THE FLESH FILES

BY STEVE SILBERMAN
How the raw materials for 21st-century medical breakthroughs are spoiled by outdated technology.

Of all the forms of woe that take root in the human genome, the cancer called Glioblastoma multiforme is one of the most merciless. It can infiltrate the brain’s white matter for months before causing any symptoms. By the time memory loss and seizures reveal the presence of an invader, there’s often little to do but minimize the patient’s suffering. Most who are diagnosed with the disease—people like the late senator Edward Kennedy—are dead within two years. 

In fall 2005, the National Cancer Institute announced that it was going after glioblastoma and several other fatal forms of cancer in a totally new way. Francis Collins, then director of the National Human Genome Research Institute, told an audience of reporters, federal health officials, and cancer survivors that the same technologies his team had employed to map the complete human genome—high-throughput DNA sequencing, lab automation, and computational biology—would now be enlisted in the fight against some of the most feared diseases in medicine.

The NCI’s new initiative would be called the Cancer Genome Atlas. By cataloging the genetic glitches that turn healthy cells into malignant marauders, the institute hoped to come up with innovative methods of detecting and treating cancer in its earliest stages as well as develop smarter strategies for prevention. The pilot phase of
the project would focus on three of the most common and treatment-resistant forms of the disease: glioblastoma, serous cancer of the ovaries, and squamous-cell carcinoma of the lungs, the classic killer of smokers.

Hopes for the atlas were extraordinarily high. NCI deputy director Anna Barker predicted that everyone in the room would remember the day as a milestone in medical history. Director Andrew von Eschenbach declared that genomic analysis would ensure that the future of cancer testing and treatment looked "no more like the past than a butterfly resembles a caterpillar." Even the normally low-key Collins—now head of the National Institutes of Health—gushed that the "planets have aligned to tackle cancer."

Just a few months later, however, NCI researchers might have wondered whether the planets had instead conspired against them. Rather than making progress by analyzing DNA from hundreds of samples of cancer tissue, the project had practically slammed to a halt. The problem was not in the data or the technology. The problem was in the flesh and blood—the tumor specimens provided by thousands of cryogenic storage facilities known as biobanks.

Located in hospitals, universities, nonprofit organizations, and pharmaceutical companies, biobanks play a quiet but crucial role in health care. Like libraries of the human organism, they archive a wide range of biospecimens—including blood, hair, sperm, saliva, plasma, whole organs, and purified DNA—to use in research and experimentation. From drug development to assisted reproduction, progress in dozens of fields would be impossible without biobanks. They are the biological back end of data-driven medicine.

When the NCI drew up its plans for the atlas, dozens of bio-repositories in the
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US assured the institute that at least 500 samples of each type of tumor could be provided easily. Once the project was under way, however, the researchers got a series of bruising reality checks.

Many biobank managers didn’t seem to know exactly what was in their own freezers. Some specimens had never been checked before they were put in storage and turned out to be unfit for analysis. Others had been obtained from patients without adequate consent, so a single sample might require months of legal wrangling before it could be used. And often, when a frozen chunk of tumor finally arrived at the lab, it was a bloody mess, riddled with dead and decaying cells or simply too small to yield enough DNA.

One bank at a major university claimed to have more than 12,000 samples of glioblastoma in its collection. Only 18 of those were good enough to use. The rate of unacceptable shipments from other institutions ran as high as 99 percent. “We originally aimed for 1,500 samples, which we were told we could get from four to six biobanks in the US alone,” says Carolyn Compton, director of the NCI’s Office of Biorepositories and Biospecimen Research, which led the collection effort. “We never made it to 500 samples of glioblastoma, even after asking biobanks all over the world. We barely got to 500 in ovarian cancer. And we never even started working on lung cancer, due solely to our inability to find the requisite samples. Meanwhile, everyone at the biobanks thought they were doing a superb job.”

In a subsequent investigation, Compton’s office discovered that an inability to obtain high-quality biosamples is a growing problem in labs, eating away at progress in medicine like an undetected malignancy. Many scientists reported that rather than pursuing the most promising leads in their research, they’re forced to design experiments around whatever tissue scraps they can get. One in five confessed that the inferior quality of the samples might be compromising their data.

Every year, billions of dollars’ worth of research into the genetic underpinnings of autism, schizophrenia, diabetes, Alzheimer’s disease, and other devastating disorders hinges on scientists’ ability to tap industrial...
trial quantities of cells and tissue. But Compton found that while our technology for decoding the inner workings of life is advancing dramatically, the protocols for collecting and storing specimens of human flesh have barely evolved in decades. At the same time, innovation in the field of biobanking has stalled for lack of funding and interest. "The science of bio-preservation is still considered an arcane, musty specialty, more akin to taxidermy than medicine. "You might have thought that doing the science would be the biggest challenge of a massive undertaking like the Cancer Genome Atlas," Compton told me last fall. "But acquiring the biospecimens turned out to be the hardest part, bar none. It's the Wild West out there."

THE FIRST FLOOR OF THE CORIELL Institute for Medical Research in Camden, New Jersey, looks less like the Wild West than a bustling spaceport crammed with flying saucers. Rows of stainless steel tanks filled with liquid nitrogen house the most diverse collection of human cells in the world. The contents of these freezers, kept in sealed Pyrex vials at -196 degrees Celsius, played key roles in a wide array of 20th-century medical breakthroughs.

The institute was founded in 1953 by Lewis Coriell, a charismatic pediatrician who pioneered methods of growing viruses and human cells in vitro. By turning test tubes and centrifuges into factories of life, he gave the young Jonas Salk a way to mass-produce polio viruses in the quantities needed to create a vaccine. Coriell also invented the first sterile air-filtration system for labs and turned a crate of foreskins donated by pediatrician and future surgeon general C. Everett Koop into a medium for growing chicken pox.

The town of Camden has changed a lot since then. Poet Walt Whitman's old cottage, a few blocks away, is a grassy oasis in a flatland of | CONTINUED ON PAGE 182
bail-bond agencies and strip malls. But the shining tanks that Coriell left behind when he died in 2001 have kept a Noah's ark of genomic data safe for decades.

The keeper of the ark is Christine Beiswanger, a cell-culturing expert with the patient manner of a middle-school science teacher. She swells with pride as she says that even if Camden were off the grid for six weeks, her beloved samples would stay frosty because of the institute's 11,000-gallon gravity-fed backup tank of liquid nitrogen. Portions of Coriell's collection have also been seeded in multiple locations, including a site in Wisconsin that bills itself as the worldwide leader in bovine genetics. "Bull semen," she says. "We went out there once. We heard more than we ever wanted to know about artificial insemination in cows. But at least we learned that our collection is safe."

In addition to human cell lines, Coriell's repository also houses tissue, plasma, serum, urine, and cerebrospinal fluid. Passed at one of the freezers, Beiswanger pulls on a blue cold-resistant glove and raises the lid. Fisting with a long hook, she catches a rack of vials and lifts it out. The nitrogen pool is eerily invisible, having the same refractive index as air. Clouds of sublimating vapor pour out and vanish halfway to the floor.

Each vial represents the trace of a human life launched into the future in hopes of a cure that will benefit others. The dozens of tanks lining this room contain cells of diabetic Native Americans, autistic Chinese, bipolar Amish, and a Latino infant who suffered from Moebius syndrome, a rare neurological disorder that causes an inability to smile, frown, or form any other facial expression.

The Coriell Institute is only one of tens of thousands of biobanks in the US. An exact number is hard to calculate, because they range in size from fridges stocked with tumors for a surgeon's personal research to a mammoth collection of tissue samples stored by the Air Force Institute of Pathology at a warehouse in Maryland.

Biobanking is an expensive proposition: Just one cryogenic freezer can suck down $2,000 worth of electricity a year. In the interests of science, it's best to archive samples and the data related to them for decades after enthusiasm for the research has faded. As a result, many biorepositories are chronically underfunded. Those in academic labs are typically paid for with money siphoned from grants and departmental expenses. Larger nonprofit institutions, including Coriell, are kept afloat by year-to-year contracts with federal agencies under the auspices of the National Institutes of Health.

A growing number of firms now market their human commodities directly to scientists online. Browsing through one of these biobazaars can be surreal for the uninitiated. Recently, the "most popular" cells available from a company called Asterand were fibroblasts removed from the milk ducts of a 45-year-old African-American woman who died of invasive breast cancer. The price of a vial containing approximately 1 million of her cells: $875 plus shipping.

Though prolific sperm donors can earn a modest living by making regular biobank deposits, tissue donors are not paid, thanks to a 1984 US law designed to prevent organ trafficking. It's technically illegal to sell human tissue at a profit under this statute, but high-volume vendors like Asterand and BioServe finesse this by charging hefty processing and shipping fees. In the age of molecular medicine, the tissue trade is booming. Asterand's annual revenue soared by 82 percent in 2008 to more than $22 million.

Lewis Coriell would not have been surprised that his cell collection in Camden gave birth to a thriving global industry. After all, he tried to persuade the US government to build a vast storehouse of human cells to support research from coast to coast. But he might have been taken aback to learn that the crude 19th-century chemistry he and his peers repurposed for bio-preservation is still the state of the art in the 21st century.

LEARNING HOW TO FREEZE and thaw human tissue without turning it into mush took scientists a very long time. One of the first cryo-pioneers was a Catholic priest named Basile Luyet, author of a 1940 book called Life and Death at Low Temperatures. For decades, he chronicled dozens of gruesome attempts to resurrect monkeys, dogs, cats, and rabbits that had been frozen in their cages or immersed in slushy water until their hearts stopped. Few of these unfortunate creatures survived the ordeal intact; their organs were turned to a rank stew by a buildup of salt and ice crystals. Shortly before his death, the priest reluctantly concluded that "it would be easier to bring back a dead body than a frozen one."

The long-awaited breakthrough was a lucky accident. In 1947, a British scientist named Christopher Polge was searching for ways to freeze, store, and revive chicken sperm, a potential boon to farmers who would no longer have to wait until their prize leghorns were in the mood. Polge tried immersing the fowl gametes in a fructose solution, which didn't work very well—until one day, mysteriously, it did. Analysis of the curiously effective solution revealed that its label had somehow been switched. The bottle actually contained glycerol, not fructose.

Glycerol seemed to be such an effective cryoprotectant that it's still employed in biobanks for preserving blood cells and fluids like saliva and urine. Another decades-old discovery is also still in use—dimethyl sulfoxide, a colorless, odorless solvent derived from wood pulp. (DMSO is weird stuff. Dip a finger in it and you will pick up a faintly oysterish flavor under your tongue a few minutes later.)

Tissue requires a different preservation method, which relies on two mainstays of Victoriana-era labs: formaldehyde and paraffin. In a typical cancer operation, the surgeon excises a lump of flesh that is suspected to be malignant. The nurse carries it to the pathology suite, where a sample is mounted on slides so a pathologist can make a diagnosis. If the patient has signed a consent form, whatever tissue is left over will be shipped to a biobank. But first, it's dipped in a diluted formaldehyde solution called formalin that acts as a fixative, arresting all metabolic processes in the cells. Then it's embedded in paraffin, which prevents oxidation.

Such tried-and-true preservation protocols have endured because, for a long time, they were all that researchers required. "Study sizes were small, investigators
sourced their samples locally, and they got what they wanted,” NCI clinical investigator Stephen Hewitt says. “There was no need for evolution.”

If you wanted to freeze and thaw red blood cells, a dash of glycerol would do the trick. Sperm? Use a 5 percent DMSO solution. Hair follicles, lymphocytes? Ditto. By 1999, Rand estimated that there were more than 300 million tissue samples stacked up in US biobanks, with 20 million additional specimens coming in every year. As new items were added to the menu, the classic recipes were repurposed over and over again with minor variations. Plasma, whole organs, stem cells?

Check, check, and check.

The industry seemed ready to accommodate any advance in medicine. When Coriell began stocking his human ark in the early ’50s, the role of DNA in heredity was just becoming clear. By the mid-’90s, doctors were scheming to hijack genes with viruses to cure congenital diseases. Keeping pace with the state of science for half a century, institutions like Coriell grew into bio-smorgasbords, serving products ranging from kidneys to dental pulp.

But with the sequencing of the complete human genome in 2003, everything changed. Our ability to probe the inner workings of cells jumped by many orders of magnitude. What the gene chip and microarray revealed—which even the trained eye of a pathologist couldn’t see—is that the traditional methods of bio-preservation play havoc with genetic material. Just as the advent of high-throughput DNA sequencing demanded factory-size shipments of tissue, it made many of our ways of harvesting and storing human specimens obsolete.

The trouble starts in the operating room. The first step in taking out a tumor is clamping off its blood supply. But cancer doesn’t give up so easily. In 2001, researchers in Florida discovered that just minutes after a tumor goes offline, its genes start switching on and off as the cells try to adapt to a world without oxygen.

While everyone’s attention is focused on the patient, those genes are rapidly altering their expression patterns. As the surgeon prepares to close the incision, the lump of deadly DNA on the back table is still hard at work.

Often the tissue destined for a biobank languishes for a couple of hours at room temperature before it’s finally dunked in formalin. In a hectic clinical environment—like Massachusetts General in Boston, where the NCI’s Compton was director of gastrointestinal pathology—a slice of diseased colon can sit around in a cooler all weekend before being fixed on Monday morning. NCI investigators have now identified more than 460 genes in colon cancer that switch on or off in just the first hour after the arteries are clamped.

In other words, the tumor that is deposited into a biobank is not really the same one that was removed from the patient. With from the trauma of a round trip to the sub-arctic regions of a freezer. Some cells get so stressed that hours after they thaw, they take themselves out of the gene pool permanently in a cascade of events known as “programmed cell death.”

In the past, a certain amount of dying off was considered just a brutal fact of life in the cryozone. But the work of Baust and others revealed that even cells that don’t outright perish experience changes in genetic expression during the freeze-thaw cycle. In fact, by keeping visible cell structures intact, cryoprotectants can lead to overestimating the quality of biospecimens, making them even more likely to corrupt genomic data.

What’s more, it turns out that formalin causes significant alterations to cellular RNA, the key to decoding the genetic mechanisms of cancer. And DMSO can have the perverse effect of amplifying a tumor’s metastatic potential, turning cancer into super cancer at the molecular level.

While news of these discoveries was trickling out in bio-preservation journals, therapeutic medicine was progressing by leaps and bounds. Many new treatments—such as using stem cells to repair bone marrow stripped out by high-dose chemotherapy and radiation—require banked cells to be thawed and injected into patients immediately. Because these protocells are so fragile, they’re often not rinsed before treatment for fear that too many will be lost. As a result, preservatives end up coursing through the patient’s bloodstream.

But DMSO can cause nasty side effects, particularly in children, including chills, nausea, kidney failure, and even cardiac arrest. So patients simply have to bear up under the onslaught of DMSO while trying to survive cancer, chemo, and radiation.

The industry’s response to these revelations was a series of cautious upgrades. Adding a dose of enzymatic inhibitors to the preservative mix can reduce unexpected bouts of cell death but does nothing to eliminate DMSO toxicity. Chilling tissue at a carefully controlled rate immediately after harvesting (using a technique appropriated from Eskimos in the early 1900s by Clarence Birdseye, father of the frozen-food industry) reduces the need for toxic anti-freeze but requires disruptive changes in
the routine of overworked hospital staff.

These incremental solutions are hardly enough to make up for a long period of complacency. “We got stuck for about 20 years,” says Allison Hubel, a cryopreservation expert at the University of Minnesota. “People assumed that things like DMSO were one-size-fits-all solutions. If you have the perception that all the big problems in a field have been solved, there’s no motivation for funding.”

Tackling unsolved problems is even harder because biobanks have been lackadaisical about establishing standard operating procedures. This is largely due to the fact that they haven’t been forced to; biobanks used for research operate outside the purview of regulatory agencies like the Food and Drug Administration. Instead, the industry supervises itself, following voluntary advisories issued by groups like the International Society for Biological and Environmental Repositories. But only a tiny fraction of US biobanks are members of that organization, which didn’t get around to publishing its first set of guidelines until 2005.

The CEO of Coriell, geneticiast Michael Christman, acknowledges an immediate need for new standards across the industry as it tries to cope with a massive tech upgrade on the research side. “Tissue banking in particular hasn’t been studied in a systematic way, and acquisition of specimens in the hospital isn’t consistent,” he says. “One surgeon might make sure that a sample is frozen right away, while another calls up the lab and says, ‘OK, send somebody over now.’ You might have specimens side by side in a bio-repository that were acquired very differently.”

Meanwhile, the ascendancy of data-driven science is putting ever greater IT demands on biobanks. In addition to the massive quantities of information generated by molecular analysis, a properly annotated sample library now requires megabytes of anonymized data about patients’ medical histories, along with a Web-enabled infrastructure capable of supporting collaborative research projects. Not surprisingly, many biobanks—with decades of legacy records still sitting in flat files in the basement—are struggling to keep up.

Finally, there are hurdles to providing labs with adequate supplies of cells and tissue that have little to do with biobanks themselves. Researchers are notoriously loath to share choice specimens, because unlike data, once they’re used, they’re gone. And though early diagnosis has been a life-saving boon for cancer patients, it has also reduced the size and quantity of tumors available for research. Only at a gathering of biobankers can you hear someone say wistfully, “The days of huge tumors are over.”

Carolyn Compton looks a little harried and jet-lagged this morning, which is understandable. The NCI’s biorepository chief has come straight off a plane from Beijing to a biobanking conference in Philadelphia and is due back in DC after lunch.

But as the trim 62-year-old steps up to the podium to offer a tart assessment of the state of the industry, her wit and energy return. “We now have the technical ability to get the right answers with unprecedented speed,” she says. “If we put the wrong stuff into the front end of our analytical pipeline, we will not only lose the war on cancer, we’ll pollute the scientific literature with incorrect data that will take us a long time to sort out. This is a crisis that requires disruptive innovation.”

The biobankers in the room hang on every word because she is one of their own. Before joining the NCI in 2005, she was a pathologist and biobank manager. Now, with millions of dollars in federal incentives at her disposal, Compton is ideally situated to bring order to the Wild West.

For decades, she was content simply to practice medicine and take care of patients. She taught at Harvard and McGill universities while running bio-repositories for major hospitals like Shriners and Mass General. A former go-go dancer for Smokey Robinson and the Miracles, she also earned a following in local gyms, teaching aerobics to housewives who had no idea that the diminutive blonde funking out at the front of the room spent the rest of her day scrutinizing tumors.

After a 2002 conference where she heard some of the top US researchers and government officials admit that the war on cancer had stalled out, she quit academia. “The consensus in the room was that a shortage of high-quality biosamples was the number one roadblock to a cure,” she says. “This hit me like a revelation. I realized that seeing patients and doing my own research couldn’t possibly be as important as removing this roadblock.” During the conference, she met with NCI deputy director Barker, who later created the Office of Biorepositories and Biospecimen Research specifically to induce Compton to come to Bethesda.

One of her first major achievements was to put the cancer atlas back on track. Compton’s team scoured hospitals, community and academic cancer centers, patient advocacy groups, surgical societies, and tissue archives worldwide for samples worthy of molecular analysis. This three-year effort finally paid off last winter, when the institute made the genetic source code of more than 300 specimens of brain and ovarian cancer available to researchers. At least 20 more tumor types will be sequenced in the next two years, with the results available free online. Recently, the NCI announced that it has identified four new subtypes of glioblastoma. In a stroke of luck, drugs currently in clinical trials for other forms of cancer target the mutations in these tumors as well. If the trials go well, the first treatments for a disease long considered a death sentence could already be halfway to market.

Down the road from NCI headquarters, the centerpiece of Compton’s plan to reboot the industry is taking shape. Here, by the end of the year, she will launch caHUB, the command center of a network designed to supply cancer labs with genomics-grade tumors and other biospecimens in industrial quantities. Jump-started by a $60 million stimulus grant, the hub will bring together pathologists, oncologists, sample providers, preservation specialists, patient advocates, ethicists, and computing experts with the common goal of dragging biobanks into the 21st century. There will be no shiny domes in caHUB’s headquarters. Storage will be outsourced to vendors that follow the NCI’s best-practice guidelines, formulated after years of probing for weak links in the so-called cold chain.

Some of Compton’s ideas seem basic. Most biobanks now evaluate the quality of a sample for the first time when it’s about to be shipped to a researcher. Gauging the quality of samples when they’re logged into the archive instead will reduce unpleasant surprises later. Compton has made clear to biobank managers that if you want the institute’s funding in the new era, you will have to play by its rules.

The reforms that will be tougher to
execute require changes in the mindset of surgeons, pathologists, and hospital staff, "Fixing this will require a new level of awareness that the tissue in the bucket is now one of the most important parts of the patient," Compton says, "because analysis of that tissue will determine all treatment decisions downstream."

One vendor that Compton is likely to tap for caHUB is a German startup called Indivumed, which has built a business based on that way of thinking. The brainchild of a ruddy Hamburg oncologist named Hartmut Juhl, the company offers biobanking and genomics analysis at 10 hospitals in the US and Europe. To guarantee the integrity of its molecular data, Indivumed assigns each patient a nurse who ensures that no more than 12 minutes elapse before a sample is fixed or frozen. The nurse also keeps meticulous records of every aspect of the process ("all very German," Juhl says), because even the patient's time under anesthesia affects the results of the analysis. Boutique biobanking like this doesn't come cheap, but it's paid for by companies like Pfizer and Sanofi-Aventis in exchange for tissue and data that help them develop new products.

Obviously, not every sample in caHUB can come equipped with its own German nurse. But the NCI's guidelines are serving as a new gold standard for the biobanking industry as a whole. Compton's campaign is also lending momentum to grassroots reform efforts far from the centers of power in Washington. Some of these are aimed at addressing problems that NCI guidelines can't solve, such as the need to move past glycerol and invent a new breed of cryopreservative agents optimized for genomics and cell-based medicine. This year, Hubel and her colleagues at the University of Minnesota launched a think tank called the Biopreservation Core Resource, dedicated to developing new ways of storing cells and tissue.

Hubel has patented a DMSO-free method of preserving fragile cells intended for treatments that require direct infusion into patients. "Sometimes even best practices aren't good enough," Hubel says. "We're here to push the envelope."

The stakes are high, as personalized medicine is finally starting to make good on its hype. In March, researchers at Johns Hopkins University announced that they're developing blood tests for cancer based on mutations in an individual's mitochondrial DNA. Drug companies are embracing genetic testing as a way to revive potential blockbuster medications that were taken off the market because they triggered side effects in volunteers with rare genetic vulnerabilities. Overall, the US market for personalized tests and treatments is expected to double by 2015.

Cancer is only one continent in the atlas of human misery. Everyday killers like diabetes and hypertension may someday yield to the diagnostic power of microRNA and other molecules we don't yet know how to look for. But getting to that future will require thawing out an industry still frozen in the past.

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