In the fall of 1953, Leroy Stevens was a 33-year-old postdoctoral researcher working in his first job as a professional scientist since graduating from the University of Rochester with a doctorate in embryology. A native of Kenmore, New York, a suburb of Buffalo, Stevens attended Cornell University before pursuing his Ph.D. After earning his degree, Stevens accepted an offer to join the Jackson Laboratory in Bar Harbor, Maine. The Jackson, an independent research institute, boasted the largest mouse-breeding colony in the world. A laboratory with such a precious resource was a wonderful place to start a career as an embryologist.

Embryology—the study of how the fertilized egg grows into an independent, free-living organism at birth—relies heavily on *Mus musculus*, the common house mouse. The mouse has long been a highly valued model system for human biology. *Mus musculus* is also a key animal model used in the study of *embryogenesis*, the astounding cellular ballet that takes the fertilized egg through the well-ordered steps of development, resulting in a mature and viable organism capable of living freely in nature.

The process of embryogenesis begins with the union of the gametes, the egg and the sperm, at fertilization. The fertilized egg (known as a *zygote*) then undergoes an ordered series of transformations that result in the development of a free-living organism. These transformations are driven by the genetic information passed to the zygote from its parental cells (the egg and the sperm), resulting in the formation of all the cells required for the zygote to develop into a biologically mature organism. In humans, this transformation process takes about 280 days. During that time, the developing fetus goes from a single cell to a fully developed newborn baby containing trillions of cells.

Stevens' work was funded by a tobacco company intent on discrediting a claim that was beginning to inflict damage on their booming business enterprise: the assertion that cigarette smoking causes lung cancer. The tobacco interests were aware of the significance of the growing body of data supporting the contention that smoking tobacco can have serious, and even fatal, health effects.

Despite convincing evidence from multiple sources indicating the presence of chemical compounds in tobacco known or suspected to cause molecular damage in the lung, the bigwigs in the tobacco industry nonetheless intended to demonstrate that tobacco itself is not a causative agent of cancer. Rather, their hypothesis contended the fault could be found in the byproducts of the paper wrapper's combustion. According to this line of reasoning, cancer was not caused by tobacco but rather by the delivery vehicle itself. Once the paper's toxic byproducts were identified and eliminated, cigarette smoking would be freed from the allegations about its potential link to lung cancer.

It was difficult to understand the reasoning behind the proposal. It sure seemed a stretch, at any rate. Was the problem due to a substance containing hundreds of chemicals and byproducts of combustion or, rather, was the cigarette paper the culprit?

We might call this hypothesis scientifically unconvincing, but that might be too generous considering the most distressing blemish on the proposal: there wasn't any credible scientific data to support it. This is a fatal flaw in any scientific argument. We should, however, acknowledge the creativity under duress (not to mention the audacity) required to put the proposal forward to the public.

We might even say the idea stank of desperation. However, the tobacco interests bought a little time by asking for more studies. Not much, however, as Stevens, an eager young investigator, dove right into the problem. His approach to evaluating the hypothesis was logical, straightforward, and easy to implement in the laboratory. He separated the tobacco from the paper and used a variety of solvents to extract the chemical constituents of the cigarette's components in isolation from each other. With these test articles in hand, he could apply the isolated extracts directly on the skin of laboratory mice and evaluate the biological impact on both the mice and their offspring.

In this way, the experiment could evaluate not only whether the test materials were *carcinogens* (cancer-causing agents) but also whether the materials were *teratogens*. Such substances cause genetic damage to the fetus in the form of congenital malformations, also known as birth defects.

The experimental hypothesis was easy to disprove (as the data available at the time foretold even before the first test tubes were mixed). In short order, the experiments clearly demonstrated that tobacco is a carcinogen, a substance that leads to the development of cancer in exposed animals.

While tumors were evident in many of the experimental animals, meticulous examination of thousands of descendants of the exposed mice did not demonstrate the presence of birth defects. However, he did find something far more intriguing. One day, while examining a young mouse derived from a lineage known as "Strain 129," Leroy came across something exceedingly rare in nature and never reported in a laboratory mouse.

This mouse had a *teratoma.* Generally found in the reproductive organs where the egg and the sperm are made (in the ovaries and the testes), these bizarre cancers are amongst the strangest phenomena known to biology. Teratomas are unique from any other kind of tumor. While tumors involve the transformation of only one type of cell into a tumor cell—liver cells (*hepatocytes*) or white blood cells (*leukocytes*), for example—a teratoma contains multiple kinds of cancerous cells. Teratomas are comprised of various combinations of skin cells, muscle cells, nerve cells, bone cells, fat cells, and sometimes even wads of hair and/or baby teeth, all jumbled up together in an alien cellular mishmash.

The earliest reference to human teratomas goes back to ancient Mesopotamia. A prophecy in a clay tablet of "a woman giving birth to a child with three legs" found at the site of the Chaldean Royal Library of Nineveh (in modern-day Iraq), one of the great cities of the ancient world, has been attributed by expert testimony to "the discovery of a benign teratoma."[i] According to this prophesy dating back more than 25 centuries, the appearance of this child will bring a time of prosperity. Given the rarity of teratomas, the fulfillment of this prophecy subjected the ancient Chaldeans to an exceedingly long wait for better times.

As a trained embryologist, Leroy Stevens recognized that he had stumbled upon something of great significance: a mouse strain that was a source of a rare testicular tumor. He knew he had to put aside the (already answered) question of tobacco carcinogenicity and focus on teratoma biology.

After two years of painstaking work in his laboratory examining thousands of strain 129 mice, Stevens had isolated for further study a representative population of mice bearing the teratoma. A photograph in Stevens' 1954 paper shows a six-month-old strain 129 mouse with a large, bulbous mass between its back legs and tail, as if a dark, furry ball were sewn to its rear end. The work demonstrated the presence of the teratoma in about one percent of the strain 129 male mice.[ii]

While one percent may seem like a small number, teratomas are far rarer in nature. Consequently, the inbred mouse strain 129 that produced one teratoma for each one hundred baby mice was considered a rich source of teratoma samples. Evidently, Dr. Stevens had made a wise decision by choosing to work at the Jackson Lab, where obtaining hundreds of mice for experimentation was a snap.

The teratomas' biological behavior in strain 129 mice was intriguing and perplexing. The mice were not born with teratomas. The tumors developed after birth as the testes matured, from as early as 8 days to as late as 210 days, a significant percentage of the test animals' average lifespan. Unlike the mouse shown in the photograph in the 1954 paper, there were no external symptoms of the teratoma's presence in most of the afflicted mice.

One day, while examining one of his teratomas under the microscope, Stevens suddenly realized (in what we might today call an "aha" moment) that most of the cells in the sample were elongated and variable in shape and overall size. These were the developed cells expected in the mouse testes. Scattered amongst the many elongated cells, he noticed another cell type that did not share the same features as the fully developed cells. These cells—and there were only a few—were smaller and rounder and lacked the elongated appearance of most of the cells in the microscopic field.

Stevens recognized that these cells had the appearance of the cells in an early embryo that had not developed into the specialized cells characteristic of a fully developed organ. Something had gone terribly wrong during the development of the fetus. He realized that these round cells must be embryonic cells that were supposed to mature into the specialized cells of the testes. Instead of undergoing development, the embryonic cells had remained in their embryonic state and had not undergone the expected biological transformations coded in their genes.

The ramifications of this finding were astonishing. Here, in a colony of laboratory mice, Stevens found a reliable source of embryonic cells. These cells were ideal for studying mouse embryogenesis—and more. Because the embryonic cells from the teratomas could be used to investigate what goes awry during the tumor's genesis and growth, he realized that this line of investigation might open a window into the mechanism of tumor formation.

In a staid manner—consistent with reports of Leroy Stevens' understated and humble nature—the last sentence in his 1954 paper specifies the importance of further work using strain 129: "It is pointed out that an inbred strain of mice in which a relatively significant percentage of males develop testicular teratomas may be an important tool in the study of some hitherto unexplored aspects of the biology of these interesting growths."[iii]

In his early experiments, Stevens found that when he minced tissue from a strain 129 teratoma, he could successfully graft the tumor into the abdomen of both male and female recipients. While the transplantation of a growing tumor was successful in all 15 attempts, the tumors in the recipients tended to grow slowly. Fortunately, 1 out of the 15 attempted transplants provided a rapidly growing tumor that could be further transplanted to new recipients, a process known as *serial transfer*. Stevens found that he could successfully transplant the fast-growing tumor, derived from a 40-day-old strain 129 mouse, every two weeks when, according to his 1954 paper, "it reaches 2 cm in diameter."[iv]

The test sample in these experiments was injected into the *peritoneum*, the tissues surrounding the abdominal organs. When tumor cell transplantation is successful in this procedure (known as *intra-*

peritoneal transfer), the tumor is established and grows in the peritoneal space. Within days, a firm, fluid-filled sac of tumor cells arises in the gut, as if the tumor has built a cocoon full of nutrients around itself to provide the molecular components required for sustained growth.

Turning his attention turned to the embryonic cells in the tumor, it seemed logical to hypothesize that these cells—endowed with a "superpower" that enables them to give rise to many types of cells during embryogenesis—held the key to understanding teratoma biology. Reasoning that "the preponderance of embryonic cells in these relatively simple tumors makes it seem likely that they give rise to the diverse types of differentiated cells found in the more complex teratomas," he began to develop a concept of how teratomas form.

Stevens proposed that a teratoma occurred because of a failure to properly execute the full developmental program of the embryo. Since the teratomas in strain 129 mice "are composed of a variety of embryonic and adult tissues which are not normally found in the testis," some of the embryonic cells must have malfunctioned in the execution of their genetic programs. Instead of producing a well-organized fetus with the appropriate types of cells and tissues throughout the organism, defects in embryonic cells created a disordered cellular hodgepodge in the testes of the newborn mice stricken with this rare tumor.

When he transplanted teratomas into the testes of adult mice, he found that he could reproduce the teratomas in mature animals. This astounding finding suggested the teratoma—or, at least, some component of the teratoma—can seed a new tumor in a fully developed animal. This work provided direct evidence that the aberrant embryonic cells in the teratoma sample were responsible for transmission of the tumor. He called these *embryonic carcinoma* (*EC*) *cells* in recognition of their ability to seed new tumors.

Seven decades ago, Leroy Stevens provided a conceptual framework for teratoma formation and a reproducible mouse model system for studying embryonic stem cells that enabled additional investigations into the nature of tumor development. The data supported the mechanistic link proposed by Stevens between molecular errors during embryogenesis and the formation of teratomas in young animals.

The reverberations of the contributions of Leroy Stevens that demonstrated the relationship between aberrant cell development and cancer are still strongly felt today. By developing the teratoma model, Stevens directly showed that genetic errors during cellular development can lead to cancer. Equally important, Leroy Stevens provided, for those who followed in his pioneering footsteps, the means for obtaining precious embryonic cells for laboratory experimentation.

These developments also provided the basis for systematic laboratory experimentation with stem cells from embryonic tissues, giving rise in the following decades to the burgeoning field of stem cell biology that today promises the possibility of replacing damaged cells with functional equivalents. As an example, stem cells implanted into the pancreas can create new pancreatic islet cells to produce insulin in diabetics. The possibilities seem endless.

Despite these outstanding contributions to biology and medical science, the name of Leroy Stevens has been lost to memory, even amongst many biologists. Looking back on his career from his home in Vermont in 1990, Stevens noted, in his characteristically unassuming manner, "This stuff was extremely

interesting, and it sure beat studying cigarette papers!"[v]

We should all be grateful for the life and work of a man of great insight, determination, and humility, Dr. Leroy Stevens, the accidental and unsung hero of cancer biology.

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[i] C Raup, Teratomas: The Embryo Project Encyclopedia (2010), *embryo.asu.edu/pages/teratomas*

[ii] LC Stevens, Jr. and CC Little, Spontaneous Testicular Teratomas in an Inbred Strain of Mice. *Proceedings of the National Academy of Sciences of the USA* 40: 1080-7 (1954).

[iii] ibid.

[iv] ibid.

[v] R Lewis, A Stem Cell Legacy: Leroy Stevens. *The Scientist* March 6, 2000.