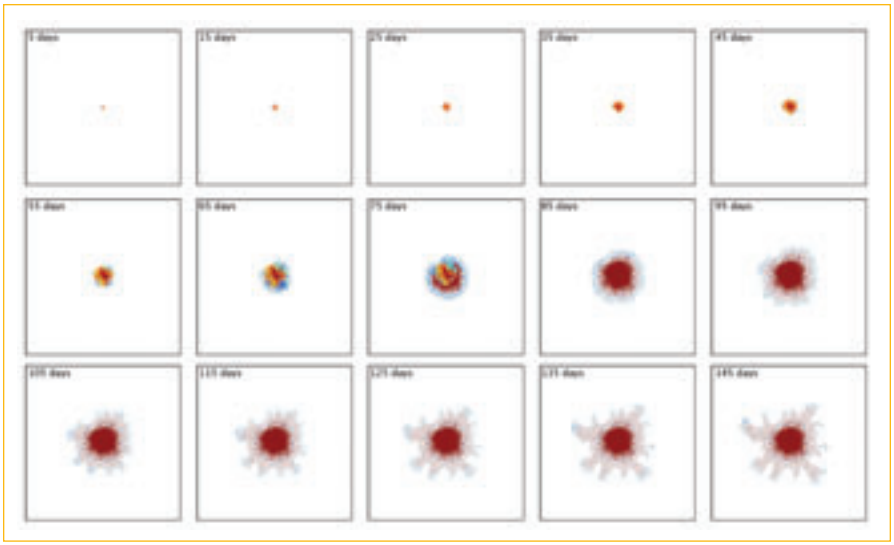
For a while, the tumor continues to grow as a cohesive ball of cells with smooth edges. At this point, the tumor is still often curable, as a surgeon can just scoop it out, says **Vito Quaranta, MD**, professor of cancer biology at Vanderbilt University and also principal investigator of the Vanderbilt Integrative Cancer Biology Program (one of the nine ICBPs).

But, eventually, some rogue cells break away from the growing tumor and invade the local tissue. To become invasive, tumor cells have to pick up certain abilities—they must escape cell-to-cell adhesion, migrate along the extracellular matrix (the surrounding connective tissue), and secrete enzymes that digest the extracellular matrix.

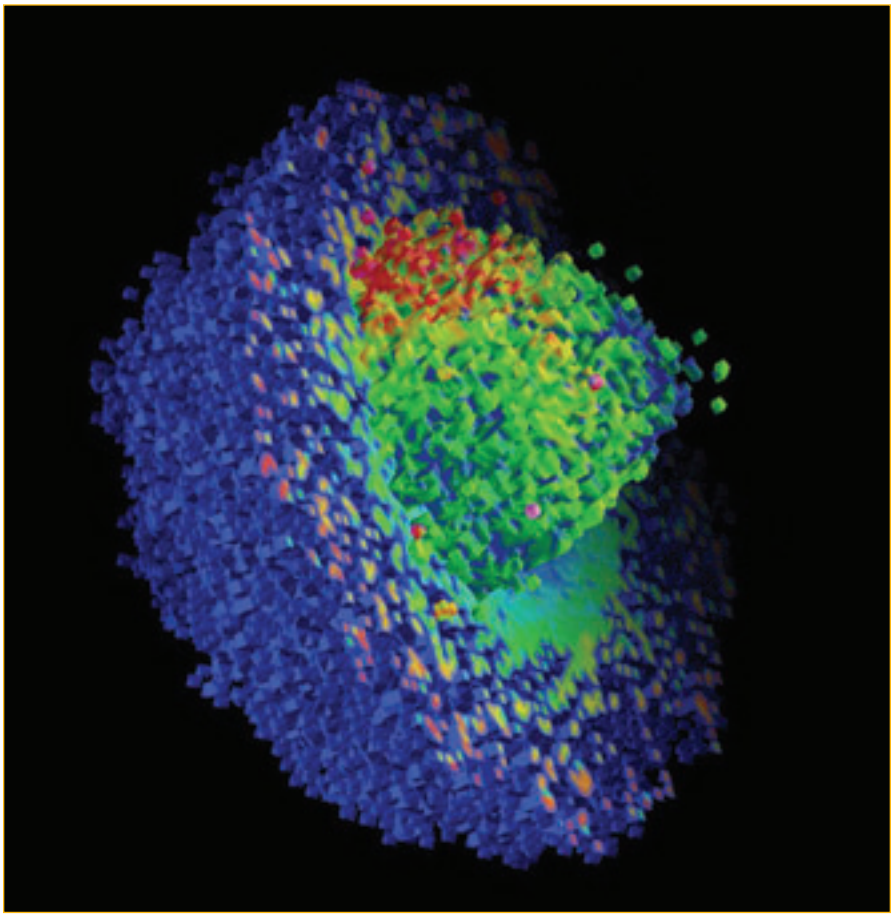
Eventually, these invading cells burrow their way into the blood or lymph systems and spread (metastasize) to distant sites, where they seed new tumors. Now it is impossible to just reach in and scoop out the tumor—and the cancer is much more deadly.

Quaranta, who is an experimentalist, collaborates with mathematician **Alexander Anderson, PhD**, senior lecturer of mathematics at the University of Dundee in Scotland, to model the process of invasion. They use a "hybrid discrete-continuum" model, which means molecules and proteins—such as oxygen and matrix-degrading enzymes—are modeled as continuous densities, but cells are modeled as individual, discrete entities that make autonomous decisions. Such agent-based models are computationally intensive, so simulations are limited to about five million cells (in contrast, a tumor may have a few billion cells).

Cells move on a two-dimensional grid that represents the changing micro-environment—including the concentrations of nutrients, enzymes, and extracellular matrix proteins. Cells have a certain probability of moving to each adjacent point on the grid (called a biased random walk). For example, cells are more likely to move to regions where oxygen levels are high. Cells are also allowed to adhere to each other, migrate, degrade their surrounding tissue, divide, even die, according to cer-



Cancer Invasion. Starting with only 50 cancerous cells, this mathematical simulation shows how a tumor grows first into a smooth ball of non-invasive cells and then—under the right conditions—into an invasive mass that fingers into the surrounding environment. Blue cells are highly aggressive; orange cells are less aggressive, and brown cells are dead. Courtesy of Alexander Anderson



Virtual Tumor. A simulation of one half of the whole living tumor cell population (outer half sphere) and the complete necrotic (dead) tumor cell population (inner sphere). Coloration relates to cell-adhesion value—cells on the outer surface of the tumor all have zero cell-to-cell adhesion. Courtesy of Alexander Anderson

THE INTEGRATIVE CANCER BIOLOGY PROGRAM

Established by the National Cancer Institute in 2003, the Integrative Cancer Biology Program (ICBP) funds efforts in computational modeling and systems biology approaches to cancer. "It's difficult to do this type of research because you have to do both experimental biology and sophisticated computational approaches. Pulling those kinds of groups together really requires a structure like a center," says **Jennifer Couch, PhD**, IT/Computational Biology Coordinator for the ICBPs. "Our vision is always that these centers will sort of form the locus for the development of a community focused on integrative cancer biology." Currently, the ICBP funds nine centers:

Todd Golub, M.D., Dana-Farber Cancer Institute, Boston, Mass.
Identifying protein kinase signatures in cancer.

Joe W. Gray, Ph.D., Lawrence Berkeley National Laboratory, Berkeley, Cailf.
Modeling signaling networks to identify patients for targeted therapeutics.

Tim H-M Huang, Ph.D., Ohio State University, Columbus, Ohio.
Epigenetic changes in cancer genomes.

Timothy Kinsella, M.D., University Hospital of Cleveland, Cleveland, Ohio.
Modeling mismatch repair defective malignancies.

Sylvia Plevritis, Ph.D., Stanford University School of Medicine, Stanford, Cailf.
Regulatory and signaling pathways in neoplastic transformation.

Joseph Nevins, Ph.D., Duke University, Durham, N.C.
Cell signaling pathways in cell proliferation and oncogenesis.

Thomas Deisboeck, M.D., Massachusetts General Hospital, Boston, Mass.
Model and simulation of multicellular patterns in cancer.

Richard Hynes, Ph.D., Massachusetts Institute of Technology, Boston, Mass.
Modeling cancer progression.

Vito Quaranta, M.D., Vanderbilt University Medical Center, Nashville, Tenn.
Model and simulation of cancer invasion.

tain parameters—which Quaranta measures experimentally—such as speed of migration and the rate of cell division. Moreover, as cells divide, they acquire mutations that make them more aggressive and invasive (better able to proliferate, migrate, and enter the surrounding tissue).

The resulting computer simulation—which shows a slice of a growing tumor—looks a bit like a weather forecasting map, Quaranta says. Virtual cells divide, move, and change colors to represent their changing phenotypes—for example, blue for highly aggressive, orange for less aggressive, and brown for dead. Depending on the conditions, tumors will either grow with smooth margins (remain non-invasive) or will finger out into the surrounding tissue (become invasive).

When they ran their model, they got a surprising result: "We found that if the surrounding environment is a smooth, easy environment, then the cells tend to be non-invasive. But if you put pressure on the cells, say by reducing oxygen or making the landscape very hard to deal with, then the tumors become invasive," Quaranta says. In gentle conditions, many different tumor cell phenotypes co-exist, but when the conditions become harsh one or two super-aggressive phenotypes prevail.

Anti-angiogenisis drugs, inflammation, even chemotherapy and radiation therapy might create conditions for aggressive phenotypes to become dominant, Quaranta says.

Their findings were published in the December 1 issue of *Cell*, a leading biology journal. Anderson says that before his collaboration with Quaranta he would never have dreamed of submitting a paper to *Cell*.

"There was a bit of a wrestling match over the exact wording. But that ultimately paid off because it produced a paper that was really aimed at their audience, and that they could understand," Anderson recalls.

"Ultimately I'm hoping this is going to be good for the math biology community, because if I can get a paper published in *Cell*, then why can't somebody else?" he adds.

Brain Tumor Revealed. Only 10 percent of glioma cells are visible on MRI (the bright white area above); a computer simulation superimposed over the MRI helps doctors visualize the rest. The yellow/pink/red areas show that glioma cells may have diffused way beyond the borders of the mass seen on MRI. Courtesy of Kristin Swanson

"It's a nice change," Alex Anderson says. "To have mathematics driving experimentation, instead of us just always playing catch up with the biology."

Quaranta says the partnership has changed his biology as well. "Our experiments now are actually driven by mathematics. So we're entering an era of mathematics-driven experimental biology that is going to be interesting to see."

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Gliomas: The Patient Level

Kristin Swanson (of the University of Washington) also works on modeling tumor invasion, but in glioma—a specific type of brain tumor that is particularly invasive and deadly. By the time a glioma mass is detectable on MRI, invasive glioma cells have already wandered far into the brain. Swanson compares it to an iceberg: the mass you can see represents only about 10 percent of the total tumor cells in the brain; the rest are undetectable, making it impossible to remove them.

Swanson's model consists of a series of partial differential equations that describe how the mass of glioma cells spreads within a virtual brain—a three-dimensional lattice complete with areas of white and grey matter (glioma cells migrate at different rates in these different tissues). Her computer simulations show the changing density of glioma cells along sections of the virtual brain—for example, red where the tumor density is high and blue where density is low.

A glioma patient's MRI reveals only the detectable part of the tumor, so Swanson uses her simulations to visualize the undetectable portion and predict how the tumor will spread.

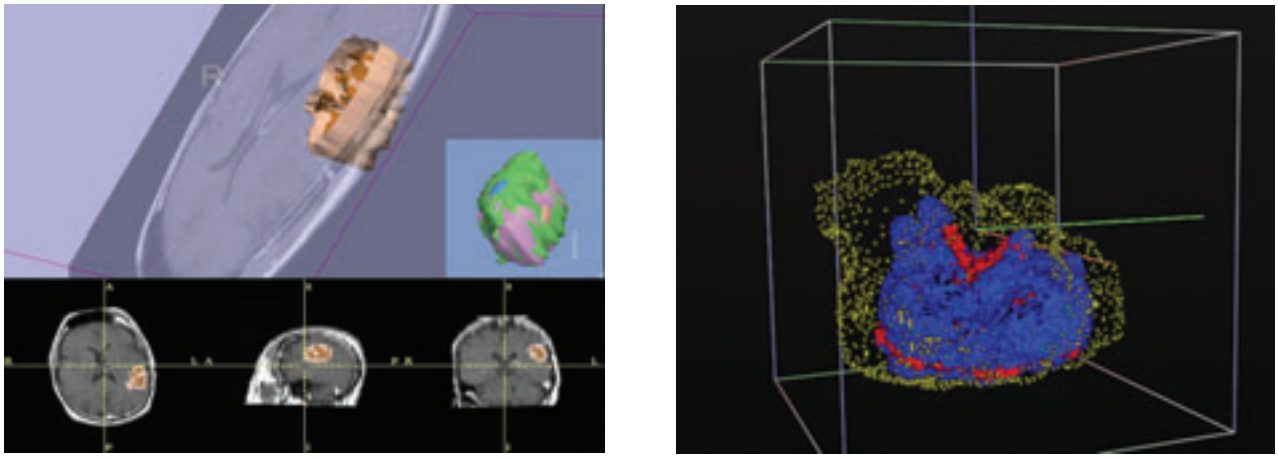
"Just using diagnostic MRI and this mathematical model, you can predict survival with very reasonable accuracy for an individual patient," she says.

Her model can also be used to run *in silico* clinical trials. "It's hard to test therapies for gliomas because patients don't live long and you can't see what's happening with most of the tumor," Swanson says. "But if you have a model for the expected behavior of an individual patient's tumor, then you can assess the success of therapy relative to the expected behavior."

Another investigator working on gliomas is **Thomas S. Deisboeck, MD**, who is assistant professor of radiology at Massachusetts General Hospital and Harvard Medical School, as well as principal investigator of the Center for the Development of a Virtual Tumor (CViT), one of the nine ICBPs. Deisboeck uses a discrete, cell-based approach, rather than a continuous approach, to predict how cells will spread through a three-dimensional virtual brain. This allows him to connect what is happening at the subcellular to the cellular and tissue levels. "Our main interest is multi-scale, multi-grid, multi-resolution modeling," he says.

His virtual cells can proliferate, migrate, die, and respond to the environment and each other. They also contain a nucleus, cytoplasm, membrane and even working biochemical pathways. The actions of particular biochemical pathway components can influence the behavior of the cells and the spread of the tumor. For example, Deisboeck is modeling how the EGFR (epidermal growth factor receptor) pathway acts as part of a molecular switch that turns glioma cells from proliferative (dividing) to migratory (invading local tissue).

Though he eventually hopes to use his models to improve patient treat-



From Patients to Molecules and Back. MRI images from a brain tumor patient (left) are used to build a 3-D* in silico *model of the growing tumor (right). Each cancer cell is represented as an autonomous agent that can move in space and change phenotypes (proliferation* = blue; *migration* = red; *quiescence* = green*). Each cell's behavior is determined by equations that represent the cell's intracellular networks, cell-to-cell interactions, and cell-microenvironment interactions. Images Courtesy of Thomas S. Deisboeck. The underlying multi-scale model was described in Zhang et al. J. Theor Biol. 244(1): 96-107,2007.

ment, his first goal is more modest—to improve diagnostics and patients' quality of life.

"What would be already a very significant achievement is if you could argue that instead of taking three MRI images say over six months, the combination of *in silico* modeling with two images would be just as informative," he says.

As principal investigator of CViT, Deisboeck's broader vision is to build an online community of cancer modelers and a toolkit for multi-scale *in silico* cancer research. CViT is creating new infrastructure, including a digital model repository that will allow people to share and combine models (www.cvit.org).

Bridging the Divide

The above examples share a common theme—a tight link between the lab or clinic and the computer. But these examples are still the exception rather than the rule. The major obstacle in bringing modeling to cancer biology remains the lack of communication between modelers and experimentalists.

On the one hand, biologists and clinicians tend to be mathematically illiterate and fearful of mathematics, says **Robert A. Gatenby, MD**, professor of radiology and applied mathematics at the University of Arizona.

On the other hand, mathematicians tend to neglect the biology, he says. "Mathematicians will set up equations and then they'll do uniqueness theorems and things like that, which are very mathematical approaches but utterly meaningless biologically. This just reinforces the biologists' opinion that this is meaningless and can't be even remotely helpful to them."

Getting these two groups to speak a common language and embrace a common objective is a major challenge. But efforts like the Integrative Cancer Biology Program are helping to bridge this divide and to train a new generation of scientists who are eager to cross disciplines.

"A lot of the students nowadays don't want to get locked into just one field; they are looking for these multi-connections between a lot of disciplines. They may be engineering majors, but they want to know something about biology," says **Daniel Gallahan, PhD**, Project Director of the Integrative Cancer Biology Program at the NIH. "That's been a pleasant surprise to me and it's something I see as a critical component for the future of this effort."

"Maybe in one or two generations, we'll have experimental biologists who are fluent in the language of mathematics," agrees Vito Quaranta of Vanderbilt University.

The Cutting Edge

Quaranta believes that a new era of cancer biology is fast approaching. "The way we do experimental oncology is going to change dramatically as these mathematics-driven simulations become more and more common place," he says.

As quantitative modeling moves from the margins of cancer biology to the mainstream, it is also presenting cutting-edge challenges for modelers.

"It's raising issues that mathematicians and modelers have never had to face before," says Philip Maini of Oxford University. For example, how do you model the mechanics of a growing tissue? How do you build multi-scale models that are accurate across different biological and time scales? How simple or complex is the optimal model?

"It's a very interesting time for graduate students and post-docs to be involved, because it's an area that's now really beginning to take off," Maini says. "Yet it isn't so far developed that you can't immediately start making inroads." □