SHAWN ROCCO/DUKE HEALTH

Neurosurgeon John Sampson places a treatment-infusing catheter into the brain of a patient with glioblastoma multiforme.

IMMUNOTHERAPY

The battle for the brain

Glioma establishes fortifications against the immune system that researchers are now learning to chisel away.

BY MICHAEL EISENSTEIN

The stars seemed to be aligned for rindopepimut in 2015. A trio of clinical studies had indicated strongly that this peptide-based vaccine elicited an immune response that could meaningfully delay the progression of glioblastoma multiforme (GBM), the most aggressive form of brain cancer. The vaccine's manufacturer, Celldex Therapeutics of Hampton, New Jersey, lobbied the US Food and Drug Administration for accelerated approval on the basis of phase II data but the agency demurred, preferring to wait for the results of a phase III trial.

Unfortunately, that trial was a failure. An interim analysis of the data in early 2016 indicated no improvement in the survival of people with GBM who received the vaccine, and Celldex terminated its development. Patients and clinicians were deeply disappointed, but not necessarily surprised. "Neuro-oncologists tend to be nihilistic in a lot of ways, just because we have seen so many treatments come and go," says E. Antonio Chiocca, neurosurgeon-in-chief at Brigham and Women's Hospital in Boston, Massachusetts.

GBM is also the most common form of brain cancer, accounting for around 50% of primary malignancies, and these tumours are almost always fatal. Surgery, chemotherapy and radiotherapy offer only short-term respite to those affected. The treatment of other tough-to-tackle cancers, including melanomas, has been transformed by approaches that unleash the body's immune response on tumours. Drugs known as checkpoint inhibitors, which can relieve the immune-system suppression that is induced by tumours, have been shown to reduce the odds of disease progression or death from melanoma by almost 50%. Unfortunately, no such revolution has unfolded for GBM. A phase III trial of the blockbuster checkpoint inhibitor nivolumab, which was developed by Bristol-Myers Squibb in New York City, yielded only more disappointment.

However, despite decades of clinical setbacks, the neuro-oncology community is optimistic that it will eventually succeed in retraining the immune system to defeat brain cancer (see 'Immune reactivation'). Researchers are learning much about the array of strategies that GBM uses to fortify itself against the immune response, as well as hitherto underappreciated interactions between the brain and immune system. "Immunotherapy is probably the only modality that can attack this problem of the tumour outsmarting the treatment," says Linda Liau, a neurosurgeon at the University of California, Los Angeles, Medical Center. "It's a treatment that can change with the tumour."

PRIVILEGE REVOKED

The central nervous system is insulated from the rest of the body in a number of ways, including the blood–brain barrier — a tightly

sealed filter formed by blood vessels that prevents unwanted biomolecules, drugs and cells from accessing the neural circuits of the brain (see page S46). The brain is not entirely sheltered in its biological fortress, however, and long-standing assumptions that it is privileged in terms of a lack of immune surveillance have been called into question.

In 2015, Antoine Louveau, Jonathan Kipnis and their colleagues at the University of Virginia in Charlottesville made a startling discovery: the existence of lymphatic vessels that allow T cells to pass from the circulation into the brain[1]. "We all went to medical school learning that the brain didn't have any lymphatics, and now we see that there are some," says John Sampson, a neurosurgeon at Duke University School of Medicine in Durham, North Carolina. This means that abnormal proteins that signal the presence of a pathogenic agent or a tumour can trigger the same immunological alarm bells in the brain as they would in other tissues, and then recruit T cells to mount a counter-attack. Other studies had already demonstrated that circulating immune cells can obtain access to the brain by unclear means — most notably, in the context of infection or autoimmune disease, but also in cancer. "There is immunological surveillance occurring," says Amy Heimberger, a neurosurgeon at MD Anderson Cancer Center in Houston, Texas.

Even the vaunted blood–brain barrier is not fully impregnable. Although large proteins are generally excluded from crossing it, antibody-based drugs — including various checkpoint inhibitors — are able to pass through and gain limited access to the brain. Sampson estimates that roughly 1% of a typical dose reaches the brain after intravenous administration, but this is probably enough to obtain a clinical effect. "If you give checkpoint inhibitors for brain metastases from melanoma or lung cancer, you can see regression," says Donald O'Rourke, a neurosurgeon at the Perelman School of Medicine at the University of Pennsylvania in Philadelphia. Collectively, these findings support the feasibility of marshalling a targeted immune response against malignancies in the brain. But O'Rourke also notes that immunotherapy regimens that can eradicate growths that have spread to the brain from elsewhere in the body fall flat when used on tumours that originate in the brain. This indicates that apparent invulnerability of GBM to attack by the immune system is not simply the result of its residence in the brain. "That's got to be something intrinsic to glioma," says O'Rourke.

GBM tumours are protected in many ways. For a start, they show extreme genetic heterogeneity, which means that the mutations that contribute to their growth can differ dramatically across such tumours. "We're talking about tumour cells that differ from their neighbours by 50–60 mutations," says Chiocca. "That makes it really tough to figure out a treatment that is so targeted that it will wipe out the entire tumour." This heterogeneity was probably a factor in the failure of the rindopepimut phase III trial. The vaccine is designed to elicit an immune response to epidermal growth factor receptor variant III (EGFRvIII), a tumour-specific protein that drives disease progression in GBM and other cancer types. But if only a subset of cells express EGFRvIII, much of the tumour will be spared.

GBM tumours also churn out chemical signals that are released into surrounding tissue to disable immune cells. These molecules include immunosuppressive proteins that are targeted by checkpoint inhibitors such as nivolumab, as well as other signals that, in parallel, can maintain immunosuppression when one of the roadblocks is lifted. In time, this inhibition can become permanent. "When you pull those T cells out of that tumour microenvironment, you can't reverse it," says Heimberger.

"When you take out a GBM, up to half the tumour is a supporting microenvironment."

Paradoxically, much of this inhibition is facilitated by other immune cells, which are recruited by the glioma for their ability to pacify tumour-killing immune cells. "When you take out a GBM, up to half the tumour is a supporting microenvironment containing tumour-associated macrophages and myeloid-derived suppressor cells," says Chiocca. "These cells tend to make the tumour stealthy so that the immune system does not see them."

KNOW YOUR ENEMY

Researchers who are developing immunotherapies are focusing on a ray of light in this darkness. "The immune response to brain tumours exists — it's just not so efficient," says Valérie Dutoit, who studies brain-cancer immunotherapy at the University of Geneva in Switzerland. "We need to do something more." The clinical trials that are in progress have therefore striven to learn from past setbacks.

For example, several therapeutic vaccines in development have been designed to reduce the chance that the tumour will evade the treatment by mutating the specific antigen that the vaccine contains. Some such vaccines rely on injections of peptides in combination with an immunity-stimulating agent called an adjuvant. Others use a type of immune cell known as dendritic cells. These are harvested from the patient and then trained to recognize a particular antigen, which facilitates a focused counter-attack by T cells when they are returned to the donor. "Dendritic cells have all of the co-stimulatory molecules that can activate T cells really well," explains Liau. This is especially important given the weakened immunity of people with GBM.

Sampson and his colleagues have devised a vaccine based on dendritic cells that can elicit a remarkably durable immune response to tumours. The core challenge of vaccine development is to find a protein that elicits such a response that is consistently expressed by tumours but not by healthy tissue. The team has focused on a protein that is expressed by cytomegalovirus — a genome-integrating virus that infects most adults at some point in their life. "It's normally latent, but comes out of latency during immunosuppressed states," says Sampson. "When it re-emerges, there's a fairly large number of T cells that have the potential to react with it." Studies in people have shown that immunosuppression induced by GBM is sufficient to reawaken the virus, and thereby enables antigen-trained dendritic cells to launch a potent response against tumour cells. In a 2017 trial of the vaccine developed by Sampson's team[2], 11 participants with GBM had a median survival of more than 41 months, whereas people who receive conventional treatments generally succumb to the disease in 15 months. Remarkably, the GBMs of 4 of those participants had not progressed after 5 years.

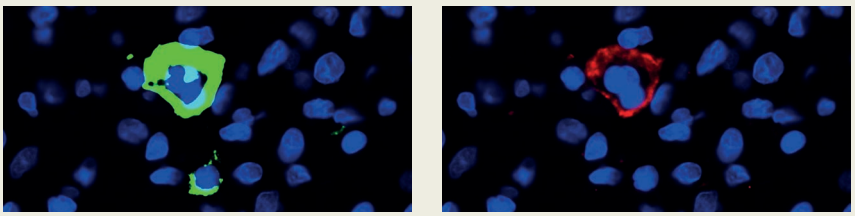
Several groups of researchers are pursuing personalized vaccines, which target multiple tumour-specific antigens identified from tissue removed during a given patient's biopsy. The European Glioma Actively Personalized Vaccine Consortium (GAPVAC), for example, is building collections of immunity-stimulating peptides that are derived from proteins found on the surfaces of tumour cells. "During the 2 or 3 months while they receive the standard of care, we were able to identify the antigens, formulate them and vaccinate patients afterwards," says Dutoit, who is participating in the consortium with her colleague Pierre-Yves Dietrich, also at the University of Geneva. "This is a good step towards personalized vaccines."

Buzz has been building around another personalized-vaccine effort that has shown hints of improved survival. The DCVax-L strategy from Northwest Biotherapeutics in Bethesda, Maryland, entails training dendritic cells with protein extracts derived from tumours. Researchers who are collaborating with the company on a phase III trial reported that 100 of the 331 people who were treated are still alive a median of 40.5 months later[3]. "Seeing that kind of long tail of survival is quite intriguing," says Liau, who is working on the study. "I'd love to see what is unique about these patients." She cautions, however, that these data are preliminary — with the trial still in progress, the treatment and control arms remain double-blinded, which makes it impossible to draw conclusions about the efficacy of the approach.

The identification of antigens that are useful for vaccines is also aiding the development of chimaeric antigen receptor (CAR) T-cell therapy, an immunotherapy that is helping to transform the treatment of certain blood cancers. CARs are engineered immune receptors that have been designed to send out signals that activate an immune response when they encounter tumour-specific proteins. By reprogramming the T cells of a person with cancer to express CARs, clinicians can essentially train those cells to hunt down and kill tumour cells. O'Rourke led an initial foray into this space,

IMMUNE REACTIVATION

Glioma cells (blue) can protect themselves from attack by immune cells such as macrophages (green) in several ways. This includes the potent inhibition of macrophages through a signalling pathway involving the cell-surface protein PD-L1 (red). The immunotherapy drug pembrolizumab blocks this pathway, enabling macrophages to go on the offensive against the tumour.



L. LIAU, R. PRINS & J. ORPILLA/UCLA NEUROSURGERY

with a first-in-human trial[4] that demonstrated intravenously infused CAR T cells that targeted EGFRvIII could safely penetrate gliomas and trigger a modest, localized immune response. O'Rourke notes that one of the ten recipients lived for almost three years after the infusion — a promising sign from a preliminary trial that was mainly designed to test safety.

However, as demonstrated by EGFRvIII-targeting vaccines, a single molecular target might not be sufficient to keep this tricky tumour in the cross hairs of CAR T cells. In that spirit, Dutoit and Dietrich are hoping to draw on their experience in identifying and classifying patient-specific GBM antigens, and are collaborating with Carl June, a pioneer of CAR T-cell therapy, at the University of Pennsylvania. Such a personalized approach, Dutoit says, could enable doctors to "offer the patients, depending on the antigen expression of their tumour, two or three CARs that may be best for them".

AWAKENING THE SLEEPERS

These antigen-specific immunotherapies might not be enough to overcome the fierce resistance of brain tumours. Not all tumour-specific proteins can spark an effective immune response, and Heimberger suggests that more leverage might be gained by finding ways to specifically kick immune cells into gear, rather than by just focusing on identifying fresh targets on tumours. Accordingly, several trials are combining vaccines or regimens involving CAR T cells with checkpoint inhibitors such as nivolumab. It remains unclear, however, whether this can overcome the suppressive effects of a tumour microenvironment that hides gliomas from the immune system.

Heimberger's team has identified a further vulnerability that clinicians could exploit: a protein called STAT3 that regulates gene expression. STAT3 is not only immunosuppressive, it also has a role in controlling the progression of cancer. "Pretty much any mechanism you can think of for tumour-mediated immune suppression is tied to STAT3," Heimberger says. Her team has helped to develop a potent inhibitor of STAT3 that can cross the blood–brain barrier, and is about to embark on a trial to see whether the inhibitor can deliver the same benefits to people who were observed in preclinical studies.

Alternatives are also emerging that can break a tumour's stranglehold on the immune system. These include oncolytic viruses, which kill tumour cells by provoking a strong but selective immune backlash. According to Chiocca, this approach exploits the fact that the immune system is more attuned to pathogenic agents than it is to tumour cells. The idea, he says, is to "wake up the immune system so it recognizes that there is a viral infection — but there's also a tumour in the midst of this viral infection". His group is working with a genetically engineered herpes simplex virus that can infect brain cells efficiently. The virus contains modifications that debilitate it until it infects tumour cells — at which point, it comes to life and fulfils its deadly mission. Chiocca's group has begun a phase I trial of the virus, with encouraging results. "We've treated nine patients so far, and it's been really well-tolerated," he says. Other oncolytic viruses that are being explored include a modified poliovirus known as PSVRIPO. Studies in mice strongly indicate that tumour-cell death from PSVRIPO effectively reawakens the slumbering immune system[5]. Even more promisingly, clinical-trial data indicate that this treatment could improve survival for people with GBM[6].

Viruses can also selectively sensitize tumour cells to chemotherapy through gene-therapy-based approaches. Liau has conducted trials of such an approach developed by Tocagen in San Diego, California. Its strategy involves reprogramming retroviruses into vectors that deliver to tumours the gene that encodes the enzyme cytosine deaminase, which converts the harmless compound 5-fluorocytosine into a tumour-cell-killing derivative. In a phase I study[7], the vector Toca 511 essentially eliminated detectable cancer in more than 20% of recipients. Liau says that although the vector–drug combination alone can kill tumour cells, experiments in animals have shown that a working immune system is crucial for achieving sustained control of the tumour's growth. She notes that the virus seems to induce an "immunologic memory against the tumour".

AN ESCALATING CONFLICT

These preliminary studies confirm that it is possible to break down GBM's daunting defences. Now, the time has come to show that such treatments can also provide meaningful benefit through more extensive, randomized controlled trials in people.

Against this backdrop, researchers are grappling with the realization that the design of clinical trials can stack the deck against immunotherapies. For example, many trials have been conducted in people with GBM that has repeatedly failed to respond to treatment. These individuals also have particularly compromised immune systems, from the effects of both the tumour and conventional treatment. "Temozolomide, the standard-of-care chemotherapy, kills off tumour cells but also kills off T cells and other lymphocytes," says Chiocca. This immunosuppression is exacerbated by the steroids that are given routinely to reduce swelling in the brains of people being treated for GBM. In this light, the ability of people with advanced disease to still mount a meaningful immunotherapy-induced response is remarkable, and more and more trials are exploring whether starting such treatments earlier might produce better outcomes. "We're now approaching patients that were newly diagnosed, as well as patients who might not need chemotherapy or might not benefit from it," says Sampson.

Liau notes that it has generally been more difficult to recruit such people because they still have treatment options remaining. However, an increasing awareness of the bleak prognosis after conventional treatment is leading those affected to consider alternative approaches. Importantly, many experimental immunotherapies seem to be remarkably safe, despite the potential risks associated with stirring up an immune response in a brain weakened by cancer. O'Rourke says that the people who received his CAR T-cell therapy did not experience the potentially fatal inflammatory 'cytokine storm' that often affects those who undergo such treatments. Moreover, he adds, trials with candidate vaccines or with checkpoint inhibitors have generally reported mild and manageable side effects — although combinations might create more risk.

But the capacity to tolerate risk is likely to be higher in people who are facing the particularly grim odds of brain cancer — and Chiocca thinks that it might be time to take the gloves off. "We've focused so much on targeted therapies, but you cannot always wipe out an enemy just with precision missiles," he says. "Especially with an enemy like GBM." ■

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CORRECTION

The Outlook article 'The battle for the brain' (*Nature* **561**, S42-S44; 2018) erroneously implied that all of the 100 people in the extended-survival subset were alive 40 months later. In fact, the figure referred to median survival for the group.