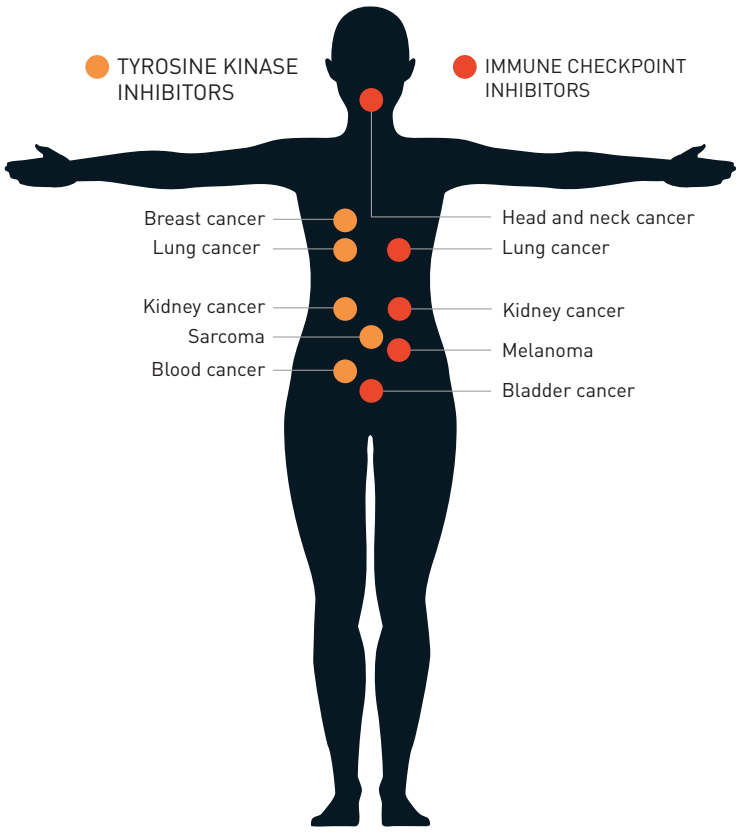
Two entirely new routes to effective cancer treatments

*Cancer has been an unwanted companion for as long as humankind has existed, and dealing with this insidious disease remains one of humanity's great challenges. The 2017 Sjöberg Prize is awarded to **James Allison** and **Tony Hunter**, whose research has laid the foundation for the development of effective cancer treatments.*

The World Health Organisation's statistics place cancer among the primary causes of sickness and death. The most recent figures, from 2012, show 14 million new cases and more than 8 million deaths. Additionally, the number of cases per year is expected to increase to 22 million over the next 20 years.

Radiation therapy and chemotherapy are blunt weapons, striking at all dividing cells and so damaging healthy tissue. Therefore, being able to encourage the immune system's own defensive cells to attack tumour cells is an attractive idea. The foundation for this type of treatment was laid by James Allison's research on the white blood cells known as T cells. These immune cells can effectively attack tumour cells after new pharmaceuticals have released the immune system's brakes.

Similarly, another attractive idea is using targeted inhibitors to turn off chains of events within the cell, ones that eventually cause cancer. This is where Tony Hunter's groundbreaking discovery of tyrosine phosphorylation has opened up for the development of an entirely new group of cancer pharmaceuticals.



Their discoveries are lifesavers. The laureates' discoveries have led to two entirely new classes of pharmaceuticals: tyrosinekinase inhibitors and immunecheckpoint inhibitors. These have proven effective in the treatment of many types of cancer.

These two research paths have a shared foundation in curiosity-driven basic research, where the researchers have identified important cellular mechanisms and their discoveries have led to new and effective cancer pharmaceuticals. However, while James Allison's research dealt with how cells interact with each other, Tony Hunter looked inside the cell to understand how they can be transformed into tumour cells.

Viruses showed the way

There are viruses, oncoviruses, that can make normal cells turn into tumour cells. Even in the 1950s, it was known that these viruses can add their own genes to cells. Researchers hoped that studying oncovirus genes would allow them to solve the mystery of what causes cancer. They wanted to use the fact that a virus has very limited genetic material, with few genes, compared to a human cell, which means that interesting genes and mechanisms should be easier to identify in a virus.

In the mid-1960s, Tony Hunter was studying biochemistry at Cambridge University, UK, working with the earliest forms of molecular biology. The genetic code had been uncovered, the structure of DNA mapped and the first protein structures were beginning to emerge. He realised that to fully comprehend how a cell or organism works, it is necessary to understand what the molecules that are active inside cells actually do.

In 1971, Tony Hunter moved to Salk Institute in the US and began to work on a polyomavirus, which is known to be able to cause tumours. During his time there, Tony Hunter learned in detail how DNA synthesis worked in the polyomavirus, but then he moved back to Cambridge. He continued to work on viruses while applying for jobs in the UK, but without success, so he returned to Salk Institute in February 1975. It was here that he would make the groundbreaking discoveries that paved the way to an entirely new form of cancer treatment.

The hunt for a new enzyme

Back in the US, he continued to study the polyomavirus. It was already known that a specific protein manufactured by the virus, T antigen, could turn normal cells into tumour cells. The question was what this protein actually did. Results from a research group studying a different virus gave him an idea – that the T antigen could be associated with kinase activities. Kinases can change other proteins by attaching a phosphate molecule to specific amino acids, which are proteins' building blocks. This change means that the shape of the protein, and thus its function, is changed. This change is called phosphorylation and is reversible; if the phosphate molecule is removed, the protein returns to its original shape.

A series of biochemical experiments demonstrated that the elusive T antigen really was linked to kinase activity, so Tony Hunter wrote a scientific article that was submitted to a journal, Cell. However, he left out one detail. At that time, it was known that two different amino acids could be phosphorylated, serine and threonine, but in his report he hadn't stated which of them was involved. Subsequently, he's written that it was important to get the article submitted quickly and that determining which amino acid was involved "could be done while the paper was being revised".

Old buffer solution led to a new discovery

To find out which amino acid had been phosphorylated, he used electrophoresis, in which amino acids pass through a cellulose gel. This method takes advantage of the way that different amino acids, over a given time, will move different distances through the gel when an electric current is added. The gel is surrounded by an acidic buffer solution and, in his experiment, he used one from a ready-mixed bottle. He analysed the unknown sample using the gel and the markers for phosphorylated serine and threonine, but the result was unexpected – the sample did not correspond to either serine or threonine.

However, his knowledge of biochemistry came to good use. There is a third amino acid that can potentially be phosphorylated, namely tyrosine. It would be sensational if he could prove it was tyrosine that had been phosphorylated, so he reran the experiment, but this time he made sure he mixed a new buffer solution. To his horror, he now obtained a completely different result – which now indicated phosphorylated threonine. He realised that the difference was the buffer solution,

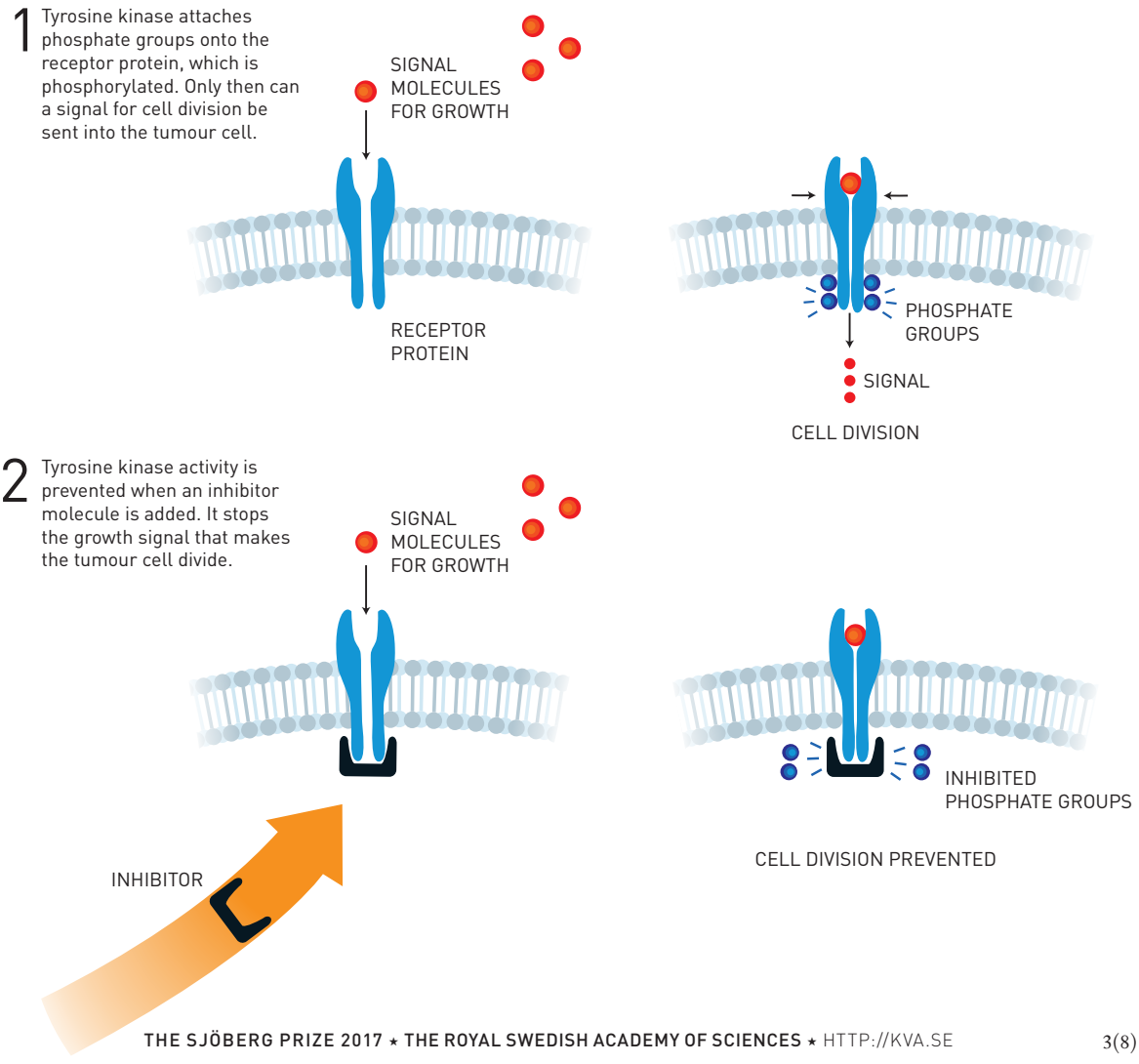
and left it to age before once again rerunning the experiment. Now he got the same result as the first time. It turned out that when the buffer had stood for a while its pH changed slightly, from 1.9 to 1.7, which led to better separation.

Turning off kinase resulted in new pharmaceuticals

The story about the old buffer solution and how the amino acid tyrosine could be phosphorylated spread rapidly in the world of research. Within a year of the study being published in Cell in December 1979, it was clear that the phosphorylation of tyrosine was an important regulatory system for numerous cellular processes, not least cell growth. In a follow-up study, published in the PNAS journal in 1980, Tony Hunter was also able to establish that tyrosine kinase activity was directly linked to normal cells turning into tumour cells.

Tyrosine phosphorylation and how the tyrosine kinase that performed this reaction could be linked to healthy cells becoming tumour cells was a paradigm shift. Researchers in the field investigated their own favourite proteins, and what emerged was that other proteins able to transform normal cells into tumour cells were also tyrosine kinases. By the end of the year, researchers had found four different types.

The idea arose of using this new knowledge to treat cancer. If a tyrosine kinase with the ability to transform normal cells into tumour cells could be turned off, this could be a way of preventing the cascade of events that lead to the formation of tumour cells. This approach gave rise to a new class of cancer pharmaceuticals, tyrosine kinase inhibitors (below).



One of the tyrosine kinase inhibitors for which Tony Hunter's discovery was the foundation is called imatinib; it has been followed by around twenty others, which block growth stimulating signals inside the cell. This effect is particularly dramatic for a form of blood cancer called chronic myeloid leukaemia, CML. Prior to this the prognosis had been extremely poor, and the available treatment was a bone marrow transplant, but patients can now live almost as long as the normal population. Tyrosine kinase inhibitors have also revolutionised the treatment of gastrointestinal stroma cell tumours, or GIST. The treatment has also had great benefits in non-small-cell lung carcinoma, even in cases in which the disease has spread.

The role of the immune system in cancer

However, we'll now leave Tony Hunter's studies of the inside of the cell and instead focus on how cells in the immune system cooperate and what these interactions mean for cancer.

The human immune system is extremely effective when it comes to finding and disarming things that differ from the body's normal tissues and cells, such as intruding microorganisms like viruses, bacteria and parasites. Cancer cells are a greater challenge. One major difference is that micro-organisms come from the outside, while cancer cells originate from the body's own cells. This makes it more complicated to identify cancer cells as harmful. The idea that the immune system should be able to find and remove tumours has been around for a long time – the immune system is very powerful and can, for example, reject an entire transplanted organ. The difficulty is being able to control the immune system's cells to obtain such an effect without damaging the rest of the body.

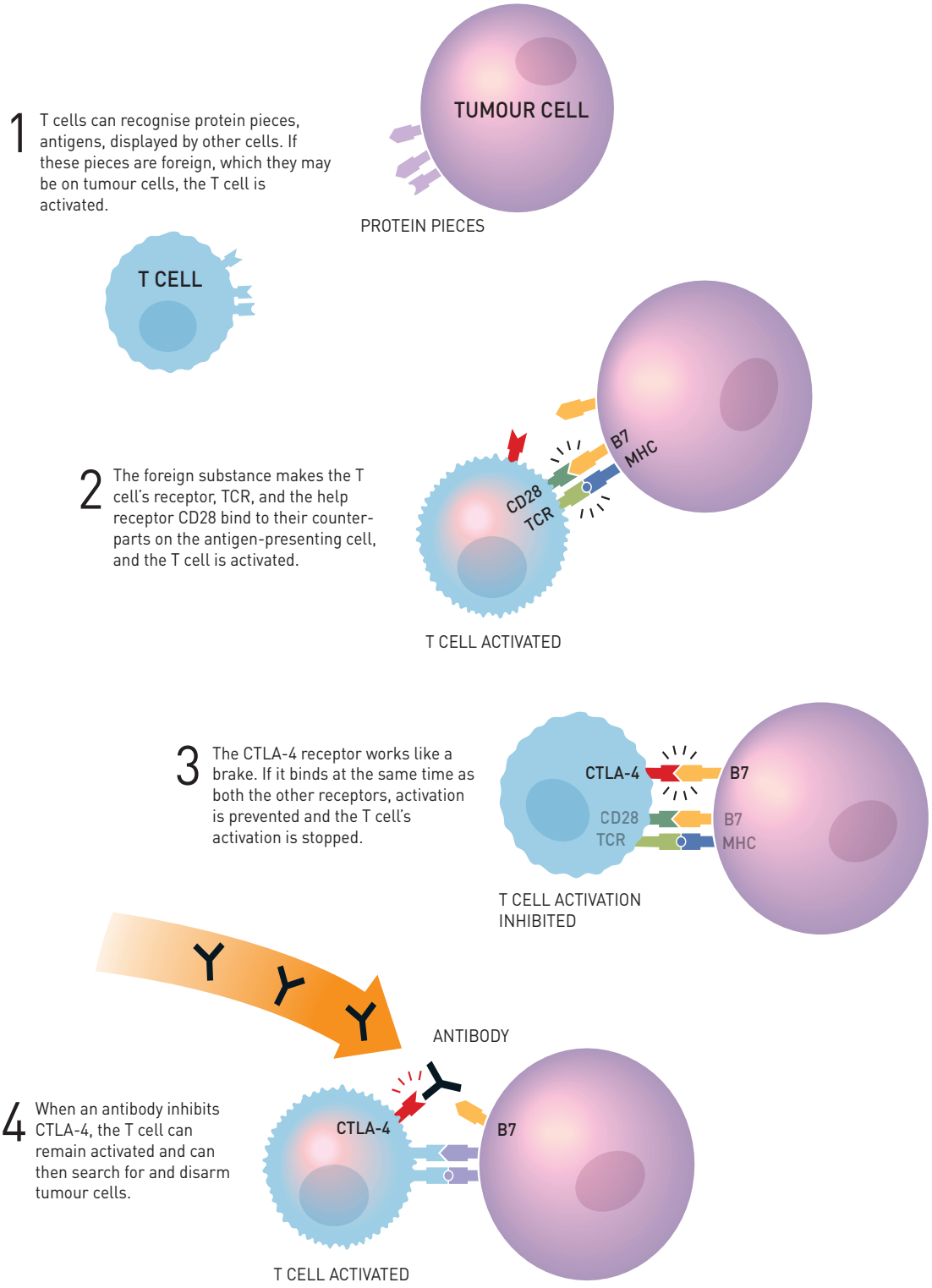
The immune system is built up of white blood cells with different functions. The ones called T cells learn to recognise things foreign to the body by assessing exposed structures on the surface of the cells. T cells can be activated when they discover microorganisms or tumorous cells, changing their function from being the immune system's guards to becoming warriors, aiming to neutralise the foreign cell.

Allison learned all about T cells

James Allison started his research career as a biochemist, but changed course and began studying the immune system at the start of the 1980s. His interest was focused on T cells and, at this time, the great challenge was to find the receptor on the T cell's surface that made it active: the T cell antigen receptor. James Allison became, in his own words, "totally hooked on understanding T cell activation".

He arrived at the University of California in Berkeley, USA, in the mid-1980s. New results from other researchers demonstrated that simply stimulating the T cell antigen receptor was not enough to obtain an activated immune response, but that an additional signal was needed. The question was what.

It turned out that the signal came from a help receptor on the T cell's surface, called CD28. This had to be stimulated at the same time as the antigen receptor – only then could the T cell become active. But there was another piece in the puzzle. This was CTLA-4, a T cell molecule that is found on active T cells, but which has a function that eluded the researchers. Some researchers, including James Allison, speculated that the CTLA-4-protein could have an inhibiting effect on the T cells' activation, something that was also proven correct. Researchers had now discovered a brake that prevented the immune system's activation (right).



Immune checkpoint inhibition. When the inhibiting signal from a surface molecule on a T cell, known as CTLA-4, is inhibited, it means that the immune system's brake is released. Only then can T cells use their full force and disarm tumour cells. Allison produced an antibody that attached to and inhibited CTLA-4 specifically. This has led to a new class of cancer pharmaceuticals –immune checkpoint inhibitors.

Easing off the brakes

Initially, James Allison did not regard himself as a cancer researcher, but rather as a researcher who conducted basic research and wanted to understand how T cells are controlled. However, he was involved in the issue of cancer, partly due to personal reasons: his mother, two maternal uncles and later also his brother were all victims of cancer. He'd seen, close up, the effects of the available treatments, radiation therapy and chemotherapy.

"So I did what I think any basic scientist should do: occasionally stop and think about the implications of your fundamental findings for application to human disease" he later wrote. What then struck him was that if T cells receive an activation signal from the T cell antigen receptor and CD28, but are simultaneously inhibited by CTLA-4, they do not have their full potential to take action against tumours, for example. However, if it was possible to block CTLA-4, the T cells' reaction may then be strong enough, he reasoned.

This was an attractive strategy. Firstly, it wasn't the tumour that was the treatment's target, but the immune system, which meant that the method could work as a universal cancer treatment. Secondly, it was not necessary to identify each tumour's fingerprint to be able to treat it; instead, it would be enough to release the immune system so that its cells could attack the tumour on their own.

Convincing experiments on mice

He has written about the first trials in 1994, in which mice in specific cages received the antibody that blocked CTLA-4, while other mice received inactive injections. When his colleague showed him the first results, he didn't believe them: the mice that had received the antibody displayed reduced tumours, while the other mice died. "It was too good to be true," he later wrote. He therefore repeated the experiment over Christmas. Now it was conducted blindly, which meant that a colleague injected the mice, while Allison studied them without knowing which mice had received which treatment. He describes how, over a two-week period, there were no differences; the tumours grew in all the mice, he noted with disappointment. But then, suddenly, something happened. In one group of mice the tumours started to shrink, and then entirely disappeared. They turned out to be the mice that had been injected with the antibody that blocks CTLA-4.

He had actually found a way to activate the immune system and get its cells to forcefully attack the tumour cells. In 1996, James Allison published his groundbreaking results in Science.

The road to clinical benefits

This success made Allison eager to produce a type of antibody that could be tested on humans. He spent a great deal of time trying to convince various pharmaceutical companies to take on the project, but there was little interest. Finally, a small biotech company was bold enough to try. In 2000, one inhibiting antibody had been produced, although it would take over a decade to refine this discovery into a pharmaceutical. Allison was an active participant on this journey.

Eleven years after he first published the results of his ground-breaking experiment, James Allison met one of the patients who had been treated with a CLTA-4 inhibiting antibody early on – from having had a couple of months left to live, several years later the woman was entirely symptom-free.

And, in 2010, astounding study results could be reported: for the first time, there was significantly increased median survival in patients with metastatic melanoma. If they received the antibody they were able to survive almost twice as long as the patients in the control group. The most striking

finding was that almost a quarter of the participants who received the antibody survived for at least two years, meaning that their life expectancy had quadrupled. Further follow-ups have shown that around one in five people enjoy long-term survival with the new treatment.

In 2011, the ipilimumab antibody was approved in the USA and in Europe for the treatment of metastatic melanoma. This has been followed by antibodies that target other inhibiting signals in the immune system; these are known as immune checkpoint inhibitors. This class of pharmaceuticals has been of decisive importance for metastatic melanoma. If ipilimumab is combined with another immune checkpoint inhibitor, the disease can be kept under control for a longer period in around 40 per cent of patients. In some patients, treatment with this type of pharmaceutical has shown good effects on numerous other forms of cancer. A few examples are non-small-cell lung carcinoma, kidney cancer, bladder cancer and head and neck cancer. Clinical trials are underway for many other forms of cancer.

Their discoveries are lifesavers

The classes of pharmaceuticals that Tony Hunter's and James Allison's research resulted in are now used to treat cancer in patients around the world. However, tumour cells may develop resistance to pharmaceuticals. Other pharmaceuticals in the tyrosine kinase inhibitor group have been developed and can be used when the first choice of pharmaceutical no longer works. With the aim of obtaining even better effects from immune checkpoint inhibitors, trials are being conducted in which this type of treatment is combined with chemotherapy, for example, or with specific vaccination against the tumour cells.

Both these classes of pharmaceuticals have great benefits, but challenges remain. One is improving the understanding of why not all treatments are effective for all patients. Another challenge is that even these innovative treatments, as with other cancer treatments, have side-effects. More research is needed to be able to help people afflicted by cancer, and James Allison and Tony Hunter remain active in this important work.

LINKS AND FURTHER READING

More information on this year's prize is available at the Academy's website: *http://kva.se/sjobergprize*

Popular science articles

Interview with Tony Hunter, Journal of Cell Biology, May 2008.
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Checkpoints. James P. Allison. Cell 162, September 10, 2015.
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THE LAUREATES

Photo: The University of Texas MD Anderson Cancer Center

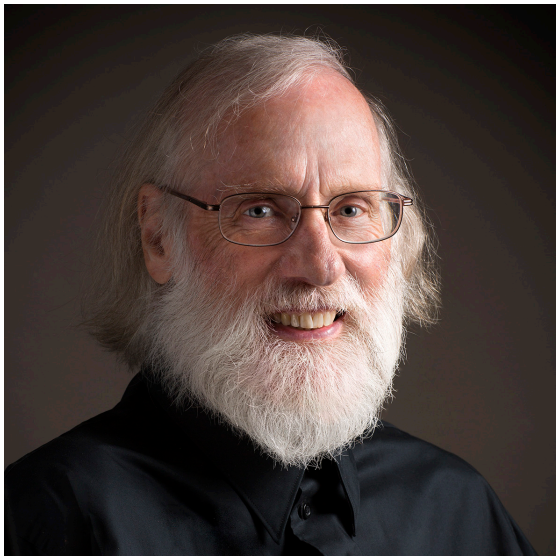

Photo: Joe Belcovson, Salk Institute for Biological Studies

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