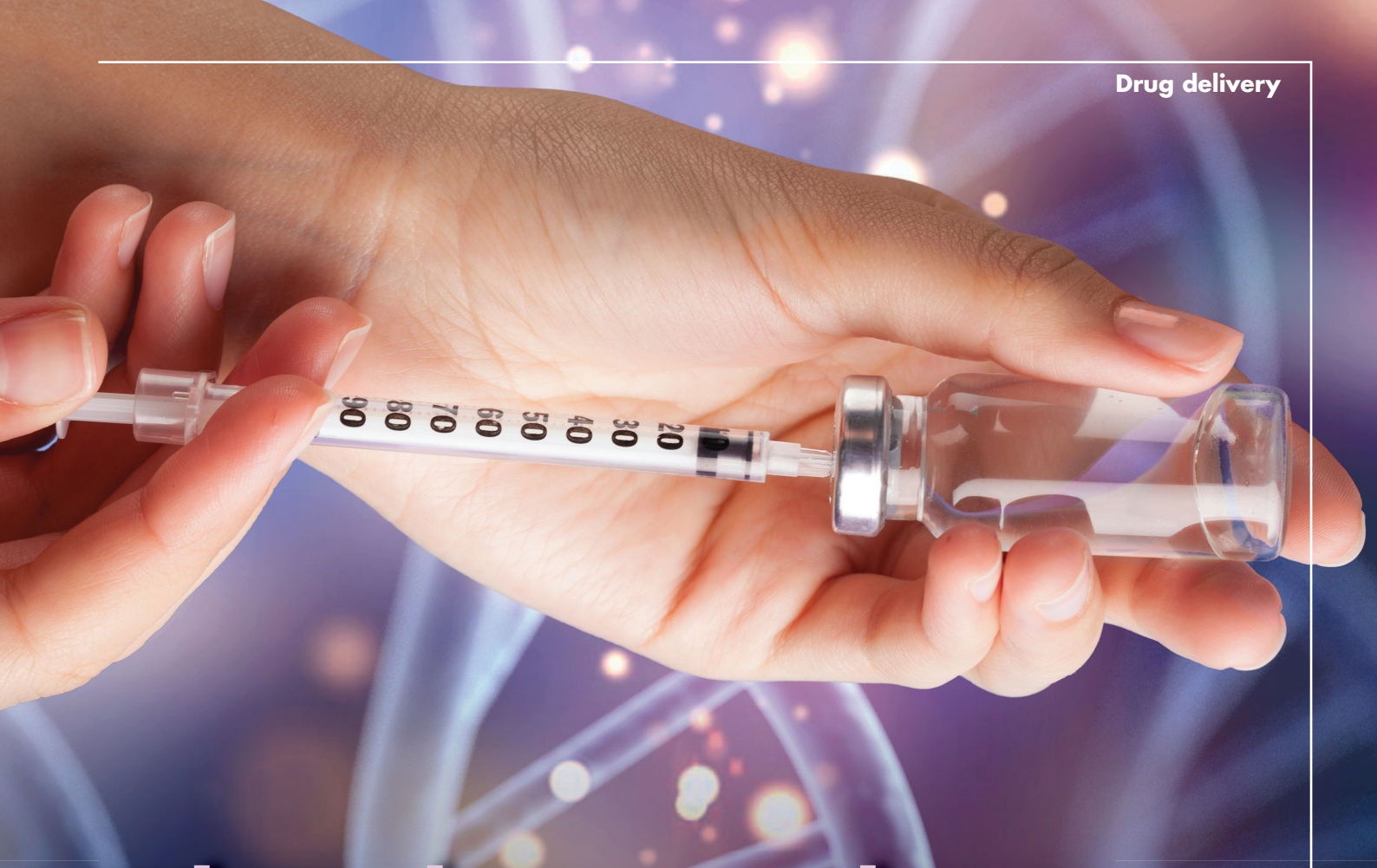


The best shot

The validation of Covid-19 vaccines will go down in history as a turning point during this pandemic, but with highly specific storage and transportation requirements, the roll-outs that followed have been anything but straightforward. The arrival of DNA vaccines could help solve some of those issues, while delivering a host of other benefits. Tim Gunn speaks to **Hong Jiang**, COO of Aegis Life, and **Luigi Aurisicchio**, CEO of Takis Biotech, to find out how their technology could be an improvement on current vaccines.

Did you think it was over? Just for a moment, maybe – as the vaccination numbers rose, or the mercury; when the lockdowns lifted; as the masks dropped?

No one could blame you. Most of last year was passed in the hope of a vaccine that might work as well as annual flu jabs, and this one began with the roll-out of two brand new mRNA products topping 90% efficacy, as well as multiple viral vector vaccines that could be shipped around the world without the need for ultra-cold logistics. For many people, the past few months have felt closer to 2019 than 2020. So, while Wall Street Investors cheered when Pfizer and Moderna revealed their phase III trial data, there's been much less enthusiasm about funding new candidates since then. "The game is over," investors told Hong Jiang, COO and co-founder of early-stage US biotech Aegis Life in spring, "we don't want another Covid vaccine deal."

Aegis Life and other developers of second-generation Covid vaccines have a very different challenge from those that went before them. The bar for initial efficacy, which was all the world cared about back in 2020, has been set higher than anyone dared to hope, raw materials and manufacturing equipment have been diverted into approved products, and people either want a vaccine that can get them back on planes and in restaurants or they don't want any vaccine at all – ever.

Tellingly, Italian company Takis Biotech's phase I/II Covid-eVax trial has been put in jeopardy by government policy. According to the country's 'green pass' scheme, only recipients of approved vaccines can enter public venues. Experimental vaccines don't qualify, so recruitment has proven almost impossible.

That's despite the fact Takis is working on what could well be a major success story for Italy and the

EU: the first DNA vaccine to reach clinical trials in Europe. Companies that reached the same point last year could hardly move without attracting funding. For example, Inovio Pharmaceuticals' DNA vaccine, which uses very similar technology to Takis Biotech's, won a $71m contract with the US Department of Defense in June 2020. But, given the speed with which most rich countries have since vaccinated their populations, the billions have stopped flowing quite so freely into new pharmaceutical technologies.

In fact, in August it was the Indian pharma giant Zydus Cadila that received the first emergency approval for a DNA Covid vaccine. What India understands better than any other country that has produced a Covid vaccine so far is that, while viral vector and mRNA vaccines easily won the race to market, two-thirds of the planet is still susceptible to Covid – and each technology has limitations that could slow the process of changing that fact.

"What we have, potentially, is a vaccine that only requires one dose, and that can be shipped at room temperature or in a refrigerator... That's what we think the world needs right now."

Hong Jiang, Aegis Life

Even within cells, most RNA molecules degrade in about two minutes. DNA, however, can be recovered from the mortal remains of creatures that died millions of years ago. That helps explain why viral vector vaccines, which use modified viruses to transport DNA into cells, are easier to store and ship than their mRNA equivalents. But those vectors can prompt immune responses of their own. Some people have pre-existing immunity to vectors adapted from viruses that naturally affect humans, while repeated use of any vector increases the chances that recipients will stop responding to it. Even under the most favourable conditions, viral vector vaccines for Covid variants take far longer to develop and produce than mRNA ones, and, as the pandemic stretches on, it's possible that vector immunity could force companies to start all over again with new viruses. Disconnected from those vectors, though, the stability of DNA molecules at higher temperatures could make them even easier and cheaper to manufacture than mRNA.

As testified by Takis' rapid pivot to a phase II trial of a booster shot specifically targeting the Delta variant, a DNA vaccine could combine the advantages and minimise the limitations of the other two approaches. Still, it hasn't turned heads in quite the same way as either did on its own. "A lot of us in the US are pretty myopic," says Jiang at Aegis Life, which also has a DNA Covid vaccine in clinical trials. "We're like, we have the mRNA, and it works, and we have the freezer infrastructure to get it everywhere in the country, so we think the problem is solved and the pandemic is over."

On one side, then, the developed world's pharma industry is slipping back towards its old inertia, with a sense that perhaps a third new vaccine technology is a bit much for one pandemic. On the other, the virus keeps mutating. If anyone's feeling complacent, they can look to the devastation wrought by the Delta variant in India – or, if they're on Wall Street, the various ways it has interrupted New York's attempts to fully reopen. Since that started, Jiang explains, US investors have changed their tune. The longer it takes to suppress Covid worldwide, they've realised, the greater the chances of a mutation that resets the vaccination counter to zero. DNA vaccines could be the best way to prime the rest of the planet's immune systems. But first, they have a lot of catching up to do.

Guns, drills and zappers

While injections of large quantities of raw DNA in saline solutions were first shown to work in animal models in the early 1990s, responses in humans and other primates have rarely proved strong enough to protect against disease. Viral vectors get around this issue by exploiting the process natural viruses use to merge with cells, splicing the DNA sequence for creating the Covid spike protein into a harmless, non-replicating adenovirus.

Cells copy these DNA molecules into mRNA strands, which catalyse the production of spike proteins the immune system can learn to recognise and combat. Moderna and Pfizer-BioNTech's vaccines engage the body's manufacturing systems one step downstream by inserting mRNA directly into cells using lipid nanoparticles tuned to sneak past the immune system and push through cell membranes.

Unfortunately, you can't just swap DNA into Pfizer or Moderna's LNPs. The dose each can contain is already limited by the toxicity of their constituent lipids, which prevent them from being used systemically, and DNA payloads instantly push them over into intolerability. Instead, most companies developing DNA vaccines have focused on using specialist delivery devices to overcome formulation issues.

Most prominently, Zydus Cadila uses pressurised steam to fire large amounts of DNA through pores in the skin with a needle-free injector. For their part, Takis and Inovio both use electroporation guns to zap intradermally or intramuscularly injected DNA across cell membranes.

In both cases, the unconventional method of administration ensures enough DNA plasmids enter cells to induce a robust immune response. Although it is the more established vaccination technology –

$71m

Value of contract awarded by the US Department of Defense to Inovio Pharmaceuticals to develop its DNA vaccine.

Inovio Pharmaceuticals

and minimises the risk of sharps injuries and the spread of blood-borne diseases – needle-free (or jet) injection, which penetrates cell membranes through sheer mechanical force, is particularly inefficient, and requires a very large dose.

Electroporation opens pores in cell membranes by subjecting them to an electrical field. The method requires smaller doses than needle-free injections for vaccine applications and is familiar in microbiology research and chemotherapy. In fact, Takis Biotech's vaccine electroporator is adapted from a large electrochemotherapy tool called a cliniporator. As the electrical conditions required for a vaccine injection are far milder and more specific than those used to fight tumours, the company's modifications have resulted in a handheld, drill-like device that incorporates four electrodes around a central hypodermic needle. By contrast, Inovio's Cellectra handhelds are designed to be placed over the injection site after a separate needle is used to deliver DNA intradermally.

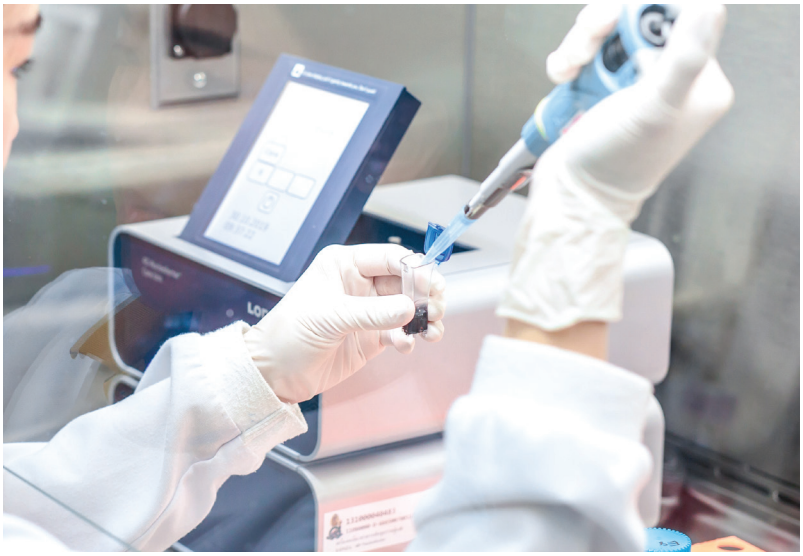
"If you do a naked DNA injection, you have a given level of expression, but if you add electroporation on top your level of expression increases by about 100 to even 1,000-fold," explains Luigi Aurisicchio, Takis' founder and CEO, adding that the technique also brings its own "adjuvant effect", prompting a heightened immune response.

In short, the only notable problem with the devices is the fact they're necessary. For all the work Takis and Inovio have done to develop portable, battery powered and easy-to-use electroporation tools, Aurisicchio admits that convincing people to use them will be difficult, particularly in the low-resource environments most in need of vaccine supplies. "DNA is very stable compared to mRNA, but you need the electroporation," he sighs. "You need a device. It's not the classical vaccine you can use in any physician's office. That's the disadvantage."

The search for solutions

Aegis Life thinks otherwise. The Entos Pharmaceuticals spinout is positioning its Fusogenix platform as a true 'second-generation' LNP-based gene delivery technology for getting DNA to its target without any extra tools. Whereas Pfizer and Moderna's LNPs deliver their payloads thanks to ionisable cationic lipids that assume a positive charge (and therefore become toxic) in contact with cells, Aegis Life's incorporate a fusion protein like those that occur naturally on viral vectors – only much smaller and stealthier.

"We like LNPs," says Jiang. "They have nice properties to encapsulate payloads and are much more flexible than viruses, but, with the positive charge on the surface, they basically barge into the cell by knocking down the door. That creates collateral damage and toxicity." In the terms of that metaphor, viral vector vaccines use fusion proteins to carefully pick the locks on cells – not causing any harm, but leaving just enough evidence to convince the immune system to upgrade its security. Contrast Aegis Life's fusion-associated transmembrane (FAST) protein – the only known fusion protein that doesn't cause immunogenicity: it enters cells with a skeleton key.



Takis and Inovio both use electroporation, shown here being in vitro, to zap intradermally or intramuscularly injected DNA across cell membranes.

As a result, the Fusogenix platform can be used to simultaneously deliver multiple genetic payloads (whether RNA or DNA) intramuscularly, systemically or orally – over and over again. Aegis Life has also found that, because its DNA survives and functions inside cells for two to three months – compared with the couple of days that injected mRNA takes to degrade – it can be used for single-shot vaccines without compromising the immune response.

Like all the approved vaccines, Aegis Life's first formulation, which is moving into phase II trials, targets the spike protein. Following it up the pipeline, though, is a vaccine that delivers DNA encoding for the spike, envelope and membrane proteins. As almost all coronavirus mutations affect the spike protein, vaccine delivery systems that can multiplex in this way are likely to be variant proof.

"What we have, potentially, is a vaccine that only requires one dose, and that can be shipped at room temperature or in a refrigerator," beams Jiang. "At room temperature, it will last for a month; and in a refrigerator, it will last for a year. It's cheap, it's easy to distribute and it only requires one shot. That's what we think the world needs right now."

Developers of other DNA vaccine candidates would make the same claim. Aegis Life is working towards a vaccine that could be delivered via a single tablet within five years, but, in partnership with Merck, Canadian start-up Symvivo already has an oral DNA vaccine delivered by bacterial vectors in clinical trials. The next race, the one that might end the pandemic – and change what it means to be vaccinated – is just getting started.