Different approaches, similar determination

Two UMMS researchers rock the scientific world

Reports of two scientific breakthroughs by UMass Medical School researchers and their colleagues continue to make international headlines, one involving a "functional" cure of a HIV-positive baby, and the other offering hope that the extra chromosome responsible for Down syndrome might be silenced. The researchers took very different approaches to tackling very different health issues—one a disease that is particularly deadly in low-resource settings, but can now be managed with expensive, life-long treatment; the other a genetic disorder with associated concerns such as congenital heart disease, leukemia and cognitive defects, with hundreds of overrepresented genes once deemed too complicated to tackle.

In fact, the only common denominators joining these two stories might be the similarly dogged determination of Katherine Luzuriaga, MD, professor of molecular medicine, pediatrics and medicine, and Jeanne Lawrence, PhD, interim chair and professor of cell & developmental biology, and the institutional culture of support and creativity at UMMS that nurtured their different pathways to success. They also both benefited from extensive National Institutes of Health funding and sustained support from colleagues on campus and around the country.

Dr. Luzuriaga, who has worked for 20 years with researchers around the world to end the HIV/AIDS epidemic, came to UMMS through a mentored fellowship. Thanks to a framework provided by the NIH and other funders, she and her collaborators may have uncovered evidence that aggressive treatment hours after birth has the potential to save the youngest HIV patients from the burden of life-long antiretroviral treatment, and she is optimistic that future research will show promise for adults as well.

In contrast, Jeanne Lawrence, PhD, interim chair and professor of cell & developmental biology, worked independently on an out-of-the-box approach to genetic research, fueled by a personal interest in improving the lives of people with developmental disabilities. While she has kept her work largely confined to the Worcester campus, the outcome of her research has wide implications, as a better understanding of the genetic pathways that underlie the syndrome may lead to a new route to gene therapy for myriad other genetic conditions, including Alzheimer's disease.

Both of these breakthroughs lay crucial groundwork for a better understanding of health issues once thought too complex to solve. UMass Medical School's institutional culture of support encourages all UMMS researchers to remain optimistic as they look for new approaches, new answers, new hope.

Katherine Luzuriaga, MD, left, and Jeanne Lawrence, PhD.

‘Functional’ HIV cure offers real hope

Katherine Luzuriaga, MD, says ‘functional’ cure of HIV in infants may transform treatment of a still-deadly disease

On the list of *Time’s* 100 most influential people of 2013 are the usual assortment of celebrity musicians, actors, athletes, politicians and businesspeople—and Katherine Luzuriaga, MD. Dr. Luzuriaga, professor of molecular medicine, pediatrics and medicine, and vice provost for clinical and translational research, was recognized along with two colleagues, Hannah Gay, MD, and Deborah Persaud, MD, for their functional cure of an HIV-positive infant. More than just a chance to hobnob at the gala reception, the doctors’ collective inclusion on the *Time* list sends an important message about science and medicine in the United States today.

“Our inclusion on the *Time* 100 list places science in the public eye and in a very favorable light,” said Luzuriaga. “Any time the popular press recognizes a scientist and the importance of the scientific process in changing our lives, it’s a good thing.” That kind of recognition could lead to a better understanding by the general public of what it means for their tax dollars to support researchers through NIH funding, as well as to encourage donations to foundations or academic institutions to support further research. And that, in turn, “can create wins for patients,” said Luzuriaga.

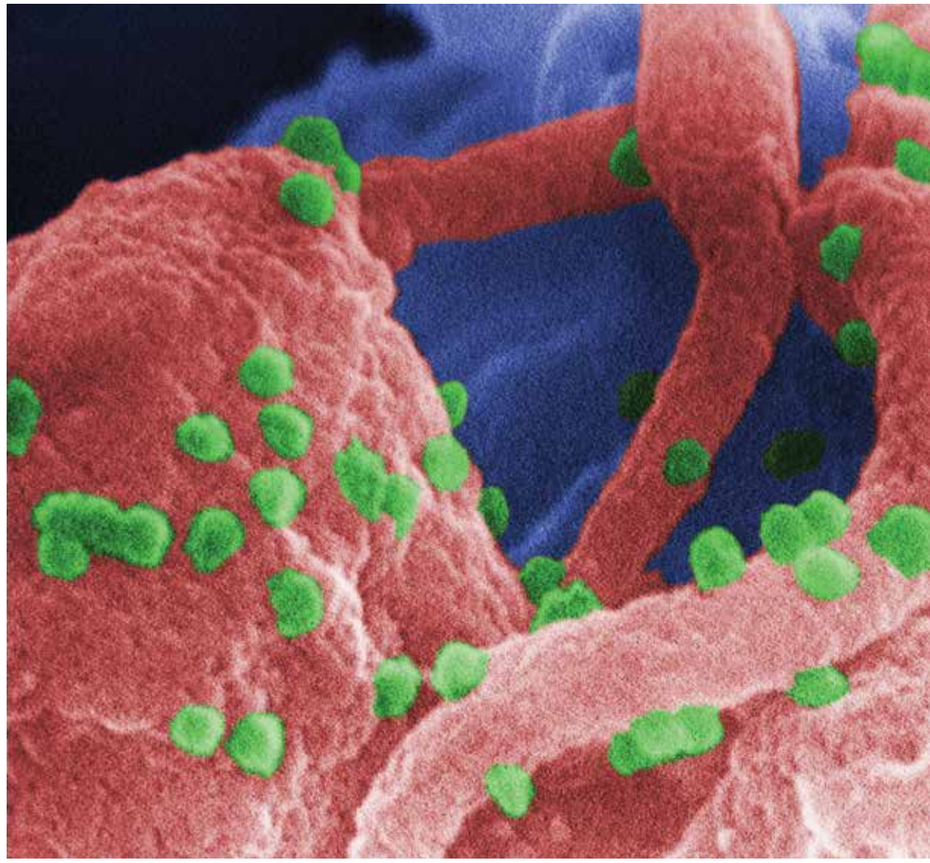
The case involved an infant born to a woman who had not received prenatal care and therefore had not been diagnosed as HIV positive before delivery. When the child was born, Dr. Gay, a pediatrician at the University of Mississippi, started therapeutic antiretroviral treatment within 30 hours of birth, even before the baby tested positive for HIV. Unlike the standard prophylactic treatment, which is administered for six weeks and followed with therapeutic doses only after an infection is diagnosed, this more aggressive approach continued until the child was 18 months old, when the mother stopped coming for follow-up visits. After five months in which no additional treatment was administered, the child’s blood was retested with standard measures. No trace of HIV was detectable; there was also no sign of HIV-specific antibodies.

Gay consulted with Luzuriaga, who immediately contacted a long-time colleague, Dr. Persaud, associate professor of pediatrics and infectious diseases at the Johns Hopkins Children’s Center. Luzuriaga and Persaud used highly sensitive molecular virology and immunology techniques to evaluate the baby’s blood for persistence of HIV. The baby has remained healthy and has not experienced an HIV rebound in follow-up. The team’s paper reporting the case was published in the Oct. 23 online edition of the *New England Journal of Medicine*.

The so-called “Mississippi baby” is just one case, but the takeaway—that ongoing treatment initiated early in an infant’s life has the potential to cure HIV infection—is significant. There is a big if, however, and that’s whether it’s possible to routinely diagnose newborns. Standard diagnostic methods use antibodies to search for infection but, because of the third-trimester maternal transfer of antibodies, that’s not a perfect approach. Properly diagnosing an infant, then, requires nucleic-acid-based detection methods to find any HIV nucleic acids in plasma,

By Sarah Zobel

Using highly sensitive molecular virology and immunology techniques, Katherine Luzuriaga, MD, showed that aggressive antiretroviral treatment of an infant just hours after birth 'functionally' cured a baby in Mississippi. At right, HIV-1 is shown as green spheres in this scanning electron micrograph. [CDC Public Health Image Library]



> "There are places in the world where this experiment can be continued to show that the result is, in fact, real, and then people can begin to think about applications that go beyond."
>
> – John Sullivan, MD

which calls for a lab with trained technicians—at a significant cost. It's not practical in many parts of the world where HIV remains unchecked so, as of now, the best chance of eliminating maternal-child transmission remains testing during pregnancy. With the mother on antiretrovirals, transmission rates drop to near zero.

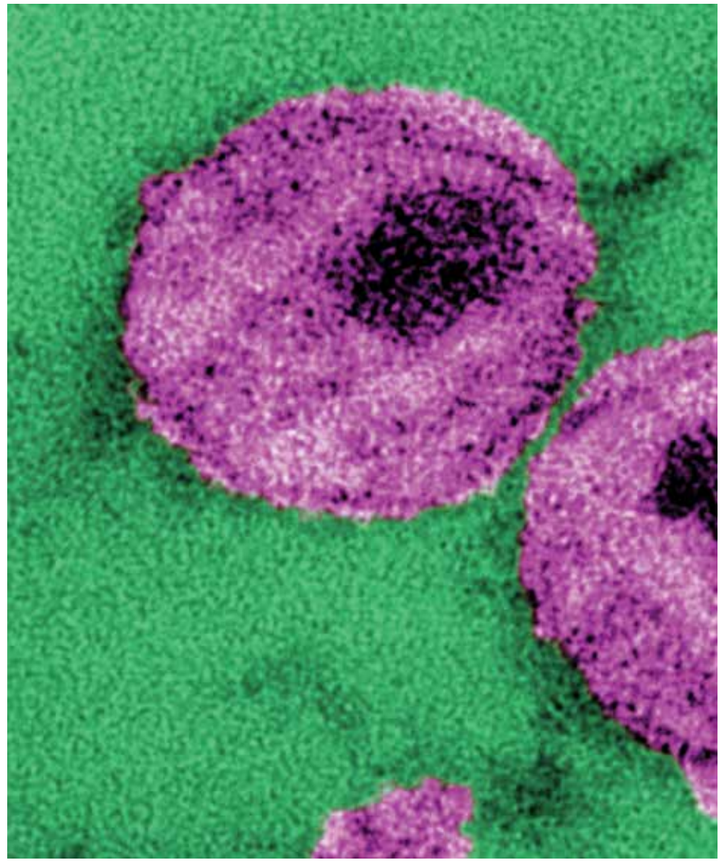
The functional cure itself was two decades in the making. In 1987, Luzuriaga arrived at UMass Medical School as a fellow in viral immunology in the lab of John Sullivan, MD, professor of pediatric immunology & infectious diseases. Together, they established a maternal-child AIDS clinic in response to the growing numbers of HIV-positive mothers and children. Through the new practice, they could directly address the speed with which signs of infection progressed in children—by age 2, more than 50 percent of HIV-positive children will be severely symptomatic. They were also part of NIH's clinical trials network and, through that, were able to conduct an initial set of studies on infants and treatments that would test their hypothesis that early treatment could alter both the clinical course and, potentially, set points of latency.

"Everything we did was going bench to bedside," said Dr. Sullivan, "and back to the bench." In 1997, with Luzuriaga as principal investigator, they published their findings in the *New England Journal of Medicine* that intervention within the first three months of life with a combination of zidovudine (AZT), didanosine and nevirapine was effective at suppressing HIV infection.

Many of the clinic's patients were from families across the region, so Luzuriaga and Sullivan established locations in Lowell and Lawrence. Many families were stressed socioeconomically so they built a team that included social workers and others to provide global assistance to families, and covered travel, phone bills and food costs, as needed. With proper adherence and careful use of AZT, the number of new patients gradually dropped. Today, the average age of clinic patients is 16, and the focus is on longer-term health issues including management of lipids and a healthy diet. After a lifetime of antiretroviral therapy, such patients may be at greater risk of diseases related to aging—in particular, coronary disease—and Luzuriaga and her team are engaged in long-term follow-up studies of the consequences of early exposure to antiretrovirals.

"We've made significant strides," she said, "but are there newer issues that they may face as they go along?"

Luzuriaga remains concerned about women and newborns who aren't treated early on. The standard recommendation is that every pregnant woman be tested for HIV; anyone who presents at labor and delivery with no documentation of having done so receives a rapid test. In the United States, Europe, Australia and Thailand, some 30 percent of infants born to HIV-positive mothers who were not treated with antiretrovirals while pregnant will be infected—the Mississippi baby is one such example. With antiretroviral therapy, less than 1 percent of infants are born infected, which translates to about 100 cases annually in the



Katherine Luzuriaga, MD, and her two colleagues, Deborah Persaud, MD, and Hannah Gay, MD, were honored at the 2013 Time 100 gala for being among the most influential people in the country. At left, this thin-section transmission electron micrograph shows the ultrastructural details of HIV virus particles, or virions. [CDC Public Health Image Library]

United States. The numbers are higher in sub-Saharan Africa, where the penetration of interventions has not been as extensive; in low-resource settings, a better infrastructure is needed to get medications to patients and simultaneously ensure their adherence.

"There are places in the world where this experiment can be continued to show that the result is, in fact, real," said Sullivan of the Mississippi baby case, "and then people can begin to think about applications that go beyond."

Luzuriaga and Persaud have recently collaborated with the NIH-sponsored International Maternal Pediatric Adolescent AIDS Clinical Trials network to develop a protocol to test whether very early potent antiretroviral therapy can clear HIV infection in infants. Data from several small adult studies also suggest that early treatment may allow some adults to eventually go off therapy and control their infection.

Collaboration will be key to learning how the virus-host dynamic plays out, and in moving from the lab to the clinic. To further those efforts, Luzuriaga was involved with the founding of the Center for Clinical and Translational Science, which spans the five UMass campuses and which she now directs. UMCCTS allows for the creation of multidisciplinary teams to address myriad medical issues; build devices that can be used in diagnosis or patient management; or take advantage of skills and equipment, such as a request for the creation of a specific protein or the use of a mass spectrometer. It is, said Luzuriaga, "an institutional attempt at building capacity for generating cross-disciplinary collaborations that will facilitate translation of basic science discoveries." The collaboration also incorporates UMass Medical School's MassBiologics, the only non-profit, FDA-licensed manufacturer of vaccines and biologic products in the United States, providing opportunities for cross-campus biologics manufacturing.

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One impetus behind the establishment of the UMCCTS was to pair UMMS with the University's Lowell-based engineering program, and an early outcome was the Massachusetts Medical Device Development Group, better known by its acronym M2D2. The not-so-veiled reference to *Star Wars* suits Luzuriaga, who said that long before she went to MIT as an undergraduate, she was very much at home in the world of science and math students.

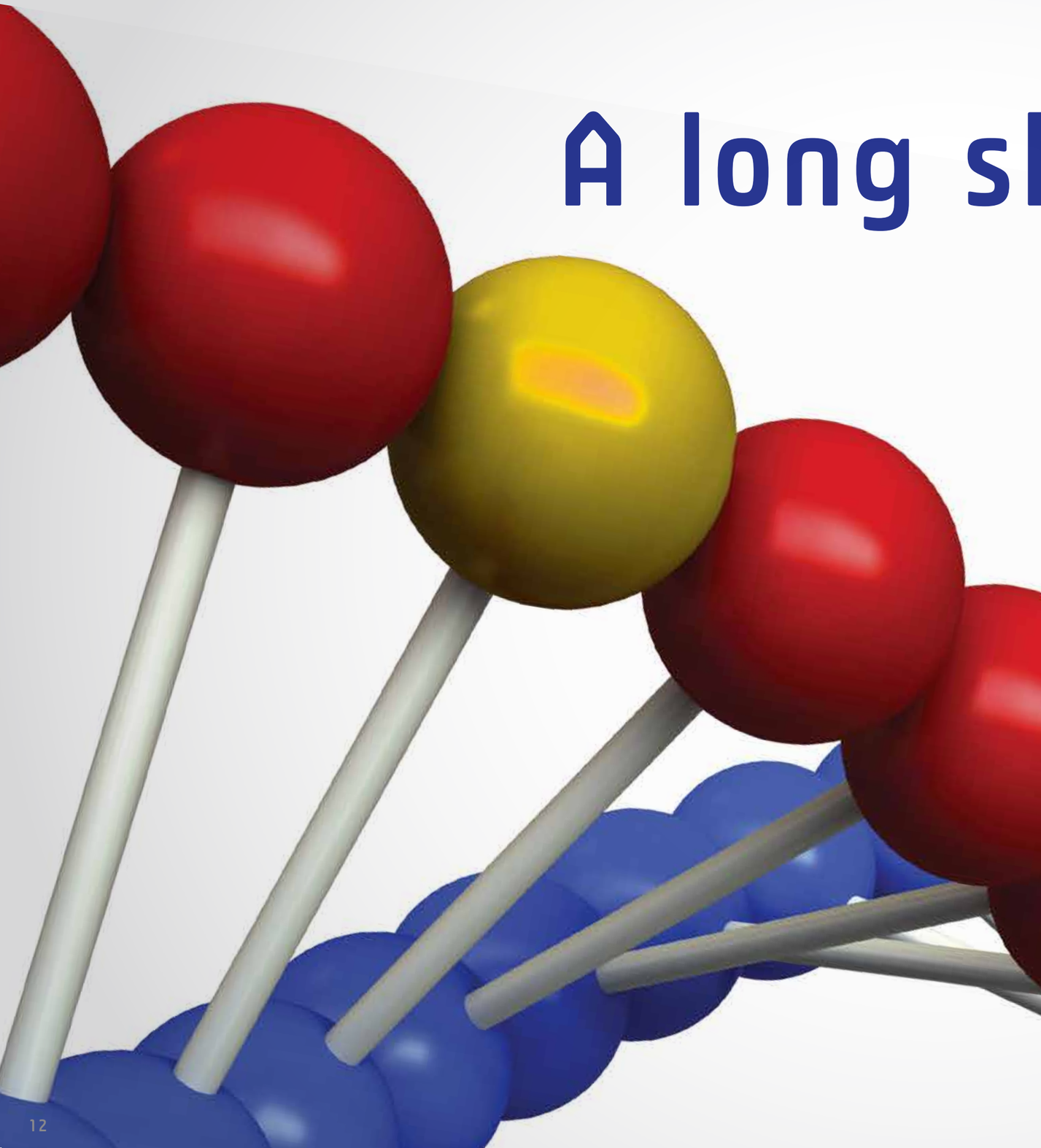
Born in Venezuela, Luzuriaga was raised in the Philippines, first coming to the United States to attend college. She'd planned to be a primary care pediatrician, but her passions for microbiology and immunology found a focus during her second year of medical school, when the first descriptions of patients with AIDS appeared in the literature. With a month to design and complete an elective—she'd spent her first two years at the University of Connecticut School of Medicine before transferring to Tufts University School of Medicine—she chose viruses and the immune system and was, she said, "hooked." She trained in pediatrics and infectious disease, and arrived at UMMS as a fellow prepared to begin research in viral immunology, specifically the Epstein-Barr virus. But the sudden rise in numbers of HIV-infected women and infants led to a refocusing of Luzuriaga's energies.

Nearly three decades later, Luzuriaga is pleased that researchers know as much as they do about HIV, observing that it is better understood than many other viral infections. She attributes that to a strong patient advocacy effort, coupled with NIH-funded advances in technology, basic understanding of HIV infection, and HIV clinical trials. Continuous NIH funding, along with backing from organizations that include the American Foundation for AIDS Research and the Elizabeth Glaser Pediatric AIDS Foundation has been key. Luzuriaga was named an Elizabeth Glaser Scholar in 1994 and an Elizabeth Glaser Scientist in 1997. She continues to collaborate with other Elizabeth Glaser scientists, including Persaud and UMMS colleague Paul R. Clapham, PhD, associate professor of molecular medicine and microbiology & physiological systems, observing that the human relationships the funding fosters have resulted in better science through collaborations. The success with Gay and Persaud was possible because they were able to move quickly, thanks to the framework provided by NIH and the other funders.

Luzuriaga's own children are 18 and 22 years old. She was pregnant with her first son alongside her early clinic patients, and notes somberly that many of those women and their children are no longer living.

"But by the time I had my younger son," she said, brightening, "we had ARVs we could use, and almost all of those kids are alive. That's been gratifying." U

A long shot pays off

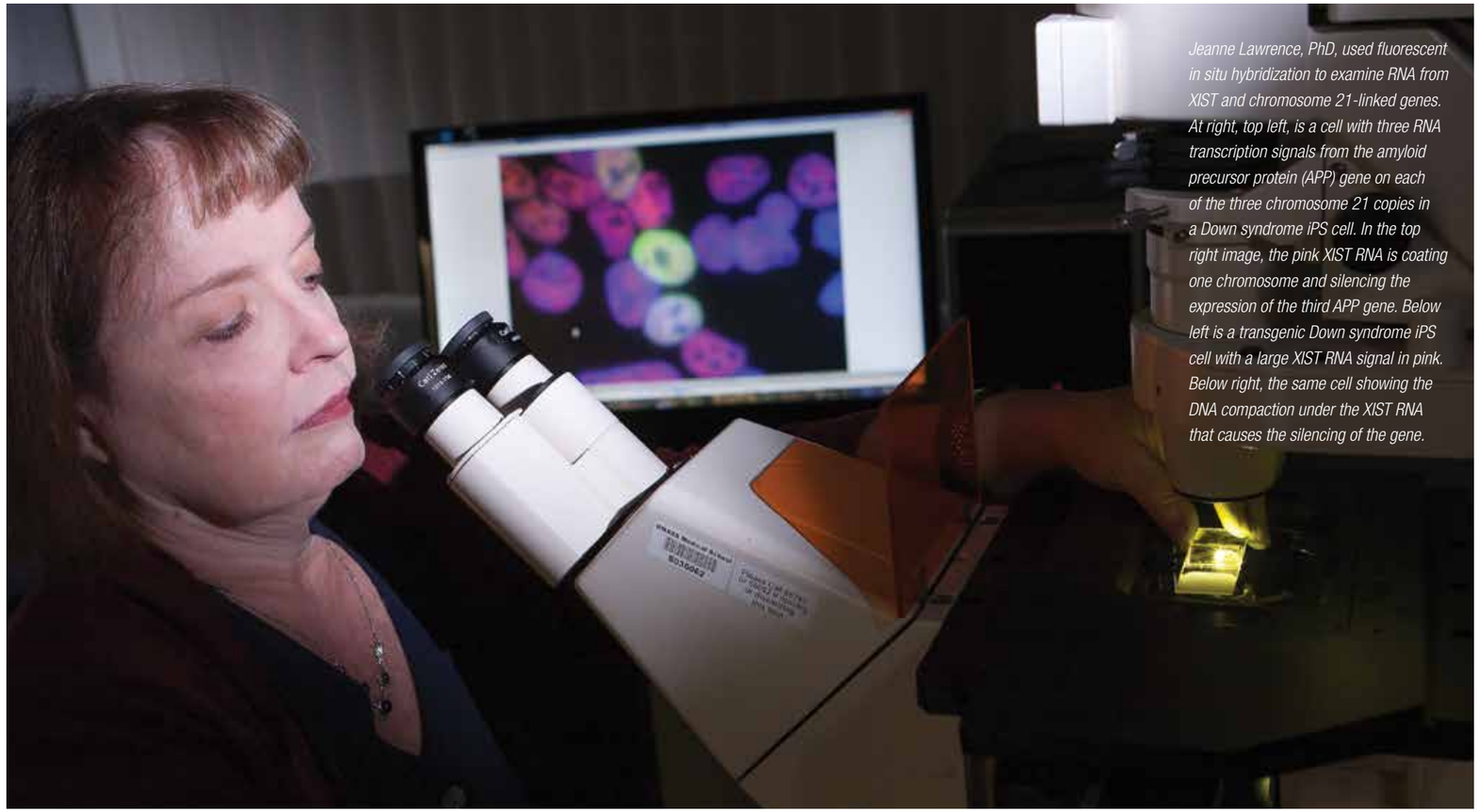


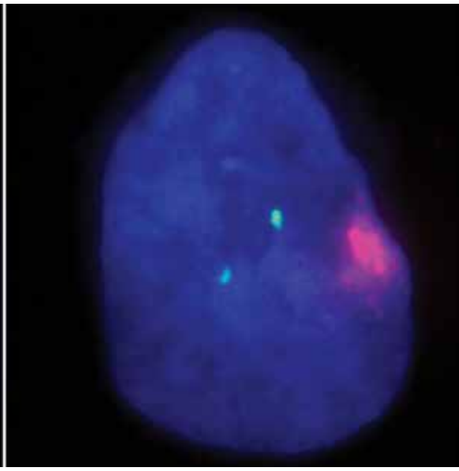
After others said it was impossible, Jeanne Lawrence, PhD, silenced the extra chromosome in Down syndrome in the lab.

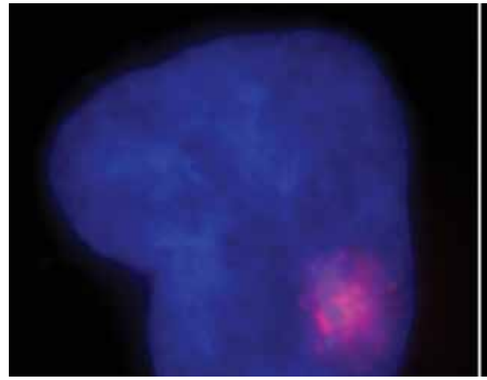
Jeanne Lawrence, PhD, is routinely described as an out-of-the-box thinker, someone who looks at the same problem others have long grappled with from an entirely new perspective—where others see the impossibilities, she said, she likes to see the possibilities. That tactic recently helped Dr. Lawrence, professor and interim chair of cell & developmental biology, prove that the extra X that's found on chromosome 21 and is responsible for Down syndrome can be silenced, a bold endeavor that other scientists either hadn't considered, or thought too challenging to try. Though it's not a cure for Down syndrome, it is a step toward one day significantly diminishing the far-reaching effects of that and other chromosomal disorders.

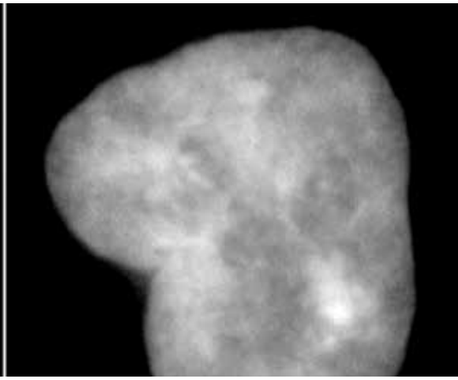
It's work that in some ways has deep and personal roots. Lawrence points to two early sources of her determination to help those born with disabilities. The first was a summer job at a local pool while she was in college. Asked if she'd be willing to give swimming instructions to eight adults with Down syndrome, Lawrence, who'd had no prior exposure to the disorder, agreed because, she reasoned, there was no reason not to. At the end of the summer, after she'd taught the group the basics and performed a few rescues of over-eager swimmers who'd headed for the deep end, the diminutive Lawrence met with the pool's director, who thanked her and said of the special request, "I asked you last because you're the smallest. But everyone else had said no, and you said yes."

By Sarah Zobel









Jeanne Lawrence, PhD, used fluorescent in situ hybridization to examine RNA from XIST and chromosome 21-linked genes. At right, top left, is a cell with three RNA transcription signals from the amyloid precursor protein (APP) gene on each of the three chromosome 21 copies in a Down syndrome iPS cell. In the top right image, the pink XIST RNA is coating one chromosome and silencing the expression of the third APP gene. Below left is a transgenic Down syndrome iPS cell with a large XIST RNA signal in pink. Below right, the same cell showing the DNA compaction under the XIST RNA that causes the silencing of the gene.

"I look back and I think, I didn't know that it was ever going to work!"

– Jeanne Lawrence, PhD

Reaching further back, to her childhood, Lawrence recalls the second influence: Patsy Sutton, a cousin who was some 20 years her senior and had cerebral palsy. Lawrence and her family routinely drove Sutton to appointments and took her swimming and on outings.

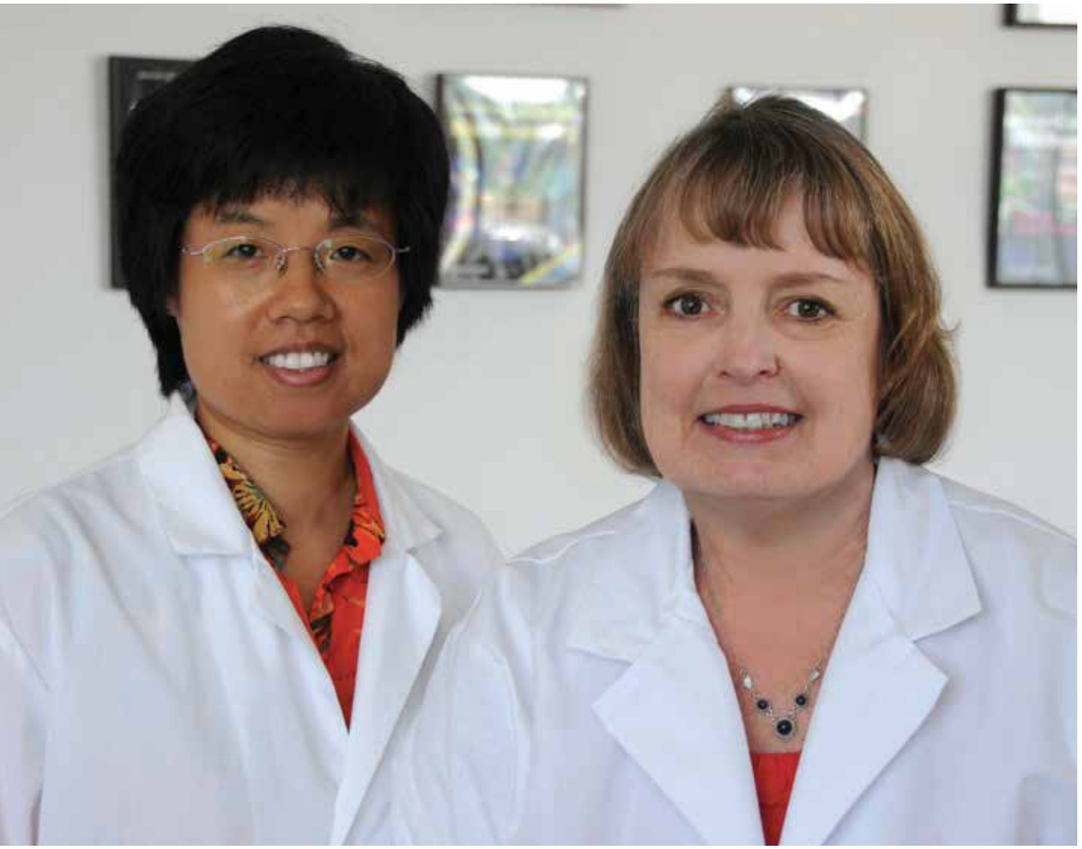
"I never thought *I'm helping someone who's disabled*," said Lawrence. "I just did it because I enjoyed her company. But today, I always have the perspective that if I'm getting up and walking around and have all my faculties, I'm actually very lucky."

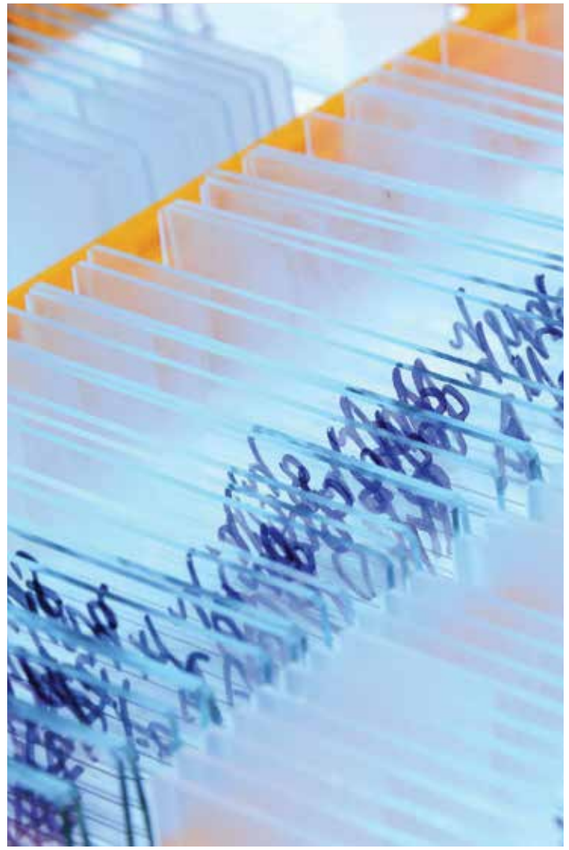
Lawrence went to Stevens College in Columbia, Missouri, intending to teach elementary school. But uninspired by the coursework, she refocused her studies on music before discovering "the intersection of science and society, philosophy and religion" in her junior year. When the dean summoned her to his office right before graduation, she was stunned to learn that she was first in her class. The prize was a fellowship to the University of Missouri, where she enrolled in her first real science courses. That led to a master's in human genetics and counseling from Rutgers, and a PhD in developmental biology from Brown. Wanting to stay involved in what she calls the "people angle" of science, upon arrival for her postdoctoral fellowship at UMMS, Lawrence quickly signed up to teach human genetics, later going on to direct the entire course. (Among other things, she lectured on the question of nature versus nurture, and was pleased to have a real-life test case in her own house when she gave birth to identical twin sons; she also has a third, older son.) In particular, she taught chromosomal abnormalities, bringing in patients with Down syndrome, sickle cell anemia, Huntington's disease and their families for special sessions designed to engage students in what it means to live with a given condition. One family whose daughter has Down syndrome attended so regularly that Lawrence was inspired to advocate for the establishment of the Patient as Educator Award, naming them as the first recipients.

At the same time, Lawrence was busy in the lab, where she was studying RNA molecules directly in cells rather than extracting them, in order to fully understand their organization and behavior. To that end, she had spent three years during her first postdoctoral fellowship attempting to develop fluorescent in situ hybridization, or FISH. At the time, it was thought that fluorescence was too insensitive to detect RNA in cells. So she spent years working without a microscope, labeling her probes with radioactivity and rapidly quantifying the radioactivity of multiple cell samples hybridized with those probes, in order to speed up the process of optimizing methods for FISH.

"I look back and I think, I didn't know that it was ever going to work!" said Lawrence of the three years she spent just studying how to make FISH more successful. Eventually, she published a quantitative analysis of in situ hybridization in *Nucleic Acids Research,* which in turn led to publication in several high-visibility journals. Her lab then extended the technique to examine RNA from XIST (X inactive specific transcript), a notably large gene that had been identified by Hunt Willard and Carolyn Brown in the early 1990s. While there was initial disappointment that the gene didn't encode a protein, Lawrence and her team determined that the gene made a unique "chromosomal" RNA that, indeed, controls X inactivation in women—what Lawrence laughingly calls the "first equal opportunity." But if XIST could silence the X chromosome voluntarily, could it also be redirected to silence a different chromosome? Some literature and studies in her own lab with Lisa Hall, PhD, assistant professor of cell & developmental biology, said that it would be possible to a degree, but no one had shown it was possible to insert XIST into a specific chromosomal site, or tested whether it would be able to silence the chromosome entirely. And if it was able to, could gene therapy control the trisomy of chromosome 21 in individuals with Down syndrome? Extrapolating from there, might scientists then be able to control the entire genome, whose various components are in a perpetual state of silencing and functioning?

The key issue unique to individuals with Down syndrome, however, is that hundreds of the genes are overrepresented, and developing gene therapy that can address a defect in a single gene has been challenge enough. Correcting an entire chromosome would seem nearly impossible. Undeterred, Lawrence and her colleagues looked to XIST.





> **"I'm a big believer in the idea that if you can show the first step, things can work out," said Lawrence. "You break through one barrier—don't worry that you have to break through *all* the barriers, just break through the first, biggest one and then see what you can figure out."**
>
> – Jeanne Lawrence, PhD

Jeanne Lawrence, PhD, right, with Jun Jiang, PhD, who successfully showed that XIST could silence a different chromosome, 19, opening the door to work on chromosome 21.

They planned to use genome editing as a sort of scissors and glue to cut and paste DNA at a specified site.

Lawrence applied for and received an Exceptional, Unconventional Research Enabling Knowledge Accelerating (EUREKA) grant from the NIH in 2008. Midway through writing the EUREKA grant, Lawrence met with Provost and School of Medicine Dean Terence R. Flotte, then newly arrived at UMMS, for an honest assessment of the project, and was relieved to get his support.

"The sheer creativity and originality of Jeanne's ideas was what made them so appealing. If you don't take crazy chances in research sometimes, you will never make a real breakthrough," said Dr. Flotte, the *Celia and Isaac Haidak Professor of Medical Education* and executive deputy chancellor.

Like so much of science, it was not a speedy process, and Lawrence found the genetic engineering aspect daunting. She contacted Sangamo BioSciences, which had developed zinc finger nucleases that could act as scissors, but the company was skeptical that inserting a gene of XIST's size would be possible. As this was very expensive technology, they agreed to collaborate, but required that Lawrence and her colleagues first prove it could be done, using the technology on a different chromosome, 19. This was a success, which Lawrence credits to Jun Jiang, PhD, instructor of cell & developmental biology, who had joined her laboratory and helped to push the project forward.

One development in stem cell biology, that of induced pluripotent stem (iPS) cells, was fortuitously timed. Lawrence and her team quickly recognized they could bypass the ethical issues surrounding embryonic stem cells but still take advantage of stem cells as an unparalleled resource. Lawrence also appreciated that working with a trisomic cell meant there was no possibility of ill effects from silencing a chromosome, since there was a spare. Lawrence's lab worked to silence chromosome 21 with XIST for five years.

By April 2013, Lawrence was ready to share on a broad scale the news that attaching XIST to a trisomic chromosome 21 would, indeed, silence it into inactivity and keep it that way, and she did so at the Global Down Syndrome Foundation's Workshop on Cognition in Down Syndrome. Shortly thereafter, the team published a paper detailing their findings in *Nature*. The professional response has been uniformly positive; in turn, Lawrence is quick to applaud those scientists who have been working in the field for decades, pleased to at last be able to discuss openly both the work and the next steps, which include reproduction of the experiment in a mouse model.

Still, there's no real cure for Down syndrome on the horizon, and there may never be. So many genes are affected that it's almost inconceivable that science could reach them in the embryonic stage. But what the research may mean is that some of the major health associated concerns—congenital heart disease, leukemia, cognitive defects and Alzheimer's—could one day be significantly mitigated, something that many of Lawrence's patient families have written to share their relief about. And in the short term, because it's now possible to compare cells with a chromosome on and then switched off, there is an opportunity to look at what cell pathologies and gene pathways underlie the syndrome, and identify targets for drug therapies, including gene interactions that might be responsible for Alzheimer's disease in the larger population.

Lawrence's lab is now busy investigating several potential applications of this "trisomy silencing" technology. She is also pursuing the basic science implications of so-called "junk" DNA, determining whether it plays a role in XIST RNA's ability to silence an entire genomic region, and expects to soon publish these findings.

The average lifespan of an individual with Down syndrome has already improved from the days of routine institutionalization, when a resulting failure to thrive was commonplace; today, many adults with Down syndrome enjoy some measure of independence, including holding jobs. But others, said Lawrence, exist just under that threshold, and she's hopeful her work's legacy will include biomedical therapies that, coupled with the existing educational therapies, will be the difference. It's a tall order, and one that Lawrence recognizes will take time.

"I'm a big believer in the idea that if you can show the first step, things can work out," said Lawrence. "You break through one barrier—don't worry that you have to break through *all* the barriers, just break through the first, biggest one and then see what you can figure out." U