Sickle Cells and a Tale of Two Hemoglobins

1 About 100,000 Americans and 5,000 Canadians are living with sickle cell disease – a severe blood disorder caused by defective hemoglobin, a crucial protein responsible for carrying oxygen.

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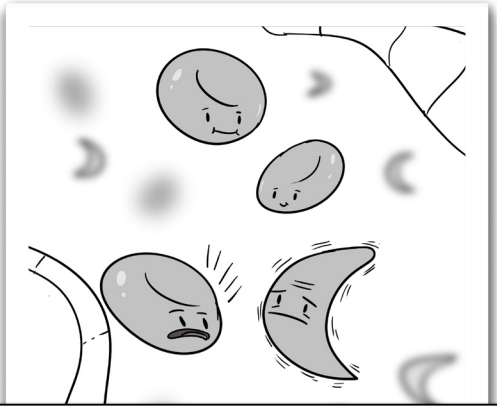

Normally, red blood cells are shaped like a donut, but the defective hemoglobin gives the cells a sickle shape, like a crescent moon. These sickle cells are stiff, sticky, and have a very short lifetime.

But humans have more than one hemoglobin gene – one is produced in developing fetuses, and after birth, babies quickly switch to the adult version. It's only mutations in parts of the adult version that causes the disease.

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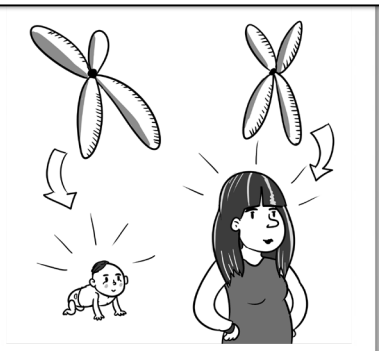
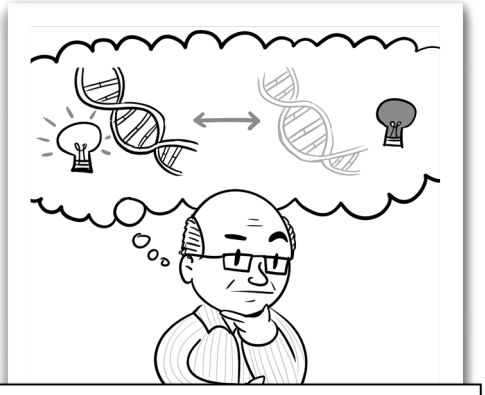
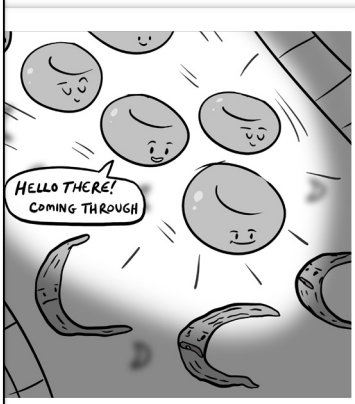
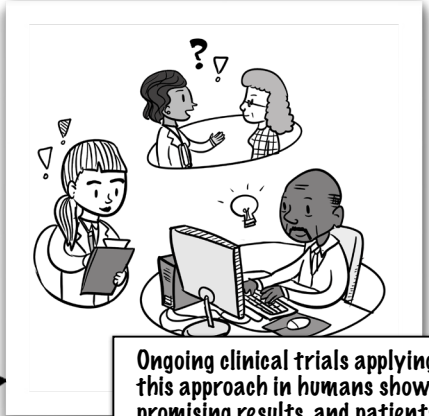




4 When Stuart Orkin discovered the molecular switch that normally turns off fetal hemoglobin production in babies, he saw an opportunity to turn this into a new treatment.

5 Orkin knew that by turning fetal hemoglobin back on, the healthy fetal version could replace the dysfunctional adult version and cure the disease. And now that he had identified the switch, he could manipulate it.





6 Ongoing clinical trials applying this approach in humans show promising results, and patients could soon be liberated from this debilitating disease

Art by Armin Mortazavi and text by Alison McAfee. October 2022

Sickle Cells and a Tale of Two Hemoglobins

A molecular switch is discovered that could lead to cures for many blood diseases.

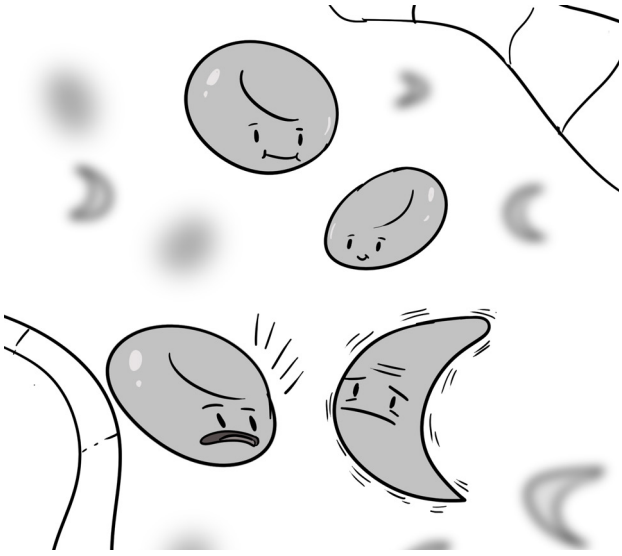
Written by Alison McAfee
Art by Armin Mortazavi

October 2022

Imagine being so familiar with the hospital that it feels like a second home – a home full of painful memories, tests, and bad news. You are scared of simple things, like swimming, because the shock of the cold water might send your body into an episode of the worst pain you have ever felt, lasting for days. You are constantly worried that you might get sick, and even a common cold could land you back in the hospital. None of this is a surprise, because since you were a youngster, you have been told that your life would probably be thirty years shorter than your peers and full of hospitalizations.

That is what it's like to live with **sickle cell disease**, an illness that affects about 5,000 Canadians and 75,000-100,000 Americans. Worldwide, the condition is most prevalent in people living in the so-called "malaria belt" – countries located in Central and South America, Sub-Saharan Africa, as well as South and Southeast Asia – with about 300,000 people born with the condition each year. But today, there is real hope for the many patients afflicted. New research by Stuart Orkin has laid the foundation for a suite of new therapeutic options. For this, Dr. Orkin has been recognized by a Canada Gairdner International Award in 2022.

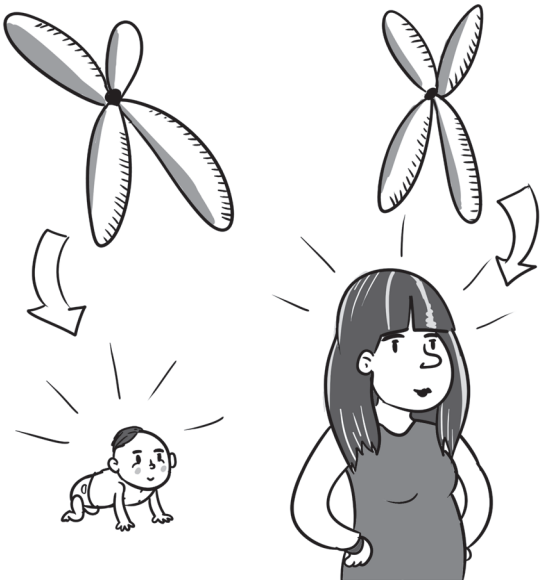
"**Hemoglobin** carries oxygen," says Orkin, the David G. Nathan professor of pediatrics at Harvard Medical School. "That's its main function, to carry oxygen and deliver it to the tissues." Hemoglobin is made up of not one protein, but two pairs of two different proteins known as the alpha and beta subunits. Sickle cell disease is caused by a single mistake, or mutation, in the beta subunits, which causes developing red blood cells to produce hemoglobin that is the wrong shape. This makes it glob together, or polymerize, inside the cells, turning them into the shape of a crescent moon instead of the normal squishy donut.



When the hemoglobin sticks together like this, it is not as good at binding to the oxygen. Even worse, the abnormal shape of the cells means that they are more likely to clog blood vessels, leading to painful episodes and other serious complications.

Luckily, humans have multiple types of hemoglobin which are normally produced at different

stages of development – embryonic, fetal, and adult hemoglobin – and it is only the mutations in components of the adult version that causes sickle cell disease. Similarly, a related disease called **beta-thalassemia** occurs when cells can't produce enough of one of the adult hemoglobin subunits.



Both of these diseases could be cured by using fetal hemoglobin as a stand-in, if only we knew how to make cells switch over to making it. Orkin, who has dedicated his career to studying diseases of the blood, discovered just that, which has led to several promising therapies.

Orkin describes his work as "solving a biological problem that has been out there for fifty years." Scientists and doctors have known about the different types of hemoglobin for a long time; indeed, as early as the 1940s, Janet Watson, a pediatric doctor in New York, noticed that very few children who later developed sickle cell disease were hospitalized as babies. She knew that, like all mammals, humans produce a different kind of hemoglobin as a fetus than as an adult, and that it takes some time after birth for the fetal version to be completely replaced – around when she would start to observe symptoms in her patients.

At a time when the exact cause of sickle cell disease was not yet known, Watson already suggested that perhaps newborns were protected from the disease because they still had their healthy fetal hemoglobin circulating in their blood. "The paucity of cases in infancy is surprising in view of the frequency of hospital admissions later in childhood," Watson wrote in 1948. "It seems likely, then, that fetal hemoglobin lacks the sickling properties of adult hemoglobin, thereby … partially protecting the infant in the first four months of life."

Later, Linus Pauling would famously describe that mutated adult hemoglobin was indeed what causes the disease, and Watson would be proven right. To astute clinicians like Watson, Orkin says, "we owe a huge debt."

But exactly how developing red blood cells switch from producing fetal hemoglobin to adult hemoglobin wouldn't be deciphered for decades. Soon after scientists invented the technology to sequence whole genomes – our complete genetic code – and scan those sequences to find relationships between genes and diseases, Orkin and his team applied that approach to look for a gene that might be responsible for the fetal-to-adult hemoglobin switch.

In a case of what Orkin describes as "dumb luck," his team and other researchers identified one gene, a lynch pin, that appeared to play a big role in how much fetal hemoglobin a person was able to produce. That gene, called ***BCL11A***, encodes what's known as a transcription factor, a type of protein that regulates gene expression, often in complex ways. Importantly, the researchers were able to show that by manipulating this specific gene in mice, they could adjust the amount of fetal hemoglobin produced, even in adult mice.

"We didn't know how many factors would be involved in the switch," Orkin says. "We thought there would be many factors with a complicated mechanism, but in mice, all we needed to do was get rid of *BCL11A* to reactivate the fetal form of hemoglobin and cure their sickle cell disease. That

Why would a human need two versions of the hemoglobin? The reason for having multiple versions, they think, is because a developing fetus needs to extract oxygen from the mother's blood, and so requires a hemoglobin that binds more strongly to oxygen.

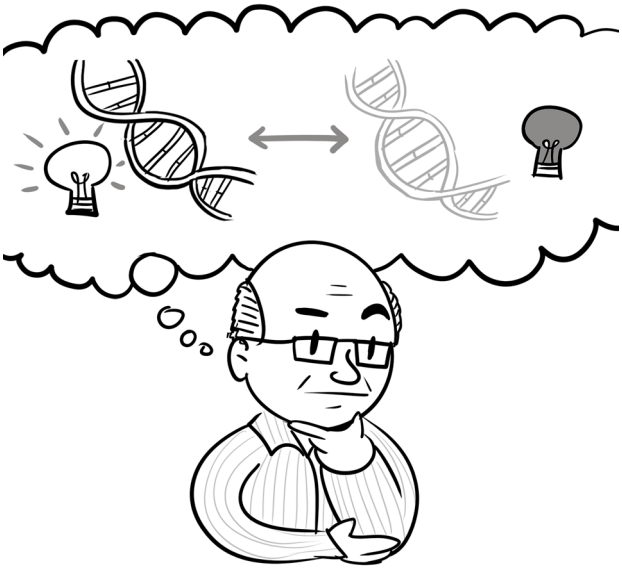
experiment showed us that if we could do something similar in a human, we would essentially have a cure."

And a new cure, we may soon have. Two companies, Vertex Pharmaceuticals and CRISPR Therapeutics, are collaborating to develop and commercialize a new therapy, based on Orkin's discoveries, that could be a huge improvement over bone marrow transplants. The companies have publicly announced that they are aiming for approval by the Food and Drug Administration by the end of 2022.

Currently, a patient with sickle cell disease can be cured by getting new blood stem cells – the cells within bone marrow that give rise to different types of blood cells, including red blood cells – and until recently, the only way to do this was to have a bone marrow transplant from a healthy donor. But this is an extremely invasive procedure, and that's only if a patient is lucky enough to find a donor with stem cells similar enough to their own for a transplant to be accepted. For most patients, the likelihood of finding a match is about one in ten.

But today, thanks to biotechnology, we now have the capability to extract stem cells from a patient with sickle cell disease, edit the *BCL11A* gene to turn back on fetal hemoglobin production, and give the cells back to the patient. This approach, which is an example of what's known as "gene therapy," greatly improves the probability that the cells will be accepted, since they came from the patient in the first place. The red blood cells that arise from the edited stem cells will have a healthy, normal shape, freeing the patient from disease.

One worry for any gene therapy is that artificially manipulating our genes might cause unexpected problems. But, before gene therapy trials for inducing fetal hemoglobin even began, nature answered that question for us: some rare people naturally keep producing fetal hemoglobin throughout their life, and they have no problematic conditions. Even a woman producing fetal hemoglobin as an adult is able to successfully carry a baby to term.



Vertex Pharmaceuticals and CRISPR Therapeutics have now treated over 70 patients with sickle cell disease or beta-thalassemia using their gene editing approach. This method is so successful in part because the editing does not have to be completely efficient – even partially turning on fetal hemoglobin is sufficient to prevent the sickling process and avoid the worst symptoms of the disease. However, the expected cost of such a therapy is astounding.

Though this service not yet commercialized, the price tag on a similar therapy, also expected to be approved for use in the US in 2022, is estimated to be in the range of $1-2 million USD. It might sound shocking, but Orkin thinks this isn't a crazy figure. "Economically, it's a good value. With the cost of care for affected people, their poor quality of life, lost wages, shorter lives, and lost productivity, $2 million is probably a bargain."

Rich countries will probably help pay for such a therapy, but the countries where the vast majority of patients with sickle cell disease live will not likely be able to afford it. Still, cheaper therapies could soon be on the horizon.

While extracting and editing stem cells has been the preferred gene therapy avenue because it enables tight control over which cells are modified, another potentially less invasive option would be to inject a patient with a gene editing formulation directly into their bone marrow. However, a worry

about such a systemic, injectable approach is that it is very hard to control which cells get edited, and editing the wrong ones could cause complications in other organs or tissues.

Fortunately, that's not a problem for *BCL11A*. There is one part of the *BCL11A* gene that specifically controls its production within developing red blood cells, and not elsewhere in the body, where it likely conducts other important jobs. Because of this, no matter which cells are edited, only the blood stem cells – and subsequently, the red blood cells – will see an effect.

The challenge, though, is being able to modify enough blood stem cells to cure the disease. These cells are rare and live in little niches within the bone marrow, so they are difficult to access with an injection. This approach is still in the research stage of development, but if feasible, an injectable option would make the therapy more accessible to patients without health insurance (or two million dollars).

The other alternative, Orkin says, are pills. "That's the value of knowing how the switch is regulated: if you know what the component parts are, you can target small molecules that work on them, and at least envision a therapy that could be given as a pill." Orkin recognizes that gene therapy is too difficult and expensive to relieve the burden of disease around the world, and pills would vastly improve accessibility. Finding such a drug, although challenging, is something he and his team are actively pursuing.

Whether all these therapies will be successful is still an open question, but one thing is clear: there are many options to investigate, thanks to the blueprint Orkin has drawn. Soon, a diagnosis of sickle cell anemia and related blood disorders may not feel like such crushing news, and new therapies could give more patients a longer, pain-free life.

While attending MIT, Orkin explains, "I thought I was going to become a physicist, but realized very quickly that of the 800 students in the class, 500 would be just as good a physicist as me." He then found a smaller pond in the burgeoning field of molecular genetics.

"It took a while to figure out what the right path was," he says. "Do what interests you. If it doesn't interest you, no amount of coaxing is going to get you very far. Science can be quite frustrating." Orkin persevered, and is now a recipient of the 2022 Canada Gairdner International Award.