Cancer's cruel chimaeras

Fused proteins that cut short the life of a young man could help to unlock some of the deadliest childhood cancers.

BY HEIDI LEDFORD

On the day that her son Max was diagnosed with cancer, Ariella Ritvo stormed into the hospital pathology lab and demanded to check the results herself. "I'm not going to leave," she told a surprised pathologist. "I have a 16-year-old lying there. I want to see it confirmed."

Confronted with a sea of cells dyed blue on the microscope slide, Ariella sized up the enemy she and Max would fight together for the next nine years: a rare childhood cancer called Ewing's sarcoma. There would be countless rounds of chemotherapy, multiple operations, mice that carried tumours grown from Max's cancer cells and several experimental drugs, including two cancer vaccines and one compound that had never before been given to a human.

In the end, the cancer would still take his life. All because of a scrambled protein.

As in most cases of Ewing's sarcoma, Max's tumour cells contained two genes that had accidentally joined together into one. The fusion protein it produces, called EWS–FLI1, is a chimaera run amok, altering the expression of thousands of genes.

Fusion proteins are a common theme in childhood cancers, from brain tumours to leukaemias. And Max's struggle with this one highlights the difficulty of tackling them in young patients. The diseases they cause tend to be aggressive, and the intensive chemotherapy treatments used to fight them can be brutal. It is hard to study paediatric cancers in general, both because they are uncommon and because of the ethical concerns involved in experimenting on children. But perhaps most maddeningly, the fusion proteins themselves, the most obvious point of attack for new therapies, have proved to be slippery targets. "There aren't too many cancers that just say, 'Here is my Achilles heel,'" says Damon Reed, a paediatric oncologist at the Moffitt Cancer Center in Tampa, Florida. "These are doing that."

Scientists are hopeful, however. In recent years, they have revealed that many fusion proteins, such as EWS–FLI1, interact with some of the cellular machinery that controls gene expression. These epigenetic controls have become a bustling area of research in adult cancers. Therapies that target them are already in clinical trials in adults. And bolstered by advances, a new initiative aims to fund a systematic study of fusion proteins in paediatric cancers.

The outlook for the field is brighter than it's been in a long time, says Stephen Lessnick, a paediatric oncologist at Nationwide Children's Hospital in Columbus, Ohio, who has studied Ewing's sarcoma for about a quarter of a century. "This has become an urgent opportunity," he says.

UNDER-STUDIED KILLERS

The first symptoms of Ewing's sarcoma are typically unremarkable. For Max, in 2007, it was a recurring backache that was impossible to differentiate from the normal aches and pains experienced by a 16-year-old wrestler with a black belt in karate.

When he developed a fever, the family assumed he had the flu. But when his breathing became laboured, Ariella decided it was time to seek help. At the hospital, clinicians drew two litres of fluid from Max's lungs. A subsequent surgical biopsy led to his devastating diagnosis.

Fourteen million people are diagnosed with cancer worldwide each year. Only about 300,000 of them are children or adolescents under the age of 19.

The rarity of childhood cancers has made them a relatively low priority for government and industry funders. As a result, the development of therapies for them has tended to lag behind that for adult cancers, says Matthew Meyerson, a cancer geneticist at the Dana-Farber Cancer Institute in Boston, Massachusetts. "And that shouldn't be true," he says.

Fortunately, cure rates are remarkably high for some paediatric cancers: more than eight out of ten children treated for cancer will live for at least five years after their diagnosis. Most are cured. Advances in the treatment of childhood acute leukaemias, for example, are heralded as among the greatest achievements of cancer research.

ILLUSTRATION BY PADDY MILLS

But paediatric cancer treatments can be aggressive. Oncologists give younger patients high doses of toxic drugs that might kill an adult, because young bodies are better at bouncing back from withering treatments. Couple that with the particular desperation that comes when a child is dying, and it's a recipe for some punishing therapeutic regimens. About 3% of children with cancer die from the treatment itself.

"You go in guns blazing to try to treat these patients," says Lessnick, who also treated Max. "It's a very, very tough disease."

Max's age put him at greater risk. Adolescents are caught in cancer limbo: their youthful resilience has begun to fade and, possibly as a result, their cure rates tend to be lower. But they are still too young to qualify for adult clinical trials, which are much more abundant than paediatric trials.

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HIGH HOPES

Max was still a toddler when, in 1992, a lab in Paris sequenced the protein product of the *EWS–FLI1* gene for the first time[1]. A year later, a team at the University of California, Los Angeles, discovered that it could be a potent trigger for cancer[2] . The work showed how FLI1, a protein that controls gene expression, takes on new properties and becomes a more potent activator of gene expression when a fragment of the protein EWS is added to it. In 1993, when Lessnick entered the field as a PhD student, the mood in his lab was ebullient. A therapy, it seemed, was just around the corner. And because EWS–FLI1 is a molecular oddity found only in cancer cells, a drug that targets it might not be as toxic as conventional chemotherapy.

"There was a clear idea that now we had the driver oncogene, and it shouldn't be that difficult to figure out how to turn it off," says Lessnick. "Twenty-five years later, we're still working on the same issue."

In that span, a small band of dedicated researchers threw everything they could at EWS–FLI1. They sought compounds to interfere with its activity, but the protein was too floppy and unstable to bind a small-molecule drug readily. They tried to shut down EWS–FLI1 expression using techniques such as RNA interference, but could not ensure that the RNA needed to silence the fusion gene would reach all the cancer cells. One cell left active might be all it takes to reseed the tumour, says Lessnick.

As attempts to directly target EWS–FLI1 failed, researchers began to sift through the hundreds of genes it regulates, in search of one that was both key to its influence on cancer and vulnerable to attack with a drug.

There was a brief flutter of hope for one such target, a protein called IGF-1R. Antibodies against it were tested in clinical trials. But they ultimately disappointed, driving back tumours in only about 10% of patients[3]. Drugs that help only a small proportion of people with an already rare disease hold little commercial interest; the companies involved ended the programme.

The field hit wall after wall. "If someone tells me something works downstream of a pathway affected by EWS–FLI1, I shut down immediately," says Reed. "We've tried that a million times."

Around 2010, as scientists began trying to improve characterization of tumours by looking at the sequences of their entire genomes, some researchers were hoping that trawling through the genomes of Ewing's sarcomas would yield other mutations that could be worthy drug targets. Instead, three different teams found the same result: Ewing's sarcomas always contained a fusion protein, most often but not always EWS–FLI1, and little else[4–6]. There were no other potential

ASHLEY WOO

Max Ritvo, pictured in 2014.

drug targets that could reach the majority of patients.

But there was a bright side to the negative results. Many adult cancers are riddled with mutations, making it difficult to tell those that drive cancer from those that are merely background changes, with no impact on tumour growth. Tumours can also have multiple triggers, complicating attempts at therapy. Simple cancer genomes, such as the ones that turned up in Ewing's, may not offer a lot of targets, but with few drivers, they should be less likely to develop resistance to an effective therapy, says Kimberly Stegmaier, a paediatric oncologist at Dana-Farber. "That's the hope," she adds.

BLUNT INSTRUMENTS

But hope does not equal progress. Treatment for Ewing's sarcoma has changed little since 1993. It generally comprises several rounds of harsh chemotherapy, surgery and radiation. In 2007, Max endured his chemotherapy in three-week cycles. The first week brought vomiting and diarrhoea, and sometimes an intestinal infection. During the second, a different set of drugs often landed him in hospital with severe anaemia. Week three was for recovery, and then the cycle started again. After four rounds of this came surgery, radiation and more drugs.

Max braved it all with characteristic good humour, save for one drug: ifosfamide. It caused him to hallucinate and he was unable to put his thoughts into words. He could only repeat two sentences: "My brain is breaking," and, "Give me the blue!" The blue was the chemical methylene blue, which counteracts ifosfamide.

When it was over, Max swore to his mother that he would never take the drug again.

He nevertheless went into remission. Free to resume life as a teenager, he spent the next few years falling in and out of love, nurturing and then overcoming a computer-game addiction and searching for truth in poetry and philosophy.

But his time as a cancer patient had left its mark. In his first year at Yale University in New Haven, Connecticut, he became obsessed with the idea that the ifosfamide had affected his memory. This fear pushed him to become a poet, he later said: he used his writing as a way to preserve the memories that he feared were breaking down.

By his final year of university, his mood had stabilized, he had co-founded a comedy troupe and his poetry had become more than just a sanctuary for his memories. But the one thing he had wanted to forget — his cancer — wouldn't let him. In 2012, his tumours came back. His mother stayed at a hotel near the Yale campus and shuttled Max from treatments to classes so that he could graduate on schedule.

Around that time, research into cancer-causing fusion proteins was entering a renaissance. While Max was at Yale, biochemist Cigall Kadoch, then at Stanford University in California, and her colleagues were studying a group of proteins that work together to modify chromatin — the assemblage of DNA and numerous proteins that help to pack and organize the genetic material in the cell. Chromatin can 'open up', allowing the genes within to be expressed, or it can be tightly wound, preventing gene expression.

In 2013, Kadoch's team had reported[7] that a protein called SS18 is part of a complex involved in the packing and unpacking of chromatin. When SS18 becomes fused to one of several SSX proteins, the resulting chimaera displaces normal SS18 from the complex, disrupting chromatin management. This, Kadoch's group found, ramps up expression of a cancer-causing gene. The work demonstrated how the fusion protein causes a childhood cancer called synovial sarcoma.

The work added to a growing body of evidence showing a link between fusion proteins and epigenetics. Researchers studying leukaemia and Ewing's sarcoma found similar links (see 'A deadly bond'). Kadoch now runs her own lab at Dana-Farber, and routinely gets requests for advice from researchers who study other fusion proteins, seeking help with the biochemical methods she used. The link she found was among the first of many between cancer-causing fusion proteins and chromatin. "Many of the fusion proteins interact with chromatin-modifying complexes to change chromatin in a way that allows gene expression to happen that shouldn't," says Scott Armstrong, a paediatric oncologist also at Dana-Farber, who has been exploring epigenetic links in childhood leukaemia.

This link, a hot area in epigenetics research, was reviving hopes for targeting recalcitrant fusion proteins. Sequencing the genomes of

adult cancers had highlighted the importance of epigenetic processes in driving cancer. Work was already under way in academic and industry labs to target epigenetic proteins in adult cancers. Paediatric oncologists now hope they can co-opt those drugs for children and adolescents.

Drugs already in development that inhibit an epigenetic protein called BRD4, for example, could find uses in treating a range of fusion-protein-driven cancers, including the muscle cancer rhabdomyosarcoma and some forms of leukaemia. The approach might also work for EWS–FLI1, which interacts with a protein involved in epigenetic regulation, called LSD1. Lessnick is acting chief medical officer at Salarius Pharmaceuticals, a company in Houston, Texas, that is developing an LSD1 inhibitor and plans to test it in Ewing's sarcoma.

DESPERATE TIMES

Max and Ariella became well versed in all of these projects and more. She runs the Alan B. Slifka Foundation in New York City, a philanthropic organization founded by her late husband that focuses on supporting the Jewish community. When Max got sick, Ariella expanded the foundation's remit to include sarcoma research.

Mother and son had also plugged into a network of families of children with Ewing's sarcoma. Ariella traded tips, rumours and science with parents as desperate for therapies as she was; some of Max's closest friends shared his disease.

In one way, the recurrence of Max's cancer was fortunately timed: at 22 years old, he now had access to clinical trials that were closed to children. Ariella was grateful for this, but the unfairness of it haunted her. "These kids don't have time to wait until they turn 18," she says. "They'll die."

In 2012, Max began a fresh chemotherapy cycle: 12 rounds in all. His doctors recommended ifosfamide again, but Max refused. Over the next four years, he tried one experimental treatment after another. His mother asked the US Food and Drug Administration (FDA) for special permission to try an immunotherapy drug that had not yet been tested in children. There was concern that the drug might not work in cancers with few mutations, such as Ewing's sarcoma, because mutated proteins are thought to stimulate immune responses. In Max, the treatment seemed only to speed the spread of his cancer.

In 2015, Ariella and Max sent his cancer cells to a company that used them to seed tumours in mice. The company then tested a battery of drugs on the tumours. The hope was that the mice could serve as stand-ins for Max — avatars. A drug that successfully battled a tumour growing in one of his avatars might send Max's tumours into retreat as well.

Max, a vegetarian, found it all fascinating and a bit disturbing: mice bearing a part of him were dying so that he might live. When an experimental drug showed promise in one of these avatars, Ariella rushed to get the FDA to grant permission for Max to take it. But the drug did nothing.

Max earned a master's degree, got married, and began work on his first book of poems — all while bouncing from one experimental treatment to the next, losing weight as his health deteriorated. By July 2016, the 1.8-metre-tall poet was down to just under 51 kilograms.

HOPE TOO LATE

As a child, Max used to climb into bed and watch cartoons with his mother every Monday. It was a ritual so sacred to him that he named the family dog Monday.

By mid-August 2016, it was Ariella's turn to come to Max's bed. She and Max's wife, Victoria, kept close watch, periodically lifting his prone torso to thump on his back, hoping to clear his chest. His rattling breaths gave Ariella nightmares, and she was terrified that Max, although unconscious, might feel as if he were drowning as his lungs filled with fluid.

On 23 August, she was holding Max's hand when his ragged breaths finally stopped. Ariella bathed her son's body, and then sat down to wait for the mortuary workers to take him away.

It was just two weeks later that advisers to the US Cancer Moonshot — an ambitious plan to accelerate the pace of cancer research — recommended an effort to tackle fusion proteins. More than just an opportunity for this particular avenue of research, paediatric oncologists hope that it is a sign that childhood cancers as a whole may be getting more attention.

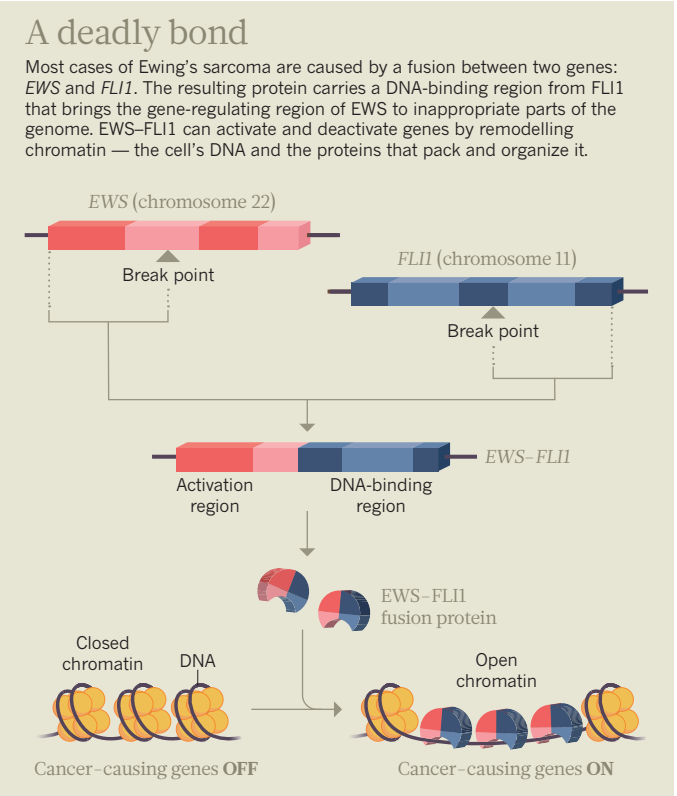
"We wanted to focus on something that was a crying need," says James Downing, president of St. Jude Children's Research Hospital in Memphis, Tennessee, and one of the researchers who put together the Moonshot recommendations.

The proposal, sketched out in brief last September, recommends a pipeline for systematically studying cancer-associated chimaeric proteins. It draws on the biochemical approach that Kadoch and her colleagues used to uncover the link between epigenetics and synovial sarcoma. And it calls for better cell culture and animal models, which have been a particular stumbling block for the field.

Lessnick holds monthly conference calls with other researchers who study Ewing's sarcoma. They share data freely, without fear of competition, he says. "There's so few of us and so much work to do."

Even after 25 years of frustration, he remains optimistic — hopeful that the attention and funding associated with the Moonshot programme could draw in researchers from other fields, such as epigenetics, with a fresh perspective. "Before, there wasn't a great way to build those bridges," he says.

During the frantic last years of Max's life, Ariella had directed the family foundation to focus on late-stage research: therapies close to the clinic, anything that might make it in time to save her son. Now, she intends to refocus its efforts to include more basic research. It's what the field needs most, she says: "And I now have the horrible luxury of time." ■



Heidi Ledford *is a senior reporter for* Nature *in London.*

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CORRECTION

The News Feature 'Cancer's cruel chimaeras' (*Nature* **543,** 608–611; 2017) should have said that mortuary workers, not paramedics, came to take away Max Ritvo's body. And the family foundation was directed to focus more of its research spending on basic science, not all of it.