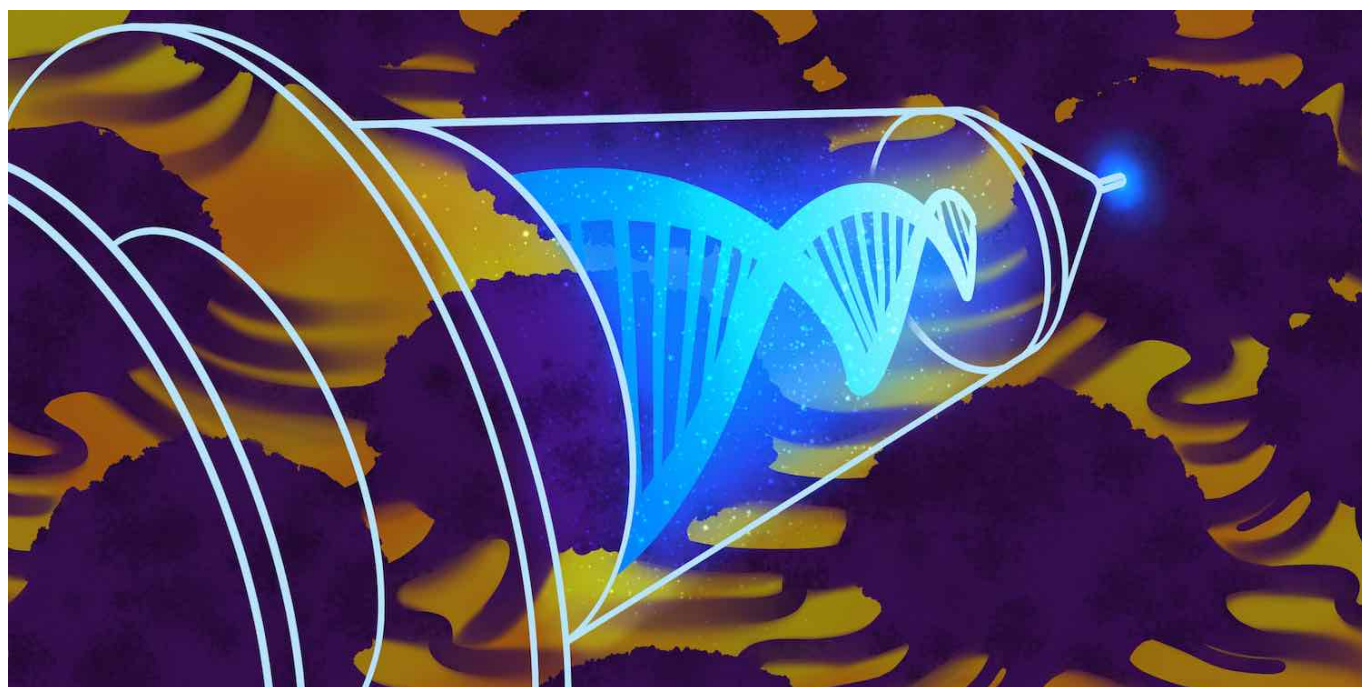


# Cancerworld

## Can gene therapy be made to work against solid tumours?

Rachel Brazil / 25 March 2021



Gene therapy to treat cancer has been on the research agenda for three decades, with the first examples having been developed in the 1990s, according to Hrvoje Miletic, Senior Consultant in Neuropathology at the Bergen/Haukeland University Hospital in Norway. “But the first clinical trials at the end of the 90s, were really disappointing,” he says. In the early phase of gene therapy, toxicity was a problem. In one case this led to a fatality, caused by immune responses to the viral vectors used to deliver potentially therapeutic genes. “The field has been dealing with these issues and modified the vectors accordingly,” says Miletic. The big challenge now, he adds, is showing efficacy. Recent successful results in treating bladder cancer suggest that gene therapies may soon start adding new tools to the cancer therapeutic armoury.

Advances in CAR-T cell therapies (chimeric antigen receptor T cell) for blood cancers have shown the power of genetic manipulation. T cells are harvested from patients and genetically modified, before being transfused back, armed with receptors that sense and target cancer cells. But extending this success to solid tumours has proved difficult, says Luigi Naldini, Scientific Director of the San Raffaele Telethon Institute for Gene Therapy and founder of biotech Genenta Science in Milan, Italy. “Unfortunately, the CAR-T cell [approach] does not work so well in most solid tumours yet, because the microenvironment in the solid tumour is very immunosuppressive,” he explains.

So far only one single gene therapy for solid tumours has gained approval – and only in China. Gendicine, a viral vector approved in 2004 to treat head and neck cancer, delivers the gene for the p53 protein. Mutations of this gene are common in cancers and the therapy attempts to replace its missing tumour suppressing role. Activated by cellular stress, it induces cell death. But a similar drug, Advexin (contusogene ladenovec), was refused approval by the US regulator the FDA in 2000, with question marks raised over the data presented.

Twenty years on, 2020 finally saw some positive news. In December, [FerGene announced results](#) from their novel gene therapy to treat bladder cancers that are unresponsive to current Bacillus Calmette-Guérin (BCG) treatment. FerGene's nadofaragene firadenovec is an adenovirus vector, that delivers the gene for the protein interferon- $\alpha$ -2b – a signalling molecule that enhances the body's own immune response. The trial of 200 patients enrolled between 2016 and 2020 showed a complete response in 53.4% of patients after median follow up of 19.7 months, with another 45.5% retaining the response at 12 months. Only 5.3% experienced disease progression during the study, which the company says makes this “a new gold standard” for treatment. The therapy is currently under consideration with the FDA.

## **Delivery mechanisms**

One of the challenges that has been holding back gene therapy is the method of delivering the useful gene. “We still rely on viruses, which are of course manipulated so they can't replicate, but still deliver the genetic cargo into the cell,” explains Naldini. Most therapies being developed use retroviral vectors, as they have the capacity to integrate genes directly into the host cells' DNA. “[The viral vectors] have been reduced to a minimal amount of sequences, so the risk that an insertion may adversely affect the neighbouring gene is much lower.”

Miletic has been developing gene therapies using lentiviral vectors, another type of retrovirus. “They have a huge advantage over [other] retroviral vectors, because they can also infect non-dividing cells... We now know that not all tumour cells are dividing, there are dormant cancer cells which are highly resistant to treatment. So with our lentiviral vectors, we are able to target both non-dividing as well as dividing cells.”

From the early trials for gene therapy, it became clear that viral vectors themselves pose the risk of stimulating an immune response, which can cause unpleasant side effects (and in one instance, death). Although these problems have been largely ironed out, some researchers have opted for gene delivery via synthetic nanoparticles. A team at Johns Hopkins University in Baltimore in the US are developing biodegradable polymer nanoparticles to house genetic material ([Choi J et al \*Nanomedicine\* 2020](#)). The most advanced system comes from biotech Genprex, whose ONCOPREX non-viral delivery system, encapsulates genes in hollow lipid spheres. The nanoparticles are taken up by tumour cells after administration at up to 33 times the rate they are taken up by normal cells. The system has been used in drug candidate REQORSA (quaratusogene ozeplasmid) carrying the TUSC2 gene, which activates signalling pathways that result in cell destruction. After early-stage trials in 50 patients showed good tolerability, the company are launching a larger clinical trial for the treatment of non-small cell lung cancer.

## **Turning cold to hot**

One of the most promising strategies so far involves delivering genes that can boost the natural immune response to cancer cells, which includes the cytokine interleukin-12 (IL-12), a small protein important in cell signalling. “IL-12 was identified years ago as an important cytokine characterised by an ability to signal inflammation very strongly,” says Daniel O'Connor, CEO of Oncosec immunotherapies in New Jersey. “You can potentially change a suppressive environment into an

immunologically active environment.”

Many tumours create an immunosuppressive or ‘cold’ environment. Patients with ‘cold’ tumours do not respond to anti-PD1/PDL1 checkpoint inhibitors. When working successfully, checkpoint inhibitors release the natural brake or ‘checkpoint’ on T cells, which in normal circumstances will stop them attacking other cells in the body. Immune checkpoint blockade therapies allow T cells to recognise and attack tumours. But up to 50% of patients with PD-L1 positive tumours show resistance or relapse after treatment because the T cells are not able to reach the tumour. Delivering cytokines directly to the tumour can change this, signalling to existing T cells and helping to create more. “The concept is to take that cold tumour and turn it hot,” explains O’Connor, “You’re taking a tumour which was previously missing those cells that could cause a proper anti tumour immune response, and changing that.”

## “Adenovirus vectors carrying IL-12 genes are injected directly into glioblastomas during surgery”

But delivering cytokines has had some teething troubles. “When it was first identified and then deployed, it was used intravenously, and it caused problems because it was very potent,” O’Connor explains. So a key advance has been finding ways to deliver the gene locally to the tumour. There are a number of different ingenious approaches to this. Biotech Ziopharm are injecting adenovirus vectors carrying IL-12 genes (Ad-RTS-hIL-12) directly into glioblastomas during surgery. Prior to this, patients are given the oral drug veledimex – an activator that turns on IL-12 production in the tumours. Ziopharm have conducted two small studies with 95 patients so far, and found success is greatest in patients who also receive dexamethasone – a steroid commonly prescribed after surgery. Patients treated this way lived a median of 16.2 months, compared with the normal life expectancy of 6–12 months.

Oncosec have developed another way of delivering genes to tumour cells without the need for viral vectors or nanoparticles. They have taken advantage of the electrotransfer method used by research laboratories to transfer DNA into cells. “When you put energy through the membrane of a cell, it causes it to become porous, and if you previously surrounded that cell with DNA plasmid, once you form those pores, the plasmids will move from outside the cell, through the membrane into the cell,” explains O’Connor.

The company uses an array of six needles that can establish an electric field to deliver the DNA into tumour cells. “Today, we treat surface lesions or subsurface lesions, [as] this deploys to 1.5 centimeters... We can get to tumours that are located subcutaneously, or tumours located on the skin,” says O’Connor. The company is also developing a miniaturised ‘visceral lesion applicator’ that will be able to reach 15 cm inside the body, allowing delivery of any gene to a much wider range of tumours.

## “We’re not using a bacteria, we’re not using a virus, we’re not using a nanoparticle, we’re using energy”

“The really significant thing about us is we’re not using a bacteria, we’re not using a virus, we’re not

using a nanoparticle, we're using energy... We skip over that part of [gene delivery], which is a large part of the reason why we don't have side effects," says O'Connor. The company has started a trial of its IL-12 gene therapy TAVO (tavokinogene telseplasmid), on its own and in combination with several anti PD-1 checkpoint inhibitors, for regionally advanced melanoma and metastatic melanoma respectively, where checkpoint inhibitors did not work alone. They are optimistic from their early results. The company is additionally starting trials combining TAVO with chemotherapy and a checkpoint inhibitor. They are also deploying TAVO against metastatic triple-negative breast cancer, a difficult tumour to treat that often does not respond to checkpoint inhibitors.

Another approach to targeted delivery is being developed by Genenta. They are using genes to deliver another cytokine, interferon- $\alpha$ , to treat patients with glioblastoma. "Interferon is one of the most powerful activators of immune response," says Naldini. "It may inhibit the formation of new vessels, you may recruit immune cells and it may activate presentation of antigen from the oncolytic cell to the immune system."

Rather than delivering the gene in vivo, the gene is transferred to harvested stem cells. After being harvested, the stem cells are modified using Temferon, a lentiviral vector containing a gene producing interferon- $\alpha 2$  (IFN- $\alpha 2$ ). When these stem cells are re-infused, they develop into white blood cells (Tie2 expressing monocytes – TEMs) that have a specific affinity for tumours. "The key part of this treatment is to make sure that the interferon is expressed only in the tumour... That's required a lot of fine tuning of the vector," says Naldini. The therapy is suitable for patients whose tumours possess a specific DNA modification that will switch on the interferon gene. A [2020 trial](#) in eight patients showed [Temferon was well tolerated](#), "We have already reached the [highest] dosing level and we have not seen toxicity related to [interferon]." Genenta has now started a phase II clinical trial in patients newly diagnosed with glioblastoma, and is also looking to develop the therapy against a broad range of solid tumours.

## Suicide genes

Suicide genes were discovered in the 1980s, when it was found that transferring genetic material from the herpes simplex virus 1 into human cells made them more sensitive to some antiviral treatments. The virus produces the enzyme thymidine kinase (HSV-TK), which is able to turn drugs such as glancyclovir into potent anti-cancer agents. In this context glancyclovir acts as a pro-drug and is only turned into the cancer killing drug when it meets the enzyme. The enzyme converts it into a molecule that mimics some of the building blocks of DNA – but when incorporated into the cells replicating DNA, it blocks further replication, leading to cell death. Genes derived from a number of viruses and bacteria have now been developed. "There are highly advanced suicide genes nowadays," says Miletic. "The sensitivity for the pro-drug has been substantially increased."

**"It's chemotherapy, but it's very targeted, because the pro-drug is only converted into the cancer-killing drug in the tumour cells"**

Miletic is working with suicide genes as a treatment for glioblastoma in his group in Bergen. The treatment involves injecting a viral vector encoding the suicide gene into the tumour, or in many cases into the cavity after surgery. Subsequently, patients are given the oral pro-drug. "It's chemotherapy, but it's a very targeted chemotherapy, because the [pro-drug] is only converted [into the cancer-killing drug] in the tumour cells," explains Miletic. The pro-drug has no impact on other areas. "It's quite a specific way of killing the tumour cells and with much less toxic side effects than

systemic chemotherapy.”

So far, however, attempts to use suicide genes in cancer therapies have not been successful. The most recent phase III trial was carried out by biotech Tocagen (now part of Forte Biosciences). They had taken their suicide gene therapy Toca 511 successfully through early clinical trials, treating patients with recurrent high-grade glioma. A retroviral vector injected into the tumour site delivered an *E coli* gene for the enzyme cytosine deaminase, which was followed by oral administration of their pro-drug Toca-FC. But in 2019, their phase III trial of 403 patients failed to meet its primary endpoint of extending overall survival compared with the standard of care.

Another company with some promising phase II clinical trial results, according to Miletic, is Candel Therapeutics, based in Massachusetts. They are conducting a registration clinical trial for aglatimagene besadenovec (ProstAtak), an HSV-TK suicide gene therapy given with pro-drug valacyclovir for treating localised intermediate- or high-risk prostate cancer alongside radiation treatment. The therapy is also undergoing additional studies in brain, lung and pancreatic cancers. More than 700 patients have been dosed to date, but whether Candel’s phase III trials will be successful remains to be seen.

Miletic is hopeful that we will be able to harness the power of suicide genes for brain cancers that are difficult to treat. “I still think that the killing effect of suicide gene therapy is very efficient,” he says. But the key, as with other gene therapies, could lie in co-opting the power of the human immune system. “If we combine it with a very effective immunotherapy, I think that we can move forward with this strategy.”

Harnessing the potential of gene therapy in cancer has been a slow and bumpy process. For O’Connor, the potential power of gene delivery *in situ* lies in achieving localised delivery and limiting off-target side-effects: “We’ve made strides, but I don’t think we have gotten to where we want to be. We’ve in some instances supplanted chemotherapy side effects with immunotherapy side effects [such as] hypertension and CRS [cytokine release syndrome].” O’Connor hopes that gene therapy might provide a truly side-effect-free solution.

For Naldini, the strategy of delivering genes that are able to harness the immune system is very promising, and he thinks gene therapy will soon have a big impact on cancer treatments. “I think we have data which shows that this can be done efficiently, safely, and also provide therapeutic benefits, at least in some types of tumours. There has been amazing progress, but I’m not surprised it took a while.”

*Illustration by Alessandra Superina*