Scientific services

2019 annual report

THE JACKSON LABORATORY

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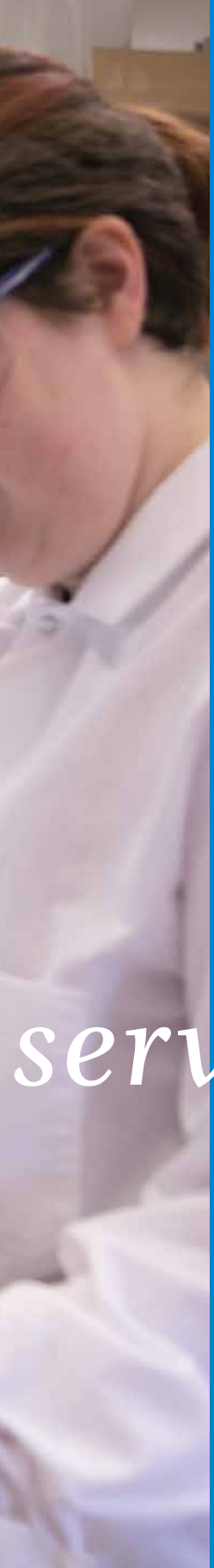
A lighthouse of



introduction

service to science . . .

Scientific Services at The Jackson Laboratory provide a unique model of how dynamic partnerships between scientists and technology platforms are pushing discovery.

Sometimes it's hard to remember what things were like in the old, old days —

say, 15 or 20 years ago — back before smartphones, social media and other wonders of information technology had woven themselves inextricably into our daily lives. How did we fill all of those hours that we now spend immersed in an increasingly virtual world? How did we stay in touch with "friends" and inform ourselves about events across the globe?

Biomedical research has seen even more dramatic changes over the same period, making it just as hard for scientists to recall how things used to be done. Two decades ago, a typical scientific group focused on a highly specific disease or biological process, usually restricted to a single type of cell or organ system. A lab usually pursued closely related questions in a single model organism, perhaps in combination with highly artificial in vitro systems. They applied complicated, finicky technologies in which they had become experts after years of intensely focused work. Methods and procedures were keys to a group's success, so they were jealously guarded. Teams developed profound expertise, but this came at a cost, a bit like the old joke of looking for a lost dime exclusively under your own streetlight. If that's where it had landed, the search could bring great rewards; if it had rolled down the street, under someone else's lamp, good luck with that.

The mid-1990s saw amazing advances in instruments capable of sequencing not only entire genomes, but comprehensively analyzing the populations of RNAs and proteins they encode. Suddenly scientists began to catch the first holistic views of the complexity of cellular molecules and the networks in which they carry out their functions. These developments were accompanied by equally important advances in microscopy and other technologies. Virtually every scientific group stood to profit, but the machines were expensive and experts were required both to run them and make sense of the massive amounts of data they produced. Twenty years ago, things had begun to crack open. The first human genome sequence was nearing completion, riding the crest of an enormous wave of technological development, and that style of doing science was being subsumed into something new. That wave was dwarfed by many subsequent ones, to the point that well-established scientists were having to change their game plan.

Twelve or thirteen years ago, the difference was articulated clearly by now JAX Scientific Director Nadia Rosenthal, Ph.D., FMedSci, FAAHMS, when I interviewed her at another institute on another continent.

The promise of the human genome is stretching by orders of magnitude with the decoding of thousands of human genomes, thousands of tumors and other tissues.

"It used to be you pursued your question in 'your' system with 'your' technology," she said. "Now you pose a question, then pick and choose from a wide menu of platforms and technologies and approaches to answer it."

The explosion of technologies and methods in recent years stimulated The Jackson Laboratory to begin setting up centralized platforms – covering virtually the entire spectrum of existing biomedical instruments and techniques and continually adapting to keep up. These services are managed by Alan Sawyer, a native of Great Britain who arrived at JAX after a career in the U.K., Germany, Italy and Australia. He is, not entirely coincidentally, the husband of Rosenthal. Their partnership is perhaps a metaphor for the marriage of services and research at the Laboratory.

"The main things you should understand about services at JAX," Sawyer says, "is that they're not just made up of people running machines on demand. Many of the heads of our services are exceptional researchers in their own right, and in every case, they're actively engaged in the design and implementation of research projects. A scientist can come to the institute with an idea, sit down with several heads of the different platforms, and together they'll develop a plan to approach whatever question the researcher is interested in."

I witnessed this during a meeting between investigator Julie Wells, Ph.D., and the heads of four services — by no means an unusual case; some projects require the participation of even more. We were discussing the "DICER" project, described in the first story in this book. Wells was talking about how the project evolved and the conversation turned to her plans for the future. She mentioned an idea and immediately all four of the Service Leads chimed in: "We could try … Maybe you could use … How about thinking … " The discussion took on a shorthand that only an expert could follow.

Muneer Hasham, Ph.D., runs a service that creates new mouse models of disease based on the highly specific tumors of individual cancer patients. He put it this way: "The PIs are the gasoline," he said. "We are the engine."

This setup means that a newly hired scientist can arrive alone, settle into an office and get started on a project immediately. It leapfrogs a lot of typical hurdles: waiting for months for the arrival of recruits, the time spent setting up the infrastructure of a new lab. Plus, you don't have to have broad expertise in all the topics that come up when investigating a disease; you can count on others. The services keep up to date on new technologies, and manage a great deal of the regulatory paperwork involved in biomedical research.

I was amazed to hear that most research groups consist of four or five people — that's a sharp contrast to a lot of other laboratories, where labs often have eight or ten or sometimes a lot more members.

"It's a completely different atmosphere than someplace like Harvard," says investigator Derry Roopenian, Ph.D., of his alma mater. "There, you may not know what's going on in the lab next door; there's very little cross-pollination. Here nobody's hiding what we're doing; nobody's isolated; we have a completely open-door policy."

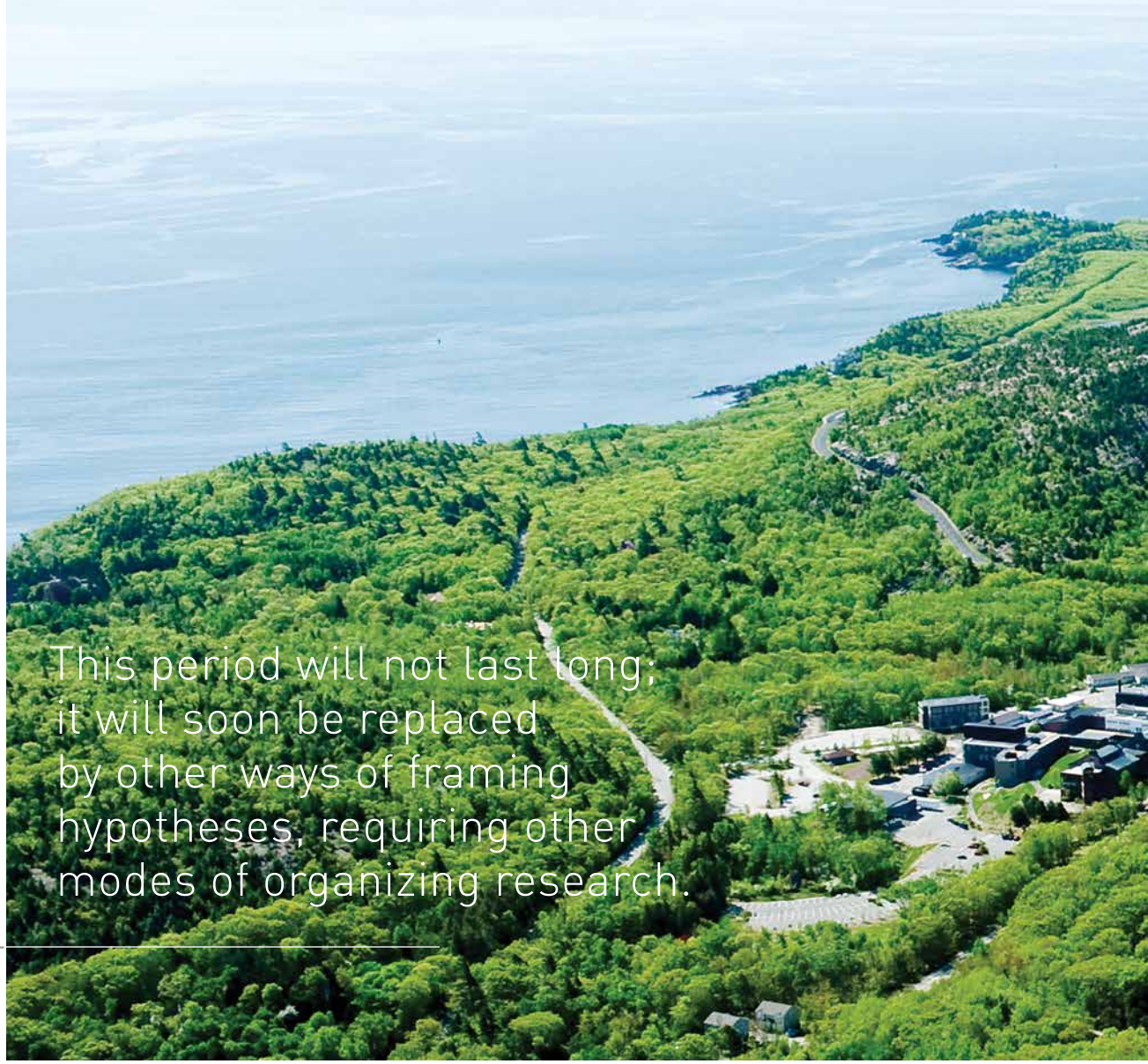
"It's an incredibly collaborative culture," Sawyer says. "Part of that comes from our isolated location – on an island, in the middle of a National Park. From the very beginning it was clear that The Jackson Laboratory would need

to be more self-reliant than places hooked into academic networks the way they are in Boston, or other locations with large universities and hospitals. Of course this has been a place where people come to work, but most of us live on the island or nearby. It's also our community."

That culture can function elsewhere — as demonstrated by the establishment of activities in a new location. Six years ago, JAX built a new building next to the UConn Health campus in Farmington, Connecticut. Senior Vice President for Research, Ken Fasman, Ph.D., explained the motivation behind the new facility.

"Our work is becoming increasingly 'translational' — that is to say, we're taking discoveries from the mouse really into the arena of clinical medicine," he says. "In the current scientific and economic climate, institutes need to take more of the steps toward that goal themselves; the pharmaceutical industry won't do it for them. This location is closer to hospitals and clinics, and we have several of the key services here."

That doesn't mean the new campus is surrounded by a city or situated in an urban environment. It sits beside a pond in a beautifully wooded area. The day of my visit, wild turkeys strolled boldly across the parking lot and, just briefly, a black bear wandered out of the tree line.



This document describes how a few specific projects have arisen in the scientific ecosphere of The Jackson Laboratory — paradigms of how it is supposed to function. At the same time it attempts to do more — to capture the sense of a very particular moment in history, when the culture of biomedicine is undergoing profound transformations. This period will not last long; it will soon be replaced by other ways of framing hypotheses, requiring other modes of organizing research. That's a natural process that occurs when scientists use models inherited from the past, apply the latest technologies to pose old questions in new ways, and confront the profound and complex barriers that still limit our understanding of processes of health and disease.

We know that fundamental concepts are still lacking, are still holding us back. The current style of thinking will be replaced by something new. This booklet can be seen as a small case study of how The Laboratory is remodeling itself to address those challenges. These projects are just examples to open the door on a unique research environment; JAX can provide many, many more.

– Russ Hodge, June 2019

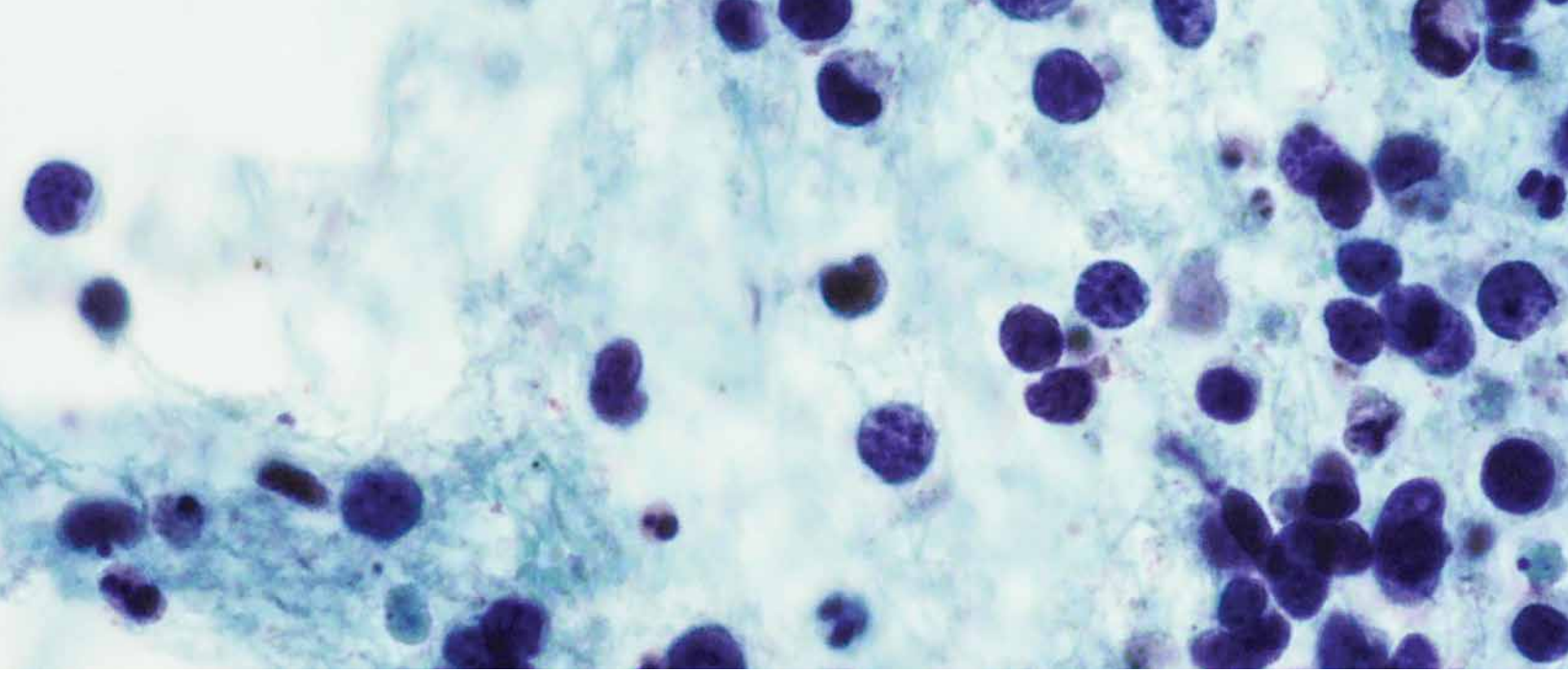
chapter 1

Orchestrating a model of lung cancer

This story began on a foggy morning on

Mount Desert Island, during a walk with a dog.

JAX CANCER CENTER STORY



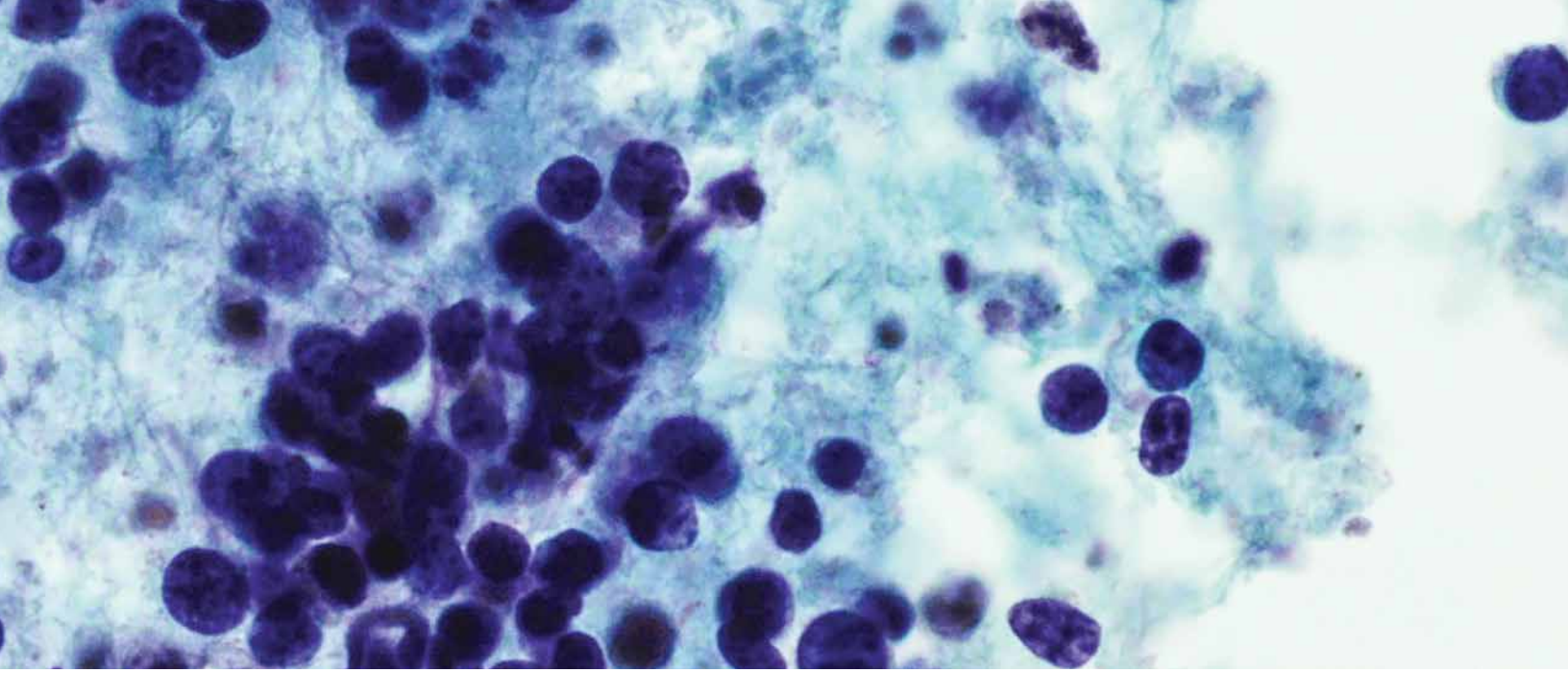
By the time they are published, scientific accomplishments usually have a polished feel, like a well-rehearsed piece of theater. This tale is more of a peek behind the curtains, from a playwright's inspiration through all the stages to a dress rehearsal. Here's a look at the process of science, at the machinery behind the scenes, at the often gargantuan efforts that are necessary before a single experiment is ever performed.

The inspiration came on a weekend. Julie Wells, Ph.D., and her husband Rick Maser, Ph.D., had set off along one of the many paths that traverse the wooded interior of the island, into the heart of Acadia National Park. For the two JAX scientists, walks with the dog provided a way to leave work behind — meetings with students about projects, grant applications and administrative details, rounds of paper submissions and revisions that sometimes seemed endless.

That day Wells was having trouble pulling it off; she was preoccupied with a problem. The theme was lung cancer, and she was butting her head against an obstacle familiar to everyone in the field. There was simply no good mouse model to study the deadliest forms of the disease, and this had hindered research for decades.

Time and time again, JAX projects have demonstrated the power of mouse models to clarify questions related to health and disease and their benefits as proving grounds for new therapies. But most of these successes have required discovering just the right mouse, or developing a new strain, or tweaking an existing one in a way that closely mimics human forms of a disease.

Lung cancer has stubbornly resisted most of these efforts. In human patients it is usually detected far too late, after a primary tumor has spread to other tissues. This metastatic phase is when it becomes deadly, making it the stage most crucial to capture in an animal model.



"We do have mouse models of lung cancer," Wells says. "Some are based on mutations in a cellular signaling molecule called KRAS, which is disrupted in many forms of cancer. Others are based on deletions of p53, a molecule that plays a role in repairing DNA and triggering the death of aberrant cells, and is also defective in a huge range of tumors. Some animals combine both mutations. They get lots of tumors in the lungs and elsewhere, but their cancer rarely metastasizes."

Wells' idea was to start with these mice and introduce yet another pair of mutations found in a specific subgroup of lung cancer patients. But her idea would probably only succeed if these additional mutations were introduced into developed animals, after birth, which posed a challenge: genetic manipulations are usually carried out in embryos consisting of at most a few cells. And these two mutations would need to be added to different cell types within the lung.

"Why don't you infect your mice with a virus?" Maser said.

Her husband, it turned out, was the perfect foil for the question. Maser and his team were experts in developing methods to hollow out viruses, replace their contents and use them as gene delivery vehicles. These techniques had long been established in cell cultures, and recently scientists have considered them a promising avenue for "gene therapies." The idea is to use viruses to carry healthy forms of genes into patients' cells, replacing defective versions they had inherited. Currently there are a number of ongoing experimental clinical trials based on this approach.

So why not use the same approach to deliver a cancer-causing gene to mice?

The project Julie Wells embarked on has become a fantastic example of the way research and Scientific Services combine efforts and brains in producing groundbreaking science at JAX. Her plan to develop a mouse with a metastatic form of lung cancer has required dozens of steps and a continual dialogue with the leads of a number of scientific service units. The process exemplifies the sort of creative, spontaneous pipeline that is becoming a hallmark of JAX research: involving a flow of molecules, cells, tissues and animals, of ideas and expertise, moving forward and back among the groups involved — all hands on deck and fully engaged in the intellectual process.

"I really doubt that I could have done this project at another institute," Wells says. "I needed the skills, time and effort of many researchers. I had a basic idea, but no clue how to achieve it. JAX is a place you can come without having to be an expert in all the disciplines and technologies you need. You can literally arrive here alone and get started on a complex project right away because you can really rely on the expertise of colleagues and excellent Scientific Services."

The current project is an example of another basic principle pursued at JAX: unusual forms of a disease can serve as a springboard to discoveries about far more common conditions. Wells' original idea stemmed from her familiarity with a rare form of lung cancer called pleuropulmonary blastoma, or PPB. It differs from most tumors in that it mainly affects infants and small children — and can be extraordinarily metastatic.

Previous studies had revealed that most patients with the disease harbor mutations in a molecule called DICER1. In some cases, the mutations appear to have occurred in their germline, as a flaw already existing in an egg or sperm cell, before they fused to create a new genome and embryo. This meant that every cell in a patient's body held one defective copy of the DICER1 gene. In many patients, at some later stage in development, the embryo or infant had acquired a somatic mutation in the second copy of the gene. This affected some of their tissues, including the lung.

The first mutation meant that the infant's cells could use the second working version of the gene to produce at least some healthy DICER1 protein. Some cells that also acquired the somatic mutation, however, would only make a shortened, less functional form of DICER1. And this would probably have devastating consequences on affected tissues, because DICER1 has crucial functions in cells.

What happens in a cell — whether it is healthy or diseased — largely depends on its ability to produce the right molecules, in the right quantities. Cells need to be flexible to maintain structure and order in spite of internal and external changes. They manage this by interpreting signals that are passed along complex biochemical pathways, ultimately activating new sets of genes. This leads to the production of different sets of messenger RNAs (mRNAs) that can be translated into proteins.

This system of gene expression is incredibly complex. Each step along the way offers the cell opportunities to intervene: to block the process entirely, or adjust the amounts of molecules that are made. For example, not all mRNAs end up as proteins. Some get tagged with smaller RNA molecules called microRNAs. These serve as markers that tell the cell the mRNA should be destroyed.

The genomes of humans and mice encode thousands of microRNAs. In most cases, they are used to tune down the amounts of proteins that are made, rather than blocking them completely, but in some cases the quantity of a particular molecule in a cell is just as crucial to its health as its presence or absence. Cancers play havoc with both the proteins a cell manufactures and how much of them it produces, and microRNAs are involved in many of these changes.

MicroRNAs begin as longer molecules that have to be whittled down to become the short tags that are attached to messengers. This is where DICER1 steps in; its name evokes the type of dicing and slicing a chef performs on vegetables in the kitchen. DICER1 recognizes the longer form of an RNA and slices out parts to leave the smaller microRNA. If it doesn't recognize the longer form or remove these extra segments, the microRNA can't be used as a tag. As a result, the cell will likely lose its ability to control quantities of crucial molecules.

This seems to be an important aspect of PPB, the aggressive form of childhood lung cancer. Losing full-length DICER1 in lung tissue may let extra proteins slip through, like releasing a brake. If this affects molecules involved in cell growth or migration, it's easy to see how this could promote tumors and metastases. The germline mutation alone ought to leave a child with one copy of DICER1, which might suffice to eliminate enough proteins to keep things under control. But acquiring a second, somatic mutation would probably set the tissue on a course toward cancer.

"Like humans, mice have two copies of the DICER1 gene," Julie Wells says. "Theoretically, modifying both of them ought to give us a model of human PPB and maybe more generally lung cancer. But PBB patients appear to get those two mutations in different ways. That's probably important to the progression of the disease, but it turned out be incredibly difficult to replicate in the mouse."

Enter Rick Maser and the idea of infecting animals with a virus. In principle, you could start with a mouse with one defective copy of DICER1 encoded in its genome — like the germline mutation in humans.

Then — theoretically, again — you could build a virus containing the copy of the second mutation. You'd use that to infect a mouse, and wait for it to deliver this gene. If it successfully replaced the second, healthy version of DICER1 with a gene encoding the defective, shortened version, the result would be like mimicking a somatic mutation.

"What Maser's construct contains and expresses are proteins and RNAs that guide the shortened version of DICER1 to its location in the genome, replacing the one remaining copy of the gene," Wells says. "We weren't trying to completely eliminate it — if cells contained no DICER1 at all, they would most likely die. The shortened

version of the protein made by this gene is still active, but has an altered activity which probably causes the cells that have it to make different miRNAs. Another technically challenging aspect of this project was that we needed to assemble all of the tumor producing mutations (KRAS, p53 and loss of one copy of DICER1) in one type of cell in the lungs. Then get the second mutation, with the shortened copy of DICER1, into a different cell type. My hypothesis is that this configuration of mutations accelerates metastasis because normally, the miRNAs act as a form of communication between tumor cells and other cell types. When you change DICER1 in these ways, you're blocking or altering that communication."

Ultimately, developing the PPB mouse model required contributions from scientific services across JAX. Wells started with an established model — animals with mutations in the molecules KRAS and p53. While the mice were prone to cancer in the lungs and other organs, the tumors rarely metastasized. The viral construct engineered by Maser changed that completely.

Conditional mutagenesis methods were first developed in the 1990s and have gone on to revolutionize genetic science at JAX and elsewhere. They permit researchers to alter or "knock out" a particular gene in nearly any tissue they desire, and only there, replacing cruder methods that eliminated a molecule in every animal cell. Those earlier methods made it difficult to study crucial genes because a gene with functions in the brain, for example, was likely to have important tasks in other tissues as well. This often made all-or-nothing knockouts fatal in the early stages of an animal's development, or at least highly disruptive of its normal biology before the role of a gene could be studied in a mature animal. Besides restricting changes in a gene to a specific cell type or tissue, the technology enables scientists to attach it to a "switch" that can be activated any time they desire.

Over the past two decades, groups across the world have used these methods to produce thousands of lines of mice harboring such genetic switches. JAX holds them in the largest collection of conditional mutant mice strains in the world, and operates an invaluable service by providing them to other labs.

These efforts produced mice prone to lung cancer that now also harbored mutations mimicking changes commonly found in human patients. Now it was time to see whether Maser's virus could give them the second mutation.

"Mice with the mutations had previously lived a long time," Wells says. "Now, those that were administered the virus developed tumors far more rapidly, experienced metastases and were dying within 11 weeks." But was the virus — and the mutation it delivered — really responsible? Answering that question required the participation of several more services and a meeting of minds among their managers.

JAX's cutting-edge Scientific Services offer what can be essentially considered a sort of hospital for mice: advanced facilities able to carry out virtually every test performed on human patients and tissues in a clinic. JAX pathologists examined the mice that had succumbed to the virus and prepared slides from a wide range of their tissues. "Rosalinda Doty, director of Pathology Services, looked at endless series of slides, trying to find abnormalities in the tissues," Wells says. "Looking for cases of lung cancer, obviously, and also metastases. And in one of the groups of mice, that's what she found. The animals had many tumors."

This still didn't answer the question of whether the virus was responsible. Proving that the tumors carried both mutations — particularly the truncated form of DICER1 borne by the virus — would be a crucial piece of evidence that it had. That task fell to Senior Manager of Histopathology & Microscopy Sciences Lesley Bechtold, and her colleagues.

"The way this is done is to incubate the tissues with an antibody that recognizes only the precise protein you're looking for," Bechtold says. "If you have just the right antibody and the protein is there, cells will give off a signal you can see under the microscope."

The form of DICER1 that had been built into the virus was a short version of the molecule that lacked specific regions that were crucial to its function. This made it similar to specific types of mutations found in PPB patients. Finding it in lung tumors and metastatic tissue required an antibody that would recognize only this form of DICER1, rather than full functioning versions of the molecule. That became the next challenge for the project.

"Most of the time scientists obtain their antibodies from commercial companies that produce them en masse, for researchers, clinicians, pharmaceutical companies and so on," Bechtold says. "The molecules are used for research and also diagnostic tests. If you want to determine whether a child has PPB, for example, you could run a test based on an antibody that a company has produced, one which is designed to recognize mutants of DICER1 that have been linked to the disease."

Some of these antibodies for human DICER1 were on the market, and companies advertised that they should work in the mouse as well. In principle that meant they ought to recognize Wells' shortened version of the molecule. She purchased two such antibodies from a company at a price tag of over a thousand dollars. Unfortunately, it was wasted money. Neither antibody would bind to her protein.

This didn't necessarily mean that the experiment with the virus had failed. The problem might well lie with the antibody itself — it might not live up to its advertising, failing to recognize something that was in fact present in the tissue. Finding out meant turning to JAX's in-house Monoclonal Antibody Service. The facility was originally set up by Alan Sawyer, now senior director of JAX's Scientific Services. His successor, Sabin Antony, is intimately familiar with the problems surrounding commercial antibodies.

"These molecules are so essential to American research and clinical labs that about $800 million is spent on them every year," Antony says. "A study recently carried out by the NIH showed that about half of that is wasted on antibodies that just don't work."

It's a major problem for the industry that has called out for innovation; years ago, this led Sawyer to set up facilities to produce effective, high-quality antibodies for international laboratories. On a practical, daily level it meant Wells had to start from scratch and get a new antibody made. Which meant, and this should come as no surprise, the participation of more JAX services.

The first step in developing an antibody is to generate lots and lots of the protein that you want the antibody to recognize, in a very pure form. At JAX this is handled by the Protein Production and Purification Service, managed by Ruth Saxl.

The most common approach is to insert an artificial gene into bacteria and turn them into protein manufacturing plants. They churn out massive amounts of a very specific version of a molecule. In this case, that meant the shortened form of DICER1 that had been built into the virus and used to infect Wells' mice. The aim was to prove that the animals had gotten it from the virus, so it had to be clearly distinguishable from the full-length DICER1 protein, normally encoded in the genome of the mice.

"The protein purified beautifully," Saxl says, "We sent it to Dorothy Ahlf Wheatcraft, who runs the JAX Mass Spectrometry Service, to make sure that it was pure enough to be injected into mice and raise the antibodies. She confirmed that was the case, so now it was time to send it to Sabin Anthony."

Anthony immunized mice with the modified DICER1 protein in the same way that you would immunize a person to give them immunity to a disease. The fact that the molecule represented an unnatural form of DICER1 meant that the mice's bodies should recognize it as foreign. In principle, this would trigger an immune response, and their cells would start manufacturing antibodies that would recognize and bind only to the altered DICER1 protein. Since these tiny immune molecules can bind to different regions of the protein, an animal often produces several antibodies against a single protein. Here Anthony's service identified and extracted 17 distinct antibodies that could potentially be used to probe the mice tumors and metastases.

The antibodies went back to Bechtold, who tried 10 of them on the tissue samples from the mice. "Two of them worked perfectly," she says. "They clearly marked only the mutant form of DICER1 that Julie Wells was interested in."

"All of this work has been a prelude," Wells says. "We were really fortunate to have received a cancer center pilot grant, which I think we were given out of a recognition of a real need. A huge barrier to our understanding of metastatic lung cancer has been the lack of a good model in which we can both probe the biology of the disease and try out new approaches to treatment."

A model needs to meet several important criteria to have much hope as an aid in understanding human health; Wells' mouse with the double mutation in DICER1 looks promising. "It's based on forms of the protein found in most lung cancer patients, and replicates both the inherited and somatic changes found in a metastatic form of the disease," she says. "Probably the most promising aspect of this is that unlike other models, these mice experience rapid tumor induction and growth, plus metastases along the lines observed in children with PPB and patients with other types of lung cancer."

Wells' mouse was conceived on a walk through the woods. Where, ultimately, will it find its niche? The new model is just a first step, she emphasizes, but her DICER1 mutants open the door on new types of experiments. The group will tag tumor cells with fluorescent markers to track their spread from the lungs to other tissues, exhaustively probing their biology along the way. "What sites do they migrate to?" she asks. "Can the number of metastases somehow be controlled? Are their locations and severity dependent on exactly what type of mutation we've introduced into the DICER1 molecule?"

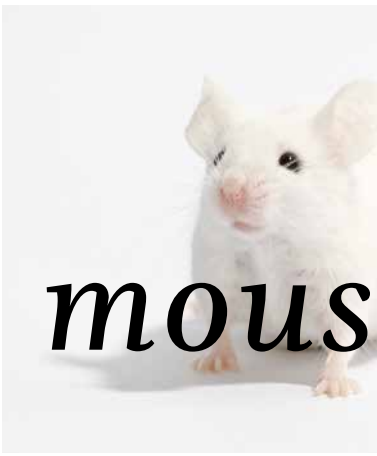
In the absence of a model, most of these issues could only be addressed through speculation, or by analogy to other tumors. Now the group has a foothold. Thanks, in no small part, to a walk with a dog.












The right mous

e for the right disease

It is no surprise that diseases strike individual mice and strains in different ways. After all, the same is true of humans: a person's genetic background influences his or her susceptibility to many diseases.

In hereditary disorders caused by single genes, it may be relatively straightforward to generate a model in the mouse, by identifying or developing animals with defects in their version of the human molecule. But in many more cases, the risk that a person will fall prey to a particular disease is influenced by many genes and environmental factors. That greatly increases the complexity of finding the best type of mouse to expose its mechanisms and develop therapies.

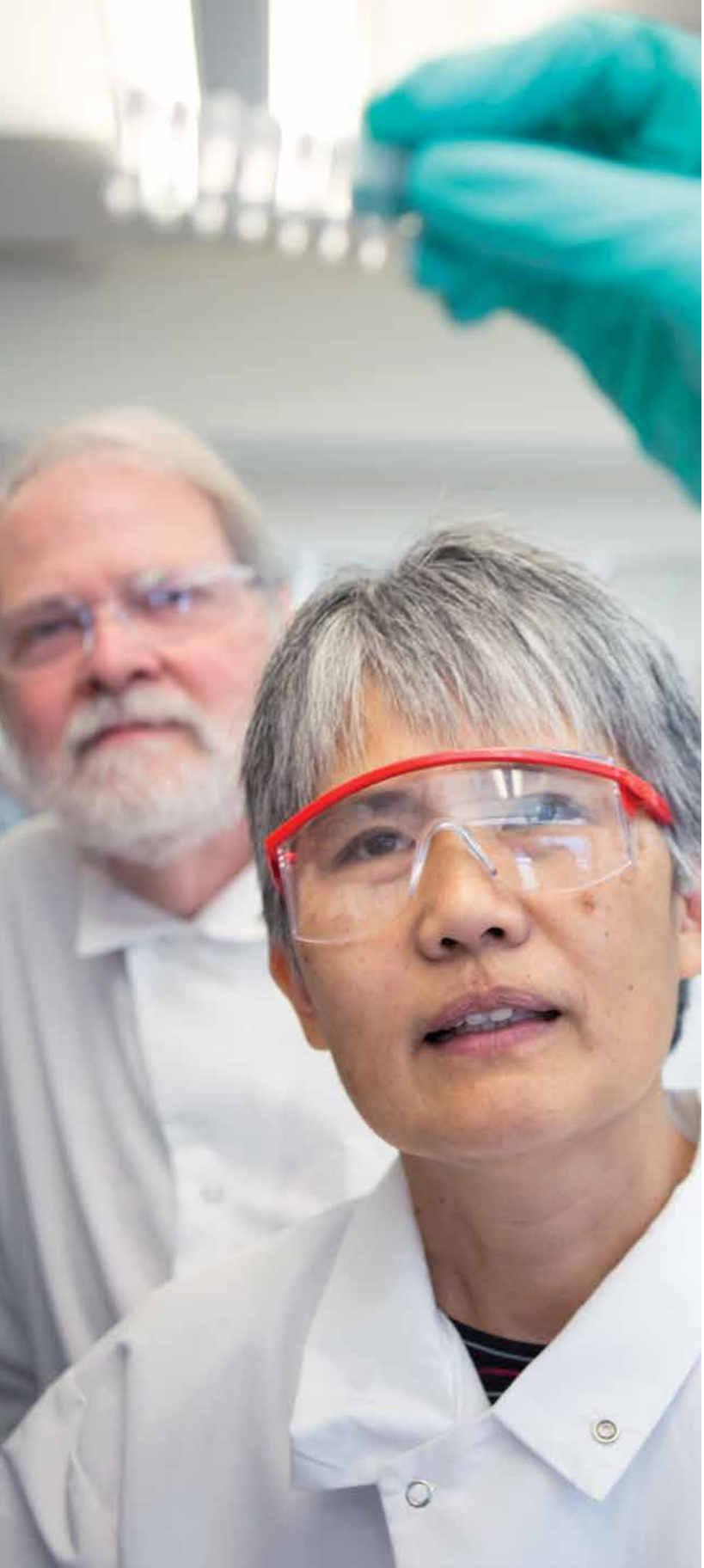
This has been a challenge faced by JAX Professor Patsy Nishina, Ph.D., in taking on a disease called Age-Related Macular Degeneration, or AMD. It's one of the most common causes of vision impairment in people over the age of 50 — the older the population, the higher the incidence. "This condition disrupts a small area in the center of the retina called the macula, which plays a crucial role in our ability to focus at high resolution under good lighting conditions," Nishina says. "For an affected person, the center of the visual field becomes a complete blur. While AMD alone doesn't cause complete blindness, it makes it very difficult for people to read, recognize faces and perform other daily tasks."

Several factors have made it difficult to develop good mouse models for AMD. One is that it mainly strikes the elderly, and most of the strains of mice used in research have lifespans of a maximum of about three years. A The JAX Blog post reviews how researchers correlate the phases of human and mice lifetimes. It turns out to be trickier than one might think.

Take, for example, "C57BL/6J" mice, developed at JAX and used widely in research around the world. During the first month of life they mature at a rate 150 times that of humans. They achieve sexual maturity at about 35 days; after that, their biology begins slowing down. The animals are considered middle-aged from about 10 to 15 months, and between 18 and 24 months they have features resembling humans from 56 – 69 years old. After that they are considered elderly. At that point differences between strains can make it especially hard to draw parallels between how a gene affects mice and the activity of related genes in humans.

A person's risk for developing AMD is not due only to several genes, lifestyle factors such as smoking and diet contribute as well. Even more factors complicate efforts to model the disease in animals — some fundamental differences between the mouse and human visual systems, for example.

"The mouse doesn't have a macula," Nishina says. "This surely has to do with evolutionary adaptations: mice are nocturnal, so vision in lower lighting conditions is more important for their survival."



Studying AMD in mice will require a model that reflects the multifactorial nature of the human disease. Particularly in such cases, not all lines of mice are equal when it comes to trying to replicate a human disease. Which would offer the best chance of success?

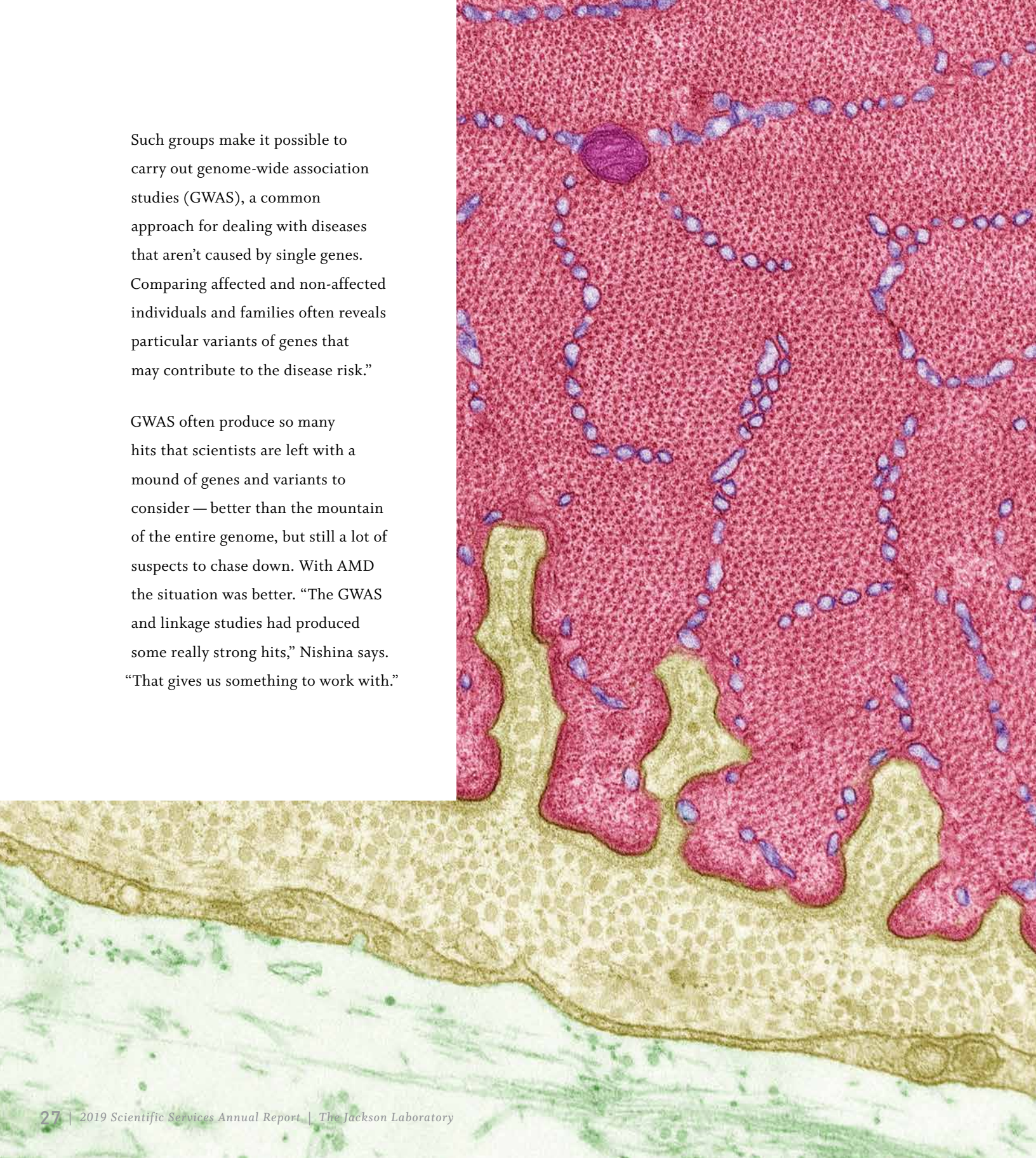
"There's no 'off-the-shelf' answer to such questions," says Grace Stafford, a bioinformatics analyst who works in JAX's Bioinformatics Core service. That team is part of the Center for Precision Genetics. As the name implies, it is a program devoted to unraveling the collusions between individuals' genomes, diseases and other factors that influence health. Stafford and her colleagues are experts at mining the relevant data.

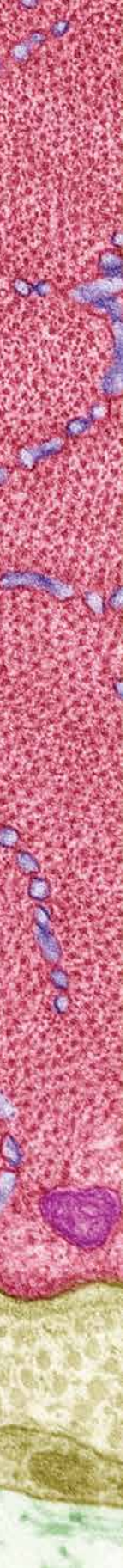
To start the search for an appropriate mouse, Stafford needed any information she could get about the mechanisms responsible for age-related macular degeneration. Despite the differences between the mouse and human eye, the basic ocular structure is very similar, and they are composed of the same types of cells. This made it likely that the fundamental processes disrupted by AMD could be emulated in the mouse, if not the disease itself.

"Here the prevalence of AMD actually helped," Nishina says. "There was a lot of data from studies of large cohorts of patients and their families.

Such groups make it possible to carry out genome-wide association studies (GWAS), a common approach for dealing with diseases that aren't caused by single genes. Comparing affected and non-affected individuals and families often reveals particular variants of genes that may contribute to the disease risk."

GWAS often produce so many hits that scientists are left with a mound of genes and variants to consider — better than the mountain of the entire genome, but still a lot of suspects to chase down. With AMD the situation was better. "The GWAS and linkage studies had produced some really strong hits," Nishina says. "That gives us something to work with."





Armed with specific genes of interest, Stafford began sifting through the data, drawing on resources such as the Gene Expression Omnibus, a database that the National Center for Biotechnology Information of the NIH has been compiling for 20 years. It serves as a clearing house of experiments that have linked the activity of genes to cell types, diseases and other biological contexts.

Stafford was looking for several specific things. First, she investigated the expression patterns of genes from the GWAS in retinal tissues — did the molecules behave differently in AMD patients than in their healthy counterparts? "The problem was, there wasn't very good agreement between different experiments," she said. "But we did find 22 genes where the results were reasonably consistent."

Armed with this list, Stafford turned to expression data on brain tissue that had been made available by the Model Alzheimer Center. She could also draw on a group of relatively new strains that JAX has been developing, explicitly for the creation of new disease models. "We were searching for strains in which retinal cells express those genes at levels similar to those of humans," she says.

The Collaborative Cross project was launched in 2011 by JAX Professor Gary Churchill, Ph.D., and his lab, and about a dozen partners from the U.S. and abroad. Its aim was to expand on highly inbred strains whose standardization played such a crucial role in 20th century biomedicine. In the era before high-throughput sequencing, inbreeding played a crucial role in eliminating genetic variability so that findings from one group could be replicated in another lab's mice. That came at a cost, making it difficult to understand how diversity within an individual and a species influenced disease. Collaborative crosses took eight highly standardized strains and bred them with each other, hoping to create a broader palette of mice with a much more extensive genetic diversity — closer to that of human beings. It produced over a hundred new founder strains whose features have been extensively studied.

Among these strains, Grace Stafford identified three that were the best matches for the gene expression levels observed in AMD. This put the ball back in Nishina's court, to take steps involving more JAX Services.

"What we're now doing is using CRISPR/Cas9 gene editing to introduce human forms of AMD-relevant genes into these strains," Nishina says. "It's the best approach we have at the moment to develop a model for a disease that we have yet to understand or find a treatment for. Once we do that, we'll have a unique model that we'll make available to the scientific community. That's a powerful part of JAX philosophy: to make new tools and offer to them to the world."

The mice that the Genetic Engineering Technology Service and Patsy Nishina's group are developing are great examples of how profoundly new methods and technologies have changed biomedical research. Current efforts to model human diseases in the mouse benefit from exquisite technologies that can manipulate animal genomes in precise ways. The CRISPR/Cas9 system is one of the latest, based on a profound understanding of the processes by which cells repair their DNA. It combines bacterial molecules with custom-designed RNAs and gives scientists a method to change precise genomic sequences. It's not yet perfect – there are a few bugs yet to work out. But CRISPR is rapidly expanding models of human disease in mice and a wide range of other species.

Such advances have moved genetic science far beyond the "wait and see" approach that dominated genetics through most of the last century. Originally the main methods of creating models were inbreeding, which tend to cluster disease-causing mutations in offspring, and methods such as radiation that introduced random mutations. Some animals developed conditions that resembled human diseases and became mainstays of biomedical research.

But even models that have been used for decades continue to yield surprises when they are examined more closely. One of the tasks of the Center for Biometric Analysis (CBA), overseen by Director Jacqui White, Ph.D., is to give scientists a deeper look at animals they develop or, in some cases, have been studying for years.

"In the past, in most places, phenotyping has been carried out in a pretty spotty, haphazard way," White says. "One of our missions is to systematize it and scale it up."

Jacqui White is a relative newcomer to JAX. She was recruited three years ago from the prestigious Wellcome Sanger Institute in Cambridgeshire, Great Britain, and now heads one of the best-equipped facilities for mouse phenotyping anywhere in the world.

She says the CBA group is divided into sub-cores that offer distinct types of phenotyping: examining mouse metabolism, behavior and physiology, and carrying out live imaging of the animals. They also offer pharmacological testing to study, for example, how animals react to compounds that have been put forward as potential drugs.

Generally, the animals examined by the CBA come from JAX scientific groups. A lab might knockout a gene in a mouse and discover that the animal develops a condition like obesity, for example. White and her team will carry out an exhaustive analysis of the animal's biology and behavior to help to determine why.

This deep phenotyping into JAX-derived animals is gene and group-centric, Whtie says, and accounts for most of the CBA's work. But the sub-cores are also participating in large-scale international efforts that work the other way around. In a collaborative effort carried out by JAX, the Sanger Institute and labs across the world, scientists are systematically knocking out mouse genes one by one and phenotyping them through highly standardized methods. The aim is to create a comprehensive collection of knowledge on the functions of mouse genes.

The latest release of data, published in June 2019, reported that the project had phenotyped 5,861 genes in 6,255 mutant lines of mice, each of which was represented by a number of animals. JAX alone had investigated 35,252 individual mice — more than any of the 10 other centers — in 1,226 lines and their healthy counterparts.

The site allows scientists to quickly scan for connections between genes, diseases and other phenotypes they are interested in. For instance, a group studying hearing loss can find up-to-date data from studies such as a recent large-scale screen carried out by members of the consortium. The partners examined 3,006 strains of knockout mice, with highly standardized tests that used several methods and examined five frequencies of sound. In a 2017 article in Nature Communication they reported their findings: 67 genes were associated with hearing loss, 52 of which had been found for the first time in this screen.

Most of this work is being carried out in the traditional highly inbred mouse strains, most of which were originally developed at JAX and are now used by labs throughout the world. But White is also contributing to a project to bring fresh blood into those strains.

"One approach to this was through the Collaborative Cross project, by combining existing lines; sadly, that hasn't produced as many new useful strains as we'd hoped," White says. "In response, Beth Dumont started an initiative to bring in new strains that had recently been acquired from the wild. Once those lines have been bred enough to be stable, we'll start crossing them into the traditional strains."

At present the scientists are working with seven strains derived from wild populations. They believe this process will introduce thousands of new variants of mouse genes that have never been studied in the lab. The animals should also provide better models for humans and their diseases; crossing new strains will alleviate some of the effects of extreme interbreeding and produce lines with more genomic diversity.

Most of this work is being carried out in the traditional highly inbred mouse strains, most of which were originally developed at JAX and are now used by labs throughout the world.

The facility and scientists continually work together to design new methods of analyzing animals. Recently Jacqui White and her colleagues have been helping JAX Assistant Professor Vivek Kumar, Ph.D., develop new ways of studying behavioral abnormalities including addiction, attention deficit hyperactivity disorders and depression. The Kumar Lab is also interested in sleep behavior, and there's a connection: sleep disruptions have been associated with abnormal social interactions and substance abuse.

"As a postdoc I studied circadian behavior and biology, and started working on methods that could be extended to other behavioral domains such as addiction," Kumar says. "In trying to dissect the genetics of the day-night cycle of mice, what we do is turn off the lights and keep the animals in darkness for 30 days. We monitor their wheel-running behavior — they are nocturnal, so they run at night. The cycle is very sensitive, and finding strains that adjusted to days that were half an hour shorter or longer was a key to identifying circadian genes."

It was interesting, Kumar remarked, that most behavioral studies were carried out during the day, simply because that's when scientists were around to do them. That meant they were performed under unusual circumstances, which included handling or other unnatural interactions. Sleep studies, for example, often isolated individual animals, whereas mice typically sleep in groups. He started to consider ways of studying mouse behavior under conditions more typical for the animals.

"Our model for this work is that an animal's genetics affect its brain circuitry, which lead to behavior," Kumar says. "This means that genetic changes trigger behavioral abnormalities through those circuits."

His lab is taking on a theme of huge social concern: substance abuse. "Every time a mouse is administered cocaine, you see an alteration of its neural circuits," he says. "The type of change depends on its genetics. Well, the effects are part of the 'reward' circuitry developed in the brain through evolution. Those circuits weren't designed for cocaine, they really evolved for other purposes. To understand what's going on in addiction, we need to go back and determine what those original functions were, and how nature manipulates them."

Like several other JAX scientists working on addiction, Kumar has not hesitated to engage the public. He has delivered a TEDx talk, given other public presentations, and serves on the board of directors at a nonprofit facility for addiction treatment and family counseling on Mount Desert Island.

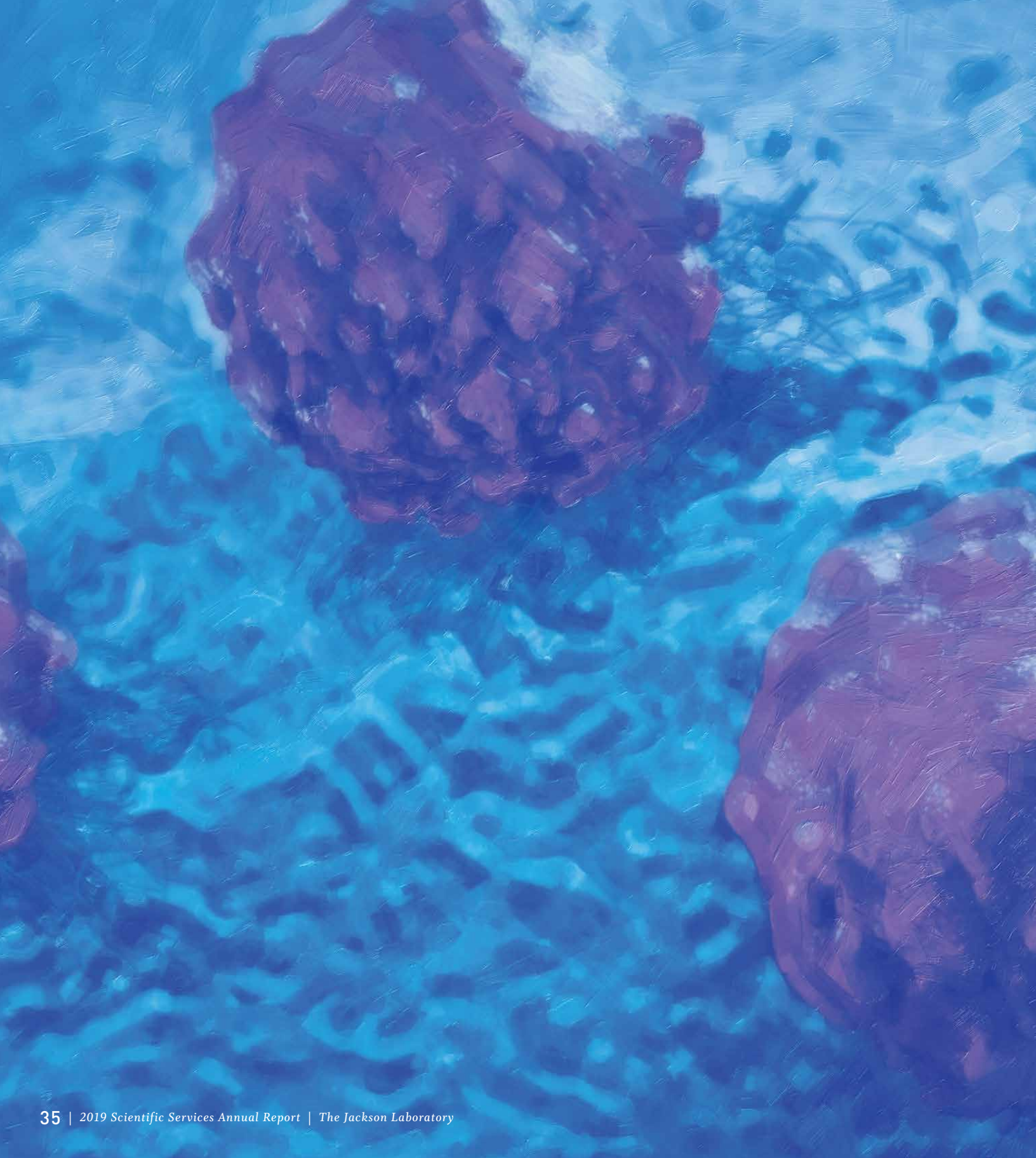
"My main message is that addiction is a medical condition," he says. "It's important to move

away from the huge social stigma attached to it. We as scientists can play a role in that process by sharing what we are learning about the neuroscience behind this terrible disease."

In June 2019, his lab received a half-million dollar grant from the National Institute of Drug Abuse to develop new technology that will automate studies of sleeping groups of mice. Films of sleeping animals will be made, then analyzed using neural networks and machine learning to score the behavior.

Once established, Jacqui White says the methods can be extended by the phenotyping center and other institutes to the analysis of pain responses and other types of behavior. "When studying pain-related behavior, some of an animal's movements are too fast to observe, or hidden from view. So, filming the mice and automating the image analysis is essential."

That type of work is very computationally intensive, Kumar says, requiring sophisticated graphical processing. Setting up a system that could continually monitor mice and score their behavior took two to three years. For the last year and a half, the group and the CBA have been collecting data. "We've now filmed almost 5,000 individual mice from about 150 strains — an hour with and an hour without cocaine," he says. "That would have been almost impossible anywhere but at JAX."



chapter 3

Sequencing cancer

Just as there is no single human genome, there is no single individual genome. A comparison of the complete DNA sequence of various cells throughout an individual's body would reveal multiple differences: from alterations of single bases to more dramatic changes that affect entire regions of the genome.

JAX CANCER CENTER STORY

Rearrangements of sequences play a normal, crucial role in the immune system; cutting out and reassembling sections of DNA is how cells generate the antibodies and receptors needed to fight off foreign molecules. Defects in that process may cause cancers called lymphomas, and disruptions lead to malignancies in other types of cells as well. Somatic mutations are changes that occur over a person's lifetime and cause genes to be damaged, lost, duplicated or shuffled to new places in chromosomes. Some of these disruptions can be repaired; those that can't may cause disease.

"Such changes in the structure of the genome are a particular hallmark of cancer," says JAX Professor Chia-Lin Wei, Ph.D. "Tumors usually begin with one or more mutations that disrupt the controls on cell replication and other essential processes. As generation after generation of new cells are created, they accumulate an increasing number of defects."

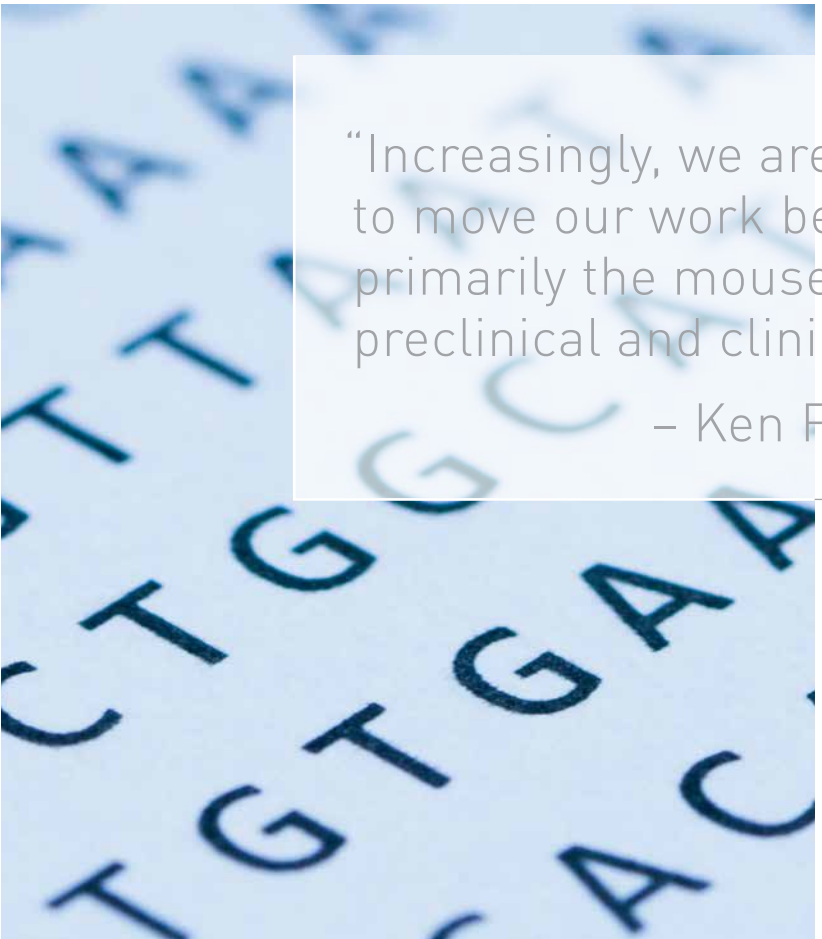
Tracking the types of changes that occur would be useful. For a cancer to survive, it must overcome intricate systems of regulation that normally recognize problems and destroy defective cells. Specific defenses have to be evaded or overpowered, and understanding how the cells achieve this is a key to understanding the origins of cancer, its progression and how many tumors circumvent therapies. Those processes can best be explored by probing the range of diversity of the cells that make up tumors and metastases. This requires advanced DNA sequencing methods and data analytic approaches, which have already yielded important insights into tumor biology. But there are still limitations, and they have hindered scientists' efforts to obtain a truly "high-resolution," letter-by-letter reading of the complete cancer genomes within a single patient.

This is one of the themes being taken on at The Jackson Laboratory for Genomic Medicine, established in Farmington, Connecticut, in 2014. "Increasingly, we are trying to move our work beyond primarily the mouse and into the preclinical and clinical context," says JAX Senior Vice President for Research, Ken Fasman, Ph.D. "There is no medical school in the Laboratory's home state of Maine. Developing the location in Farmington creates new opportunities for networking with hospitals, clinics, the University of Connecticut, and well-developed biotech and pharmaceutical partners."

These interactions are enhanced through a number of joint appointments held by JAX staff. Wei is the Director of Genome Technologies at The Jackson Laboratory. For years she has been working at the forefront of advancing sequencing technologies and projects which apply them to complex, fascinating biological

questions — ranging from the deep evolutionary ancestry of plants, to chromatin conformations in neuronal development, to the "microbiomes" of bacteria, fungi and viruses associated with brain diseases, to fundamental questions about tumors.

"To really understand the types of changes that occur during cancer, we need to obtain a very high-resolution view of the comprehensive genetic makeup of the cancer cells," she says. "And that means overcoming a major limitation of classical sequencing methods – which provide massive, high accuracy DNA sequences, but usually only for less than 300 bases at a time."



"Increasingly, we are trying to move our work beyond primarily the mouse and into the preclinical and clinical context."

– Ken Fasman, Ph.D.

DNA has often been described using the metaphor of a text — which captures many fundamental aspects of the basic structure of a genome. The DNA in our cells is arranged in a linear string of nucleotides, or bases, wound up and packed into 23 pairs of chromosomes. Mutations and other events alter the code by deleting, adding or rearranging single bases or entire segments, sometimes cutting them from one region and inserting them in another, sometimes plugging them in backwards, sometimes adding extra copies.

One huge challenge in reading an entire genome is that sequencing methods can only read short stretches of the code with very good accuracy. Most of these techniques depend on enzymes that synthesize clonally amplified DNA by incorporating each of the four fluorescent labeled nucleotides supplied in specific order, and followed by imaging them with high-resolution microcopy.

The process is like chopping up hundreds of copies of this text into random fragments, each containing just a few words or random segments of sentences. Reconstructing the whole requires identifying overlapping bits in different fragments and then building outward to assemble the whole text. That's particularly complicated for the text of the genetic code, which only contains four chemical letters, especially when lots of regions consist of small, repetitive patterns. Assigning such segments to the right place can be a computational nightmare, which would be greatly simplified if you could obtain much longer reads. That would increase the chances of placing fragments in the right positions. To use another metaphor: imagine trying to assemble a huge, colorful puzzle bearing an abstract image — the smaller the pieces, the longer it will take.

Assembling all the pieces became a huge computational task that was handled by Ankit Malhotra, Ph.D., and his colleagues. He had participated in many other major sequencing efforts, including the 1000 Genomes Project — work built upon the single "consensus sequence" decoded around the turn of the century to decipher the genomes of 1,000 individual healthy humans, giving scientists their first glimpse of the true genetic diversity of our species. The project revealed that individual sequences diverge far more than nearly anyone would have predicted.

Expanding that work to cases like the diversity of tumor cells within a single patient poses new challenges that would be best solved, again, by obtaining longer reads. "Some methods manage this; unfortunately, they tend to be less accurate," Wei says. "On the other hand, while in the past shorter reads have been more accurate, it has been harder to reproduce the way they are assembled into whole genome sequences. That's a methodological trade-off for which, at the moment, there's no perfect solution."

Progress is being made, though, through emerging technologies. Among many promising methods, she adopted nanopore sequencing. This is a new approach to reading sequences. Strands of DNA are directed toward pores in a surface, then threaded through. The pores are generally protein channels obtained from bacteria. In some nanopore platforms, proteins are used to slow down the DNA as it moves through the pore. That's important because of the way the method reads the sequence.

As DNA is threaded through, the sequencer measures the electrophysiological properties of the channel. Each of the four nucleotides that make up DNA has a unique charge, so it changes the properties of the pore. The electrical readout gives the sequence, a bit like watching a ticker tape, or a text scroll across a screen.

Wei says the method has the potential to deliver reads that are thousands times longer than the short segments that can be decoded by more standard technologies. "There's still a lot of room for improvement," she says. "Bringing it in to the laboratory and applying it to interesting questions gives us an opportunity to develop it and add our own innovations. That is becoming a major focus."

"We have established a group that is constantly looking at emerging technologies that will be useful for the Laboratory," says Alan Sawyer, director of Scientific Services. "And we have several funding structures that permit us to acquire them. DNA sequencing is fundamental to virtually every type of research. Nanopore technology has become robust enough that when Chia-Lin Wei proposed we add it to the Genomic Services, it seemed like a hugely important move to make."

With the technology in hand, postdoc Liang Gong, Ph.D., and a talented computational scientist Chee Hong Wong, M.S., in the Wei Lab began a systematic investigation of the structure of cancer genomes. Their study provided the much-needed concrete evidence of the superior performance that nanopore long-read sequencing offers over current short-read sequencing for the comprehensive detection of genomic structural variants, and does so in the biomedically relevant context of a breast cancer cell genome.

"We really didn't know what to expect from this high-resolution view," Wei said. "Cancer amplifies every problem related to genome assembly. You not only have to deal with the specific features of an individual, but also variations between different lineages of cells that — by nature — scramble up that unique genome in all kinds of ways."

Even with longer reads, a huge computational effort was required to pinpoint the positions of sequences in the context of the individual genomes. The scientists developed a structural variation detection pipeline called "Picky" that was designed to detect and classify the full spectrum of structural variants in long-read data. Like many of the innovative methods and computational tools developed here, Picky has proven useful beyond JAX. The scientists have provided it for free, and it is now being used across the world to analyze sequence data obtained through nanopore and other types of experiments.

The study yielded several new insights into the enormous number of changes that had occurred in the breast cancer cell lines. "In a single line we found over 30,000," Chia-Lin says. They were of all types: sequences had been deleted, inserted, duplicated, inverted, translocated…"

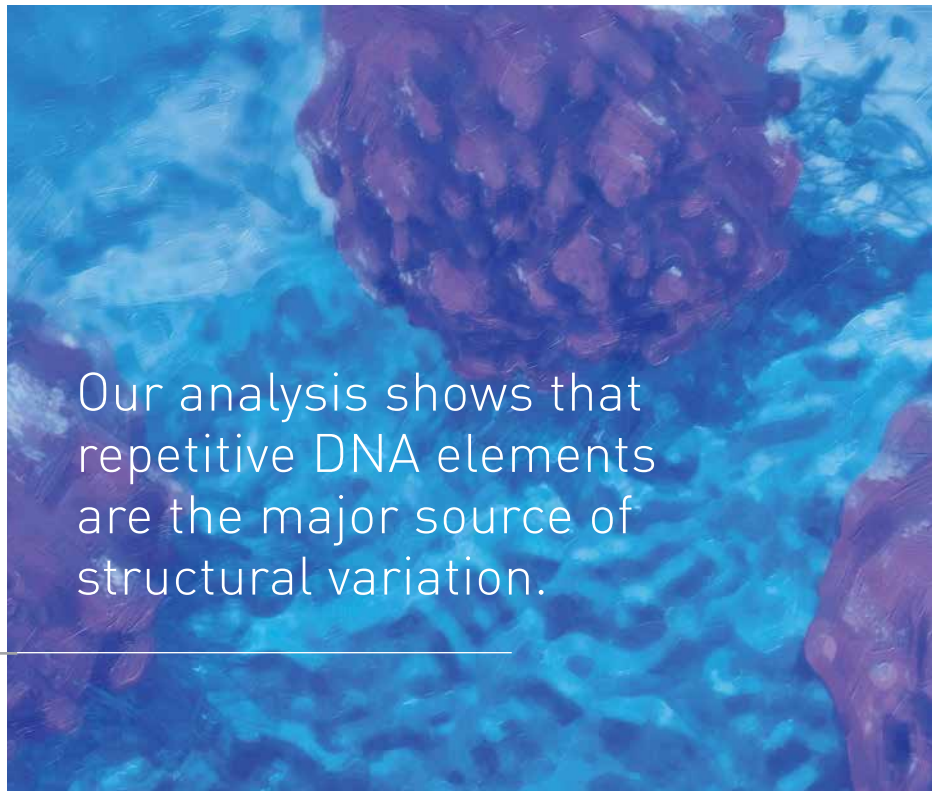
A large proportion of them had not been detected in earlier studies of cancer genomes, based on methods that produced shorter reads. "A huge number of them occur in regions containing short, repetitive sequences," Wei says. "our analysis shows that repetitive DNA elements are the major source of structural variation. That makes sense because those are precisely the regions of the genome that are hardest to assemble. You would predict that such changes would be missed, but until now we had no idea just how many that might involve."

Another type of change that had previously been hard to detect was extremely common: microinsertions involving just a few nucleotides. Their locations were particularly interesting. They are very common at highly active sites in the genome, involved in the regulation of genes. Such sites are also susceptible to

disruptions called breakpoints — regions where sequences have been removed or inserted from other regions in the genome.

These regions are crucial to health because they determine when and how genes are activated to produce the molecules a cell needs. During most of a cell's lifetime, its DNA spreads through the nucleus in loose strands that come together and drift apart. Genes are drawn into loops with other strands, which provide docking sites for molecules involved in transcribing sequences into RNAs. For this to happen, those regions need to be accessible — rather than tightly packed into a form that prevents their transcription. It may be this accessibility, the scientists believe, that makes such regions susceptible to mutations.

The types of alterations discovered by the project may turn out to be extremely important in diagnosing cancer or understanding its progression in patients. "It's important to have shown that structural variants tend to affect crucial, regulatory regions of our DNA," Wei explains, "and that so many are small, and hiding in highly repetitive regions of the sequence. All of these changes can affect the health of a cell, and a huge proportion of the patterns we have seen could only have been achieved with this extremely detailed view of cancer genomes. We'll surely find more as we learn to expand the methods to even longer sequences with higher accuracy."



The diversity of cells within a tumor is a theme that has also occupied one of Chia-Lin Wei's colleagues on the Farmington campus, Jeff Chuang, Ph.D., head of a computational group whose central focus is likewise cancer.

"The heterogeneity of a patient's cancer cells is thought to be related to the success of treatments and overall outcomes," Chuang says. "A higher degree of diversity is probably related to metastases and resistance to therapies. The more types of cells in a tumor, the better the chances are that some of them will have characteristics that help them evade a treatment, or escape a solid tumor and invade a new tissue. The question is how you assess that diversity — whether current approaches are giving an accurate picture."

Researchers have developed several algorithms to detect mutations in cancer sequence data. In 2018, Javad Noorbakhsh, Ph.D., and other members of Chuang's team tested them against each other. They applied four mutation-predicting tools to a set of 4,722 samples from the Cancer Genome Atlas, a project run by the National Cancer Institute. The methods produced different results, and the scientists found that a common practice for scoring mutations was skewed by the number of copies of a gene found in cancer cells. "It turns out that these copy numbers are more relevant to the clinical outcomes for patients than the score itself," Chuang says.

This is only one of the group's many projects to link sequence data to cancer. "What particular

mutations, gene expression patterns and other features of cells influence the survival of a tumor?" asks Chuang. "What characteristics of specific forms of cancer are associated with better or worse patient outcomes? What determines whether a patient's cancer can be successfully transferred to a mouse, where we can ask new questions and try out treatments? What features of tumor cells permit them to metastasize to new tissues, and how do they evolve resistance to therapies?"

In some cases, the information needed to answer such fundamental questions about the disease may already exist, in massive amounts of data that have been collected in resources such as the Cancer Genome Atlas and studies based on them. A number of "biobanks" have been established based on material from tens of thousands of tumors, healthy tissues and specimens from patients' relatives that have been collected by hospitals and clinics over the years.

Whether that data becomes meaningful depends on researchers' ability to pose the right questions and translate them into algorithms. But the sheer quantity of information has been an obstacle. "A single study may involve hundreds of terabytes of data," Chuang says, "amounts that simply can't be downloaded. And processing it — even on an immense computer farm — would take months and months."

Biology isn't the only discipline facing such a data deluge, and recent years have seen

the development of solutions based on cloud computing. These efforts use vast arrays of computers networked through the Internet to handle and run algorithms on huge datasets.

Preeti Bais, Ph.D., a computational scientist in Chuang's group and part of the Computational Sciences Scientific Service, had been confronting the problem in the algorithms she was writing related to cancer immunology. "The cloud project actually began with the arrival of a high school student," Bais says. "JAX runs a program with the Farmington schools, where pupils come and get involved with some of our projects."

A couple of years ago a student came along named Sherry Jingyu Zhang, who was highly motivated to do programming. At the end of the short-term project with the school, she asked about the possibility of a longer-term internship. Chuang and Bais decided to put her to work on a new, cloud-related project. The work was funded by a grant from the National Institutes of Health (NIH).

Over the course of a few months Bais and Zhang wrote an algorithm for the "Seven Bridges" Cancer Genomics Cloud of the NCI, set up by Amazon. The task was based on a project from Chuang's group: to scan sequences of the tissues from cancer patients for signs that their immune systems were trying to combat tumors.

"Cancer cells produce new, mutant forms of proteins that are unfamiliar to the body and which the immune system can potentially recognize," Bais says. "If that happens, immune cells produce antigens against the tumor. Theoretically you can read the sequences of those molecules in the cells' genomes — the trick is to pick them out against the background of all the other antigens a person's body is producing."

Detecting tumor-specific antigens requires culling through the output generated by highly sensitive, next-generation sequencing experiments — that's where the massive amounts of data and computational challenge come in.

"Writing the algorithm itself wasn't the biggest challenge," Bais says. "We managed it in a couple of months. But we were beginners at cloud-based computing, which meant we had to learn how to integrate the data from all different kinds of platforms and then figure out how to run it."

The initial analysis involved a few dozen patient tumors and was so successful that the scientists immediately began getting requests from other institutes. As one of the first cloud computing projects funded by the NIH, it was regarded as a trailblazer.

"The ecosystem that JAX has built has been so vital," Chuang says. "The Laboratory's resource and technology investments have been a soil for my lab's ideas to germinate and flourish. We couldn't have developed our concepts for scientific cloud computing without the prescient investments JAX has made in the Computational Sciences, IT, high-performance computing and expertise in the form of scientific engineers."

chapter 4

Sometimes in science, it's personal.

A dialogue between diabetes and the immune system: from Neanderthals to the microbiome.

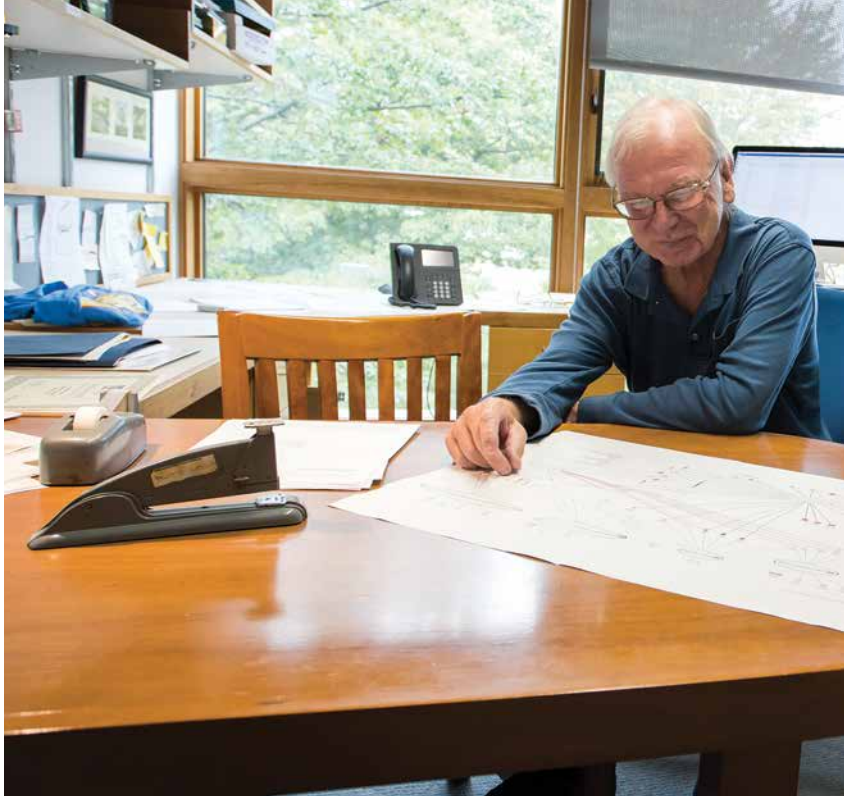
"I come from a family that's been hit by diabetes," says JAX Professor Dave Serreze, Ph.D. "In the current generation, I have a nephew with the disease. Yes, that was certainly something that motivated me to follow a particular path in research."

He's talking specifically about his work on Type 1 diabetes, also known as juvenile diabetes, due to its tendency to strike hard at an early age. In this condition, a person's body loses the cells in the pancreas that produce insulin. Without this essential hormone, cells are unable to absorb the glucose and other carbohydrates they need as nutrients. Blood sugar levels skyrocket, and without treatment the body wastes away. Since the 1920s the solution has been to administer insulin; prior to that, the disease was nearly always fatal at an early age. Type 2 usually develops later, and differently: the body still produces insulin, but cells become insensitive to it. If caught in its early stages, it can usually be treated through a regimen of diet and exercise.

Both forms run in families. Previous work by Serreze's group, and others, reveals a powerful hereditary component to Type 1. However, the disease has been called a geneticist's nightmare. "Studies have linked 60 to 70 regions of human chromosomes and probably over 100 genes to this form of diabetes," he says. "In most cases we're talking about normal variants of genes commonly found in the population – it's only in combination that they reveal their dark side."

One of the most important models for research into the disease has been the non-obese diabetic (NOD) mouse, a strain developed by a group in Japan and first reported in 1980. Coincidentally, that's the year George Snell, Ph.D., of The Jackson Laboratory was awarded a Nobel Prize for fundamental discoveries concerning the human immune system.

Dave Serreze, whose association with JAX goes back to the early 1980s, calls Snell an important mentor. His own research has straddled the interface between diabetes and immunity. A key accomplishment of his lab has been to expose the genetic basis for a complex interplay of immune cells in the origins of Type 1 diabetes.



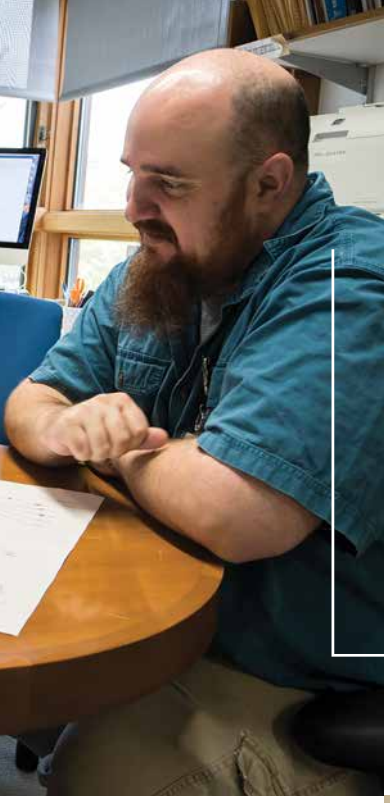
Data from 2014 placed the world-wide incidence of Type 1 at around 30 million. To find so many cases of a fatal, hereditary disease that strikes the young seems like an evolutionary conundrum, unless the genes that contribute to it might somehow be helpful in some way, Serreze notes. That's the case with sickle-cell anemia, another dangerous disease that partially protects its victims against malaria.

Understanding how this might be the case for diabetes requires a close look at the biology of Type 1 diabetes. It has long been recognized as an autoimmune disorder, in which the beta cells in the pancreas that produce insulin are destroyed by the immune system.

"In the NOD mouse model and very likely humans, this is accomplished by T cells, which normally target foreign cells and pathogens," Serreze says. "In diabetes Type 1 they mistakenly recognize molecules on beta cells and wipe out this crucial population of pancreatic cells."

In 2016, working with the NOD mice, his lab discovered that this process has an additional step. Dave's team and others had known for years that another component of the immune system, B cells, played some role in diabetes. A lack of these cells in mice offered nearly complete protection from the disease. It has taken years to work out the details of why this happens. The group found that certain B cells expressed specific antibody molecules on their surface that are capable of binding proteins from beta cells termed antigens. The B cells take these antibody-bound proteins up and process them into fragments that are subsequently presented to autoreactive T cells, which are activated and begin to kill beta cells.

What specific molecule was triggering this response? The best way to answer this question was to extract B cells from pancreatic tissue and study the antigens they were reacting to. Members of the Serreze Lab extracted tissue from the pancreas; the next step was to isolate the immune cells. This was accomplished using a method called flow cytometry.



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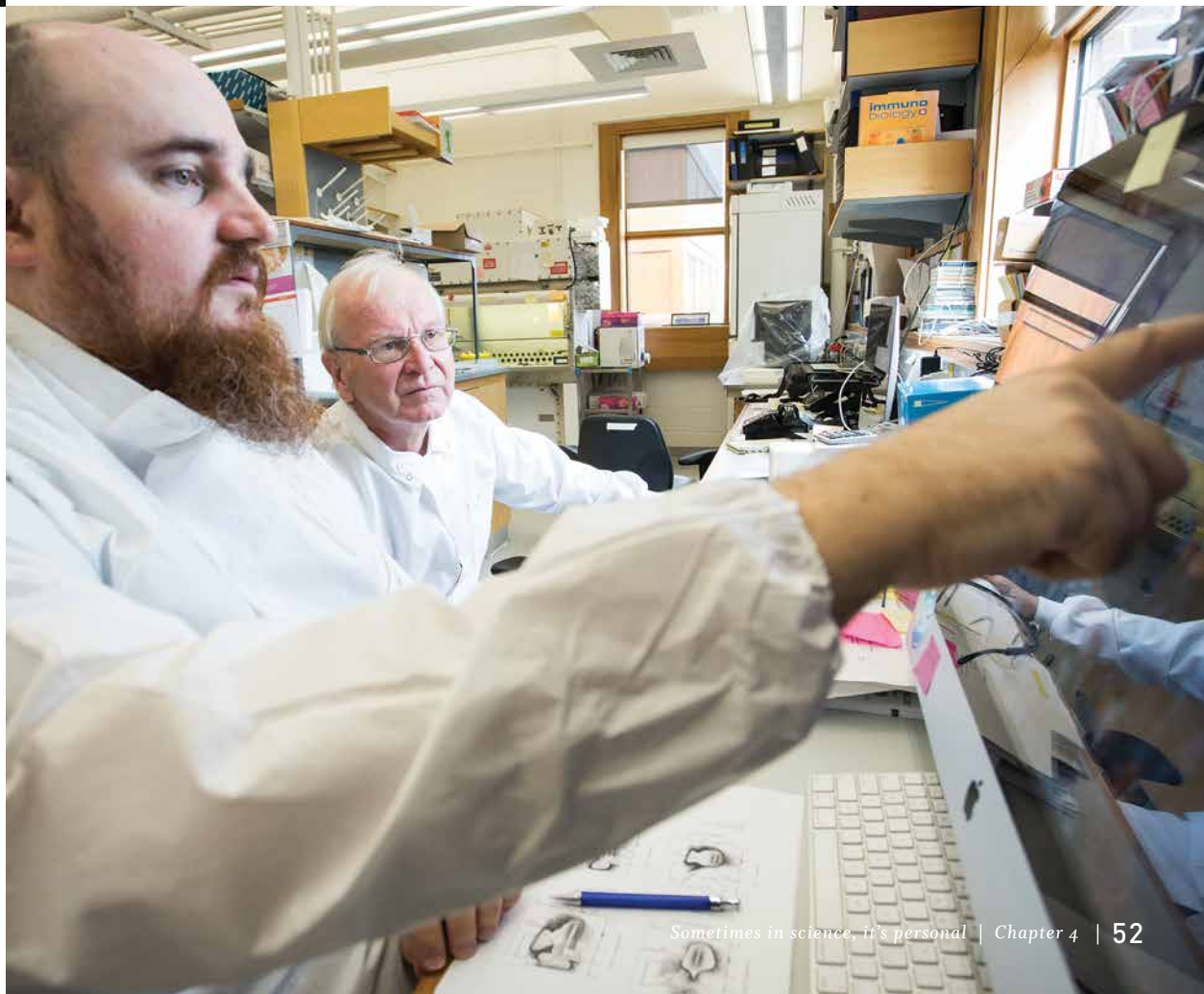


Will Schott has been Manager of JAX's Flow Cytometry Service for five years — a position he attained after almost a dozen years of working with the technology. Prior to that, he worked in the laboratory of Affiliated Scientist Edward Leiter, Ph.D. — on diabetes. Leiter was originally responsible for introducing the NOD mouse to The Jackson Laboratory, and over a long career he generated other mouse models for diabetes. Dave Serreze had been a student in his lab.

Flow cytometry has been essential in unravelling the connections between diabetes and immunity. "One of our major uses of these instruments is to detect specific cells and sort them by types," Will says. "In this case the job was to find precise populations of B cells, labeled with fluorescent antibodies."

The technology is based on passing single cells in a very fine stream through a laser beam; the fluorescent markers give them distinct optical

properties which permit them to be separated. It's another example of a platform that has seen amazing technical advances over the past few years. Today's machines can measure dozens of features of cells simultaneously, and are so sensitive that they can isolate a single unique cell in a population of hundreds of thousands. Combined with other technological advances, these improvements are finally giving scientists the capability of peering into the biology of single cells — even very rare ones.

Flow cytometry has played a crucial role in probing the biology of diabetes. In the 2016 study by the Serreze Lab, it was essential in identifying an antigen on beta cells that B cells were responding to. "It wasn't what we expected — not one of the most likely suspects," Dave says. "It turned out that these cells strongly recognized a protein called peripherin, which is normally expressed by neurons, including those in the brain."

The situation was puzzling because antibodies against peripherin are sometimes found in the bloodstream of healthy humans and mice. "This made us wonder whether it was really this molecule that the B cells were responding to," Dave says. To find out, the scientists isolated a DNA sequence in B cells that infiltrated the pancreas — the code for the antibody that allowed the cells to bind peripherin. They built this sequence into the genome of another strain of NOD mice, with the help of the JAX Genetic Engineering Technologies Service.

Observations of this strain revealed that their B cells invaded the pancreas at a higher rate and triggered a quicker onset of Type 1 diabetes. Once again, flow cytometry was brought into the picture, this time to compare the B cells of normal NOD mice with those bearing the peripherin antibody. They found significant differences in the way the cells developed in the two lines.

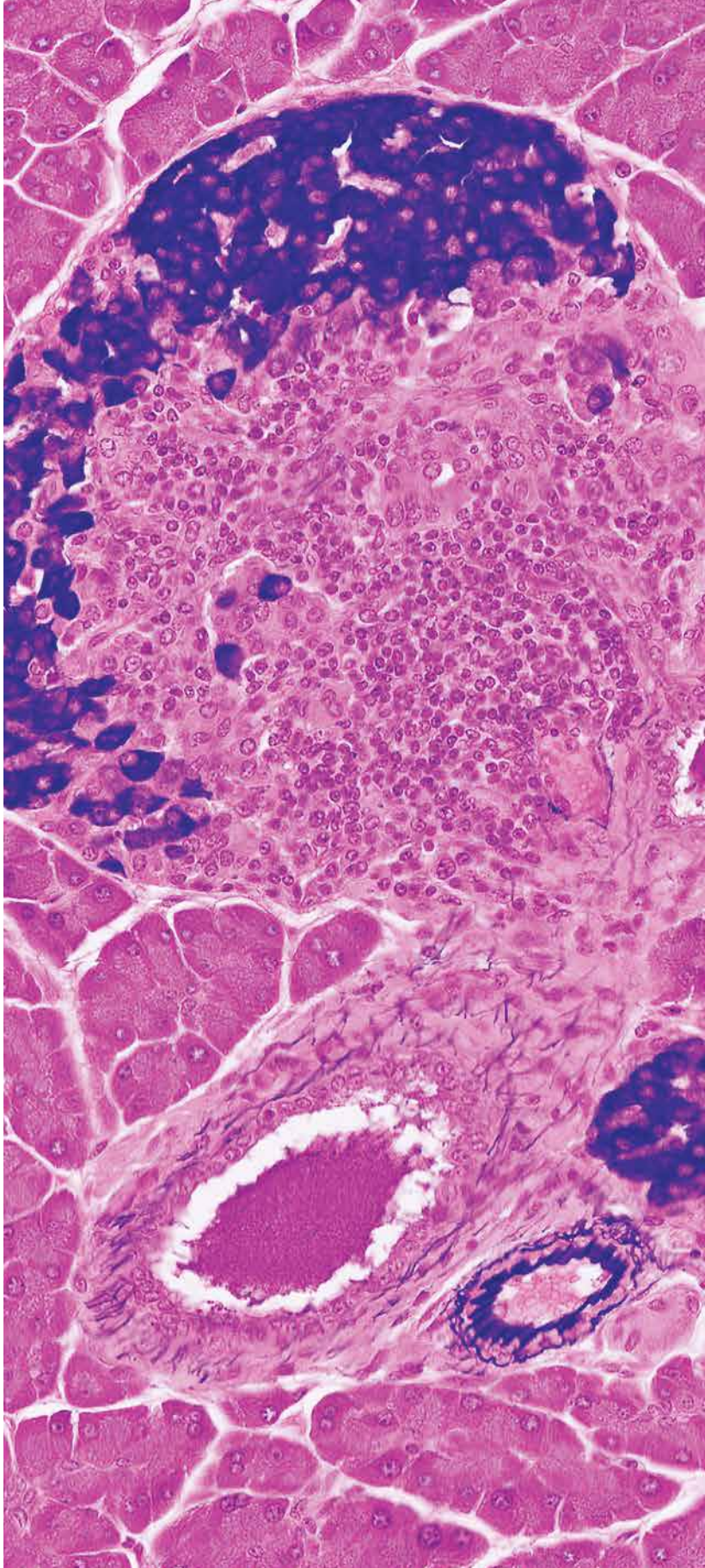
"The comparison showed that B cells bearing the peripherin-autoreactive antibody were undergoing changes indicating they were being activated — as normally happens to B cells when they recognize a foreign antigen," Serreze says. "This is the step at which they can stimulate autoreactive T cells to become activated and launch the attack on pancreatic beta cells, and the result is Type 1 diabetes."

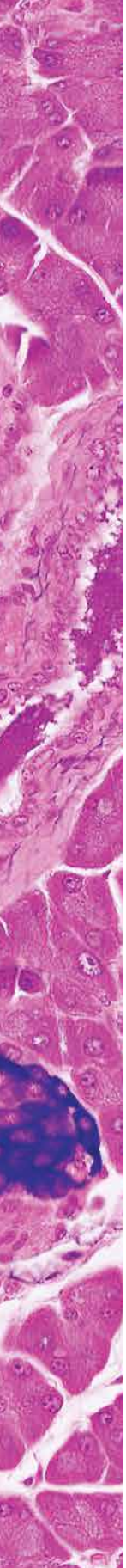
So not only had the lab confirmed that B cells played an important role in the disease — they had pinpointed a part of the mechanism by which they were doing so. At least in these mice, one component of the autoimmune reaction in Type 1 diabetes was a response to peripherin.

A look at the global distribution and history of diabetes may provide more insights into the disease. Worldwide, the highest rates of Type 1 are found in Finland, where its incidence is about 400 times that of China, whose rates are among the lowest. Not only is the disease more prevalent, but the rate of new cases is increasing.

"Just step across the border into a former western region of Russia called Karelia [currently divided among the northwestern Russian Federation and Finland] and you find a population that is very closely related, from a genetic standpoint," Serreze says. "But there the incidence is about half. For this reason, it is very unlikely that genetics can explain the difference in disease frequency." Which means that an answer has to be sought elsewhere.

A potential culprit, he thinks, might be the microbiome, the rich ecosphere of microorganisms that inhabits our gut, skin and other niches of our bodies. Its composition and how immune systems deal with it depend on environmental, lifestyle and even





geopolitical factors. Certain strains of bacteria appear heavily in one population but not the other, and their presence may alter the body's sensitivity to factors that contribute to autoimmunity.

And recently, insights into both types of diabetes have emerged from a more unusual source, DNA extracted from fossils. Over the past 20 years, the laboratory of Swedish geneticist Svante Pääbo, Ph.D., in Leipzig, Germany has managed to recover DNA and reconstruct genomic sequences from the remains of archaic humans — Neanderthals and Denisovans. Neanderthals inhabited Eurasia from about 400,000 to 40,000 years ago, while closely related Denisovans spread across Asia and toward the south and east. These analyses have clarified many aspects of their relationship to our species and shown that some interbreeding occurred between the groups, meaning that variants of some modern human genes can be traced back to them.

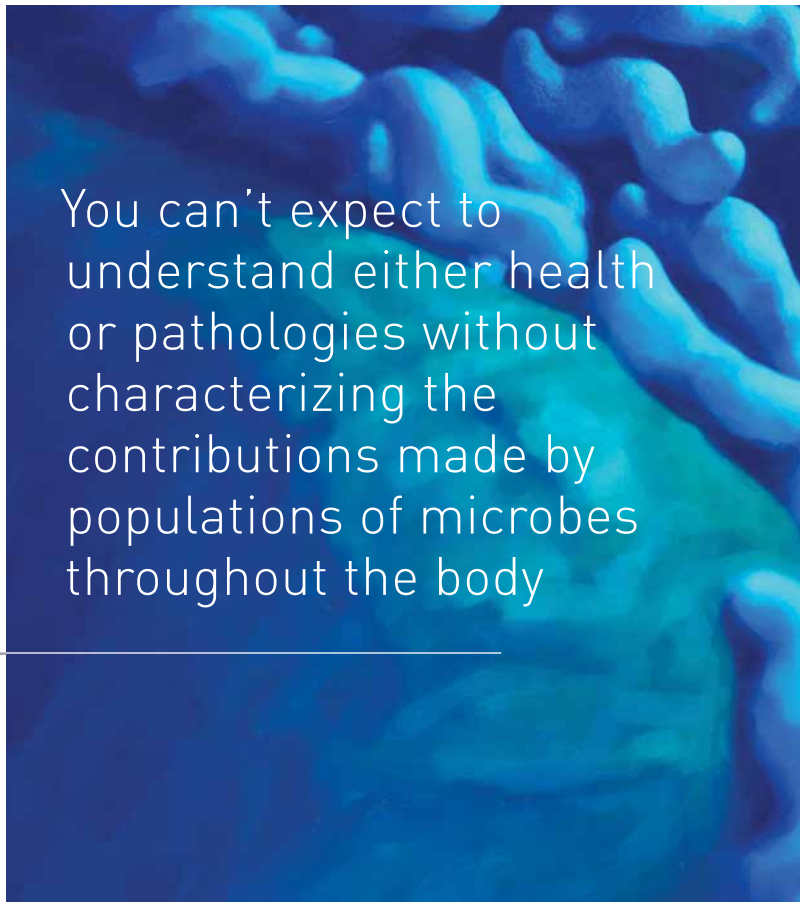
Those include molecules which have immune functions, particularly interesting from an evolutionary point of view. As populations develop variations and gradually diverge into new species, they occupy new environmental niches and encounter new pathogens. That usually places pressure on their immune systems.

Enough samples of archaic human DNA have now been sequenced to draw some conclusions about the evolution of genes which encode major histocompatibility complex (MHC) proteins. These are molecules expressed on the surface of B cells and other cells of the immune system to which antigenic protein fragments become bound for subsequent display to T cells. Particular variants of MHC molecules also play key roles in autoimmune conditions, such as Type 1 diabetes.

Dave Serreze says there is increasing evidence that a particular form of MHC associated with diabetes is a Neanderthal legacy. "On average, about 3% of the DNA of a Northern European is derived from Neanderthals," he says. "But about 50% of the variants of MHC class 1 come from them. To me that suggests some huge, infectious event that placed enormous pressure on that gene — a sort of immunological 'asteroid strike.'"

If so, it's another example of how understanding ancient gene functions can yield crucial insights into the disruptions they cause in the present day.

Other factors that change an individual's risk for disease lay much closer at hand, in the microbial populations that inhabit our bodies. Microbiome research has exploded over the past decade, thanks to the huge advances in the speed and sensitivity of sequencing. The motivation for large-scale studies has been clear: a person's body is host to 10 times as many bacteria than it has cells. They preferentially inhabit niches that are exposed to the environment, such as the gut, the skin and mouth. They play crucial roles in all aspects of our health, says George Weinstock, Ph.D., professor and director of Microbial Genomics at JAX.



The focus of his lab is the other form of diabetes — Type 2 — and particularly the dialogue between the disease and the ecosphere of thousands of microscopic species that share our bodies. "You can't expect to understand either health or pathologies without characterizing the contributions made by populations of microbes throughout the body," Weinstock says. "That's true for humans, and it's equally true for mice."

The controlled environments in which laboratory mice are bred and raised offer scientists an opportunity to manipulate interactions between communities of microbes and their hosts. A number of basic principles are emerging: microbiomes are passed between parents and offspring; "bugs" (what are "bugs"?) in one part of the body are different than those found in another. And bacteria play a significant role in a much wider range of diseases, developmental processes, and lifestyle phenomena such as aging than previously suspected.

One major study Weinstock and his colleagues have been involved in, a collaboration with Stanford University and several international labs, has been devoted to studying

connections between the microbiome in the rise of Type 2 diabetes. Their initial results, published in Nature earlier this year, tracked individuals over four years, including both healthy and prediabetic subjects.

"This project has taken a truly comprehensive look at the biology of 106 individuals, from their genomes to extensive molecular profiling, the infections and inflammations they've experienced, the vaccinations they received, their immune responses, the development of diabetes and how their microbiomes have responded to all of these factors," Weinstock says.

The work has produced a massive data set that is being mined for some intriguing insights into the interactions between hosts and their microbiomes. While there were significant differences between all the individuals, some features did distinguish the healthy group from those with pre-diabetic symptoms. Depending on whether the subject's body responded normally to insulin or was resistant gave different responses to respiratory infections. The immune response in the latter group tended to be weaker, occurred later and was more likely to progress to chronic inflammations.

"In general, we identified significant differences in their pathways and responses that may turn out to be crucial for new diagnoses and treatments that consider the contributions and responses of the microbiome," Weinstock says.

The diverse effects of microbiomes on health mean that they can skew results and need to be taken into account in designing experiments. They can diverge widely even in mice belonging to the same strain, which has been a challenge for labs working at different sites. For example, in 2016 four universities were carrying out preclinical studies in a single strain of mice, on what had looked like a promising therapy for Type 1 diabetes, only to obtain widely divergent results.

This year a study published in *Nature Biotechnology* reported a similar issue in a study of salmonella infections in mice. Scientists at Vanderbilt University and the University of California had obtained animals from four vendors — all belonging to the classic "C57BL/6" strain that Director Clarence Cook Little brought with him when founding The Jackson Laboratory. All of the mice were infected when administered high doses of salmonella bacteria, but only animals obtained directly from JAX were affected by low doses.

The reason turned out to lie in the microbiomes of the mice. The strains from other vendors turned out to be infected with low levels of an endobacterium that offered them some protection against salmonella. Mice from JAX didn't have it, but in some cases acquired the bacterium after being housed with the other animals.

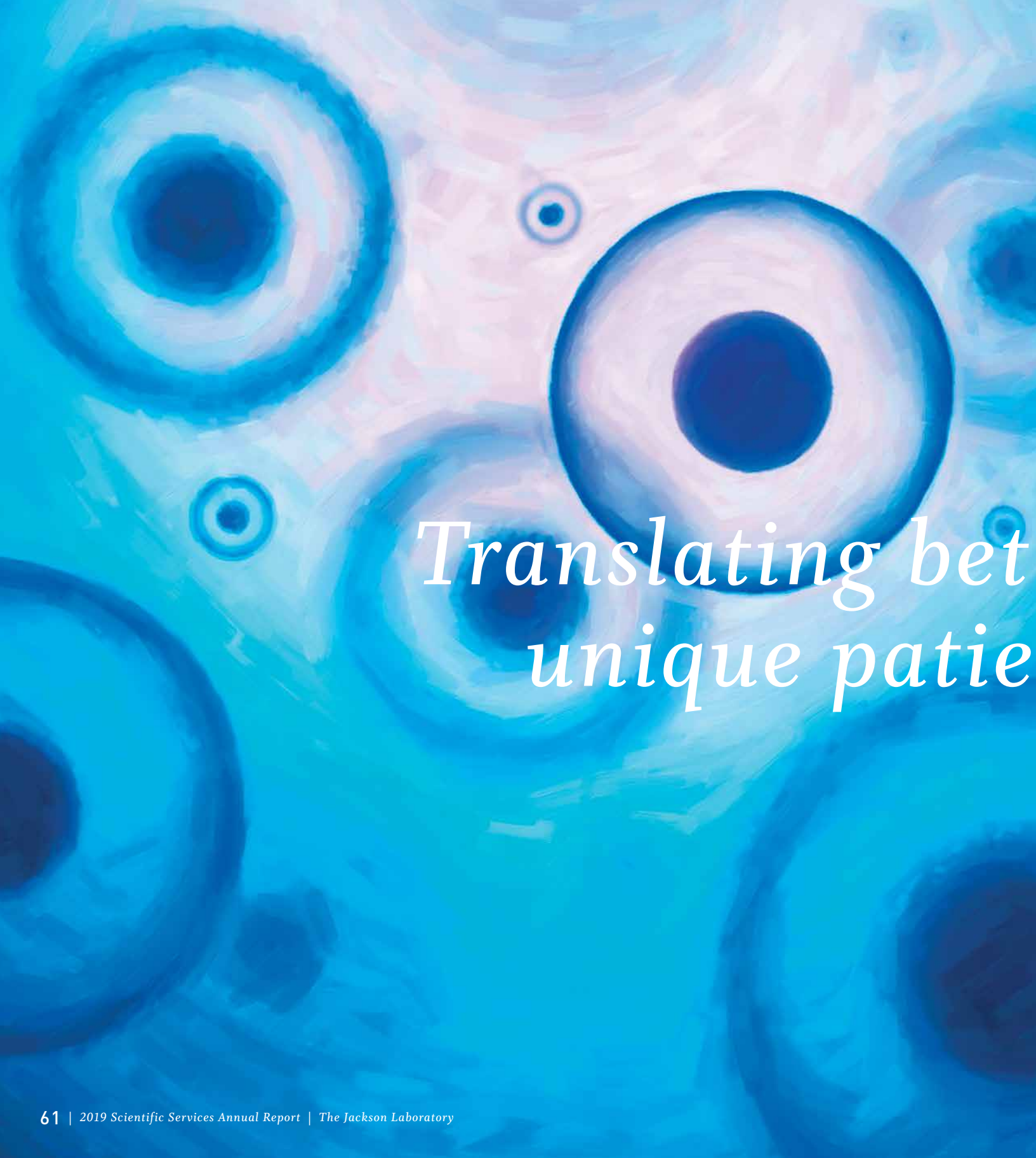
Our diets play a crucial role in the composition of our microbiomes; George Weinstock and his colleagues have contributed to an increasing body of evidence that intermittent fasting has positive effects on the immune system and processes related to aging.

It may also influence multiple sclerosis (MS), the dreaded neurodegenerative disease. Yanjiao Zhou, M.D., Ph.D., in the Weinstock Lab, will explore this connection in a five-year grant for a total of almost 2 million dollars from the National Institute of Neurological Disorders and Stroke.

Evidence that MS might be connected to the intestinal microbiome has emerged from a number of studies, including cohorts of identical twins — cases in which one develops the disease and the other does not. This is a clear indication that the causes of a disease are not exclusively genetic. For MS, transferring microbes from an affected twin to mice raised in completely sterile environments caused some animals to develop inflammations that resembled the disease.

There is a connection between what a person eats and the microbiome. Microbes are both taken up in food and receive sustenance from it, which means that different diets affect them in different ways. This adds an important dimension to studies linking lifestyles to disease.

"Current treatments for MS that do not address the microflora of the gut may be inadequate," Weinstock says. "Our approach will be to study the effects of periodic fasting on mice with experimental autoimmune encephalomyelitis, a model for MS. If we find that this modulates the microbiome in a way that affects the disease, the model will provide a testing ground for new therapies."



Translating bet unique patie

chapter 5

ween single cells, nts and mouse models

Imagine using Google Earth to zoom far into the body, down to the "street view" of the neighborhoods where cells live. You'd find them chatting about the weather, trading news and gossiping. Imagine what a cell might say.

NCI Designated Cancer Center

JAX CANCER CENTER STORY

Its molecules serve as both memories and a chemical language, written in scripts such as proteins, which it releases in conversations or prints on its outer membrane. There the messages are captured by other cells, using a sense that combines touch and taste.

Cells can be classified in many ways — as human or mouse, as muscle cell or neuron, as healthy or diseased — yet they retain unique features that make them individuals. A great deal of disease research is devoted to defining the origins and characteristics of cells, and conversing with them. Much can be gained by understanding how they are altered in diseased states, and by convincing them to change.

But this requires hearing what individual cells have to say and until recently, it has been hard to pick single voices out of a crowd. That's finally happening, says Paul Robson, Ph.D., who heads the Single Cell Biology Laboratory on the Farmington, Connecticut campus, with a satellite lab in Bar Harbor. His group is capitalizing on a range of new technologies to explore the amazing diversity of cells.

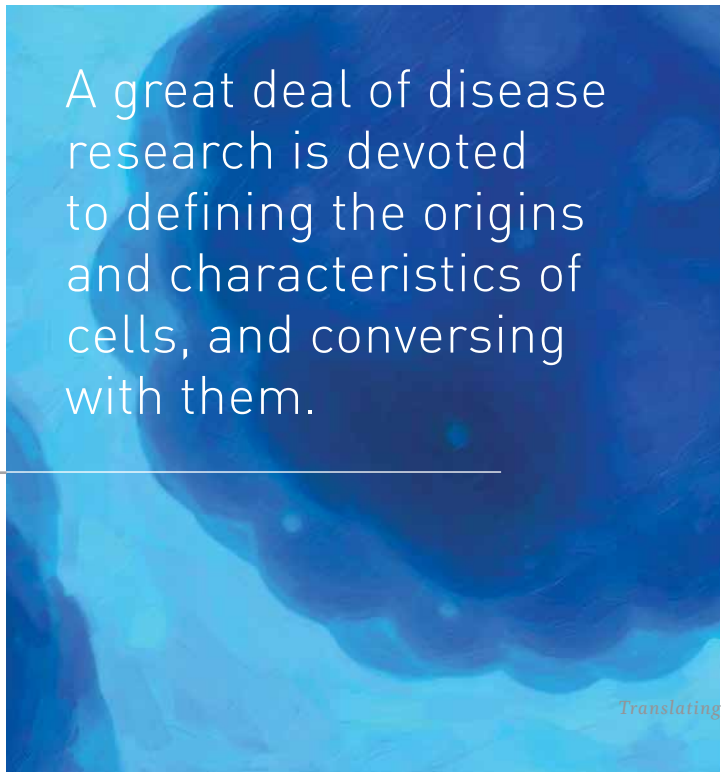
Robson's lab is one of the best examples of how the JAX environment combines services with science. "We are offering unique and vital services to a wide range of groups, pushing technologies while working on quite diverse scientific questions," he says. "At the same time, we're pursuing our own research program. It's a yin and yang situation that poses some challenges."

Five years ago found Robson far away on another continent, happily working at the Genome Institute of Singapore. "I remember a moment at 2 a.m. sitting outside by the pool having an absolutely fascinating telephone conversation with a scientist on the other side of the planet," he says, "talking about the potential of new technologies for some of our projects. That was Karolina Palucka, M.D., Ph.D., who was moving her group from Baylor Institute for Immunology Research in Dallas to The Jackson Laboratory's new site in Farmington."

It was one of many conversations that eventually led to Robson's recruitment to JAX. Now, five years on, his lab is engaged in extensive collaborations with Palucka and several other groups across the Laboratory. His group has quickly developed expertise and implemented a number of very new methods such as "droplet-based" sequencing and cellular barcoding based on antibodies. "The way we are set up lets us acquire new technologies and put them into the hands of researchers very quickly," he says.

Among the results have been much better "stratifications" of cells — in other words, recognizing subtypes among populations that have been lumped together in the past. Very recently, in a collaboration with the group of Alexander Jackson, Ph.D., at the University of Connecticut, Robson and his colleagues intensely scrutinized populations of nerves cells in the lateral hypothalamic area of the brains of mice. This region is highly conserved in function between mouse and human. It plays a crucial role in a range of behaviors including wakefulness and sleep and has been implicated in conditions ranging from addiction to mood disorders, obesity and other neuropsychological disorders. The project revealed that the area contains many more distinct subtypes of neurons than previously known — each of which may make unique contributions to disruptions of mental and cognitive functions.

Another project, carried out with the group of Ephraim Trakhtenberg, Ph.D., at UConn Health, started in a casual conversation at a faculty get-together. "We've known that vision involves highly specialized populations of retinal ganglion cells (RGCs) that link the eye to the brain," Robson says. "There's one, for example, that is basically devoted to telling a mouse that a hawk is circling high overhead. It was the theme of one the first drop-sequencing papers in 2015, but they weren't able to distinguish subsets of RGCs very well. Ephraim said he'd developed a method of enriching these cells. I told him that we were able to obtain high-quality transcriptomes of single cells. Putting those two things together, we have now been able to molecularly describe about 40 very highly specialized RGC sub-types."



The list of applications continues; Robson's team has assisted in a collaboration between JAX Assistant Professor Adam Williams, Ph.D., and a group from Yale, zooming in on specific T cells involved in allergic reactions.

Robson's lab has an important role in a $2.8 million dollar grant from the National Institute of Allergy and Infectious Diseases, awarded to JAX researcher Jacques Banchereau, Ph.D., whose aim is to discover why flu vaccines become less effective in the elderly. What happens to immune cells as they age is one of Banchereau's interests, and it's an area that stands to profit immensely from the single-cell approaches Robson can provide.

Banchereau had arrived at JAX from the Baylor Institute for Immunology Research in 2014 as one of the first recruits to the Farmington campus. He immediately set out to convince his colleague Karolina Palucka to move northeast as well. Subsequently, Robson's arrival has been a boon to both labs.

"What Banchereau and his collaborators hope to do in this grant is to distinguish between subsets of immune cells – those that mount a response to a vaccine versus those that don't," Robson says. "That will require isolating the cells in the first place, then thoroughly characterizing, with an unbiased approach, the differences between the types."

Can old immune cells be taught new tricks? Maybe so, if they can be heard as individuals, and the complex grammar by which they communicate can be decoded.

Immunology is a central theme of research on the Farmington campus — it is the focus of the labs of both Jacques Banchereau and Karolina Palucka, and Paul Robson's interests overlap their work in several ways. Robson's expertise in defining cellular heterogeneity in solid tumors is essential to a major grant recently awarded to Palucka's lab.

"The topic is a type of tumor called triple-negative breast cancer (TNBC)," Palucka says. "The name refers to cases that cannot respond to current therapies targeting hormone and growth factor receptors; for example Herceptin. TNBC is a complex disease with several subtypes, and we have yet to clarify the role genetic alterations and immune cells play in the development of these tumors. Additionally, these tumors go on to metastasize, and while this progression is the cause of most fatalities, we have yet to understand the underlying molecular and cellular mechanisms."

Tracing the lineages and genetics of such cells could reveal the biological hurdles that they have to overcome to achieve this, Palucka says, and that might help pinpoint weaknesses that could be exploited through therapies. But the potent cells that metastasize may be quite rare, and isolating them and unraveling their features will require the precision of single-cell technologies.

The search for new therapies links the themes of cancer, immunology and vaccines addressed in the labs of Palucka, Banchereau and many others. A number of promising new approaches to tumor treatment are being developed on the principle that in most cases, mutations that might lead to tumors are recognized by a person's immune system. This suggests that it might be possible to vaccinate patients against their tumors. It would require training immune cells to treat cancerous cells as foreign, in much the way vaccines stimulate a response against pathogens.

For years Palucka and Banchereau have been working on an approach based on dendritic cells, which play crucial roles in the way vaccines promote immunity against other types of diseases. One of the advantages of these cells, Palucka says, is their high flexibility and a diverse range of subtypes.

Such innovations are benefiting immensely from the advent of highly sensitive single-cell analyses such as those being developed in Paul Robson's group. They are also drawing on contributions from many other JAX services: Monoclonal Antibodies and all of JAX's protein-based platforms. They bring together diverse sequencing methods, offered through the Genome Technologies, and require enormous computational expertise provided by the Computational Science Service.

This work is jumping forward through new, precise genetic engineering technologies, particularly CRISPR/Cas9, which is handled in JAX's Genetic Engineering Technologies, headed by Bill Buaas, Ph.D. "One thing that has so long made the mouse the premiere model for biomedical research," says Alan Sawyer, senior director of JAX Scientific Services, "is the broad and very powerful palette of methods that have been developed for engineering its genome. CRISPR is taking that toolbox to the next level."

The dialogue between immune mechanisms and disease is highly nuanced, with a huge molecular vocabulary and a highly complex grammar of biological mechanisms. There are points in both cancer and autoimmune diseases when cells seem to start speaking different dialects: new terms are introduced and old modes of understanding break down. These effects are amplified across species, where subtle differences between humans and mice pose a serious barrier. Species' immune systems are under enormous evolutionary pressure and become highly aligned with the unique biological niches they inhabit — a major reason why many discoveries made in the lab have been devilishly hard to translate into the context of clinical medicine.

A particular problem for the use of mouse models to study human cancers comes from the fact that most of such research requires mice with compromised immune systems. That has been necessary because otherwise, a mouse would reject human tumor cells simply because those cells — whether normal or cancerous — are foreign to the mouse immune system; a humans would reject cells from another species in the same way. That means cancer would never develop in the mouse, making it impossible to study the progress of the disease or later stages such as metastases, or to try out new types of treatments.

Immunodeficient mice allow the the growth of human tumors but lack a functional human immune system that is a critical component of new immunotherapies. "We're finding an increasing number of ways of getting around this by 'humanizing' mouse immune systems," Palucka says. "One way we're doing this is to transplant human immune components into the mouse." Several years ago the Schultz Lab (overseen by Lenny Schultz, Ph.D.) took this strategy using a non-obese diabetic line of mouse, which was particularly receptive to the human stem cells that produce blood. The animals develop a range of human blood and immune cell types. Another lab pursuing this approach is that of Muneer Hasham, Ph.D., who runs the PDX Research and Development Core. The strains they are generating have become essential for research into cancer, infectious diseases, allergies, inflammations and rejections of tissue in the aftermath of transplantations.

PDX stands for patient-derived xenografts, or PDXs — the flip side of immune transplantations. Here cells or tissues from patients' tumors are transplanted into the mouse – generally giving it a very faithful version of an individual's disease.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Muneer Hasham has been actively pushing the technology by developing PDX models in humanized mice. "Initially xenografts could only be performed in severely immune-compromised mice," he says. "But we're taking this combined approach with Palucka and other groups. These new models are giving us a chance to work out the details of immune responses to tumors in a much more natural situation."

Many PDX models replicate the main characteristics of patient tumors, but the disease progresses in similar ways. This has permitted scientists to peel apart aspects of cancer biology, such as how particular forms of genes contribute to distinct stages of the disease, or the features that distinguish "cancer stem cells" from the much less potent cells that make up most of a tumor's mass.

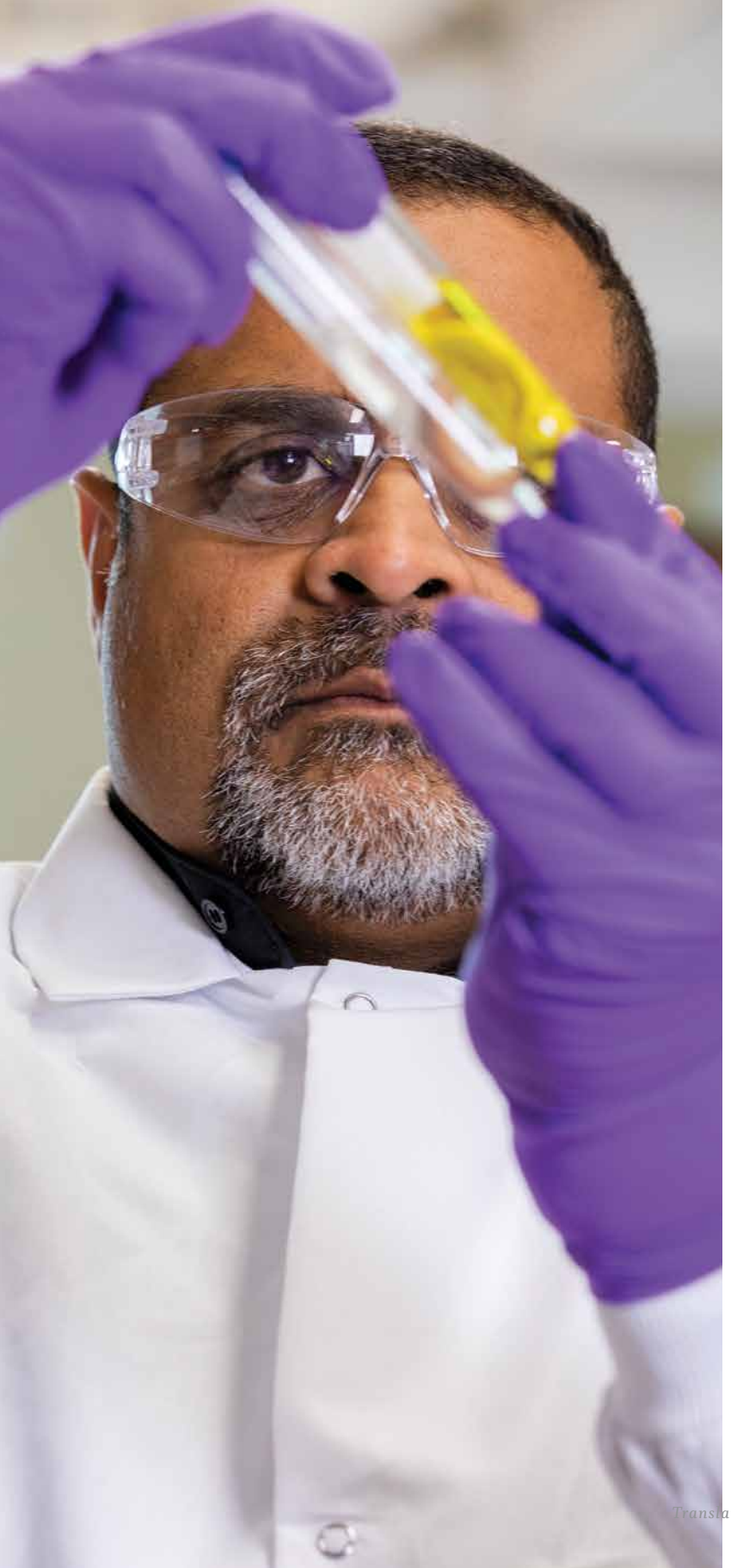
"It's not only the disease that takes a similar course in animals and patients," Hasham says, "but the models often respond to treatments in similar ways. They're helping us understand why patients respond so differently to classical therapies."

Generally, once a successful PDX has been established, this means that a number of animals can be developed with the same tumor. This gives scientist-physicians a unique proving ground for diverse therapeutic approaches. "Even some quite unusual ones," Hasham says. "There have been cases where researchers have thrown a battery of FDA-approved substances at PDX mice and found something that had a positive effect on the animal's health – even if it had originally been intended to treat a completely different condition. If your mouse responds to an approved drug, you can go back and try it in the human as well. In a number of instances there have been very positive results."

Not all tumors lend themselves to the approach; that has been the case for metastatic lung cancer, for example — a problem that the lab of Julie Wells, Ph.D., has taken on in a different way (Wells story). Still, JAX currently has over 500 lines of PDX models on hand, live or cryopreserved, which are made available to scientists across the world.

The development of PDX models is a service of JAX's facility in Sacramento, Calif., which was established more than a decade ago to expand the laboratory's mouse breeding and research services from a West Coast base. The station has about 375 employees and offers a variety of other in vivo services.

By chance, the first day of my visit to Bar Harbor was the last for one of Hasham's collaborators. Derry Roopenian, Ph.D., was "sort of" retiring, and I had been temporarily given his old desk. We met in the afternoon, when he came by to pick up the pictures of his family and remove a few last odds and ends. Most scientists I know don't retire the same way as other professions — there are continuing collaborations, a backlog of manuscripts, and other ends to tie up.



Hasham and Roopenian have used PDX mice to probe a hypothesis about tumors that was originally formulated nearly a century ago by Dr. Otto Warburg and has resurfaced from time to time. Warburg was a German physician and biochemist who won a Nobel prize in 1931 for his work on cell metabolism. His studies of the biology of tumors revealed that they required enormous amounts of energy and derived it by breaking down glucose through anaerobic processes that differ from those of healthy cells. They achieve this in a series of biochemical steps called the glycolytic pathway, and Warburg believed it represented a potential weakness that might be exploited in therapies against the disease.

One problem with this approach has been the difficulty of interfering with glycolysis specifically in tumor cells, without affecting healthy tissue. Doses of inhibitors usually need to be high to maintain their effects, which may ultimately be toxic. And most attempts to develop combination therapies that would more specifically target tumors have been disappointing.

"We recently showed that a compound called 2-deoxy-D-Glucose, or 2DG, could reduce the size of tumors in mice," Roopenian says. "2DG affected several different types of tumors, but it wasn't a perfect solution, because there were still unwanted effects. That's when Hasham had the idea of combining it with another substance called DIDS, developed by our former JAX colleague Kevin Mills, Ph.D."

"DIDS targets a different aspect of tumor biology: the ability of cells to repair themselves when they have suffered genomic damage," Hasham says. "That's a crucial function in cells which have already become disrupted through cancer and are accumulating lots of new mutations and other structural problems with their genomes."

Enter a mouse with a patient-derived form of chronic lymphatic leukemia (CLL). Hasham and his lab showed that CLL tumors had specific features that were perturbed by DIDS. Combined with 2DG, lower doses of the two substances could reduce tumors more effectively than standard therapies, without affecting healthy processes in the animals.

"We all know about the adverse effects of chemotherapies," Hasham says. "What we're seeking are treatments that hit cancer without completely derailing a patient's quality of life, or causing other types of damage that could lead to further complications down the road. The great advantage a researcher has here at JAX lies in the Scientific Services. There are cores established here that exclusively focus on delivering specific processes. So without PDX R&D, Flow Cytometry, Histopathological Sciences and Microscopy cores, this work would have taken an enormous effort and funds on my end."

Like most of the other service providers and users at the Laboratory, Hasham says the structure is immensely helpful because it makes things much simpler for scientists. "Instead developing all this expertise within a group and needing 20 partners, you can work with two," he says. "And another function we provide, which is especially important when you work with animals, is that we help scientists with all of the regulatory procedures they need to deal with."

The project is a good example of how a new dimension can be added to results acquired somewhere else at JAX — at some point in its history — and integrated with the institute's high-caliber services. "This type of 'institutional memory' is crucial to the way we do science, and it adds real value to our work," Roopenian says.

Ideally, cancer and other diseases could be detected at a stage before they become truly disruptive and affect systems throughout the body. That idea brings this report full circle, once again to the group of Julie Wells, and a collaboration between her lab, that of Carol Bult,Ph.D., and Hasham's.

The theme is another important use of PDX animals: in developing new forms of diagnosis for cancer and other diseases. Tumors constantly shed cells — which may become metastatic — and molecules, with which they engage in a dialogue with surrounding tissue and the immune system. Those may include abnormal microRNAs or unusual amounts of these small regulatory molecules. Upon release they circulate through the bloodstream, where they may be taken up by other cells or degraded.

"Blood is the easiest material to take from a patient," Wells says, "and it may contain tumor-specific factors early in the course of a disease, before they spread and become truly disruptive. That's particularly important in lung cancer, for example, which is usually quite advanced and metastatic before it is diagnosed."

A lung cancer signature might be found through the presence of specific, unusual molecules, or it might be read from changes in the amounts of microRNAs or other factors present in the blood. To find out, the scientists carried out a careful examination of the microRNAs in the tumors and serum of ten PDX models of lung cancer. Wells says the results, which are still being analyzed, look promising for several reasons. "What we've found suggests that microRNAs do have potential as markers and diagnostic tools. And the fact that these molecules influence the expression of target genes — including some that are crucial to the biology of tumors — suggests that they could be used in therapies as well." A crucial step in doing so, she says, will surely be to explore their effects on animal models derived from patient tumors.

An inte

chapter 6

erview with the director

with Alan Sawyer and Russ Hodge

Russ Hodge: You're trying to develop a new model of how research and services ought to work together. How would you describe the recipe, and how have your experiences fit together?

Alan Sawyer: I don't know if I'd call it a new model — it's not entirely new to me, but JAX increasingly recognizes the value of the efficiencies that can be gained by providing services through core facilities, what we call Scientific Services. You get more bang for the buck with centralized Services. Research Group sizes can be much smaller and do not have to include all the necessary areas of expertise. The way the core facilities are structured at JAX has changed over the past five years. One of these changes involves each Service having "faculty partners" who provide expertise and help the Service Leads run the facilities. Since I arrived I've put much more emphasis on users' group committees. These now consist of two faculty members, one from each JAX campus, and a "power user," as well as the people from the facilities. So that's become the functional unit to administer the Services. The users' group committees meet three times a year, and then there's a fourth open meeting for all of the Services, to ensure cross referencing and to eliminate redundancies.

How do you decide whether something should be a service or not?

First you need to identify a critical unmet need. Then you have to decide whether it can be done by somebody else outside of JAX or if we can do it more effectively in-house. As an example, I started a Protein Production and Purification Service because I was running the Monoclonal Antibody Service at the time and we needed somebody to make proteins for us — that was a limiting step. I went to my manager and said that we needed a protein production facility and I knew somebody internally who could do it. They basically said, "Okay, prove it." I applied for one of the internal innovation grants and using those funds we compared results from four different companies and two external core facilities against the results of an internal candidate who would head up the Service. We looked at different metrics — the quality of the product, turnaround time, communication, flexibility, feedback and cost, of course. We commissioned companies to make the same five proteins, recorded how long it took, checked them for contaminants using mass spectrometry, and so on. In the end it turned out that the best quality proteins were made by our internal candidate, and they were the quickest, and the cheapest too. That was an example of an internal

demand, from one Service that needed another Service to carry out upstream work to feed it.

Other Services are set up when our scientists identify a strategic need. Some of this will be top-down. The most notable and recent example is our Single Cell Biology Service; this was recognized as a strategic need by our president and CEO Ed Liu, M.D. He followed that up by recognizing that what we also needed was a cell screening core facility, and this is currently being set up inside the Single Cell Biology Service.

When you started in science 30 years ago, at that time there were really virtually no such things as core facilities. That has to do with the fact that in the early phases, the expertise to do procedures had to come from within a scientific group — there weren't really off-the-shelf solutions for things. Then came a phase where there had to be a combination — you had facilities, but every assay or method had to be tailored to fit the specific question or problem you were trying to address. Have we now emerged from that? Nowadays it's almost unthinkable for a lab to do without facilities, now, because there are simply too many technologies you need to master... What I've heard from scientists at JAX is that basically they can arrive at the lab alone and jump in and start to work right away, because the expertise is there and the collaboration with the facilities works so smoothly... Is that the future?

It's an interesting point, but the evolution might actually go another way. If you look at PCR… this might have been done as a core Service at some stage. But it has become so trivial to run PCR reactions now, you can just buy a machine for your lab and stick in your samples. As the technologies improve, some parts of a Service's offerings may end up back in the lab as automation and miniaturization render the need for specialist knowledge obsolete. In fact, one of our Services' functions is to push the technology to get it to that point. In microscopy, for example, we now have slide scanners where you just drop off your slides and you don't need to do anything; it will just

"... JAX increasingly recognizes the value of the efficiencies that can be gained by providing services through core facilities..."

The future is now: Construction of new state-of-the-art space for Scientific Services in the Snell building.

dump all your image data into a folder. You never even need to go near a microscope.

Look five or ten years down the road… Do you think this type of facilities operation will continue, or will it be at the level that it some sort of huge company is required to push and handle it?

We don't necessarily need to be the place where a particular facility is absolutely at the very front edge of development… Maybe it's all right to be "fast followers." Look at sequencing, for example. There the technology isn't being advanced in private labs; it's happening in massive companies. Microscopy, on the other hand, is still being pushed by microscopists. So it depends on the platform, and the state of the art. We're looking all the time for what we can outsource — what's been commoditized, and what can we ask someone else to do. Usually the costs are still prohibitive.

At the European Molecular Biology Laboratory, where we were colleagues back in the early 2000s, EMBL's thinking was that we needed to have technology development in the context of people pushing science, at the edge. Hasn't this been the case for JAX's mice — where the institute as a whole has evolved this function of providing "mice for the world?" Wouldn't the concept of Jackson as a "place that provides mice" as a service be quite a bit different from the real state of affairs?

JAX needs a research program to keep producing mice that sell. There are only a few faculty at JAX whose efforts are specifically devoted to developing new mouse models. Normally the models get developed here as part of the faculty's research — we don't really know if there will be commercial value. This is a great aspect of JAX: the mice follow the research, rather than the research following the mice. It usually starts with an interesting question, someone saying, "I can probably map this disease to this gene by interbreeding these mice." From there, somebody else over in the business side will say, "Hey, this animal might be marketable." So we develop that whole technology and produce some excellent research from it, and the commercial side runs with it. And ultimately, the Scientific Services are there because there's a need that comes from the academics. Very seldom do we have a "build it and they will come," strategy. What I say to scientists, when we establish our core budget every year, is that what we do has to be driven by the science; if it isn't, we aren't going to buy it. We're not just acquiring gizmos because they're on the market somewhere. There has to be a PI who says, "I really need this Service in order to do my research." And then we'll ask, okay, what technologies could we exploit or develop to be able to address that particular question. So they'll come to us with a scientific problem and we have to solve it.

You're standing at this really interesting intersection between tech development, basic science, the pharmaceutical industry, and the world of medicine. All of those strands are subject to various kinds of economic and social pressures, and they're highly dynamic. Many of them have nothing to do with science, but rather with how healthcare is organized, or how the shareholders of companies aim to increase their profits. How do you adapt to this environment? How do you fit into JAX's view of itself in this matrix?

Some years ago JAX made a strategic decision to get more translational, which led to the development of the Farmington campus, where we started to work on human cells and human diseases. It all has to do with human diseases in the end — we've been using the mouse as an analog for a long time, and have basically been trying to develop better and better analogs. But Farmington is a more direct translational effort. There are lots of ways we fit in there. We've developed a clinical lab, for clinical sequencing that's organized outside the central Scientific Services. And we are moving closer to the bedside.

JAX is also part of a number of important consortia, devoted to oncology, dementia, diabetes and other areas. So we are definitely moving in this clinical direction, which has lots of implications for the types of facilities we need, where they are located, and how they are organized.

Where are you headed?

The short term is pretty exciting. We have embarked on a construction program that consolidates many of the core Services onto two floors directly adjacent to each other. The Snell 2 building is being modernized into state-of-the-art lab space that has been custom designed for each of the facilities while at the same time being flexible enough to repurpose and remodel in the future for different usage if we need to. This is scheduled for completion in the Spring of 2020.

In addition to this we are seeing the build out of the cell screening platform, an extension and expansion of our flagship long-read sequencing workshop and we have engaged Professor Andy Greene, Ph.D., to expand and integrate our proteomics platforms more fully into the faculty's research. It's a pretty exciting time to be in Scientific Services at JAX.

I think in the long term, a sort of collectivist approach to the kinds of scientific questions we are trying to address is going to be more effective than a highly individualistic approach. And this is what we're transitioning to — a new kind of teamwork. It's coalescing the Scientific Services and their technologies with the PIs to such an extent that they interdigitate. It's hard to tell where the research ends and where the Service starts. Science is no longer a matter of applying your main instrument to your one model system to work on a single pathway. It's choosing from a whole palette of methods and technologies — whatever's appropriate to the question at hand, wherever your science leads you. For each problem you assemble a unique recipe to reach a solution. Along the way some technologies will be commoditized and you may make the strategic decision to outsource them, but there will always be new ones, where scientific expertise and excellence will be crucial.

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About the author

Russ Hodge has been writing about molecular biology for over two decades. He launched the Office of Information and Public Affairs at the European Molecular Biology Laboratory in Heidelberg, Germany and is currently Science Writer at the Max Delbrück Center for Molecular Medicine in Berlin. He has written eight popular books on the life sciences, hundreds of articles and over a dozen innovative reports for research institutes. He is also an accomplished artist and musician. His work on theory, practice and humor in science communication can be seen at www.goodsciencewriting.wordpress.com.

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