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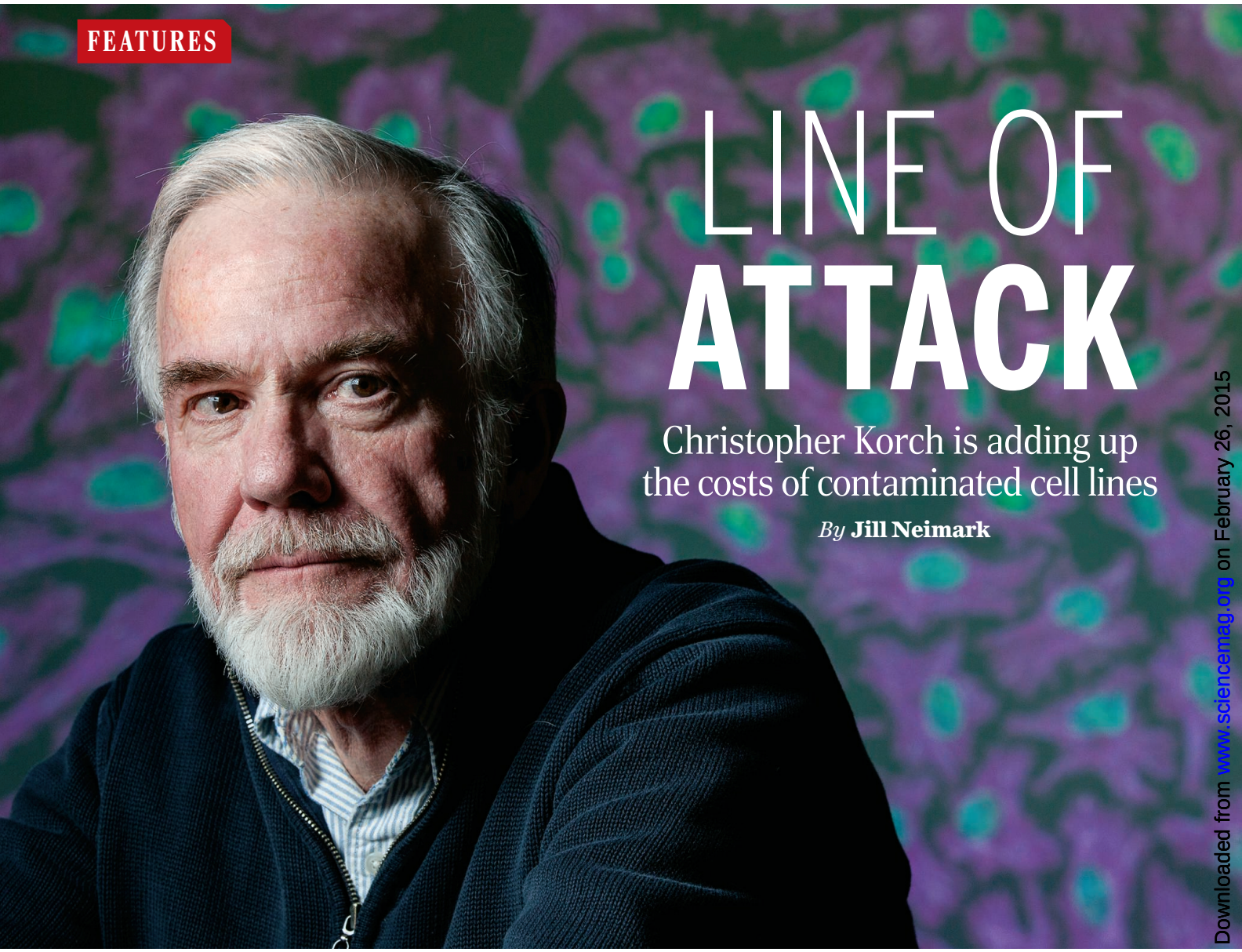

LINE OF ATTACK

Christopher Korch is adding up the costs of contaminated cell lines

By **Jill Neimark**

"I call myself a corrector," says University of Colorado geneticist Christopher Korch. What Korch passionately wants to correct is the contamination of laboratory cell cultures, a problem that has bedeviled biomedical research for more than half a century. Over the past 15 years, he has published on 78 widely used cell lines that turned out to be overgrown with other cells. Thyroid lines were actually composed of melanoma cells, prostate tissue was displaced by bladder cancer, and normal uterine cultures turned out to be nothing but breast cancer, casting doubt on countless studies of basic biology and disease.

And yet until recently he has felt more like a voice in the wilderness than a catalyst for change. "All too often, scientists have ignored my findings," Korch says. "Not one of my published papers has led to a retraction by a journal or scientist. Less than 10 corrections have been issued, when each false line I discovered affects the conclusions of hundreds or thousands of papers."

Now Korch has a band of allies and, he hopes, a novel way to persuade recalcitrant biologists: Zoom out from individual cases of contamination to show the big picture. After a year of intensive data gathering and analysis, he believes he has for the first time begun to quantify the damage done to the scientific enterprise by contaminated cell lines. "We're looking at tens of thousands of publications, millions of journal citations, and potentially hundreds of millions of research dollars," he says.

Many widely studied cell lines continue to be overrun by HeLa cancer cells (displayed behind Korch).

A few scientists who have seen a preliminary draft of Korch's white paper, which is now under review at a journal, have been moved to set changes in motion. "What impresses me about Dr. Korch's analysis is that the problem is more pervasive than I might have predicted," says Ferric Fang, a University of Washington, Seattle, microbiologist who recently co-authored a study estimating the amount of National Institutes of Health (NIH) funding wasted on a decade's worth of papers that had been retracted.

Fang, who edits the journal *Infection and Immunity*, says he will present Korch's findings to the leadership of the American

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Society for Microbiology, which publishes 13 journals, including his. But Fang cautions that "not all research using misidentified cell lines necessarily represents wasted effort." Some studies may still yield useful information, he says, adding "I am wary of sensationalizing the problem with large dollar amounts that are imprecise."

Korch acknowledges it's impossible to determine an exact dollar amount, but he feels that his financial estimate is actually conservative. His goal, he says, "is to give an idea of the enormity of the problem. We need to rattle the cage of complacency to get the attention of scientists, granting agencies, journals, and universities."

A TALL, GENTLE SWEDE with silvery hair, a neatly trimmed beard, and—according to his wife—the patience of Penelope, Korch arrived in Aurora, Colorado, in 1995, after several decades of steady but unspectacular genetics research in Sweden, France, Norway, and the United States. He was soon asked to direct the university's DNA sequencing facility, and became one of the world's experts in analyzing cell lines to determine their true genetic identity. Korch, now 70, uses a standard method called short tandem repeat (STR) profiling, which looks at specific DNA sequences that vary in number from one individual to the next. The technique, which the FBI and other law enforcement groups have long used to genetically fingerprint DNA from blood and other tissue at a crime scene, can also distinguish cell lines that come from different individuals.

"Christopher is definitely the CSI Crime Scene hero of science," says Paul Bunn, founder of the University of Colorado Cancer Center. And as the director of the sequencing center, Bunn says, "he was utterly free to pursue his obsession without pushback."

Immortal cell lines start as tissue samples that are coaxed, sometimes with great difficulty, to grow and multiply indefinitely in nutrient-rich plastic flasks. Cell lines are assumed to retain the properties of the original tissue, functioning as a living, physiologic test tube to explore the most important questions in basic biology and biomedicine, such as how cancers and normal tissues respond to drugs.

A cell line's immortality, however, is both its great strength and its most striking weakness. It allows experiments to be repeated again and again to confirm a finding. But as those same cell lines are passed from person to person and lab to lab over many decades, they can be contaminated by other cells. The interlopers can outgrow the original cells, ultimately displacing the authentic culture completely. Based on his and other investigators' research, Korch estimates that about 20% of cell lines are contaminated.

A tale of two impostors

Christopher Korch estimated the impact of research on two cell lines, HEp-2 and INT 407. Due to contamination long ago, both are now widely acknowledged to be composed of cancer cells called HeLa.

5789
ARTICLES
in **1182** journals may have used HEp-2 inappropriately, producing an estimated **174,000** citations

1336
ARTICLES
in **271** journals may have used INT 407 inappropriately, producing an estimated **40,000** citations

$713
MILLION
Estimated amount spent on the original articles published on **INT 407** and **HEp-2**

$3.5
BILLION
Estimated amount spent on subsequent work based on those papers

Researchers have mostly ignored or denied the problem. In the 1970s, biologist Walter Nelson-Rees aggressively exposed impostor cell lines and pushed for regular authentication, earning so much vilification for his efforts that he left science altogether. And less than a decade ago, cell biologist Roland Nardone took up the fight, chastising journals and funding agencies for not requiring that cell lines be tested to verify their identity (*Science*, 16 February 2007, p. 929). He, too, made little headway.

Now Korch has joined the fray. In 2012 he joined a newly formed volunteer global organization of 20 scientists, the International Cell Line Authentication Committee (ICLAC), dedicated to cleaning up the cell line literature. Chaired by Australian cell biologist Amanda Capes-Davis, ICLAC curates a free, online database of misidentified lines, which now number 475. Members meet by teleconference, publish articles on cell culture practices that minimize contamination risk, and add notations on impostor lines to PubMed Commons, an online tool that allows participating scientists to add a comment below any abstract indexed on PubMed. The group also offers assistance to journals and scientists trying to authenticate cell lines. Says Capes-Davis: "We don't use picket lines, bash down doors with a pickax, or brandish signs; we use data to convince our colleagues." Thanks in part to ICLAC's efforts, 28 journals now require cell line authentication, and some institutions do as well.

Starting in 2013, Korch also set out to quantify the damage from misidentified lines. His first case studies are HEp-2, thought to have originated in 1955 in a sample of a squamous cell laryngeal cancer, and INT 407, cultured in 1957 from the finely minced jejunum and ileum of a 2-month-old embryo. In 1967, geneticist Stanley Gartler unmasked both lines as HeLa, the most studied and rapaciously aggressive cancer cell line in biology, which must have contaminated and displaced the original cells around the time they were first cultured. Yet HEp-2 has been extensively used to study laryngeal cancer, while INT 407 is widely accepted as a model for normal intestinal cells.

Cutting his laboratory time to 2 days a week, Korch spent most of the next year at home, compiling and quantifying a mountain of data, to measure the footprint that the two contaminated lines have left in the scientific literature. Each has multiple monikers, he found. (HEp-2 is also HEp 2, Hep2, Hep-2c, Hep 2c, Hep2c, H.Ep.-2, H. Ep.-2, H.Ep. #2, H. Ep.-2, or H. Ep. #2. INT 407's aliases include Intestine 407, Henle 407, INT407, and INT-407.) Once he had all their iterations, he searched PubMed, Google Scholar, Web of Science, and many other journal databases from publishers such as Stanford University's HighWire, the American Society for Microbiology, and Elsevier. He found that HEp-2 has been used in more than 5700 published articles, under its cloak-and-dagger disguise of laryngeal cancer. INT 407 has been used in 1300-plus published articles, in its fraudulent identity as normal intestine.

In some cases, Fang contends, the mistaken identity does not undermine the

findings. "The first paper to describe how *Salmonella* invades host cells was made in 'INT 407' cells," he says, explaining that they were thought to be a good model for the intestinal cells the bacterium attacks. "This is one of the most important papers in the *Salmonella* field and has been cited more than 600 times." Even though the cells are HeLa, not intestinal cells, Fang see no reason to question its conclusions. "*Salmonella* uses this same mechanism to invade a wide variety of cells."

In other cases the cells' identity is critical. Last October, *Current Microbiology* published a study of a gene that allows another food-borne pathogen, *Listeria*, to invade cells. The study compared how the bacterium invaded three ostensibly different cell lines: INT 407, thought to be a model of the pathogen's intestinal target, and HEp-2 and HeLa. In truth, the study tested a single cervical cancer cell line, HeLa, three times.

"We used three different cell lines because we hoped to find a general mechanism that would work in many tissues," says Radosław Stachowiak of the University of Warsaw, one of the study's authors. "I was aware there were some issues with some cell lines, especially INT 407, but did not know about the scale of the problem." He goes on to echo many scientists who have a hard time believing a line is false. "I have to admit it is hard to accept all these lines are HeLa in fact, given their different appearance and even growing conditions. Since it turns out we tested only in HeLa we don't know if the mechanism is restricted to HeLa alone." Stachowiak has contacted the journal to request that it add a correction to the paper.

KORCH FINDS that even after scientists know their favorite cell line is contaminated, they may keep studying it. A typical case is ECV304, believed to be a good model for blood vessel cells because it was thought to originate in a sample of umbilical vein. In fact, a team led by geneticist Wilhelm Dirks of the German biobank DSMZ discovered in 1999 that it is a bladder cancer. More than a thousand papers have been published on ECV since then, and in 2011 two researchers explained why they have stuck with the cell line, in a letter in *The Journal of Biological Chemistry*. Wen-Cheng Xiong of the Medical College of Georgia in Augusta and her colleague Sylvia Simon of CNS and Pain Innovative Medicines in Södertälje, Sweden, acknowledged that the line was misidentified but said the bladder cancer cells have features of endothelial tissue, such as blood vessels. "[I]n the absence of an ideal model, we believe that ECV304 cells remain useful in studies of endothelial functions," they concluded.

To ICLAC, such sentiments are profoundly annoying. "This is typical of the wishful thinking adopted by scientists trying to evade the truth," fumes experimental pathologist John Masters of University College London, an ICLAC member. Korch is a bit more charitable. "They look at their line and are convinced it's still a valid model, because its behavior seems to match their expectations."

Take the case of Korch's fellow Swede Anita Sjölander, whose work has been a focus of ICLAC for 2 years. Her group at the Faculty of Medicine at Lund University in Malmö, Sweden, published 41 articles on INT 407 between 1988 and 2011, consistently referring to it as normal intestine even though Gartler had exposed it as HeLa 2 decades earlier. In 2001, Masters and colleagues confirmed the mistaken identity in 250 "blinded" samples of cell lines, including INT 407, from the major U.S. and European cell banks and five cancer research institutes. STR genotyping showed that "every known stock of INT 407 is HeLa," he says.

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> **Christopher Korch**, University of Colorado

Yet Sjölander was not convinced. She had tested the cells extensively over the years, and their behavior and morphology convinced her they were genuine intestinal cells. "All that functional testing, though interesting, is unreliable," Korch says. "For example, intestinal cells have microvilli. But so do HeLa cells."

Korch and ICLAC kept up the pressure on Sjölander. "I don't mind if the scientists get mad at me. I'm friendly, but I'm not afraid," Korch says. He often sent along Swedish greetings before urging her to get STR fingerprinting done on her samples. Sjölander explained that she had done so but told him that the results were not conclusive. So Korch persisted, pushing her in December 2014 to request a re-evaluation of the data that he himself could review. The results clearly identified her stock as HeLa, Korch says, and Sjölander now agrees that her batch of INT 407 cells are impostors. Sjölander studies mediators of inflammation in the intestine, but her 41 studies using INT 407 are not applicable to the intestine, Korch says. "She should add a correction to all her INT 407 papers." (Sjölander declined to answer when *Science* asked if she planned to correct or retract her papers, beyond saying that cell line contamination "is a serious problem and we treat it as such.")

SJÖLANDER'S INT 407 WORK suggests how big a shadow contaminated cell lines can cast on the literature. Korch extrapolated from the average citation rate of Sjölander's INT 407 papers to calculate the broader impact of the published work on the cells. "I calculated there may be as many as 40,000 citations that refer to work (directly or indirectly) using the impostor cell line INT 407 over the last half century, all referring to it as normal intestinal epithelium." HEp-2 is worse: Korch estimates that as many as 174,000 papers cite HEp-2 studies that may not be valid.

Then there's the financial cost. Masters estimates that $100,000 is the typical cost of an average cell study. Using that figure, Korch says $713 million has been spent on published work involving just HEp-2 and INT 407. The tally could be as high as $2.8 billion, if the per study value is $400,000—the figure that Fang and coauthors used in their 2014 analysis of the cost of retracted articles. "That's only two lines," Korch says. "The consequences to research based on all the 475 impostor cell lines on the ICLAC website are nearly inconceivable."

Will Korch's new study of the costs wake the scientific community out of a half century of inertia? Or will he go the way of Nelson-Rees, Nardone, and others who have sounded the alarm about cell line contamination and gone unheeded? "We at NIH agree entirely that this is a serious issue and one that the time has come to really address," says Jon Lorsch, director of the National Institute of General Medical Sciences in Bethesda, Maryland, who reviewed Korch's analysis. Recently, Lorsch, along with Francis Collins, the director of NIH, and Jennifer Lippincott-Schwartz, the 2014 president of the American Society for Cell Biology, issued another call for biologists to take cell line identity seriously, hinting that NIH may require grant applicants to verify their cell lines (*Science*, 19 December 2014, p. 1452).

All of this makes Korch optimistic at last. "I see the floodgates beginning to open, actually," he says. "Scientists everywhere are starting to demand reproducibility. I hope my work is one extra push in the right direction. We all want pyramids of literature built up solidly on sound foundations." ■

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