To stay young, kill zombies

Killing off cells that refuse to die on their own has proved a powerful anti-ageing strategy in mice. Now it's about to be tested in humans.

BY MEGAN SCUDELLARI

Jan van Deursen was baffled by the decrepit-looking transgenic mice he created in 2000. Instead of developing tumours as expected, the mice experienced a stranger malady. By the time they were three months old, their fur had grown thin and their eyes were glazed with cataracts. It took him years to work out why: the mice were ageing rapidly, their bodies clogged with a strange type of cell that did not divide, but that wouldn't die.[1]

That gave van Deursen and his colleagues at Mayo Clinic in Rochester, Minnesota, an idea: could killing off these 'zombie' cells in the mice delay their premature descent into old age? The answer was yes. In a 2011 study[2], the team found that eliminating these 'senescent' cells forestalled many of the ravages of age. The discovery set off a spate of similar findings. In the seven years since, dozens of experiments have confirmed that senescent cells accumulate in ageing organs, and that eliminating them can alleviate, or even prevent, certain illnesses (see 'Becoming undead'). This year alone, clearing the cells in mice has been shown to restore fitness, fur density and kidney function[3]. It has also improved lung disease[4] and even mended damaged cartilage[5]. And in a 2016 study, it seemed to extend the lifespan of normally ageing mice[6].

"Just by removing senescent cells, you could stimulate new tissue production," says Jennifer Elisseeff, senior author of the cartilage paper and a biomedical engineer at Johns Hopkins University in Baltimore, Maryland. It jump-starts some of the tissue's natural repair mechanisms, she says.

ILLUSTRATION BY PAWEŁ JOŃCA

This anti-ageing phenomenon has been an unexpected twist in the study of senescent cells, a common, non-dividing cell type first described more than five decades ago. When a cell enters senescence — and almost all cells have the potential to do so — it stops producing copies of itself, begins to belch out hundreds of proteins, and cranks up anti-death pathways full blast. A senescent cell is in its twilight: not quite dead, but not dividing as it did at its peak.

Now biotechnology and pharmaceutical companies are keen to test drugs — known as senolytics — that kill senescent cells in the hope of rolling back, or at least forestalling, the ravages of age. Unity Biotechnology in San Francisco, California, co-founded by van Deursen, plans to conduct multiple clinical trials over the next two-and-a-half years, treating people with osteoarthritis, eye diseases and pulmonary diseases. At Mayo, gerontologist James Kirkland, who took part in the 2011 study, is cautiously beginning a handful of small, proof-of-concept trials that pit senolytic drugs against a range of age-related ailments. "I lose sleep at night because these things always look good in mice or rats, but when you get to people you hit a brick wall," says Kirkland.

No other anti-ageing elixir has yet cleared that wall, and for a few good reasons. It's next to impossible to get funding for clinical trials that measure an increase in healthy lifespan. And even as a concept, ageing is slippery. The US Food and Drug Administration has not labelled it a condition in need of treatment.

Still, if any of the trials offer "a whiff of human efficacy", says Unity's president, Ned David, there will be a massive push to develop treatments and to better understand the fundamental process of ageing. Other researchers who study the process are watching closely. Senolytics are "absolutely ready" for clinical trials, says Nir Barzilai, director of the Institute for Aging Research at the Albert Einstein College of Medicine in New York City. "I think senolytics are drugs that could come soon and be effective in the elderly now, even in the next few years."

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THE DARK SIDE

When microbiologists Leonard Hayflick and Paul Moorhead coined the term senescence in 1961, they suggested that it represented ageing on a cellular level. But very little research was done on ageing at the time, and Hayflick recalls people calling him an idiot for making the observation. The idea was ignored for decades.

Although many cells do die on their own, all somatic cells (those other than reproductive ones) that divide have the ability to undergo senescence. But, for a long time, these twilight cells were simply a curiosity, says Manuel Serrano of the Institute for Research in Biomedicine in Barcelona, Spain, who has studied senescence for more than 25 years. "We were not sure if they were doing something important." Despite self-disabling the ability to replicate, senescent cells stay metabolically active, often continuing to perform basic cellular functions.

By the mid-2000s, senescence was chiefly understood as a way of arresting the growth of damaged cells to suppress tumours. Today, researchers continue to study how senescence arises in development and disease. They know that when a cell becomes mutated or injured, it often stops dividing — to avoid passing that damage to daughter cells. Senescent cells have also been identified in the placenta and embryo, where they seem to guide the formation of temporary structures before being cleared out by other cells.

But it wasn't long before researchers discovered what molecular biologist Judith Campisi calls the "dark side" of senescence. In 2008, three research groups, including Campisi's at the Buck Institute for Research on Aging in Novato, California, revealed that senescent cells excrete a glut of molecules — including cytokines, growth factors and proteases — that affect the function of nearby cells and incite local inflammation[7,8,9]. Campisi's group described this activity as the cell's senescence-associated secretory phenotype, or SASP[7]. In recent unpublished work, her team identified hundreds of proteins involved in SASPs.

In young, healthy tissue, says Serrano, these secretions are probably part of a restorative process, by which damaged cells stimulate repair in nearby tissues and emit a distress signal prompting the immune system to eliminate them. Yet at some point, senescent cells begin to accumulate — a process linked to problems such as osteoarthritis, a chronic inflammation of the joints, and atherosclerosis, a hardening of the arteries. No one is quite sure when or why that happens. It has been suggested that, over time, the immune system stops responding to the cells.

Surprisingly, senescent cells turn out to be slightly different in each tissue. They secrete different cytokines, express different extracellular proteins and use different tactics to avoid death. That incredible variety has made it a challenge for labs to detect and visualize senescent cells. "There is nothing definitive about a senescent cell. Nothing. Period," says Campisi.

In fact, even the defining feature of a senescent cell — that it does not divide — is not written in stone. After chemotherapy, for example, cells take up to two weeks to become senescent, before reverting at some later point to a proliferating, cancerous state, says Hayley McDaid, a pharmacologist at Albert Einstein College of Medicine. In support of that idea, a large collaboration of researchers found this year that removing senescent cells right after chemotherapy, in mouse models for skin and breast cancer, makes the cancer less likely to spread[10].

The lack of universal features makes it hard to take inventory of senescent cells. Researchers have to use a large panel of markers to search for them in tissue, making the work laborious and expensive, says van Deursen. A universal marker for senescence would make the job much easier — but researchers know of no specific protein to label, or process to identify. "My money would be on us never finding a senescent-specific marker," Campisi adds. "I would bet a good bottle of wine on that."

Earlier this year, however, one group did develop a way to count these cells in tissue. Valery Krizhanovsky and his colleagues at the Weizmann Institute of Science in Rehovot, Israel, stained tissues for molecular markers of senescence and imaged them to analyse the number of senescent cells in tumours and aged tissues from mice[11]. "There were quite a few more cells than I actually thought that we would find," says Krizhanovsky. In young mice, no more than 1% of cells in any given organ were senescent. In two-year-old mice, however, up to 20% of cells were senescent in some organs.

But there's a silver lining to these elusive twilight cells: they might be hard to find, but they're easy to kill.

OUT WITH THE OLD

In November 2011, while on a three-hour flight, David read van Deursen and Kirkland's just-published paper about eliminating zombie cells. Then he read it again, and then a third time. The idea "was so simple and beautiful", recalls David. "It was almost poetic." When the flight landed, David, a serial biotech entrepreneur, immediately rang van Deursen, and within 72 hours had convinced him to meet to discuss forming an anti-ageing company.

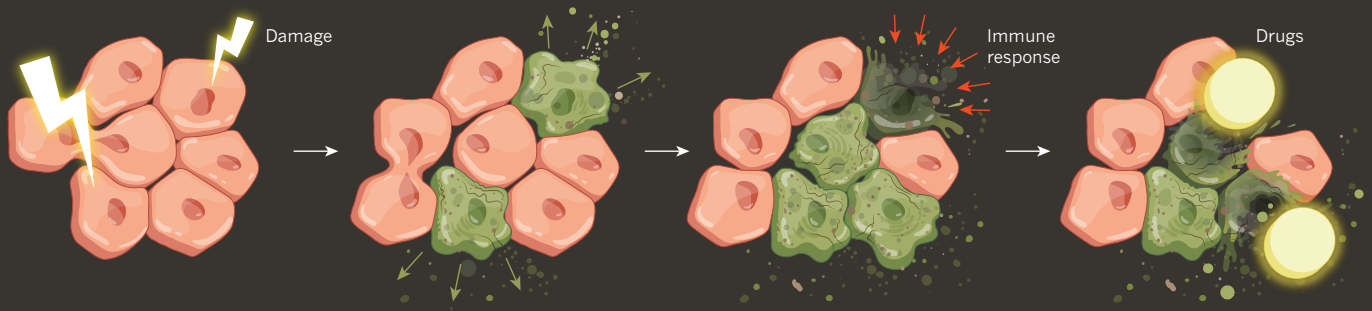
Kirkland, together with collaborators at the Sanford Burnham Medical Research Institute in La Jolla, California, initially attempted a high-throughput screen to quickly identify a compound that would kill senescent cells. But they found it to be "a monumental task" to tell whether a drug was affecting dividing or non-dividing cells, Kirkland recalls. After several failed attempts, he took another tack.

Senescent cells depend on protective mechanisms to survive in their 'undead' state, so Kirkland, in collaboration with Laura Niedernhofer and others from the Scripps Research Institute in Jupiter, Florida, began seeking out those mechanisms. They identified six signalling pathways that prevent cell death, which senescent cells activate to survive[12,13].

Then it was just a matter of finding compounds that would disrupt

BECOMING UNDEAD

Damage or disease can lead a cell down the path to senescence. Scientists are still finding out how cells behave once they get there — and how to get rid of them.



THE TRIGGER
Damage or disease, along with signals from other cells during development, can induce senescence.

SPITTING OUT SIGNALS
Once senescent, cells stop dividing and belch out proteins such as cytokines, which attract immune molecules.

CLEAR OR CLOG
The immune system can kill senescent cells and allow tissue to regenerate. But in diseased or ageing tissue, senescent cells build up.

ZOMBIE KILLERS
Drugs in development turn off a cell's survival tricks to clear senescent cells from joints, blood vessels or the eye.

those pathways. In early 2015, the team identified the first senolytics: an FDA-approved chemotherapy drug, dasatinib, which eliminates human fat-cell progenitors that have turned senescent; and a plant-derived health-food supplement, quercetin, which targets senescent human endothelial cells, among other cell types. The combination of the two — which work better together than apart — alleviates a range of age-related disorders in mice[14].

Ten months later, Daohong Zhou at the University of Arkansas for Medical Sciences in Little Rock and his colleagues identified a senolytic compound now known as navitoclax, which inhibits two proteins in the BCL-2 family that usually help the cells to survive[15]. Similar findings were reported within weeks by Kirkland's lab[16] and Krizhanovsky's lab[17].

By now, 14 senolytics have been described in the literature, including small molecules, antibodies and, in March this year, a peptide that activates a cell-death pathway and can restore lustrous hair and physical fitness to ageing mice[3].

So far, each senolytic kills a particular flavour of senescent cell. Targeting the different diseases of ageing, therefore, will require multiple types of senolytics. "That's what's going to make this difficult: each senescent cell might have a different way to protect itself, so we'll have to find combinations of drugs to wipe them all out," says Niedernhofer. Unity maintains a large atlas documenting which senescent cells are associated with which disease; any weaknesses unique to given kinds of cell, and how to exploit those flaws; and the chemistry required to build the right drug for a particular tissue. There is no doubt that for different indications, different types of drug will need to be developed, says David. "In a perfect world, you wouldn't have to. But sadly, biology did not get that memo."

For all the challenges, senolytic drugs have several attractive qualities. Senescent cells will probably need to be cleared only periodically — say, once a year — to prevent or delay disease. So the drug is around for only a short time. This type of 'hit and run' delivery could reduce the chance of side effects, and people could take the drugs during periods of good health. Unity plans to inject the compounds directly into diseased tissue, such as a knee joint in the case of osteoarthritis, or the back of the eye for someone with age-related macular degeneration.

And unlike cancer, in which a single remaining cell can spark a new tumour, there's no need to kill every senescent cell in a tissue: mouse studies suggest that dispatching most of them is enough to make a difference. Finally, senolytic drugs will clear only senescent cells that are already present — they won't prevent the formation of such cells in the future, which means that senescence can continue to perform its original tumour-suppressing role in the body.

Those perks haven't convinced everybody of the power of senolytics. Almost 60 years after his initial discovery, Hayflick now believes that ageing is an inexorable biophysical process that cannot be altered by eliminating senescent cells. "Efforts to interfere with the ageing process have been going on since recorded human history," says Hayflick. "And we know of nothing — nothing — that has demonstrated to interfere with the ageing process."

Fans of senolytics are much more optimistic, emboldened by recent results. Last year, van Deursen's lab went beyond its tests on super-aged mice and showed that killing off senescent cells in normally ageing mice delayed the deterioration of organs associated with ageing[6], including the kidney and heart. And — to the joy of anti-ageing enthusiasts everywhere — it extended the animals' median lifespan by about 25%.

Successful results from mouse studies have already lured seven or eight companies into the field, Kirkland estimates. At Mayo, one clinical trial has opened, pitting dasatinib and quercetin in combination against chronic kidney disease. Kirkland plans to try other senolytics against different age-related diseases. "We want to use more than one set of agents across the trials and look at more than one condition," he says.

If eliminating senescent cells in humans does improve age-related illnesses, researchers will aim to create broader anti-ageing therapies, says David. In the meantime, researchers in the field insist that no one should take these drugs until proper safety tests in humans are complete. In rodents, senolytic compounds have been shown to delay wound healing, and there could be additional side effects. "It's just too dangerous," says Kirkland.

Van Deursen says that continuing to answer basic biological questions is the field's best shot at success. "Only then will we be able to understand what ageing really is, and how we can, in an intelligent way, interfere with it." ■

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1. Baker, D. J. *et al. Nature Cell Biol.* **10,** 825–836 (2008).
2. Baker, D. J. *et al. Nature* **479,** 232–236 (2011).
3. Baar, M. P. *et al. Cell* **169,** 132–147 (2017).
4. Schafer, M. J. *et al. Nature Commun.* **8,** 14532 (2017).
5. Jeon, O. H. *et al. Nature Med.* **23,** 775–781 (2017).
6. Baker, D. J. *et al. Nature* **530,** 184–189 (2016).
7. Coppé, J.-P. *et al. PLoS Biol.* **6,** e301 (2008).
8. Kuilman, T. *et al. Cell* **133,** 1019–1031 (2008).
9. Acosta, J. C. *et al. Cell* **133,** 1006–1018 (2008).
10. Demaria, M. *et al. Cancer Discov.* **7,** 165–176 (2017).
11. Biran, A. *et al. Aging Cell* **16,** 661–671 (2017).
12. Zhu, Y. *et al. Aging Cell* **14,** 644–658 (2015).
13. Fuhrmann-Stroissnigg, H. *et al. Nature Commun.* **8,** 422 (2017).
14. Kirkland, J. L. & Tchkonia, T. *EBioMedicine* **21,** 21–28 (2017)
15. Chang, J. *et al. Nature Med.* **22,** 78–83 (2016).
16. Zhu, Y. *et al. Aging Cell* **15,** 428–435 (2016).
17. Yosef, R. *et al. Nature Commun.* **7,** 11190 (2016).

CORRECTION

The News Feature 'To stay young, kill zombies (*Nature* **550,** 448–450; 2017) omitted the journal name in reference 4. The reference should have been: Schafer, M. J. *et al. Nature Commun.* **8,** 14532 (2017).