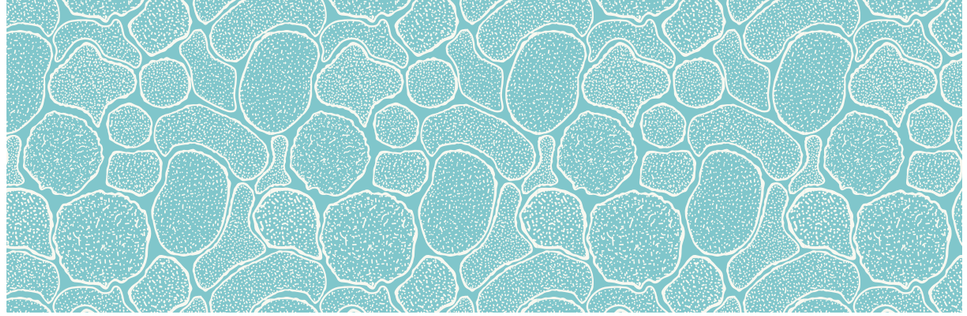
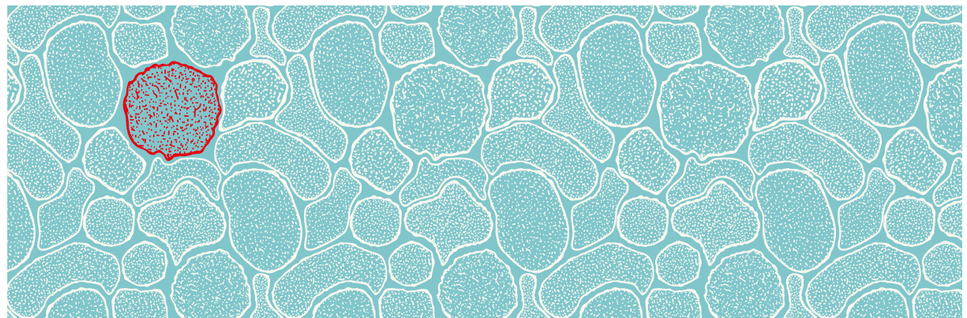


WHY DON'T IMMUNE CELLS FIGHT CANCER?

Using cutting-edge genomics and creativity to stop immune cells from switching off upon entering tumours

By Dan Rubinstein
Photographs by Hadas Parush



Daniel Kirschenbaum's wide-ranging scientific curiosity found a home in Ido Amit's lab at the Weizmann Institute of Science. The research group is a leader in the development of single-cell genomic analysis — a method for isolating individual cells and quantifying their genetic composition to better understand their condition and properties.

There are two ways to look at brain cancer, suggests Daniel Kirschenbaum, a neuropathologist and immunology researcher at the Weizmann Institute of Science. The disease is traditionally described as the uncontrolled growth of cells, but it can also be seen as the failure of the immune system to identify and attack these cells.

"Our immune system is equipped to fight cancer," says Kirschenbaum, who followed his wide-ranging scientific curiosity from a hospital in Switzerland to a trailblazing immunology lab at Weizmann as an Azrieli International Postdoctoral Fellow. "We have cells that are tailored to do this. But if they fail to do so in the early stages, the cancer just grows."

In fact, in glioblastomas, one of the most common and aggressive types of brain tumour, nearly half of the mass is made up of immune cells. If somebody with cancer gets the flu, immune cells flowing in their blood will usually fight off the virus. "But cells that are supposed to fight the tumour go inside, switch off and essentially become harmful," says Kirschenbaum. "They're supporting a tumour with factors and nutrients.

"If we can stop this, we can help people," he continues. "We can treat their cancer and improve and prolong their lives."

Kirschenbaum was interested in a broad spectrum of science while growing up in Budapest, from chemistry and biology to psychiatry and genetics. But mostly, he was fascinated by the brain. He wondered how physical changes to the "hardware" of an organ you can touch led to cognitive phenomena, such as the changes to personality and perception experienced by stroke patients. He was curious about how people developed free will or the illusion thereof — the capacity to make decisions and perform actions — as their brains developed. Ditto the emergence of language in individuals.

Despite these diverse interests, becoming a doctor felt like a pragmatic choice. After medical school in Hungary, however, he didn't like the clinical aspect of his job at a hospital in Germany. So Kirschenbaum shifted to a neuropathology residency at University Hospital Zurich, where he enjoyed analyzing neurosurgical samples to diagnose tumours and neurodegenerative diseases and completed a PhD. Afterwards, unsure of his next move, he went on holiday in Israel and arranged informal meetings with several research groups.

Immunologist Ido Amit's lab at Weizmann, one of the places where Kirschenbaum gave a talk, is a forerunner in the development of single-cell genomic analysis — a method for isolating individual cells and quantifying their genetic composition to better understand their condition and properties. This technique, which has been revolutionizing immunology for the past decade, can be applied to cells from cancerous tumours and used to create maps of cell states: some healthy, some diseased, some in transition.

Kirschenbaum didn't know much about immunogenetics when he visited Israel, but Amit offered him a postdoctoral position nonetheless. "Many of our group's members don't come from our main areas of research," says Amit, who was struck by Kirschenbaum's passion for technologies that can be used to discover fundamental biology, as well as his intellect, leadership traits and open-mindedness. "As time goes on, people who are in a particular field tend to put themselves in a box, while people who come from the outside bring fresh ideas and perspectives. I like that."

"It was something to switch on my curiosity and excitement," Kirschenbaum says about accepting Amit's offer and moving to Israel in June 2020. "It was a very intuitive decision, and usually these decisions work the best in my life."

Since then, in close collaboration with his lab colleagues, two of whom are also Azrieli Fellows (see "Science as a Team Sport," page 27), Kirschenbaum has been attempting to answer a confounding question: What makes functional immune cells "switch off" when they enter a tumour? What happens after one hour? After two hours? Three? And so on. "To understand this," he explains, "you need to introduce a temporal dimension into studying a dynamic process that changes over time. But when we do single-cell analysis, we just take a snapshot. We don't know what came first and what came second. It's just a mixture."

Kirschenbaum's focus at Weizmann is a method to add "time signatures" to maps of cell states. He calls this technique "zman-seq": "zman" is the Hebrew word for time, while "seq" is short for sequencing. Still a work in progress but already quite promising, it's used to show precisely when cells change states and which genes are responsible for the transition from functional in the blood to dysfunctional in a tumour. Identifying these pathways will allow researchers to develop and test drugs that can alter the trajectory of immune cells, so they do what they are supposed to do upon encountering malignant cells.

"Daniel's technology to move from snapshots to movies allows us to see where the 'bad character' is," says Amit. "Now we can come up with innovative ideas for therapies that prevent that character from changing the immune cell."

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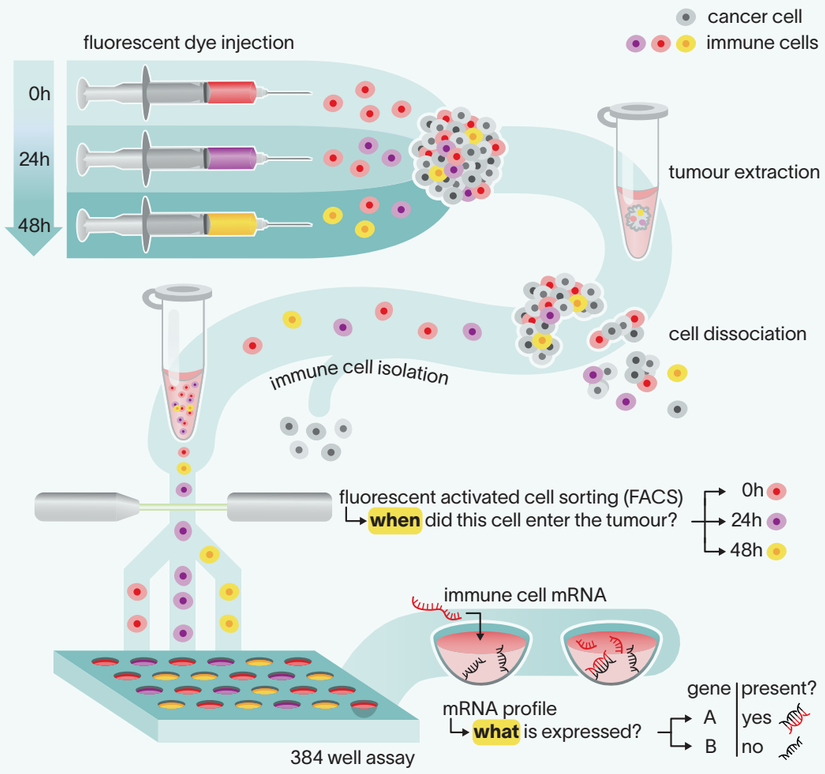
CELLULAR SLEUTHING

After inducing brain tumours in mice, Daniel Kirschenbaum injects fluorescent dyes of different colours into their vascular systems over several days. Immune cells in the blood pick up these colours and enter the tumours. Kirschenbaum then extracts the tumours and dissociates the cells, a three-hour lab protocol that involves centrifuges, filters and digestive enzymes. The cells are then put through a FACS machine, which deposits single cells into 384 individual wells on a plastic plate and records their fluorescence values, allowing him to decode the time signature of each cell. Since each of the wells is pre-coded with genetic barcodes, he can determine which cell each molecule in the experiment is associated with. This genomic data (which can also be described as the mRNA profile of each cell) is clustered and analyzed. Understanding precisely when immune cells change states and which genes are responsible for the transition from functional in the blood to dysfunctional in a tumour will allow researchers to develop and test drugs that help immune cells do what they are supposed to do.

Diagram by
Sarah Nersesian/Designs That Cell



"The problems that really turn on my curiosity are very fundamental, unsolved scientific challenges that require a technical solution," says Kirschenbaum. "I want to help solve big issues. That's what keeps me running. Ido's lab is like this: tackling fundamental problems, resulting in methods that people can start to use around the scientific community."

For more than a year, Kirschenbaum tried various techniques to overlay temporal information atop the molecular data collected from conducting single-cell analysis of immune cells inside tumours. Finally, he zeroed in on an approach that's conceptually fairly simple but technically rather complex.

Working with a cohort of mice, he induces tumours by injecting a few specific cells into their brains. Two weeks later, he injects a coloured fluorescent dye into the vascular systems of the mice. The immune cells in the blood pick up this colour — say, red — and enter the tumour. The next day, he injects a different colour — green — and new immune cells take on *that* colour and enter the tumour, along with some red and red-green cells. On day three, Kirschenbaum introduces a third colour, and the cells that enter the tumour are one of the three colours or some combination thereof. "If you follow these steps, you have cells in the tumour with different colour combinations," he explains, "and by separating the cells by colour, you can understand when they entered the tumour."

To differentiate by colour, Kirschenbaum first extracts the tumour and dissociates the cells, a three-hour lab protocol that involves centrifuges, filters and the application of digestive enzymes to disintegrate the matrix around the cells so individual cells can be isolated. The cells are then put through a FACS (fluorescence-activated cell sorting) machine, which deposits single cells into 384 individual wells on a plastic plate and records their fluorescent values, allowing him to decode the colour or time signature of each cell. Next, using enzymes that can read an RNA sequence, he amplifies the genetic material of each cell to obtain a stronger signal and puts them into a next-generation sequencer. Since each of the 384 wells is pre-coded with genetic barcodes, he can determine which cell each molecule in the experiment is associated with in addition to its time signature. This transcriptomic or genomic data (which can also be described as the mRNA profile of each cell) is clustered and analyzed by computational scientists in Amit's lab, and Kirschenbaum and his colleagues interpret the results together.

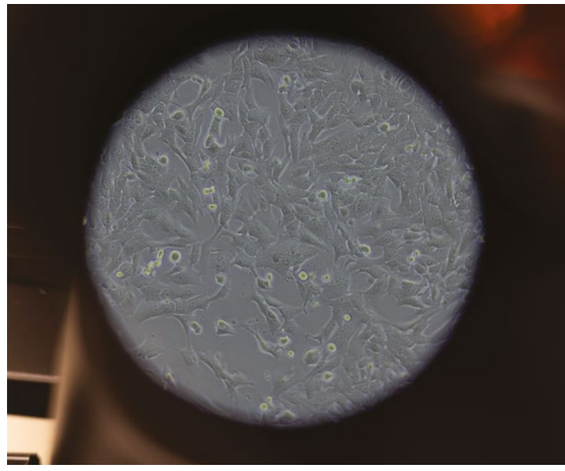
"The fluorescent profile of the cells represents their exposure to the temporally defined injections of fluorescent dyes," he explains. "Using this method, we know the transcriptomic profile and the temporal profile of every single cell we sorted. Based on the temporal signature we can now order the cells in time. This way we see how the transcriptomic profiles of single cells change in sequence across time."

Parallel to this research at Weizmann, two different drugs have shown in tests the potential to modulate how immune cells interact with cancer cells, and one is close to clinical trial. But researchers don't understand exactly how they work, says Kirschenbaum. Using the zman-seq method, "we'll be able to see — beautifully — how certain cells respond to this drug across time," he says. "We'll be able to draw arrows and look at how cells change compared to the non-treated ones. We'll be able to see and understand pathways. This is very concrete." If an immune cell enters a tumour and a certain gene is not expressed, for example, and several hours later that gene is highly expressed, researchers will be able to discern its importance.

"It provides a fresh approach to developing better therapies," Amit says about zman-seq's potential to illuminate the environment in which immune cells become pathological. "Daniel's technology to move from snapshots to movies allows us to see where the 'bad character' is. Now we can come up with innovative ideas for therapies that prevent that character from changing the immune cell."

These days, while writing a paper about his method, Kirschenbaum is also trying to make it more effective. He'd rather use genetic barcodes than colours to time-stamp immune cells, because the range of useable colours is limited, whereas the number of genetic sequences is endless, plus these labels would last longer and provide more precise, higher-resolution pictures.

Zman-seq and any iterations that emerge can be used with all tumours, not just brain cancer, and this research fits with the overarching goal of developing novel immunotherapies in Amit's lab. Yet Kirschenbaum remains somewhat restless. The work satisfies his curiosity. He finds it very stimulating and inspiring. "But at the same time," he says, "it makes me think about next steps and new directions."



Kirschenbaum (top) and his lab mates, including Azrieli Graduate Studies Fellow Yonatan Katzenelenbogen (bottom), are focused on tackling fundamental problems and developing methods that people can start to use around the scientific community.

Kirschenbaum (centre) enjoys brainstorming with Truong San Phan (left), an Azrieli International Postdoctoral Fellow, and Katzenelenbogen (right), a PhD student and Azrieli Graduate Studies Fellow.

Science as a Team Sport

When Daniel Kirschenbaum joined Ido Amit's immunotherapy lab at the Weizmann Institute of Science, he joined a bustling group of nearly three dozen researchers who use single-cell genomic technologies to better understand diseases such as cancer, Alzheimer's and multiple sclerosis. But even though Kirschenbaum had significant lab experience at University Hospital Zurich, where he worked as a neuropathologist before moving to Israel as an Azrieli International Postdoctoral Fellow, he still needed somebody to show him the ropes. Yonatan Katzenelenbogen, a PhD student and Azrieli Graduate Studies Fellow who had been in Amit's lab for two years already, embraced that role.

"This is a big lab, which can be hard to navigate," says Katzenelenbogen, whose research focuses on developing genomic technologies to diagnose and provide precision medicine for cancer patients. "Since we routinely work with a broad range of tools and models, it's necessary to master a large number of techniques, lab-related protocols and devices. As part of his training, I showed Daniel how to use our flow cytometry device, which sorts cells for further processing, and how to use our unique molecular techniques to create a full transcriptomic RNA library from each individual cell."

Not only did Katzenelenbogen support his colleague on the technical side of things, the two creative thinkers also bounced ideas off one another, which helped Kirschenbaum work toward his zman-seq method. "I can come up with ideas very quickly," says Kirschenbaum. "If you give me a problem, a technical issue, they just come. But you have to combine generating ideas with trashing them, because if you try all of your ideas you won't get anywhere. Even if you have very interesting ideas, you have to see the flaws immediately, which happened through my discussions with Yonatan.

"You cannot swim without water," adds Kirschenbaum. "You need a counterforce, someone to give resistance, so it stimulates you to think critically."

About eight months after Kirschenbaum started at Weizmann, Truong San Phan, an Azrieli International Postdoctoral Fellow from Germany, joined the lab. "Daniel was doing large experiments and needed a hand," says Phan, whose research focuses on trying to find out what makes chronic inflammatory diseases chronic. "I helped with any kind of work that had to be done: handling samples, handling tissue, isolating brain cells." The pair talked while they worked, easing the tedium of the manual labour and providing further motivation for both.

"The questions we're asking in this type of research are getting more complicated; the challenges are getting bigger and bigger," says Phan. "Projects cannot be done by one person. It's always a team effort. And the harder the question, the more people we need."

"Science is not an individual sport, like tennis," affirms Amit. "It's more like football, where you can only be great if you learn to work as a team and people are passionate about one another's research. This is the DNA of our lab. We focus not on prizes but on achievements. If you think of big human achievements — like landing on the moon — most are the result of a team effort. If we want to achieve great things, we can only do it as a team."

'I can come up with ideas very quickly. If you give me a problem, a technical issue, they just come. But you have to combine generating ideas with trashing them, because if you try all of your ideas you won't get anywhere.'

The atmosphere in Amit's lab can be credited, in part, to the culture that he nurtures; most of his students and postdocs have two or three or even four projects on the go, so there are what Katzenelenbogen calls "overlapping dependencies," reducing competition. "That's super important in our field," says Katzenelenbogen. "It increases creativity, because we're always brainstorming with each other. This creates more than work-related collaboration. It creates friendships that make it really fun to arrive at work in the morning, which is essential for success."

Moreover, this teamwork is emblematic of the non-hierarchical, merit-based system that's typical of research in Israel. It doesn't matter whether somebody is a PhD student: they can still show a postdoc what to do and get into a deep conversation about science. "Then, after you discuss a big idea, there are no excuses — you just do it," says Kirschenbaum. "If there's a reagent missing, you just go to another lab one floor above and ask if they have it. There's no need to postpone the work while waiting for your supplies to arrive. If you want to do something, you just do it." ▲●■