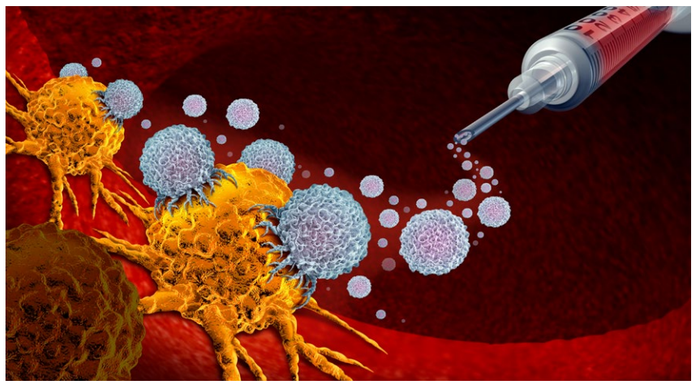
A new concept in vaccination targets experimentally induced antigens

The development of a vaccine for cancer has long proven to be a challenge. This vaccine would need to target neoantigens expressed in tumour cells, which vary widely between patients, or aren't produced in high enough numbers. To tackle this challenge, Dr Eli Gilboa's team at the University of Miami is working on a different approach to vaccination. By inducing the presentation of ubiquitous cryptic antigens by suppressing mediators of antigen processing in tumour cells like TAP (transporter-associated with antigen processing), and stimulating an immune response against these antigens, the tumour cells can be destroyed without need to target tumour-resident mostly mutation-generated neoantigens.

At this stage of the coronavirus pandemic, vaccinations are on everyone's mind. For the past few months, the race to develop a vaccine for COVID-19 has dominated science news all over the world. However, that hasn't been the only vaccine in the pipeline. Dr Eli Gilboa at the University of Miami has been working to develop a vaccine for a disease that affects almost forty percent of men and women in the US every year: cancer.

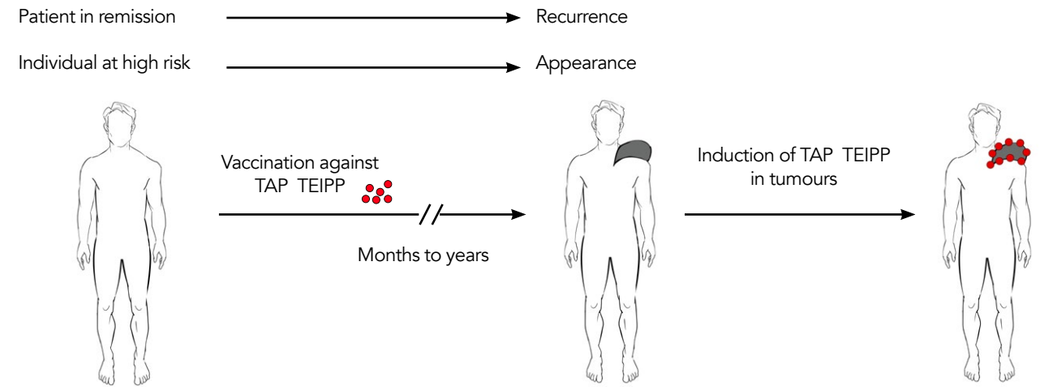
Developing a vaccine for cancer has proven difficult in the past because unlike diseases like COVID-19, it is not caused by a virus or by bacteria. Instead, it is caused by mutations in the DNA of the body's own cells. This then poses a problem; how can we teach the immune system to tell the difference between cancerous tumour cells and healthy cells? To answer that question, Dr Gilboa's research has focussed his research on the molecules that naturally trigger the body's immune response: antigens.



THE ROLE OF NEOANTIGENS

Antigens are molecules on the surface of cells that alert the immune system to attack if it is unfamiliar with them. The most potent antigens in a tumour cell are antigens that are unique to the tumour, namely they are not expressed in normal cells. Such antigens generated mostly by random mutations in the genetically unstable tumour cell are called neoantigens. Studies have shown that immune responses targeted to the rare neoantigens expressed at high frequency in most tumour cells, called "clonal" neoantigens, are best able to inhibit tumour growth.

Unfortunately, targeting them isn't at all simple, for two main reasons. The first is that neoantigen production is caused by random mutations in the DNA of the tumour cell and therefore they can vary widely in structure. Each cancer patient will have different neoantigens, and most neoantigens vary among the patients' tumour cells; only a very small proportion of said neoantigens are present at sufficient high frequency, "clonal neoantigen", to elicit an effective anti-tumour immune response. This would mean that each vaccine would need to be personalised to the patient by identifying their specific clonal neoantigens, which would be both very expensive, labour intensive and we still don't know how to do that. The second is that between 70 - 85% of cancer patients either don't produce or produce too few neoantigens for a vaccine to be successful. Therefore, the majority of cancer patients wouldn't benefit from this style of vaccination, even if it could be done successfully.



Stimulating an immune response in patients in remission or individuals at high risk of developing cancer against cryptic antigens by suppressing mediators of antigen processing like TAP (transporter-associated with antigen processing), and inducing them once tumours recur or develop, respectively. Reproduced from: Garrido, G. *et al.*, 2020. Vaccination against Nonmutated Neoantigens Induced in Recurrent and Future Tumors. *Cancer Immunol Res.* 8(7). pp. 856-868. https://doi.org/10.1158/2326-6066.CIR-20-0020.

So, if most patients don't produce enough clonal neoantigens, how else can we target tumour cells for vaccination?

MARKING CELLS FOR VACCINATION

To overcome this issue, Dr Gilboa's team are taking a different approach. Instead of targeting neoantigens that already exist in the patient' tumours, they are experimentally inducing tumour cells to produce a different set of antigens that are shared among all tumour cells, corresponding to the rare tumour-resident mutation-generated "clonal" neoantigens. To do this, they are suppressing specific mediators in the antigen processing pathway of the cells, like the peptide transporter TAP (transporter-associated with antigen processing). All cells in which TAP is suppressed, or downregulated, would then present new antigens on their surface that they wouldn't normally produce, essentially neoantigens, in effect marking them as better targets for the immune system.

The vaccine would even be suitable for patients who don't express any clonal neoantigens, making it a universal cancer vaccine.

But stimulating the production of these antigens in the tumour is only one half of the process. The immune system will also have to be taught to recognise these antigens as foreign and therefore target them for destruction. That's where the vaccination comes in.

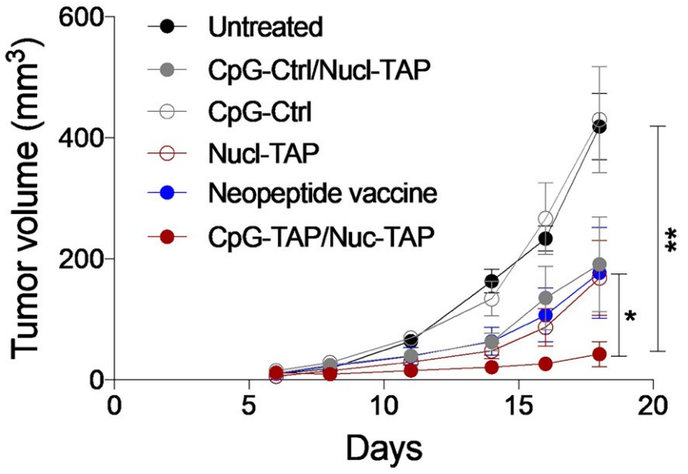
However, it isn't a vaccination in the way you might think. Instead of injecting a patient with a portion of the disease-causing agent, as would be the case for a vaccine against a bacterial or viral illness, TAP production is downregulated in specific cells of the immune system, known as professional antigen-presenting cells. By downregulating TAP production, the same antigens will be presented in both these and the tumour cells, which allows the immune system to recognise the tumour cells as harmful and attack them.

This thereby programmes an immune response against any cells that display these antigens, meaning that if the cancer were to recur in future the immune system would recognise the antigens if TAP were downregulated in these cells again.

TURNING OFF THE TAP

Based on the seminal studies of Thorbald van Hall and his colleagues at the University of Leiden in Netherlands, at the core of this vaccination method is downregulating TAP in both the tumour cells and in antigen-presenting cells, namely cells called dendritic cells. So, how do they do it? The key player here is a small, interfering RNA (siRNA) that is specific to TAP.

RNA is a type of nucleic acid. There are many different types of RNA that each play a different role in biological processes, from mRNAs that copy genetic information from DNA in order to create blueprints for protein



Pre-clinical trials using mouse tumours show that vaccination against TAP-downregulation induced antigens is effective against a wide range of tumours and was more effective than vaccination against tumour-resident mutation-generated clonal neoantigens. Reproduced from: Garrido, G. *et al.*, 2020. Vaccination against Nonmutated Neoantigens Induced in Recurrent and Future Tumors. *Cancer Immunol Res.* 8(7). pp. 856-868. https://doi.org/10.1158/2326-6066.CIR-20-0020.

production in a process called translation, to microRNAs that suppress other types of RNA in order to help regulate gene expression. siRNA is another type of RNA that operates within a cellular pathway known as the RNA interference pathway.

In this pathway, siRNAs interfere with the expression of specific genes by degrading mRNA molecules following translation, which prevents them from relaying the genetic information needed for the production of certain proteins. Therefore, creating an siRNA that is targeted to TAP can prevent its production by degrading mRNA that carries the code to make it, and thereby inducing the generation of new antigens whose production is not regulated by TAP.

The next step is getting this siRNA into the tumour and dendritic cells. To do this, the siRNA is joined with an oligonucleotide, a small molecule made up of individual nucleotides, the same type of molecule that makes up DNA and RNA. These oligonucleotides will form an aptamer, a binding agent, that targets nucleolin, a molecule that is expressed on the surface of some cells. As nucleolin is widely expressed on the surface of most, if not all, tumour cells but not normal cells, it is a broad target for the delivery of the therapeutic siRNA-oligonucleotide conjugate to tumours in the body. Likewise, to target the TAP siRNA to dendritic cells it is fused to another oligonucleotide that binds specifically to a receptor expressed on dendritic cells.

THE FUTURE OF VACCINES?

Pre-clinical studies using mouse tumours have already found that vaccination against TAP-downregulation induced antigens was effective against a wide range of tumours, each with different origin and biology. It was in fact more effective than vaccination against the neoantigens that resulted from mutations in each tumour, and was found to be safe, with no measurable toxicity, though in mice.

With the world's attention turned to vaccines, a revolution in their design may be on the horizon.

Vaccinating against antigens induced by TAP-downregulation overcomes the limitations that targeting clonal neoantigens pose: the need to personalise each vaccine and the small number, or complete lack, of neoantigens expressed. This style of vaccination requires only two, easily synthesizable substances and results in tumour cells presenting the same set of new antigens, replacing the need to identify patient-by-patient rare clonal neoantigens. This means that the vaccine would also be suitable for patients who don't express any clonal neoantigens, making it a broadly available cancer vaccine, unlike the previously explored personalised one.

TAP is just one of several mediators of antigen processing that could act as a target for downregulation and induce the overproduction of antigens to vaccinate against. Others include ERAAP and Invariant chain. Interestingly, downregulation of TAP and ERAAP stimulate a response from immune cells called $CD8^+$ T cells, whilst inhibiting Invariant chain stimulates other cells called CD4 T cells. As such, developing strategies in which both TAP or ERAAP and invariant chain are downregulated simultaneously could result in a greater and more effective immune response.

GOING BEYOND CANCER

The possibilities of this method of vaccination do not end with cancers. Some pathogens, viruses like CMV, EBV or HSV, naturally downregulate the production of TAP when they infect cells. In these cases, TAP would only need to be downregulated in dendritic cells in order to trigger an immune response and therefore destroy the infected cells. Even in the case of viruses like HIV, in which TAP is not downregulated, siRNAs can be targeted to these cells in order to mark them for the vaccination.

So, with the world's attention turned to vaccines, a revolution in their design may be on the horizon. This prototype of a universal vaccine, that could be used to treat such a wide range of diseases from cancers to infectious diseases that have previously eluded cures, could pave the way for new approaches to treatments that overcome the need for personalised medicines.

Behind the Research

Professor Eli Gilboa

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Research Objectives

Dr Gilboa's research takes a multi-pronged approach to cancer immunotherapy using nucleic acid-based drug and drug delivery platforms.

Detail

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Bio

Eli Gilboa received his Ph.D. in molecular biology at the Weizmann Institute, Rehovot, Israel. He was an Assistant Professor in the Department of Molecular Biology at Princeton University from 1980-1986, and served as an associate member of the Memorial Sloan-Kettering Institute from 1986-1993. He then joined Duke University Medical Center as the Joseph and Dorothy Beard Professor of Experimental Surgery and Immunology and Director of the Center for Genetic and Cellular Therapies overseeing the development and clinical implementation of novel gene- and cell-based therapies. In 2006 Dr Gilboa joined the Sylvester Comprehensive Cancer Center and the Department of Microbiology & Immunology, Miller School of Medicine, University of Miami as the Joe Enloe Dodson Professor of Microbiology & Immunology and Director of the Dodson Interdisciplinary Immunotherapy Institute.

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Sylvester Comprehensive Cancer Center, University of Miami, Miller Medical School.

Collaborators

Greta Garrido, Brett Schrand, Agata Levay, Thorbald van Hall

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Personal Response

Once developed, how feasible is it that this style of vaccine can be rolled out globally for readily available use, similar to the current flu vaccinations?

" Quite feasible. The tumour and dendritic cell targeted TAP siRNA are short nucleic acids that, unlike antibodies, can be synthesised in a cell-free chemical process, thereby simplifying the manufacturing process and cost of goods. "