Centre for Personalised Medicine

PRINT EDITION

The Gene'Zine

OXFORD UNIVERSITY
PERSONALISED MEDICINE SOCIETY

Building Your Personal Armour Against Disease

OUPM

MT 2022

DISCLAIMER: ALL GENES EXPRESSED ARE OUR OWN

CONTENTS

MT22

WELCOME

EDITOR-IN-CHIEF OF ‘THE GENE 'ZINE’
NIAMH OWENS

This third issue of The Gene 'Zine promises to be a riveting read. From Parkinson's to Psychiatry to Pharmacy, our writers have expertly navigated a range of therapeutic advancements in the field of Personalised Medicine.

With a vast array of technologies explored and articles delivered in an engaging and informative way, you will soon find yourself immersed in the exciting developments proposed and looking forward to their impact in the immediate future.

A huge thank you to all who have contributed to the issue - it is a pleasure to work with a team of such talent!

EDITOR-IN-CHIEF OF 'THE GENE 'ZINE'
DAHRIA KUYSER

I'm super excited to introduce the third issue of The Gene 'Zine, although the previous issues are a hard act to follow!

In this issue our talented writers have brought some light on a wide range of topics, from organoids to newborn screening to some potential futures of Parkinson's treatment. There's sure to be something to interest you regardless of which areas of personalised medicine pique your interest.

Thanks again to all of our writers and editors for their patience and hard work putting all of these articles together!

MEET THE EDITORS

Smaranda Codreanu

Hi. I am Smaranda, final year medical student. I have big dreams of becoming a surgeon who manages to keep an active interest in research and academia. For me this means being constantly challenged, always curious and ready to ask questions fearlessly. My fields of interest include thoracic oncology, fungal infections in transplant patients and AI/VR training and learning for surgical trainees. When I am not doing medicine-related activities, I enjoy ballroom dancing, spending time with my three dogs and hiking.

Olivia Fisher

Hi, I'm Olivia and I'm a Merton postgraduate studying MSc Genomic Medicine. I am a qualified pharmacist, and I am passionate about how genetics can improve treatment outcomes for patients. I enjoy reading and writing about pharmacogenomics, direct-to-consumer testing, and new personalised treatments. My Master's research project will be focussing on how small-scale genetic testing can improve treatment outcomes in primary care settings. I am very excited to be a writer and editor this year!

Megan Perry

Hi, I'm Megan, a second year Biochemistry student at Trinity. I'm really interested in all areas of personalised medicine and its ability to treat conditions that are otherwise difficult to cure. I'm looking forward to the future of this field and its potential to drastically improve lives.

MEET THE WRITERS

DEVON DARLEY

Hello, I'm Devon a 1st-year Biomedical Sciences student at New College. Curious, I have always enjoyed learning and writing about the hidden biological world that surrounds us. With a deeply set passion for the personalization of medicine, I have a particular interest in epigenetics, specifically transgenerational epigenetic inheritance, and phage therapy.

MICHAEL MILAD

OUPM PRESIDENT

Hi, I'm Michael, a fifth year medical student at GTC - I am excited about the potential Personalised Medicine has to revolutionise health, but also the ethical dilemmas that can arise! The Gene'Zine provides an incredible platform to share recent advances, as well as discuss the challenges that exist within the field.

TOMASZ SZELIGOWSKI

Hi! I'm Tom - I'm currently an FY1 doctor in the Oxford deanery but was previously a medic at Teddy Hall. I'm mainly interested in ophthalmology and find the application of personalised medicine to ophthalmic conditions absolutely fascinating! I also previously researched microbiome changes in patients with schizophrenia which is another area of personalised medicine with great potential to improve our current management options for a great number of conditions.

HOSSAMELDIN SABER

Hossameldin, a community pharmacist and writer, sees potential in precision medicine to overcome the challenges society faces every day regarding medication errors and polypharmacy.
Addressing the case, he equipped himself with experience and CE programs aiming at postgraduate studies in clinical pharmacogenomics.
He also acknowledges the responsibility of pharmacists to bridge the gap between the theory and application of this new field, and also, communicate with other healthcare providers to deliver good precision medicine practice.

REBECCA HOWITT

Hi I'm Rebecca and I'm a 5th year medical student at Queen's. I am particularly interested on the application of personalised medicine to paediatrics and am looking forward to writing on the topic of newborn screening for the magazine.

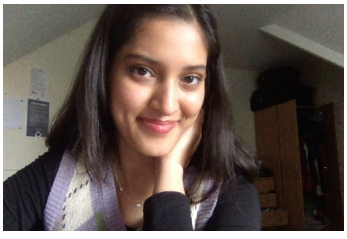
ELSTON D'SOUZA

Hi! I am Elston, a DPhil student and a Computational Research Assistant at the Big Data Institute and St. Hugh's College. My research uses computational methods and large datasets to look at the causes behind rare genetics disorders such taht we can help guide effective clinical diagnoses and therapeutics.

TAISYA VOLODINA

Taisya is the fourth year medical student at Ludwig Maximilians University in Munich. She is working on her doctoral project at the nexus of genetics and oncology and is fascinated by the development of oncological field towards personalized therapy and diagnostics.
As a medical student she is looking forward to having the opportunity to implement new technologies in the clinical setting.

NITYA GUPTA



HI! I'm Nitya and I am a MSc Genomic Medicine Student at St. Anne's. I am enthusiastic about the application of AI and machine learning to the field of genomics and how it can impact drug discovery as well as patient diagnosis and treatment. In my masters research project I will be investigating vaccination response kinetics using single-cell omics.

WHAT'S STOPPING ASOS FROM SOLVING PERSONALIZED MEDICINE?

by Elston D'Souza

Living with a rare disease is difficult. Some would describe it as dealing with the feelings of uncertainty of finding a diagnosis. And most go a lifetime knowing full well that there might never be a cure.

From a scientist's perspective, rare and orphan diseases are one of the grandstanding challenges in clinical medicine. This is partly because we will never have enough documented cases to study them meaningfully.

However, despite the misleading name, rare diseases are not rare. That is the irony. Despite each syndrome or disorder being individually rare, approximately 1 in 10 people suffers from one rare condition or another [1].

What makes tackling these diseases particularly challenging is that no single treatment can scale. The handful of documented cases, in some way, capture the absolute cutting-edge definition of personalised medicine. So that begs the question, how can we possibly treat such disorders? Especially, n-of-1 trials, where a drug or a therapy is developed and tested to treat a single individual.

Creating new therapeutics with ASOs...

Over the past few years, antisense therapy has become one of the many ways to treat a handful of rare genetic conditions. Antisense therapy is a relatively old idea from over two decades ago that uses molecules called antisense oligonucleotides (ASOs). These ASOs are typically designed to treat genetic disorders that involve a mutation that leads to abnormally high or low amounts of genes expressed. Currently, there are over 50 ASOs in clinical trials in treatments for the more familiar rare disorders such as Huntington's disease, Alzheimer's disease, Prion disease, Parkinson's disease, and Duchenne muscular dystrophy.

ASOs truly came to be at the forefront in 2019 in the remarkable case of Mila Makovec. Dr Timothy Wu's lab developed milasen, in a record-breaking 10-month development process to treat, now the 11-year-old, Mila. The first person (of hopefully many) to ever have a drug created specifically for them [3].

So how do ASOs work?

Genetics tells us that genes encode molecules called mRNAs. mRNAs are the template that helps cells create proteins, which in turn carry out the vast majority of a cell's essential functions.

CREDIT: UNKNOWN

Many genetic diseases are caused by mutations that can lead to abnormally high levels of a certain gene's mRNA. One potential way to treat these kinds of conditions is to reduce the amount of mRNA back to normal levels. This process is known as gene silencing and forms the basis of the idea behind how ASOs work.

ASOs are negatively charged molecules that are carefully crafted to bind to the mRNA almost like a zipper: this is because they have a sequence complementary to the mRNA. This then allows it to be degraded through naturally occurring cellular processes, effectively reducing the level of proteins that could cause disease.

What's holding ASOs back?

ASOs are cleverly personalised and highly-specific drugs. But, like many other drugs, ensuring that they don't have unintended effects inhibiting other key cellular processes is a near-universal concern for drug designers that complicates their development especially in considering their dosage and composition.

However, the greatest technical challenge inhibiting their widespread use has been delivering them effectively to the cell or target tissue, as they have difficulty overcoming the lipid bilayer [5,6]. For instance, ASOs targeted towards treating Huntington's disease (which is a genetic disorder primarily localised to the brain) have to overcome the 'brain-blood' barrier and due to their highly delicate composition cannot be simply delivered through either an injection or a pill.

What does the future hold?

ASOs are not panaceas. They are very suitable for certain types of disorders such as those that are neurological [7] or early-onset developmental in nature, which usually are a result of a single genetic mutation.

With every therapeutic success such as milasen, there are many failures. In recent memory, the results from two different ASO trials treating Huntington's disease showed essentially no benefit. Other attempts, such as an ASO candidate [7] that aims to treat a certain form of ALS were scrapped as well. Whereas another candidate [8] to treat different form of ALS that was meant to reduce levels of the SOD1 protein failed to show promise after Phase III trials.

Regardless, any personalised drug, ASOs in particular, suffer from regulatory issues. One particularly thorny example is a consequence of the EU's Orphan and ATMP regulations [10]. ASOs that can treat rare diseases can bypass many of the regulatory processes that many usual drugs are required to abide by. However, n-of-1 ASOs are notably excluded from these regulations. Regulatory processes aim to ensure the safety and efficacy of drugs but cost money and most crucially time. But, as n-of-1 trials are targeted toward treating developmental or neurological disorders, early treatment is crucial in many instances like in the case of milasen.

Increasing funding for translational ASO research and streamlining regulatory processes are crucial factors for antisense therapy to flourish in the near future. Whether they can be the go-to tool in the arsenal in the fight against rare genetic disorders is an answer we will likely have to wait for.

References & Further Reading

1. https://rarediseases.info.nih.gov/about/
2. Bennett, C. F. & Swayze, E. E. RNA targeting therapeutics: Molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annual Review of Pharmacology and Toxicology 50, 259–293 (2010).
3. Cross, R. Milasen: The drug that went from idea to injection in 10 months. Cen.acs.org (2019). Available at: https://cen.acs.org/business/Milasen-drug-idea-injection-10/97/i42. (Accessed: 9th June 2022)
4. https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=dd70cd5f-b0fc-4ba4-a5ea-89a34778bd94
5. Liou, S. Antisense gene therapy. HOPES Huntington's Disease Information (2014). Available at: https://hopes.stanford.edu/antisense-gene-therapy/. (Accessed: 9th June 2022)
6. Gagliardi, M. & Ashizawa, A. T. The challenges and strategies of antisense oligonucleotide drug delivery. Biomedicines 9, 433 (2021).
7. Silva, A. C. et al. Antisense oligonucleotide therapeutics in neurodegenerative diseases: The case of polyglutamine disorders. Brain 143, 407–429 (2019).
8. Biogen and ionis announce topline phase 1 study results of investigational drug in C9ORF72 amyotrophic lateral sclerosis. Biogen Available at: https://investors.biogen.com/news-releases/news-release-details/biogen-and-ionis-announce-topline-phase-1-study-results. (Accessed: 9th June 2022)
9. Biogen | Investor relations. Available at: https://biogen.gcs-web.com/static-files/b2154d4e-f69f-49d4-9a61-e834387293ea. (Accessed: 9th June 2022)
10. Bennett, C. F. & Swayze, E. E. RNA targeting therapeutics: Molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annual Review of Pharmacology and Toxicology 50, 259–293 (2010).
11. Oligonucleotide API market. Future Market Insights Available at: https://www.futuremarketinsights.com/reports/oligonucleotide-api-market. (Accessed: 9th June 2022)

NEWBORN SCREENING FOR SPINAL MUSCULAR ATROPHY

by Rebecca Howitt

What is Spinal Muscular Atrophy?
Spinal muscular atrophy (SMA) is a neurodegenerative condition that arises due to the loss of both copies of the SMN1 gene. The SMN1 gene encodes the survival motor neuron protein, which is essential for motor neuron health. There are four different types of SMA (see Table 1) with type I being the most severe, whereby affected infants develop low muscle tone ('floppiness') and delayed motor milestones within the first 6 months of life. Eventually, the respiratory muscles also fail, resulting in fatality by the age of 2 years. Whereas type IV does not develop until adulthood, and has much milder symptoms. The differences in SMA types arise due to varying copy numbers of the SMN2 gene. The SMN2 gene usually produces a non-functional protein, but ~10-15% of transcripts produce a full-length survival motor protein (SMN). In the absence of SMN1, the low level of SMN produced by SMN2 is able to compensate slightly. Hence, with more SMN2 copies, there is a greater level of SMN production, improved motor neuron survival and lower disease burden.

The Power of Newborn Screening
Newborn screening is done via a heel-prick blood test on day 5 after a baby is born. Currently 9 conditions are screened for in the UK, including sickle cell anaemia and cystic fibrosis, and by identifying these early, babies can begin treatment. This same principle applies to SMA, which is already screened for in several countries, including Belgium, Germany, Japan and the USA. Genetic testing looks for a deletion of exon 7 of the SMN1 gene, which is the most common causative loss-of-function mutation in SMA. By detecting SMA early, novel disease-modifying drugs that halt disease progression can be given before symptoms develop. The two licensed treatments for SMA are onasemnogene aveparvovec and nusinersen, which are one-off injections of gene therapy treatments that act to prevent neuronal death. Giving these at the presymptomatic disease stage, prior to high levels of motor neuron death, greatly improves the outcomes for babies with SMA. Otherwise, the treatment is not initiated until a child develops diagnostic symptoms such as muscle weakness, and at this point, the motor neurons that have already died cannot be salvaged by gene therapy. Therefore, newborn screening enables a much higher rate of neuronal survival and a lower level of SMA disease burden. Excitingly, the STRONG research group based in the Department of Paediatrics, Oxford have just begun a pilot study of newborn screening for SMA in the Thames Valley area, with the hope that it will eventually be introduced into routine testing in the UK.

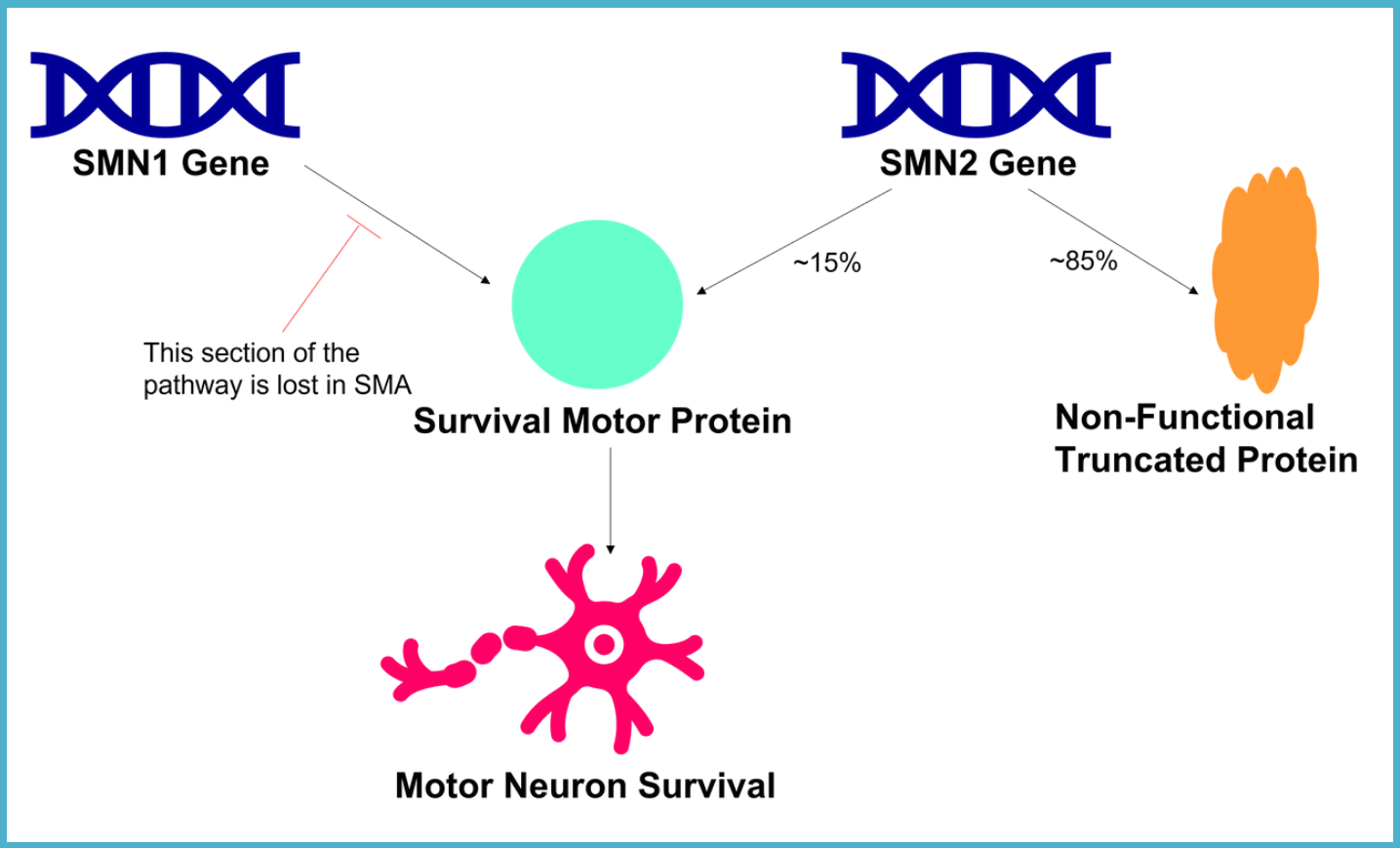
Challenges and Ethical Conundrums
As mentioned earlier, there are four types of SMA (types I-IV) each with differing levels of severity. Type I is the most common type and manifests very early in childhood with devastating consequences, whereas type IV SMA (which accounts for <2% of cases) is characterised by mild symptoms that do not develop until adulthood. Whilst all types have a SMN1 gene deletion (which can be picked up by newborn screening) in common, it is the number of SMN2 gene copies that influences the disease severity. Therefore, following a positive bloodspot result, subsequent tests can be performed to measure SMN2 copy number, with additional copies being associated with a milder form of SMA. However, SMN2 copy number cannot perfectly predict SMA type, leading to a difficult conundrum in

Type	Age of Onset	Level of Neurodisability	Life Expectancy	SMN2 Copy Number
I	0-6 months	Cannot sit	<2 years	2
II	<18 months	Cannot stand	20-30 years	3-4
III	18 months – 30 years	Can stand, may need mobility aid to walk	Normal	3-4
IV	>30 years	Able to stand/walk alone	Normal	4-8

how to report and treat cases identified by newborn screening where a patient has a SMN1 deletion but ⩾4 SMN2 copies. They may have type III SMA and become symptomatic in childhood, or have type IV and remain asymptomatic for many years to come.
This raises an ethical dilemma as to whether it is right to inform a child and their family that they have a chronic health condition that will not develop until they are older. Especially given that the child may not be able to access gene therapy treatment if they have ⩾4 SMN2 copies and are asymptomatic, it can create potentially detrimental levels of health anxiety. Moreover, The European Society of Human Genetics advises against screening children for conditions that manifest in adulthood, and instead recommends waiting until the individual is old enough to give informed consent. However, given the number of SMN2 copies cannot predict the exact age of onset, this line is blurred, and indeed there is global discrepancy in how this issue is managed. For example, in an Australian pilot study, only SMA cases with ⩽3 SMN2 copies were reported as positive (as only these would be eligible for treatment), whereas in Massachusetts, USA, two babies with 4 SMN2 copies were diagnosed with SMA via newborn screening and given gene therapy treatment. This highlights another of the biggest challenges in genomics and personalised medicine – global inequality in access to diagnostics and treatment.

References & Further Reading

1. Dangouloff, T. *et al.* Newborn screening programs for spinal muscular atrophy worldwide: Where we stand and where to go. *Neuromuscular Disorders* 31, 574–582 (2021).
2. Glascock, J. *et al.* Treatment Algorithm for Infants Diagnosed with Spinal Muscular Atrophy through Newborn Screening. *Journal of Neuromuscular Diseases* 5, 145–158 (2018).
3. Kolb, S. J. & Kissel, J. T.. Spinal Muscular Atrophy. *Neurologic Clinics* 33, 831–846 (2015).
4. Müller-Felber, W. *et al.* Infants Diagnosed with Spinal Muscular Atrophy and 4 SMN2 Copies through Newborn Screening-Opportunity or Burden? 1. *Journal of Neuromuscular Diseases* 7, 109–117 (2020).
5. Nicolau, S., Waldrop, M. A., Connolly, A. M & Mendell J.R. Spinal Muscular Atrophy. *Seminars Pediatric Neurology* 37, 100878 (2021)
6. https://www.ox.ac.uk/news/2022-03-11-first-uk-pilot-study-newborn-screening-spinal-muscular-atrophy-launched-oxford



FROM LENSES TO ADENOVIRAL VECTORS

by Tomasz Szeligowski

CREDIT: HTTPS://WWW.GENOMICSEDUCATION.HEE.NHS.UK/BLOG/BACK-TO-THE-FUTURE-OF-OPHTHALMOLOGY-5-PREDICTIONS/

In the 19th century, Hermann von Helmholtz revolutionised ophthalmology with the direct ophthalmoscope - a device which gave ophthalmologists sight by allowing them to look inside the eye. Since this discovery, diagnosing retinal diseases has relied heavily on describing their abnormal appearance on examination. The emergence of genetic testing allowed us to understand that many conditions previously grouped together due to similar appearance were in fact caused by diverse gene mutations. Thus, patients could be stratified into increasingly precise diagnoses, each with unique features and possible treatments. This extended to both congenital eye diseases, and acquired conditions where many genetic risk factors contribute to the complex risk profile. But knowing the genetic causes of eye diseases is clearly not enough - after all, a patient who comes to see their ophthalmologist may not be interested in whether their condition is caused by mutations in retinoid isomerohydrolase or guanylyl cyclase 1. They come to have their sight restored, thus inspiring research in gene therapies.

The concept of manipulating human genes for medical purposes has, for a long time, existed in the realm of science fiction. Even when it started coming closer to reality, the problems associated with it, both in terms of creating efficient gene delivery systems and patient safety, cast doubt on how feasible it was as a treatment strategy. A true breakthrough came in 2017, when the FDA approved Luxturna - the first licensed gene therapy product in history. It was designed to treat a subtype of Leber congenital amaurosis - a family of inherited retinal conditions caused by a number of mutations, where impaired photoreceptor function leads to early loss of vision. Luxturna uses a viral vector - a genetically engineered virus which harbours a functional copy of the defective gene and has the ability to infect non-dividing retinal cells to induce production of the functional gene, and hence restore photoreceptor cell function. The virus is injected directly beneath the retina, allowing precise delivery to its target site. This milestone invention sparked renewed interest in gene therapies, and put ophthalmology at the forefront of gene therapy research. But why ophthalmology? And what can we expect in the future?

Why ophthalmology?

Delivering genes to human cells is a difficult task. It requires producing an effective vector which will deliver the genetic product to precisely targeted cells and maintain long-term gene expression, as well as avoiding anti-viral immune responses which can not only prevent the intervention from working, but also potentially trigger destructive inflammation in the target organ. The unique features of eyes make them the perfect candidate for gene therapies. First, they are one of the so-called “immune privilege sites” where immune responses are naturally dampened through a variety of mechanisms, as inflammation could result in clouding of the visual pathway. Another crucial feature is that the eye is simply easy to reach - it is one of the few organs we can look directly into, allowing precise delivery of vectors. Finally, its lack of lymphatic drainage limits the escape of vector viruses into the bloodstream where they might encounter the immune system, while the fact that retinal cells do not divide ensures long-term expression of introduced genes.

Where next? - Ophthalmology in the age of precision medicine

Although gene therapy is still in its infancy, it is a busy area of research. Its success will depend on parallel advances in two fields: gene therapies themselves and genetic testing. As mentioned before, eye conditions often have many possible mutations leading to similar presentations, making precise genetic diagnosis essential for identifying treatment targets. Once targets are identified, flexible and robust vectors will be necessary to allow efficient production of personalised vector constructs to match specific subgroups of patients.

This in turn will be crucial in overcoming a great obstacle in gene therapies - their cost. Importantly, replacement of defective genes is only the beginning. There is growing interest in the use of advanced gene modification systems like CRISPR-Cas9 which allow direct repair of mutations at the nucleotide level. This system relies on a protein capable of cutting DNA at precise points, and an RNA construct which guides the cutting protein to its correct location. Thanks to this, the CRISPR-Cas9 system can be used in a variety of ways including inducing small deletions to inactivate abnormal genes, inserting DNA sequences, or even modification of individual nucleotides thanks to the addition of special DNA editing enzymes. Thus, these approaches require an even deeper understanding of the patient's genetic make-up as they depend not only on the knowledge of the genes affected, but also the specific sequences of affected DNA regions.

An especially exciting new avenue for gene therapies will be the treatment of acquired conditions, for example age-related macular degeneration (AMD) which is a leading cause of blindness in the developed world. The aetiology of AMD is extremely complex and large genetic studies identified many genes associated with its development, each contributing only a fraction of the overall risk. The most promising approach in gene therapy for AMD involves targeting the contributing physiological pathways, in particular the VEGF molecule responsible for formation of abnormal retinal blood vessels. Currently, VEGF is targeted with regular eye injections of anti-VEGF antibodies that inhibit its function, but this treatment is uncomfortable for patients, and associated with risks each injection. A clinical trial currently underway offers a solution to this problem by introducing the anti-VEGF antibody gene directly into the retina, thus allowing its prolonged expression. The available results already show great promise with stable visual function and a 96% reduction in the need for anti-VEGF injections. Increasing the availability of genetic testing may mean that in the future, AMD patients will be routinely tested for variants contributing to their disease, which may in turn inform which pathophysiological pathways could be targeted to stop the progression of their disease.

The future for gene therapies is bright, and ophthalmology will likely continue to lead the way in this field. However, developing treatments for previously untreatable conditions is not the only outcome; availability of gene therapies will undoubtedly widen general access to genetic testing. This change will improve diagnostic precision and as a result truly launch ophthalmology into the era of personalised medicine.

References & Further Reading

1. Gordon K, Del Medico A, Sander I, Kumar A, Hamad B. Gene therapies in ophthalmic disease. Nat Rev Drug Discov. 2019 Jun;18(6):415-416
2. Guimaraes TAC, Georgiou M, Bainbridge JWB, Michaelides M. Gene therapy for neovascular age-related macular degeneration: rationale, clinical trials and future directions. Br J Ophthalmol. 2021 Feb;105(2):151-157
3. Moraru AD, Costin D, Iorga RE, Munteanu M, Moraru RL, Branisteanu DC. Current trends in gene therapy for retinal diseases (Review). Exp Ther Med. 2022 Jan;23(1):26.
4. Dhurandhar D, Sahoo NK, Mariappan I, Narayanan R. Gene therapy in retinal diseases: A review. Indian J Ophthalmol. 2021 Sep;69(9):2257-2265.

WEARABLES - BETWEEN BIG DATA AND MANAGING PARKINSON'S ONE STEP AT A TIME

by Smaranda Codreanu

Globally, due to an increase in life quality and expectancy, neurological impairments seem to have increased in prevalence. Parkinson's disease is the second most common neurodegenerative disease, affecting over 6 million people worldwide, especially people over the age of 60. Over time, the cause was identified as the destruction of the substantia nigra in the nervous system. However, no precise biomarkers can be used routinely to assist in diagnosis, thus the diagnosis is based on the symptoms of muscle rigidity, tremor, insomnia and postural instability. Treatment includes a gradual progression of different drugs and increasing the dose accordingly as the body gets used to the medication. The key feature is that the treatment is supposed to delay the onset of symptoms and preserve the patient's autonomy for as many years as possible. Of course, the practitioner sets dates for regular check-ups, but sometimes these dates are too far apart from each other and the optimal control of the diseased cannot be reached. Thus, the moment when the symptoms (fluctuation, wearing-off phenomena) worsen cannot be pinpointed, leading to a lack of satisfaction on both sides.

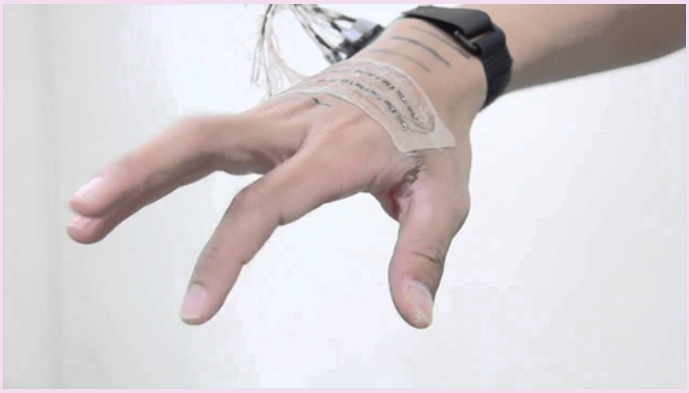
In the era of technology, we use the internet to call our friends across the globe and a multitude of apps and smart devices to track everything from how long we sleep to how many steps we are walking a day. So why can't technology be the solution for better monitoring Parkinson's? We have the proper devices to regularly check the gait, tremor and sleep in Parkinson's disease, providing a tool for decision-making in the clinical setting by capturing data from everyday activities in an objective manner. The ability to remotely capture behavioural data and use it to optimize treatment strategies could make a great difference in the quality of living.

So far even with big players in this game like Intel and the Michael J. Fox Foundation and smaller teams from research labs all around the globe, we've yet to reach a consensus on how to best approach the monitoring of Parkinson's. Most smart devices have tried different approaches to monitor the gait and tremor, starting from wearing just a smartwatch to wearing an assembly of multiple sensors placed on the thigh and trunk. These sensors are then tied down to an accelerometer to measure step length and frequency and a gyroscope is worn on the wrist to track the tremor. The newest additions go as far as using insoles for plantar pressure and step monitoring. In the end, they all aim to be unobtrusive, and cost-efficient, with a longer battery life, to increase patients' compliance.

If you think that all these seem too good to be true, you are most definitely right. All the studies have rather small samples of patients (think dozens), only include patients with early onset or mild symptoms and the algorithms used by the devices often have trouble distinguishing between tremor and intention movements. Throw into this mix sensitive medical data, high costs and moderate compliance at best and you might understand why we have had ongoing debates on how to approach wearables for the better part of the last 8 years.

Although it seems like I have painted a rather dire image of the current state of the art technologies on monitoring Parkinson's, the bottom line is that the wearables have proven to be efficient on small study samples and they allow us to collect objective longitudinal raw data for the first time. Moreover, distinguishing between tremor and intention movement will be easier for the algorithms as they have larger sets of data. Based on this, the algorithms can learn actively from the subject's habits and activities and can thus offer a personalised approach to monitoring from the comfort of their own home. The practitioners and researchers are easily pinged in the scenario of worsening symptoms or abnormalities from the baseline. This is followed by a sooner check-up and adjustment of treatment where it is needed, ensuring improved life quality for the patients.

We have a very long way to go in order for all these technological advances to be standardised and approved by FDA and EMA for daily use on a larger scale, but we are making progress one step at a time by asking ourselves: how can we do better for patients suffering of a disease without a cure in sight.



References & Further Reading

1. Adams, J.L., Dinesh, K., Snyder, C.W. et al. A real-world study of wearable sensors in Parkinson's disease. npj Parkinsons Dis. 7, 106 (2021). https://doi.org/10.1038/s41531-021-00248-w.
2. Channa A, Popescu N, Ciobanu V. Wearable Solutions for Patients with Parkinson's Disease and Neurocognitive Disorder: A Systematic Review. Sensors (Basel). 2020 May 9;20(9):2713. doi: 10.3390/s20092713. PMID: 32397516; PMCID: PMC7249148.
3. Rovini, E.; Maremmani, C.; Cavallo, F. A Wearable System to Objectify Assessment of Motor Tasks for Supporting Parkinson's Disease Diagnosis. Sensors 2020, 20, 2630. https://doi.org/10.3390/s2009263

PERSONALISING PARKINSON'S

INDIVIDUALISATION OF DEEP BRAIN STIMULATION TARGETING

By Jenny Park

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease after Alzheimer's disease. In the UK alone, more than 145,000 people are living with PD, and over 10 million individuals are diagnosed with this condition worldwide. These numbers will increase with the rise of the ageing population — an unsettling future in the absence of a current "cure" to halt its disease progression. Nonetheless, personalised approaches in advanced treatments like deep brain stimulation (DBS) have vast potential to improve treatment of Parkinson's disease.

What is Parkinson's Disease (PD)?

First described by Dr. James Parkinson in 1817, symptoms of Parkinson's disease include tremor at rest, slowness of movement, rigidity of the extremities and neck, and minimal facial expressions. Compounding their motor complications, PD patients also report non-motor symptoms: anxiety, dementia, depression, bladder and bowel problems, and sleep problems. Primary motor defects are linked to a significant loss of dopaminergic neurons in the brain. These neurons play a central role in transmitting signals in brain pathways responsible for inhibiting and initiating movement. In fact, up to 60-70% of dopaminergic neurons are lost when a patient starts to exhibit motor dysfunction. Dopaminergic neurons are concentrated in a brain region called the substantia nigra compacta, which is Latin for "black substance". This is particularly fascinating because the substantia nigra of a healthy brain displays black pigment, unlike a Parkinson's brain that has depleted most of its dopaminergic neurons (Figure 1).

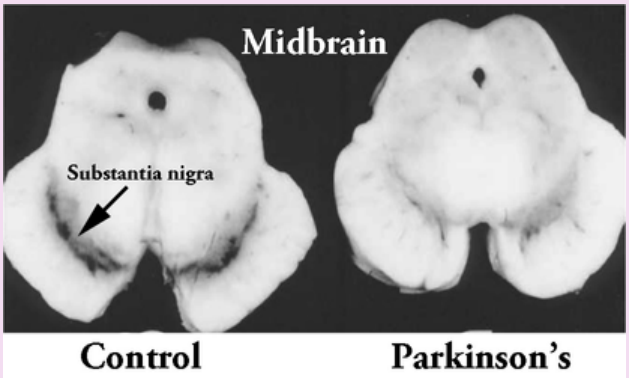


Figure 1: Contrasting pigmentation in substantia nigra (Control vs PD)

CREDIT: HTTPS://SCIENCEOFPARKINSONS.COM/2019/12/03/TAU/

An Effective Treatment for Parkinson's: Deep Brain Stimulation (DBS)

No miracle drug has yet been discovered for Parkinson's, but scientific breakthroughs have led to highly effective treatments, including deep brain stimulation. Deep brain stimulation is a surgical procedure lauded for its dramatic clinical benefits for motor problems in Parkinson's disease patients. Electrodes are implanted in the brain along with a pulse generator implanted in the chest wall. Then, an electric current is generated through a connected lead wire to stimulate the targeted deep brain tissue to alleviate violent tremors or other motor symptoms (Figure 2). The two common targets in which neurosurgeons place these electrodes are either the subthalamic nucleus (STN) or the internal segment of the globus pallidus (GPi).

While effective, there is an increasing demand to further improve the existing system of neuromodulation. No single patient is alike; therefore, a personalised approach on DBS technology enables the tailoring of electric stimulation to an individual's symptoms. Several studies have examined ways to adopt methods that are fine-tuned to the unique clinical spectrums presented by each patient.

A Fully Individualised Approach in DBS Targeting

One approach in individualised DBS targeting is called the "fully individualised template approach". This method adopts a symptom-specific approach where DBS targeting is based on the specific symptoms exhibited by the patient. First, the individual patient is studied using functional neuroimaging (fMRI or PET) in order to visualise the patient's symptom network. In 2019, Barcia et al. utilised the fully individualised template approach: while provoking the patient's symptoms during the scan, Barcia et al. acquired fMRI data that could be used to identify the regions associated with the symptoms. This data is then interpreted to estimate potential neuromodulation targets that would have maximum improvement. Though this study was done in patients with Obsessive Compulsive Disorder, this method may be used to identify optimal DBS targets. Even though there are limited published examples that use the fully individualised approach for Parkinson's disease patients, this approach is still promising because it increases the efficacy of DBS stimulation on specific target sites in the brain and the debilitating symptoms of PD. In a fully individualised approach in DBS targeting, there is no involvement of a group template or an average template obtained from a set of subjects. Instead, only the patient's preoperative data is used to generate the optimal anatomical target. The benefit of this method is in its ability to minimise

variability in the efficacy of DBS since traditional DBS targets have often led to unsatisfactory therapeutic outcomes for patients.
However, there are limitations that need to be addressed to use this strategy. The individual differences observed in the fMRI scans may be attributed to noise from the MRI machine rather than the unique differences in each patient's brain. Further steps need to be taken in order to reach widespread usage of symptom-specific DBS targeting.

The Future of DBS
Despite these hurdles, the individualisation of DBS targeting promises an exciting future in DBS targeting. When future studies develop standardised protocols that optimise target sites for individuals, it will enhance the quality of treatment for those suffering from the debilitating symptoms of PD.

References & Further Reading

1. Mishima, T., Fujioka, S., Morishita, T., Inoue, T. & Tsuboi, Y. Personalized medicine in parkinson's disease: New options for advanced treatments. *Journal of Personalized Medicine* 11, 650 (2021).
2. Perlmutter, J. S. & Mink, J. W. Deep Brain stimulation. *Annual Review of Neuroscience* 29, 229–257 (2006).
3. Rajamani, N., Horn, A. & Hollunder, B. Outlook: Towards personalized connectomic deep brain stimulation. *Connectomic Deep Brain Stimulation* 527–542 (2022). doi:10.1016/b978-0-12-821861-7.00009-9

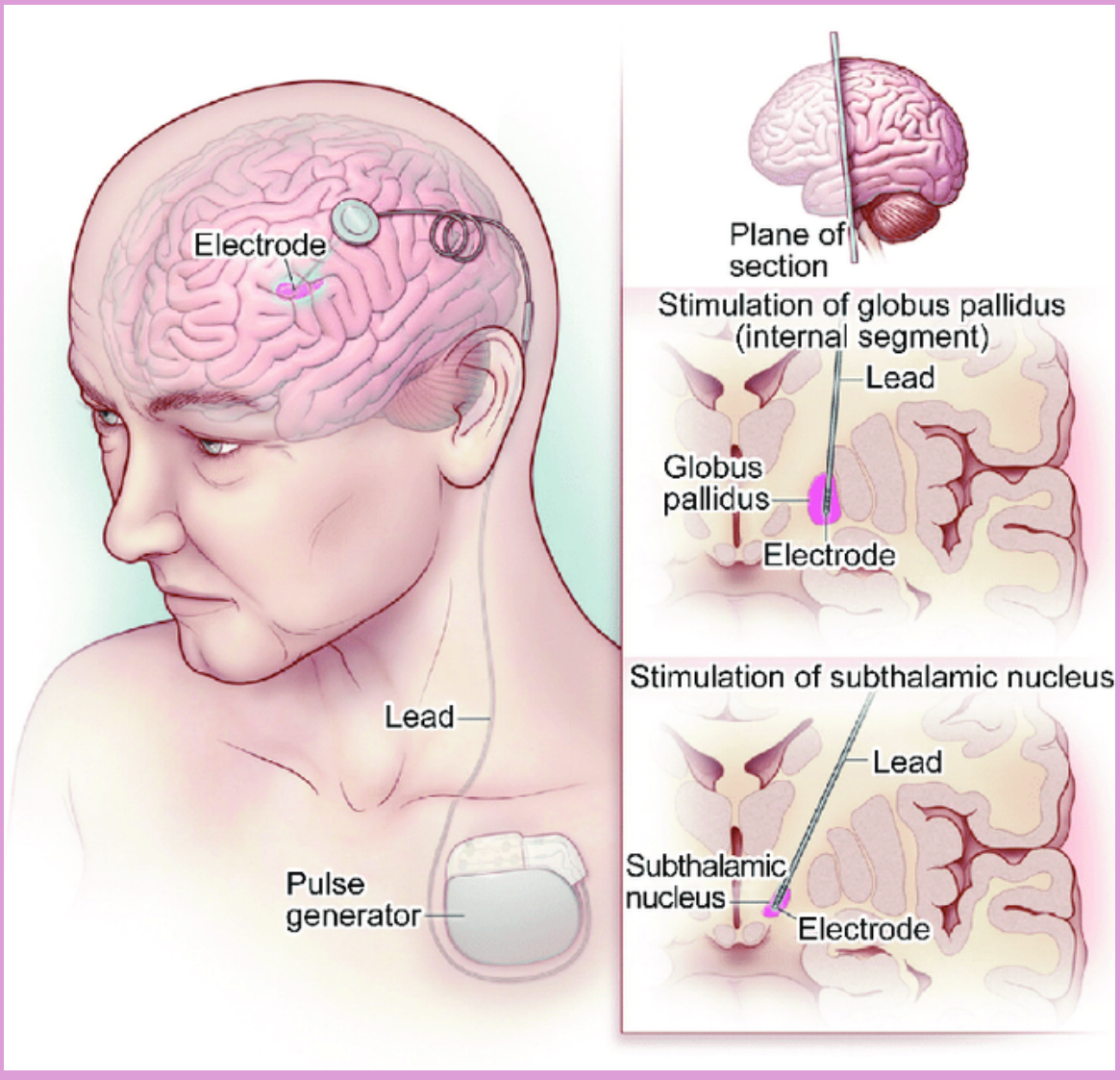


Figure 2: Stimulation on subthalamic nucleus and globus pallidus internal segment

CREDIT: EDELMAN *ET AL.*, 2015

USHERING THE FUTURE OF PSYCHIATRIC RESEARCH WITH OPTOGENETICS

by Elston D'Souza

Rene Decartes, once said that the mind exists separate from the body back in the 16th century. This may be why psychiatric disorders are among the most difficult to both diagnose and to treat. Many other disorders can usually be tested or imaged, whilst most psychiatric conditions still rely on individual consultations rather than examining the brain directly. To allow for a better understanding of the brain's circuitry especially when disrupted during psychiatric disorders, Francis Crick - one of the founders of the structure of the DNA molecule suggested that neuroscience needed to have a switch that could turn specific neurons on or off.

In the brain, the electrical activity of cells called neurons and how they project to their nearest neighbours, help regulate behaviour. When there are deficits in these activities, we think they are the root cause of various psychiatric disorders. However, the exact details behind how these neural activities work has eluded scientists for many years. Lately, a promising technology called optogenetics may help us understand the pathophysiology behind many disorders.

How does optogenetics work?

Optogenetics uses molecules that convert light into electricity inserted into neurons through novel gene-therapy techniques [1]. Once in place, researchers can shine light on neurons, letting them switch each neuron effectively on or off. This can let researchers look at what the end result of behaviours is if there are certain neurons turned on, and conversely which neurons are essential, such that they would lead to disorders if turned off. The promise of this technology is the precision at which researchers are able to target individual neurons.

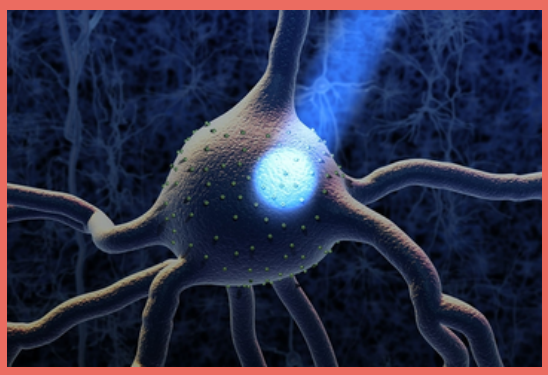
Finding the aetiology of anxiety

We live in a time where mental illness is on the rise, with nearly 1 in 5 individuals suffering from a mental illness [2]. But like many disorders, the underlying mechanism behind even the most common illnesses such as anxiety are unclear.

While we know that fear and anxiety is primarily controlled by the amygdala, we were unclear of the details until a certain experiment with mice in 2011 showed that when the bed nucleus of the stria terminalis region of the amygdala was involved with anxiety-related behaviours [3]. For example, when optogenetics was used to stimulate these neurons, the mice were calmer, and when inhibited they displayed anxious behaviour as well as a higher respiratory rate.

References & Further Reading

1. https://psychscenehub.com/psychinsights/optogenetics-in-psychiatry-2/
2. https://digital.nhs.uk/data-and-information/publications/statistical/adult-psychiatric-morbidity-survey/adult-psychiatric-morbidity-in-england-2007-results-of-a-household-survey
3. https://www.nature.com/articles/nature09820
4. https://www.youtube.com/watch?v=Nb07TLkJ3Ww
5. https://news.stanford.edu/features/2014/optogenetics/



CREDIT: MCGOVERN INSTITUTE FOR BRAIN RESEARCH

Where are we currently at with optogenetics?

Currently, optogenetics is still used in ongoing research into other mental disorders such as depression, but also relatively rare disorders such as schizophrenia and bipolar disorder. However, many of these studies are based on mouse models and have yet to be translated to humans. Thus, any inferences made are contingent on the evolutionary similarity between humans and mice.

The major reason why is primarily because in order to stimulate neurons, gene therapy must be used to transfer genes encoding photosynthetic machinery from algae, fungi or bacteria. Gene therapy, whilst gaining momentum, is still in its infancy in terms of regulatory development. Furthermore, we are still unaware of the consequences of injecting foreign bodies such as the effect on the immune system [4].

How can we diagnose and treat people better using optogenetics?

However, the future does look promising with optogenetics. We know that many psychotropic drugs that are used to manage psychiatric disorders work by modulating the level of neurotransmitters which in turn modulate the electrical activity within the brain. However, these drugs rarely are specific and can affect the biochemistry of various regions within the brain, in turn potentially a lack of effectiveness. Through optogenetics, we can hone in on the specific neurons we know cause the disorder and target molecules within those circuits leading to better drugs that are specific to the patient, potentially reducing side effects [4].

Alternatively, we can use optogenetics directly through stimulating the specific neurons, in situ, or in place, where light can be used directly to stimulate a specific subset of neurons as a part of a therapy. However, the flexibility of optogenetics means that the neuron doesn't necessarily have to be in the brain, but other peripheral nerves such as within muscles. Despite initially being positioned to understand the brain, optogenetics could be used to help muscles contract in disorders such as cerebral palsy [5].

Optogenetics has come light years since Prof. Deisseroth at Stanford trialled the idea that opsins from green algae (aka pond scum) are at the heart of optogenetic technology to turn on and off neurons. We are still uncovering the various uses to apply optogenetics not only to understand the basis of psychiatric disorders but also treating them [5].

THE AI DOCTOR WILL SEE YOU NOW

by Michael Milad

Artificial intelligence (AI) has recently received significant hype, with promises that "they" will drive our cars, do our work, or potentially spell the end of civilisation. Despite all this excitement, few can accurately explain what AI is. Nick Bostrum, a philosopher at the University of Oxford, categorised three major groups of AI:

1. Narrow AI – this is the AI we are most familiar with – it uses a previously defined algorithm to continually improve, restricted to a specific function. So, whilst a narrow intelligence machine can beat the best chess player in the world, it still has an IQ of zero as it fails to do anything else.
2. General AI – a level of AI that has not yet been reached. At this level, the machine could one day have the same cognitive abilities as a human being, able to effectively reason, argue, memorize, and solve issues.
3. Artificial Superintelligence – a theoretical, potentially dystopian machine which exceeds the combined cognitive capacity of humanity. Figures such as Stephen Hawking, Bill Gates and Elon Musk have expressed how an artificial superintelligence could escape human control, take a treacherous turn that eventually results in the extinction of humanity. The fear of reaching this level is what is driving Musk to integrate AI with the brain through Neuralink, ensuring symbiosis with superintelligence rather than competition.

These definitions explain the multifaceted nature of AI, which is best defined by the complexity and cognitive ability of the algorithms. Narrow and general AI are most likely to revolutionise medicine in the upcoming decades, and should be on the radar of aspiring and current clinicians.

Narrow AI has already shown promise in revolutionizing medicine. Researchers at the John Radcliffe have developed an AI system which is more accurate at diagnosing heart disease than doctors in over 80% of cases. Across the Atlantic, researchers at Harvard University have developed an AI-assisted diagnostic tool that can detect potentially lethal blood infections: they trained the machine on a series of 100,000 images, garnered from 25,000 slides. The system was able to recognise bacteria with a 95% accuracy. Given that a recent workforce census determined only 3% of NHS histopathologists have enough staff to meet clinical demand, AI would aid alleviate shortages.

Cogito, a behavioural analytics company, has been using AI-powered voice recognition to analyse and improve customer service interactions across a range of industries. They have delved into the healthcare industry with their recent "Cogito Companion" App, which tracks a patient's behaviour, speech, and interactions. It does so by monitoring a patient's phone for both passive and active behavioural signals, such as location data that can indicate when a patient hasn't left their home for a long period, and communication logs, that indicate they haven't spoken to anyone for several weeks. Integrated apps such as this demonstrate the potential role of AI in personalised medicine: algorithms can monitor patients, observing behaviour as well as reminding them to take medication. Furthermore, general intelligence could learn about the daily habits of each patient, fitting in reminders at times that will maximise adherence. This could all improve not only medical outcomes, but patient's subjective experience of care.

However, there remains a lot of uncharted territory when it comes to a machine handling our health. For example, whilst robotic tools are currently valuable parts of surgical practice, they make take a more independent role, operating without the direct instruction of a surgeon. What happens if a mistake arises: can a patient sue a robot for malpractice? Traditionally, medical malpractice is thought to be the result of negligence on the part of the doctor. However, the concept of negligence, especially for narrow intelligence, is an awareness inherently lacked by AI. If not the robot, who takes the blame – the doctor overseeing the company manufacturing it, or the specific engineer that designed the algorithm? Another major issue is security – with medical apps likely to take a larger role in our healthcare, who will store, and control, all this data? These questions must be addressed soon if AI is to be fully integrated into care.

We must also remember that narrow AI relies on the data that we input and that abstractions which are logical to humans are not to machines. For example, dermatologists often use rulers to measure lesions that they suspect are cancerous, and the ruler then features in any photo taken of the lesion which is added to the patient's medical record. When a series of these images are presented to the machines, they learn to associate the presence of a ruler in a photo of a malignant lesion and the resulting algorithm is more likely to say a lesion was cancerous if a ruler was present, regardless of the appearance of the lesion itself. This means the algorithm may be failing to recognise malignant skin lesions in photos when there is not a ruler. Algorithms may also inherit our bias, resulting from a lack of diversity of the input, such as patient data, used to train AI: white men still dominate within clinical and academic research, as well as accounting for most of the patients involved in clinical trials.

Despite these issues, AI will become prominent in medicine, which places the role of the physician in question.

Will doctors remain relevant when general Intelligence can answer all questions and recommend more effective treatment in a fraction of the time it takes a human? The answer is yes, but physicians will have a remarkably different duty: instead of focusing their time and attention on diagnostics and admin, doctors will be at the side of the patient, providing the human touch of medicine.

It is important to remember that humans are the ones ultimately driving the change in AI – rather than fearing the future, physicians should be prepared to build it.

CREDIT:
HTTPS://WWW.FLICKR.COM/PHOTOS/30478819@N08/50959554757

Self-regulated DNA Nano carriers- A programmable drug release for better therapy in cancer and other diseases.

Author
Sadia Adnin Oyshi

Affiliation
B. Pharm (4th year)
East West University

Abstract

Optimized drug release is a huge contemporary challenge, specifically in the case of cancer patients. A perfect personalized drug delivery can bring about an immense impact in overall therapeutic effect. If the drug delivery system is related to a specific individual's DNA sequence, medicinal delivery can be vastly improved. Thus, this study focuses on self-regulated DNA nanocarriers, specifically indicating a self-regulated buffer system that can be programmed. It mainly follows Le Chatelier's principle to regulate nucleic acid molecular buffers for the chemotherapeutic drug, doxorubicin, and the antimalarial agent, quinine. This process can result in potent sustained drug release, enhancing therapeutic effect and minimising adverse effects.

Introduction

DNA sequences lie at the root of our individuality. Thus, ensuring optimized drug delivery is impossible without engineered compatibility with these genes in drug discovery. In the case of cancer patients, especially, it is a huge challenge. At present, only around 50% of these patients have a fighting chance of optimized drug delivery. Developing self-regulated DNA nano-carriers which specifically indicate efficient drug delivery with self-regulated programmable nucleic acid molecular buffers may just be able to bring about miracles for these patients. This study shows best results to this effect in the case of chemotherapeutic drug, doxorubicin, and the antimalarial agent, quinine. It can bring about a sustained drug effect whilst avoiding adverse effects in areas besides the target organ. Additionally, the nano sizing of the molecule ensures sustained drug release. Hence, by modifying pharmacokinetic and drug properties, we can both decrease toxic effects and ensure therapeutic optimization in a safe and efficient manner.

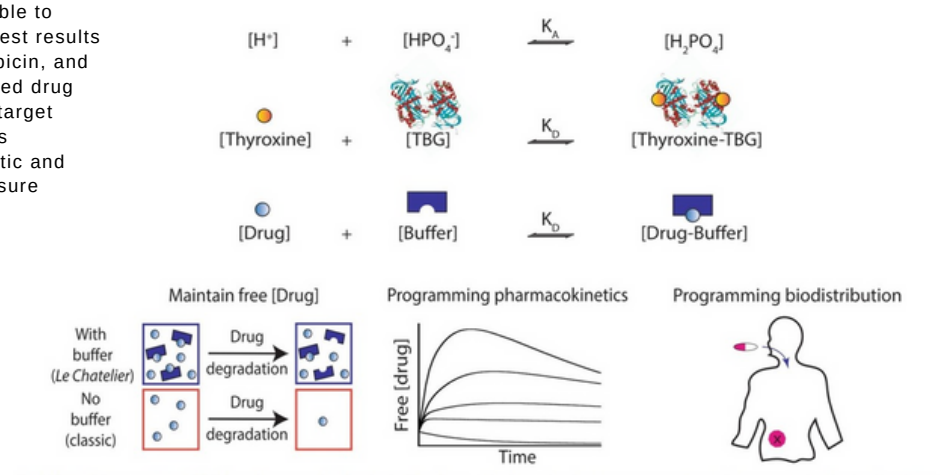


Fig. 1 | Drug delivery systems (DDS) based on natural molecular buffers. Top: Nature employs molecular buffers like HPO_4^- and TBG to maintain a constant concentration of active biomolecules using *Le Chatelier's* principle for H^+ and thyroxine, respectively. Bottom left: Similarly, we can engineer self-regulated molecular buffers to sequester a large reservoir of inactive drugs and to maintain the free active drug concentration at a precise concentration despite drug degradation over time by releasing the bound drugs. Bottom middle and right: We hypothesize that drug pharmacokinetics and biodistribution can be modulated by tuning the molecular properties of the buffer.

Methodology

The buffer system was made based on programmable DNA chemistry to carry the drug molecules.

• For quinone buffer, employed quinone binding DNA aptamer KD (dissociation constant) 90 nM.

• For doxorubicin buffer, employed A DNA binding sequence that displays KD 130 nM.

• The buffer exhibit sits optimal buffer capacity β when the free drug concentration matches the dissociation constant value: β max quinine = 135 ± 21 nM and β max doxo = 128 ± 7 nM

• To maintain a desired concentration of a free drug programmed buffer, the first approach consists of varying the KD of the buffer.

o For the quinine aptamer, introduced site-specific mutations that reduce the affinity for quinine .

o Since doxorubicin binds duplex DNA through intercalation in GC base pair, mutations could not be used to tune their KD. To circumvent this limitation, explored and found that specific G-quadruplex sequences (D1 and D2) displayKD that are 4 and 27-fold higher than the original D0 GC duplex DNA35,36.

• For maintaining free drug concentration, the molecular buffers were used as drug reservoirs and, by increasing its concentration, prolonged the therapeutic exposure of a drugs by up to ninefold.

• For pharmacokinetics profile:

o Degradation or elimination: regulation molecular buffer is generally done in kidney with cassettes. In this case, low concentrated DNAase was used for increased half-life.

Modifying chemical properties: chemically modified DNA backbones were made like phosphorothioate or a G-quadruplex sequence which increased half-life (tested with mice serum)and modified drug like properties, i.e. hydrophilic or hydrophobic balance properties

Experiment and Analysis

Both in vivo and in vitro experiments were performed.

- In vitro: HeLa and HCT116 cell lines were obtained from ATCC (Manassas, USA). Both cell lines were authenticated based on morphology and PCR assays with human-specific primers. Both cell lines were negative for mycoplasma.
- In vivo: Animals were housed inside an SPF (specific-pathogen-free) animal facility, exempted from the majority of known pathogens for murine species.

Toxicological effect:

1. Blood evaluation

2.Biomarkers analysed: troponin T, N-tergamma- glutamyltransferase natriuretic peptide (NT-proBNP), urea, creatinine, overall and conjugated bilirubin, alanine transaminase (ALT), aspartate transaminase(AST), alkaline phosphatase (ALP), and gamma-glutamyltransferase

Histopathological evaluation: Organs have been harvested ex vivo and immediately transferred in pre-classified Biopsy Cassettes. The cassettes have subsequently been transferred in jars containing 10% formalin answer and have been maintained at a constant for forty-eight hours at room temperature.

Results and Discussion

This study found that nano carriers extended the therapeutic impact of the drug and decreased its dosage throughout the entire course of treatment. It can be directed towards specific areas wherein the drug has maximal targeting therapeutically, causing reduction of adverse effects. In animals, doxorubicin was maintained for an extended period 18 times greater inside the blood, with a reduction in cardiotoxicity whilst maintaining the mice's overall health as evidenced with the maintenance of weight gain.The mixed outcomes confirmed the sustained pharmacokinetic effect of doxorubicin produced through the study's programmed self-regulated buffers and decreased several unwanted physiological results of doxorubicin, like weight reduction and cardiomyocytes vacuolation, whilst simultaneously improvingparameters such as coronary heart rate

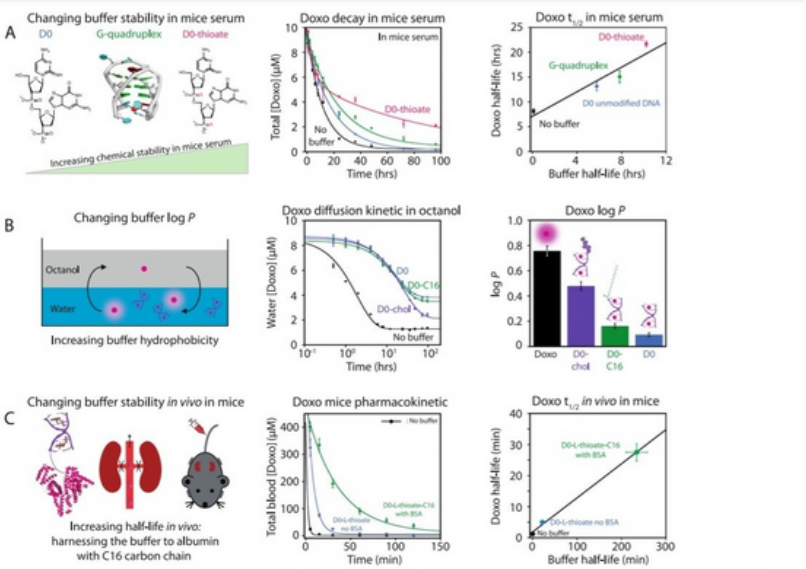


Fig: 4 Optimizing the pharmacological properties of doxorubicin through chemical modifications of its buffer. A Increasing the chemical stability of doxorubicin in mouse serum by increasing its buffer chemical stability (left panel) resulted in increasing the doxorubicin half-life (middle and right panels). Black line: no buffer; blue line: D0 buffer; green line: G-quad buffer; pink line: D0-thioate buffer. Total doxorubicin concentrations were determined using HPLC-fluorescence measurements, while DNA buffer half-lives were determined using SYBR green fluorescence (supplementary Fig. 12). Data in the middle panel were fitted with equation E19—see Supplementary Information) and a linear regression was used for the right panel. For each condition, $n = 3$ independent experiments. Data were presented as mean values ± SD. **B** Programming the doxorubicin partition coefficient log *P* (left panel) by modifying the hydrophobicity of the buffer resulted in different water-to-octanol diffusion (middle panel), which changed the doxorubicin log *P* value (right panel). Black line: no buffer; blue line: D0 unmodified buffer; green line: D0-C16 (D0 with C16 moiety); purple line: D0-chol (D0 with cholesterol moiety). Data in the middle panel are fitted with equation E19—see Supplementary Information. Data and error bars from the right panel are derived from the fitting of the middle panel. For each condition, $n = 3$ independent experiments. Data were presented as mean values ± SD. **C** Increasing the doxorubicin blood circulation time using molecular buffers (left panel) in IV-injected CD-1 mice. Both molecular buffer D0-L-thioate (blue line) and D0-L-thioate-C16 (green line) increased doxorubicin half-life in vivo (middle and right panel) compared to non-buffer (black line). Data in the middle panel fitted with equation E19—see Supplementary Information. Data and error bars from the right panel are derived from the fitting of the middle panel. For all conditions, $n = 6$ and data are presented as mean values ± SD.

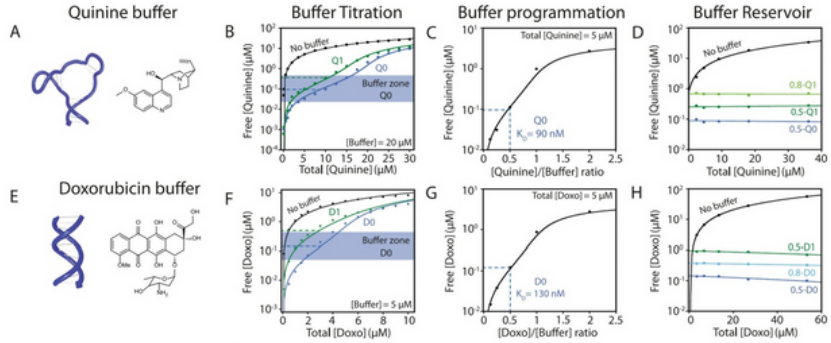


Fig. 2 | Programming the buffer capacity of molecular buffers. Quinine (**A**–**D**) and doxorubicin (**E**–**H**) buffers maintain the concentration of free quinine and free doxorubicin near their K_D values even in a large drug concentration range. **B**, **F** Increasing the dissociation constant of the buffer, K_D, increases the concentration of free drug proportionally with buffer variants Q1 and D1 for example (black line: no buffer added; green line: D1 and Q1 buffer variant; blue line: D0 and Q0 buffer variant). Data fitted with equation E5—see Supplementary Information. **C**, **G** The free drug concentration can also be precisely programmed by varying the [Drug]/[Buffer] ratio. Data fitted with equation E5—see Supplementary Information. **D**, **H** The free drug concentration can be maintained over large variations of total drug concentration by maintaining a constant [Drug]/[Buffer] ratio (black line: no buffer added; light green line: 0.8-Q1; green line: 0.5-Q1 and 0.5-D1; blue line: 0.5-Q0 and 0.5-D0; light blue line: 0.8-D0). Data fitted with linear regression. All data were obtained using fluorescence measurements (Supplementary Fig. 1), and the errors were obtained from the fit.

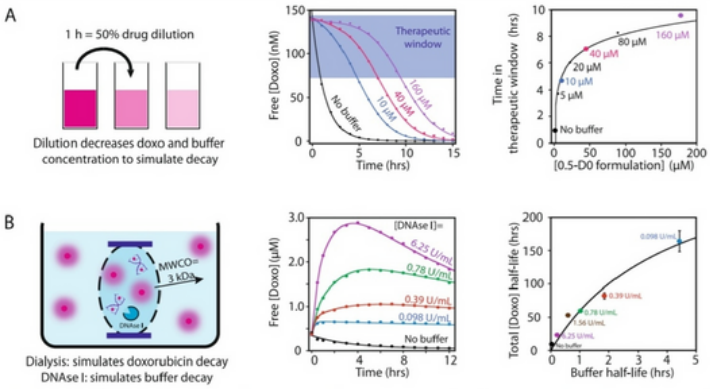


Fig: 3 Programming the doxorubicin pharmacokinetic profile using molecular buffers. (**A**, left panel) Simulating doxorubicin and buffer degradation using serial dilutions. (**A**, middle and right panel) Large reservoirs of drug/buffer formulation of 0.5-D0 maintain the free doxorubicin concentration in an arbitrarily selected therapeutic window depicted by the blue square between 75 and 150 nM for a much longer period despite many dilution cycles (for middle panel, black line: no buffer; blue line: 10 μM reservoir; pink line: 40 μM reservoir and purple line: 100 μM reservoir). Data in the middle panel are fitted with equation E11 and data in the right panel with equation E14 (Supplementary Information). (**B**, left panel) Simulating doxorubicin clearance using a dialysis cassette and buffer degradation using DNAse I nuclease activity with formulation 0.5-D0. (**B**, middle panel) The pharmacokinetic profile of doxorubicin (10 μM) can be shaped by modifying the stability of its DNA buffer (D0) using various concentrations of DNAse I (black line: without DNAse I; blue line: 0.098 U/mL; red line: 0.39 U/mL; green line: 0.78 U/mL; purple line: 6.25 U/mL and pink line: 100 U/mL). Data were collected for 24 h and fitted with equation E18—see Supplementary Information. (**B**, right panel) The doxorubicin half-life can be increased by increasing the buffer half-life (data fitted with a Michaelis–Menten equation). For right panels **A**/**B**: each data point is derived from the fitting of a single doxorubicin release kinetic from middle panels A/B and all error bars were derived from the same fitting. Data were presented as mean values ± SD. All data were obtained using fluorescence measurements.

Conclusion

This new tool can not only bring about enhanced therapeutic effect by personalised delivery but, additionally, the high programmability of the DNA and protein chemistries can be utilized to design these carriers to precisely deliver a wide range of therapeutic molecules. Furthermore, it can be combinedwith human-designed liposomic transporters that are being employed to deliver drugs at different rates. These contemporary technologies in combination could ensure a future wherein therapeutics are delivered with optimized efficacy and efficiency, whilst minimizing adverse effects.

References - This poster has been made utilising the work of the following:

•Desrosiers, A. et al. (2022) "Programmable self-regulated molecular buffers for precise sustained drug delivery," Nature Communications, 13(1). Available at: https://doi.org/10.1038/s41467-022- 33491-7.
•Ktori, S. (2022) DNA-basednanotransporters deliver precise concentrations of drug, GEN. Available at: https://www.genengnews.com/topics/dr ug-discovery/drug-delivery/dna-based- nanotransporters-deliver-precise-concentrations-of-drug/ (Accessed: November12, 2022).
•Wang, Y. et al. (2022) "Chemically modified DNA nanostructures for Drug Delivery," The Innovation, 3(2), p. 100217. Available at: https://doi.org/10.1016/j.xinn.2022.10021 7.
•Guan, C., Zhu, X. and Feng, C. (2021) "DNA nanodevice-based drug delivery systems," Biomolecules, 11(12), p. 1855. Available at: https://doi.org/10.3390/biom11121855.

THE RISE OF PERSONALISED NUTRITION

by Nitya Gupta

"What am I going to eat for my next meal?". Though this question is seemingly simple, the answer has serious `consequences'. In the short term, the food we consume impacts energy levels, focus and mood, but long term, it affects lifespan, inflammation, memory, and the risk of chronic disease, including heart disease, diabetes, and cancer.

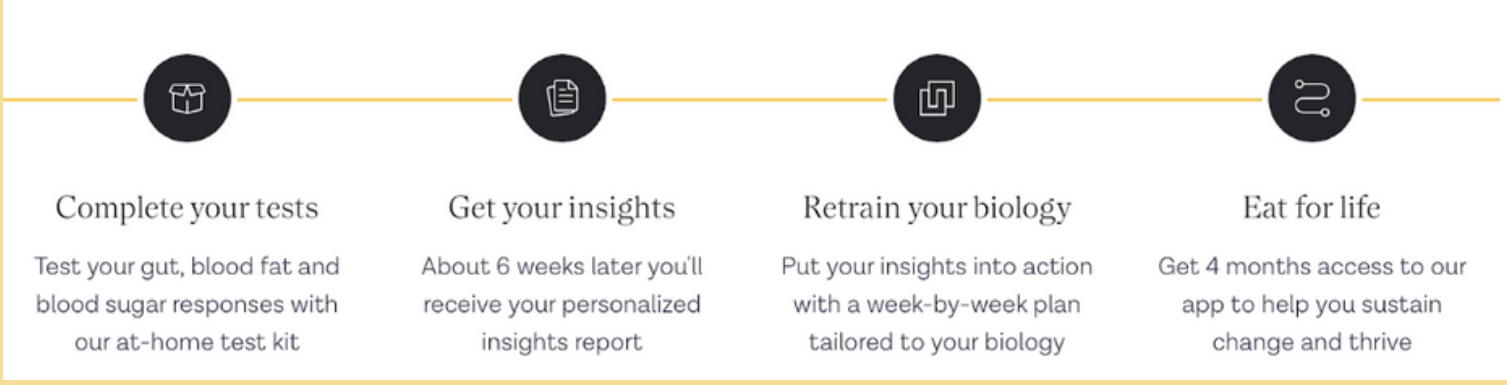
We are influenced in our dietary decision-making by cravings, perceived satiation, health, and the latest diet trends, whether that's fasting for 14-18 hours per day, having low-carb and high-fat meals or eating nothing but mildly seasoned potatoes. These trends are seasonal and offer opposing advice, and while they may be effective in promoting weight loss, they can have adverse consequences. Furthermore, standard nutritional guidelines are based on population averages, and these one-size recommendations do not fit all.

The company Zoe is rooted in the philosophy that everyone's response to food is unique, and their studies have recorded tenfold variations in responses to the same meal. These differences result from microbiome and nuclear DNA variation and lifestyle characteristics. Zoe aims to help people understand their metabolism and makes personalised recommendations to naturally improve gut health and reduce inflammation. It was formed as a collaboration between researchers at King's College London, Harvard University and Stanford University.

Zoe accomplishes this by measuring three key variables: blood sugar levels, blood fat responses, and the species making up your gut microbiome.

Why sugar and fat?

It is normal for blood sugar to rise and fall after a meal, however, dramatic and frequent blood sugar spikes after eating can overwhelm the body's natural responses. Blood sugar rushes are followed by crashes leading to hunger caused by all the insulin pumped out to clear the glucose. In the long run, continuous glucose spikes and high triglyceride levels (the alternate energy substrate to glucose) can increase heart disease risk. High blood sugar impairs blood vessels' ability to dilate, encouraging the build-up of fatty plaques leading to their hardening and narrowing. Furthermore, the glucose rollercoaster also contributes to chronic-low grade inflammation linked with the development of type 2 diabetes, heart disease and Alzheimer's.



Why gut microbes?
We are what we eat, or more specifically, our microbiome is. Unlike DNA, for which any two people share at least a 99.9% similarity, every gut microbiome is unique, and even twins share only 34% of species. Gut bacteria have certain macronutrient requirements, and the food we consume will promote the expansion of certain species and restrict others.

A healthy microbiome is one with a large diversity of beneficial microbes. These microbes are essential for effective nutrient metabolism, immune system regulation and protection against pathogenic invasion. Zoe has identified a set of 'good' and 'bad' gut bacteria and linked them with specific foods and metabolic health. Eubacterium eligens, for example, promotes the production of anti-inflammatory molecules, increases polyunsaturated (healthy) fat, and lowers insulin secretion. It thrives in diets high in zucchini, spinach, tomato, nuts, fish, and seafood but doesn't in those with potatoes or fast food. Prevotella copri is another 'good' bacteria that may aid in blood sugar control and likes eggs and dark chocolate.

Why Zoe?

Zoe takes the data collected from the real-time glucose sensors, blood fat finger prick tests and microbiome measurements from stool samples and uses a specialised AI algorithm to generate a report about your metabolic and gut health. It then creates personalised scores for any meal you eat, taking your metabolism and microbiome into account.

Zoe's PREDICT studies are the first of their kind. They involved randomised, mixed meals of varying macronutrient combinations that reflect real-world diets. In addition, while previous studies solely focused on glycemic outcomes, Zoe considers triglyceride levels to detect dysregulated fat responses. Zoe's algorithms are also trained to make meal-specific predictions as though an individual typically responds comparably to different meals of similar macronutrient profiles, this is not always the case. While one participant had an exaggerated glycaemic response to a banana but a normal response to a cookie, another experienced the opposite.

They found that genetics, contrary to the researchers' expectations, was not a predominant predictor of metabolic responses. Instead, gut-microbiome diversity, meal timing, exercise, sleep and circadian rhythm were identified as key determinants of postprandial triglyceride and glycaemic responses.

To characterise the ecology of your microbiome, Zoe performs metagenomic sequencing on stool samples and recommends foods that uplift populations of under-represented 'good' microbes in your gut. Zoe puts an emphasis on plant diversity as most 'good' bugs thrive in such diets. Fermented foods, such as kimchi, kombucha, and yoghurt, are often recommended as they are rich in probiotics (living beneficial microbes).

The AI models devised for UK populations performed well when applied to an independent US cohort despite the difference in the environment, validating the prediction model. However, both cohorts were comprised of healthy young adults of European ancestry, so further validation is required in cohorts of non-European ancestry, older adults and people with diseases that affect metabolism.

Overall, Zoe makes a convincing argument against diet culture and promotes eating in line with your biology for a guilt-free healthy lifestyle. 82% of current members reported having more energy, 83% no longer felt hungry, and the average weight loss among users was 4.3 kgs. It has demonstrated the power of personalised nutrition as a strategy for disease prevention, specifically diabetes, heart disease and other chronic health problems and in maintaining cardiometabolic health.

References & Further Reading

1) Berry SE, Valdes AM, Drew DA, Asnicar F, Mazidi M, Wolf J, et al. Human postprandial responses to food and potential for precision nutrition. Nature Medicine. 2020; 26 (6): 964-973. 10.1038/s41591-020-0934-0.
2) ZOE—Understand how food affects your body. https://joinzoe.com/ [Accessed Nov 18, 2022].

PHAGE THERAPY IN THE ORAL MICROBIOME

by Devon Darley

Oral Microbiome

Revered for its complexity, the oral microbiome provides the human body with natural protection. As our body's second largest and second most diverse microbiome, the oral cavity hosts fungi, viruses, protozoa, and over 700 unique species of bacteria. Initially identified in 1674, when Antony van Leeuwenhoek observed his dental plaque under a microscope, the oral microbiome is now understood to be much more than the "many very little living animalcules, very prettily a-moving" that van Leeuwenhoek first described. The human microbiome is composed of both a core and a variable microbiome. The core microbiome consists of organisms consistently detected across the population, while the variable microbiome is responsive to an individual's unique environment and genetics. Together, these two versions of the microbiome help to maintain our health in a state of equilibrium. With colonisation of the oral cavity beginning from birth, its microbiome plays an integral lifetime role in our overall systemic health.

Naturally present in the oral microbiome, *Streptococcus mutans* causes dental caries (tooth decay) when it forms what is known as a biofilm on teeth, demineralizing the enamel and breaking down the dentin. Simply put, biofilms are an assemblage of cells, (more specifically, communities of bacteria), that reside within an exopolysaccharide—also known as an extracellular polymeric substance (EPS)—, matrix. The assemblage of cells are irreversibly associated due to the enclosing EPS matrix, and therefore cannot easily be dispersed from one another.

Resulting from an imbalance in the oral microbiome's state of equilibrium, periodontitis (gum disease), is caused by two bacteria, *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Together, these bacteria destroy both the tissue and alveolar bone surrounding a tooth, ultimately resulting in tooth loss.

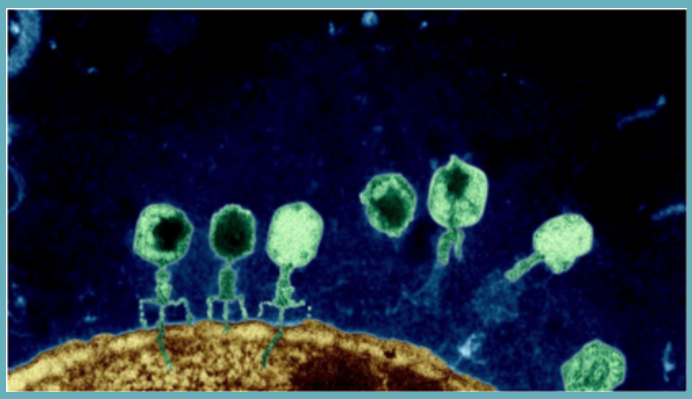
Perhaps even more alarming, bacteria in the oral microbiome are in part responsible for our overall systemic health. Certain nasty pathogenic bacteria are linked to major health problems such as cancer, arthritis, Alzheimer's, coronary heart disease, diabetes, and premature birth. In cancer, it is believed that *Porphyromonas gingivalis* and *Fusobacterium nucleatum* chemically induce oral squamous cell carcinoma tumorigenesis. The oral bacteria *P. gingivalis* and *F. nucleatum* are in part responsible for heightened levels of inflammation throughout the body. Heightened inflammation significantly worsens rheumatoid arthritis. Alzheimer's patients suffering from *F. nucleatum* periodontal disease saw their memory ability decline six times faster than those without gum disease. *S. mutans*, the same bacteria that causes dental plaque, is also linked to atherosclerotic plaque, the type of plaque that builds up in arteries and results in coronary heart disease. When infecting the tissue surrounding a tooth, *P. gingivalis* causes an increase in blood sugar levels, which can lead to the development of diabetes or the worsening of diabetic symptoms. *F. nucleatum* has been linked in mothers to blood and placenta infections that result in premature births and low birth weights.

Mitigation of Harmful Oral Bacteria

Integrally linked to overall health, discovering a way to control and prevent the buildup of harmful bacteria in our oral microbiome is of critical importance. While antibiotics spring to mind as a useful tool in the fight against unfavourable bacteria, their countless flaws make them unsuitable for the task. Firstly, antibiotics are non-specific, indiscriminately killing various strains of bacteria. As a healthy oral microbiome plays a role in maintaining overall health, it is important that only targeted harmful bacteria are killed, and helpful bacteria are left to preserve equilibrium. Secondly, and perhaps even more crucially, antibiotics are unable to penetrate the biofilms that the majority of bacteria in the oral microbiome are protected by, rendering them useless against biofilm-based infections. Finally, as we enter into a new age of antibiotic resistance, antibiotics are becoming universally less effective. When ingested, an antibiotic kills *nearly* all of its targeted bacteria. Oftentimes, any surviving bacteria contain a mutation making them immune to the antibiotic. With competition eradicated, the resistant bacteria quickly replicate, taking over the environment. These mutant bacteria are known as "antibiotic-resistant" bacteria. Once infected with antibiotic-resistant bacteria, the go-to treatment of antibiotics is rendered useless.

Bacteriophages

Mercifully, there is another option to kill bacteria: bacteriophages. Bacteriophages, colloquially known as phages, are naturally occurring viruses that selectively infect specific strains of bacteria, and are present in large quantities across most parts of the planet. In aquatic biomes, there are estimated to be 104 - 108 complete virus particles (virions) per ml, and in terrestrial biomes, there are an estimated 109 virions per 1g. To date, over 14,244 different bacteriophage genomes have been sequenced.



BACTERIOPHAGES INFECTING E. COLI

Whilst each bacteriophage is composed of either DNA or RNA wrapped within a protein capsid, there are two specific types of bacteriophages, "lysogenic" phages and "lytic" phages. Lysogenic phages integrate their DNA into the host bacterium's genome and reproduce with each generation of bacteria before entering the lytic cycle. Lytic phages immediately enter the lytic cycle, hijacking the bacterial replication machinery to produce new phages before lysing the cell.

To be infectious, a phage must first bind to a specific surface receptor on the bacterium. Attaching to a host bacteria is a highly specific process that involves the matching of complementary receptors. These receptors are usually either surface components of the bacterial cell, such as a sugar transporter, or a sex pilus. Receptor specificity determines which bacteria a phage can infect. Some phages are strain-specific while others can infect a range of related bacterial strains.

Once bound to a complementary receptor, the next stage of phage infection is injection. In order to infect, bacteriophages must inject their genetic material into the bacterium. Roughly 96% of bacteriophages are equipped with a tail that specializes in breaching the bacterial surface. Using the T4 phage as an example, the tail consists of a baseplate with tail fibres attached and a contractile sheath surrounding a non-contractile tube. The sheath itself is made of around 138 copies of gene product (gp) 18, colloquially known as tail sheath protein, arranged in a loose helical structure. When infecting a bacterium, tail fibres attach to the host cell, which causes a change in the baseplate's shape. When the baseplate changes from a hexagonal dome-shape to a planar star-shape, the sheath contracts to less than half of its original length, thus condensing and tightening its helical structure. As the helical structure condenses, the gp18 molecules that form the helix slide over one another, which considerably increases the overall diameter of the sheath. With the sheath contracted, the inner tube is driven through the surface of the bacteria, forming a pore in the bacterium's cell wall peptidoglycan degradation. Once the pore is formed, the phage's genetic material, usually in the form of DNA, is injected into the bacterium's cytoplasm.

If lysogenic, the phage integrates its genetic material into the host's genome, in the form of an endogenous prophage. After being incorporated into the genome, the prophage is reproduced with each cell division. Whilst the lysogenic cycle is harmless to the host, once the host is subject to stressful environmental conditions, the phage enters into the lytic cycle.

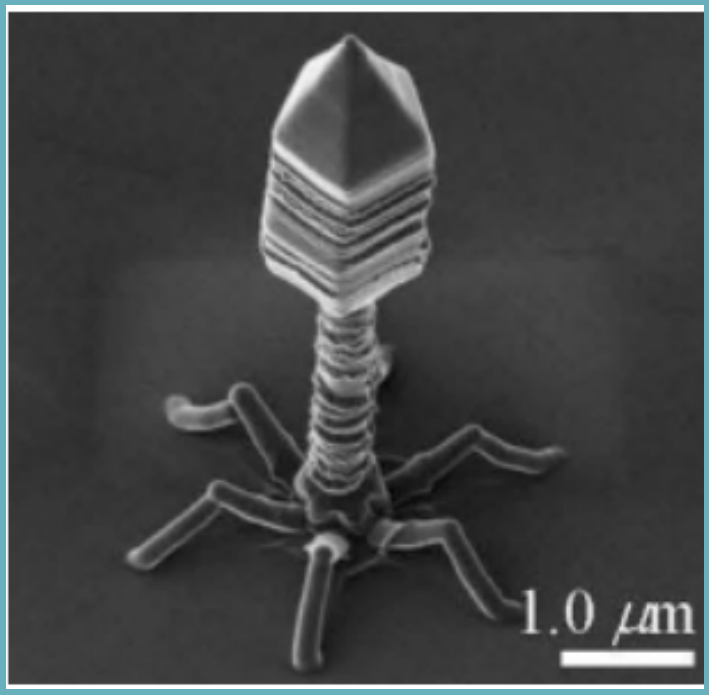
The lytic cycle begins when the process of transcription and translation are redirected from the bacterium's genes to the phage DNA. In order to hijack the bacterium's transcriptional machinery, phages usually target both RNA polymerase (RNAP) and regulatory transcription factors. Whilst there is great diversity in the specific techniques used to modulate transcription machinery between different bacteriophages, most target either transcription initiation or elongation and termination. Again using a T4 phage as an example, the promoters for early T4 phage genes, or T4 early promoters, closely resemble the σ70-dependent promoters for the host bacterium's genes. RNAP, however, seems to have a higher affinity for T4 early promoters than for the promoters of its own genes. Once RNAP has interacted with the T4 early promoters, it is modified such that premature termination of cytosine- containing DNA is induced. Whilst the bacterium's DNA naturally contains cytosine as one of its four bases, the T4 phage contains 5-hydroxymethyl cytosine instead, which does not induce premature termination of transcription. Through this process, the T4 phage is able to redirect the bacterium's transcription mechanisms from bacterial genes to phage genes.

With phage DNA being transcribed and translated, viral nucleic acids and proteins are produced. For the synthesis of phage copies, a series of different proteins are required. To begin, multiple copies of a capsid protein and, a scaffolding protein, are made, which come together to form the capsid head of the phage. With the head assembled, the viral genome is replicated and translocated into the capsid head via a packaging complex that utilizes ATP hydrolysis. After scaffolding proteins are removed from the head, and the head has 'matured', proteins for the tail are created. Requiring a wide variety of specific proteins, once complete, the tail is connected to the head. Copying of the bacteriophage is then complete.

Depending on the family of phage, after anywhere from tens to thousands of viral copies are produced, lytic proteins become active to lyse the bacteria and release the phage copies into the surrounding environment. In order to hydrolyze the bacterial peptidoglycan cell wall, multiple phage late proteins —, such as lysins, holins, and murein synthesis inhibitors—, are produced and activated. With its cell wall lysed, the bacterium dies.

Phage Therapy

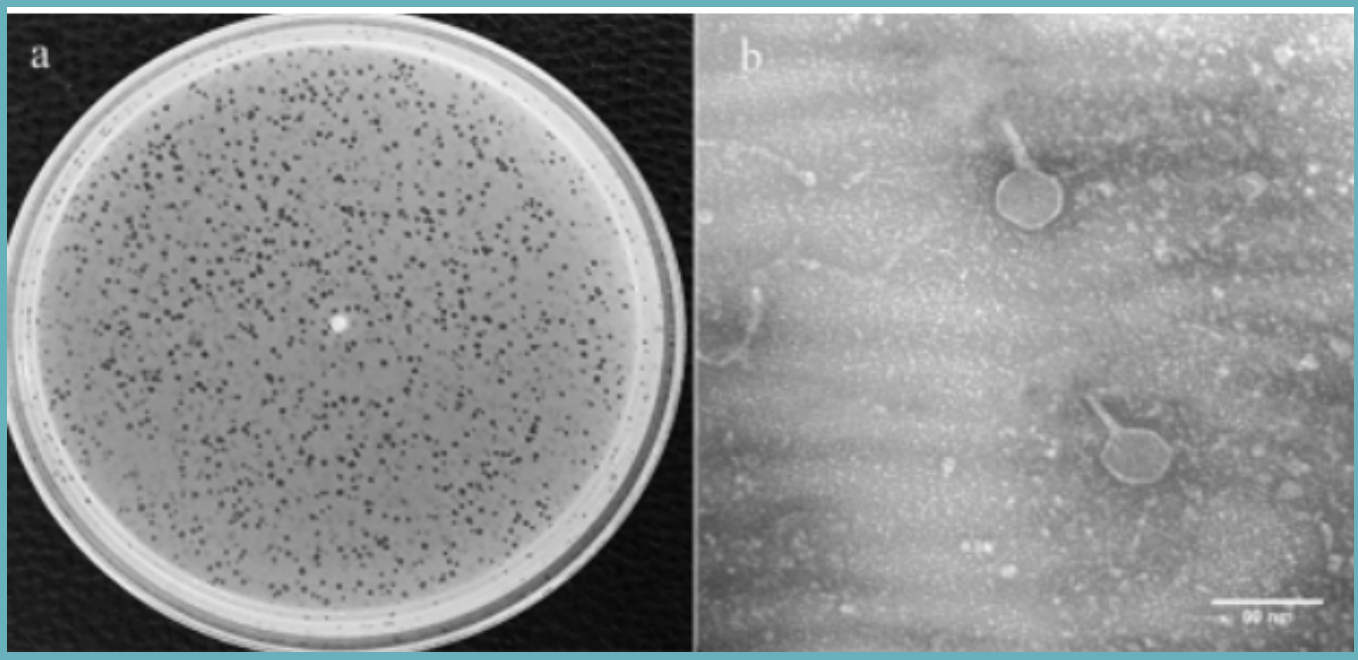
As well as their virtue of specificity, bacteriophages are also capable of penetrating biofilms. Bacteriophages have EPS depolymerase on the exterior of their capsid which degrades the EPS and breaks up bacterial biofims. Once the biofilm has been broken down by EPS depolymerase, the bacteriophage can access and infect the bacteria within the biofilm. Naturally specific, able to degrade biofilms, and largely impervious to bacterial resistance, bacteriophages are the perfect candidate to target harmful bacteria lurking in the oral microbiome.



A BACTERIOPHAGE AS SEEN UNDER A SEM

Use of bacteriophages to combat specific bacteria in humans, an idea known as phage therapy, isn't new. In fact, phages were used to kill bacteria almost a decade before the discovery of penicillin. In 1915, Frederick Twort discovered bacteriophages and used them to successfully treat patients with cholera and dysentery. Following the discovery of penicillin in 1928 by Alexander Flemming, less emphasis was placed on phage therapy, and after the USSR continued research on phage therapy in the years following World War II, the West abandoned it entirely for disdain of its communist connections.

Although once slurred, phage therapy has the exciting ability to revolutionize how we protect the oral microbiome. Unique bacteriophages can be employed to specifically target the harmful bacteria Streptococcus mutans, Porphyromonas gingivalis, and Fusobacterium nucleatum. Already, the bacteriophages SMHBZ8 and FnpΦ02 have been isolated to infect S. mutans and F. nucleatum respectively.



NOVEL BACTERIOPHAGES ISOLATED FROM MULTI-DRUG-RESISTANT E. COLI

Every individual has a unique oral microbiome, one that may give rise to equally unique health problems. The ability to screen for different bacteria,— and prescribe bacteriophages to fight those thatwhich are harmful—, could revolutionize our approach not only to personalized oral health but also to the broader concept of personalized systemic healthcare. Today's healthcare predominantly focuses on treatment rather than proactively seeking to cure underlying health issues. As knowledge and application of science and technology advances, tomorrow's healthcare should predominantly focus on targeting the root cause of health issues. With its harmful oral and broad systemic health implications, one such pressing issue to tackle is the importance of controlling the bacteria present in the oral microbiome through the use of phage therapy.

References & Further Reading

- Deo, P. N., & Deshmukh, R. (2019). Oral microbiome: Unveiling the fundamentals. Journal of oral and maxillofacial pathology : JOMFP, 23(1), 122–128.
- https://doi.org/10.4103/jomfp.JOMFP_304_18
- Marsh, P. D., & Bradshaw, D. J. (1995). Dental plaque as a biofilm. Journal of industrial microbiology, 15(3), 169–175. https://doi.org/10.1007/BF01569822
- Harper, D. R., Parracho, H. M. R. T., Walker, J., Sharp, R., Hughes, G., Werthén, M., Lehman, S., & Morales, S. (2014). Bacteriophages and Biofilms. Antibiotics, 3(3), 270–284. https://doi.org/10.3390/antibiotics3030270
- Summers W. C. (2012). The strange history of phage therapy. Bacteriophage, 2(2), 130–133. https://doi.org/10.4161/bact.20757
- Aksyuk, A. A., Leiman, P. G., Kurochkina, L. P., Shneider, M. M., Kostyuchenko, V. A., Mesyanzhinov, V. V., & Rossmann, M. G. (2009). The tail sheath structure of bacteriophage T4: a molecular machine for infecting bacteria. The EMBO journal, 28(7), 821–829. https://doi.org/10.1038/emboj.2009.36
- Kilian, M., Chapple, I., Hannig, M. et al. The oral microbiome – an update for oral healthcare professionals. Br Dent J 221, 657–666 (2016). https://doi.org/10.1038/sj.bdj.2016.865
- Chhibber-Goel, J., Singhal, V., Bhowmik, D., Vivek, R., Parakh, N., Bhargava, B., & Sharma, A. (2016). Linkages between oral commensal bacteria and atherosclerotic plaques in coronary artery disease patients. NPJ biofilms and microbiomes, 2, 7.
- https://doi.org/10.1038/s41522-016-0009-7
- Berger, D., Rakhamimova, A., Pollack, A., & Loewy, Z. (2018). Oral Biofilms: Development, Control, and Analysis. High-throughput, 7(3), 24. https://doi.org/10.3390/ht7030024
- Binder Gallimidi, A., Fischman, S., Revach, B., Bulvik, R., Maliutina, A., Rubinstein, A. M., Nussbaum, G., & Elkin, M. (2015). Periodontal pathogens Porphyromonas gingivalis and Fusobacterium nucleatum promote tumor progression in an oral-specific chemical carcinogenesis model. Oncotarget, 6(26), 22613–22623.
- https://doi.org/10.18632/oncotarget.4209
- Ben-Zaken, H., Kraitman, R., Coppenhagen-Glazer, S., Khalifa, L., Alkalay-Oren, S., Gelman, D., Ben-Gal, G., Beyth, N., & Hazan, R. (2021). Isolation and Characterization of Streptococcus mutans Phage as a Possible Treatment Agent for Caries. Viruses, 13(5), 825. https://doi.org/10.3390/v13050825

CREATING FRANKENSTEIN'S MONSTER:
THE RISE OF ORGANOIDS IN PERSONALISED MEDICINE

by Megan Perry

Models are vital for drug discovery in personalised medicine. Before a drug can be approved, it must succeed in several trials starting with 2D cell cultures, then using animal models, and finally testing on humans. Yet the time, money, and labour required for these stages delays progress in drug discovery and restricts the development of drugs specific to patients. In order to increase the rollout of novel and personalised medicines, a new approach is required: organoids.

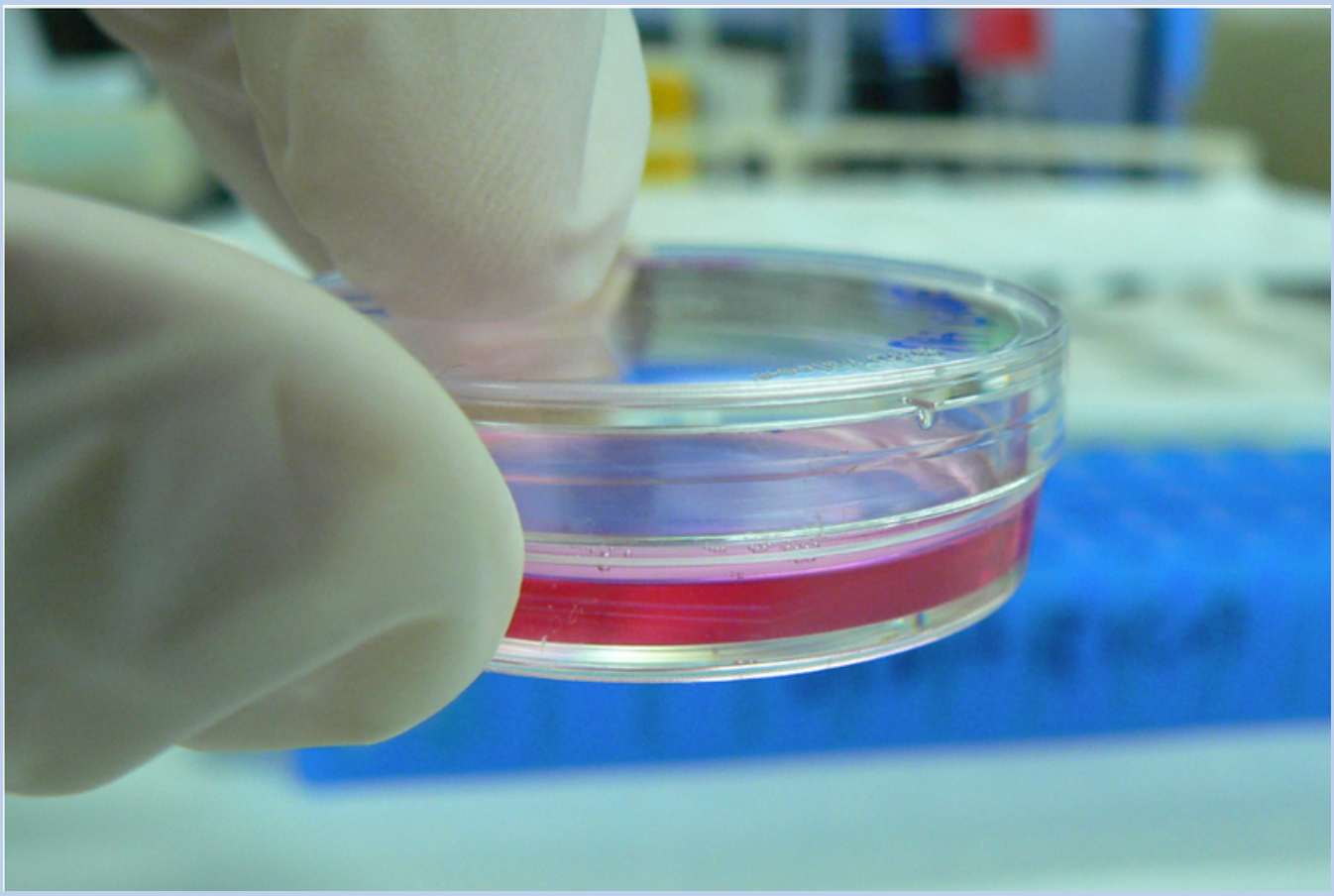


IMAGE CREDIT: HTTPS://WWW.FLICKR.COM/PHOTOS/KAIBARA/30752682O0/IN/PHOTOLIST-5FKY6W-DWPAK9-HRQPSZ-2MEODM6-2INF4E8-QB7DLP-A5BHV-A5BHY-SS8E25-2N4EDTM-A5BHZ-2IUT6R3-2KRRFSP-QN8M5-2KZQ4A8-8KFZQM-JVNECL-AXFIY-2J1YGHR-NK7OHG-2HPHVB5-DKYRU9-NNDMHV-JR9GC-9NNBVA-EDXV9P-NWT6P-2IG5DCK-NDKYAF-CXWSV6-AKIAVP-AKMP9H-DNF1DP-NMQSXK-AKIABD-RECQ-AKIAJX-AKMPCO-AKMP7G-NHUZUJ-GTLAGZ-NWT4H-CKSCHW-AKIAWZ-AKIAUT-RAOVSO-CEFS29-4DDQJS-2HFQR7D-2HFPUMF

2D cell cultures

For patient-specific 2D cell cultures, cells can be extracted in a biopsy from patients, then are grown in vitro in a culture medium. The medium contains both nutrients required for cell survival, as well as growth factors (signalling molecules that promote cell replication). Most adult cells can only divide for 40-60 generations before naturally dying. This is disadvantageous for high-throughput industrial methods so, instead, cells are often 'immortalised'. This process involves genetically altering the cells so that they can divide indefinitely when supplied with growth factors. Given 2D cell cultures require few nutrients and can double their population size every day, they are relatively quick, cheap, and easy to develop. Hence, they are used as the first stage in drug screening.

Yet, 2D cell cultures are not so useful for studying drug effects in tissues, as these contain a large non-cellular component: the extracellular matrix (ECM). The ECM—made of many different proteins—acts like a scaffold for cells in tissues, with both structural as well as signalling roles. 2D cell cultures are a monolayer so cannot recapitulate the three-dimensional environment provided by the ECM. Thus, key cell-matrix interactions are missing, which reduces the accuracy of drug response predictions.

Animal models

Animal models are the other major model used in medicine. Unlike 2D cell cultures, animal models can integrate cell-matrix interactions and other large-scale communications, such as between organs. For personalised medicine, two main animal models are used: genetically engineered mouse models (GEMMs) and patient-derived xenografts (PDX). GEMMs can be produced by various genetic engineering methods, such as microinjection of the DNA of interest into the mouse embryos. Meanwhile, PDXs are generated by transplanting a section of patient tissue, such as a tumour, into immunocompromised mice. Then, the patient-specific tumour is able to grow in the mouse and drugs can be tested on the model before being used for patients.

Unfortunately, animal models are expensive, labour-intensive and raise ethical concerns for the animals' welfare. The mice can take several months to develop, which extends the time taken in preclinical trials and slows down the drug discovery process. Moreover, despite the high genome similarity between mice and humans, there are still differences and, as such, mice cannot predict human drug responses with full accuracy.

Organoids as models

Organoids are 3D cell cultures that resemble 'mini-organs'. The key difference from 2D cell cultures is the addition of a matrix, which allows cells to self-organise into miniature organ-like structures, up to a few millimetres in diameter. The most commonly used matrix is 'Matrigel', a natural mixture of ECM proteins secreted from mouse sarcoma (i.e. cancerous) cells.

Organoids are derived from stem cells. Whereas in 2D cell cultures it is useful to have specialised cells and just immortalise them to keep them replicating, organoids use stem cells so that the process of organ development can be recapitulated in vitro. Stem cells are unspecialised cells that self-replicate and can differentiate into specialised cells, so are the origin of all cells in the body. Some stem cells have a greater potency (ability to differentiate into different cell types) than others. Embryonic stem cells can develop into any cell type in the body, whereas adult stem cells are limited to a select few types. Additionally, induced pluripotent stem cells can be generated from adult non-stem cells via reprogramming them to express (produce) Yamanaka factors, a set of four proteins found in embryonic stem cells. This reverses the ageing process so the adult cells become embryonic-like and can be used in cell cultures instead of actual embryonic stem cells.

Organoids can be developed using more or less potent stem cells, but the associated protocols are different. For all organoid development, the addition of a matrix is vital. Aside from this, the growth factors used to stimulate cell division and differentiation vary depending on the stem cells used and the desired organ to be developed.

Advantages of organoids

Organoids have many advantages over the other current models used in research. Patient-derived organoids take 4-6 weeks to develop, so are slower than 2D cell cultures but significantly quicker than animal models. Similarly, the expense of developing organoids lies in between the other two models. Aside from these differences, organoids have a greater potential for predicting patient responses. Compared to 2D cell cultures, organoids have the advantage of spatial resemblance to organs and the presence of the matrix to provide accurate cell-matrix interactions. Compared to animal models, organoids can be made entirely from patient-derived cells so contain only the human genome, which allows them to better predict patient responses than from mice.

Moreover, studies using organoids have shown that they have a very high success rate for

predicting patient drug responses. In 2018, Vlachogiannis et al. compared the response of patient-derived gastrointestinal tumour organoids with the response observed in patients to treatments such as chemotherapy. The results were highly promising: the organoids had an 88% positive and a 100% negative predicted value. Simply, 88% of the organoids that responded to treatment were matched with a response in patients—so there was a small proportion of false positives where the organoids responded but the patients didn't. For all of the organoids which had no response, the original patient also didn't respond to treatment—so there were no false negatives where the organoid didn't respond but the patient did. In particular, the absence of false negatives is reassuring, as this avoids the potential dismissal of drug targets in initial screening that could later be successful in patients.

Areas for improvement

Unfortunately, organoids face barriers before they can become more widely used. As organoids are a relatively recent discovery (first created in 2009 by Sato et al.), there is yet to be a standardised approach for their development. Various labs have produced organoids via different methods, which limits the reproducibility of the protocols. Standardisation is an important step in the path to wide acceptance of organoids as models.

Secondly, an area for improvement in cancer organoids is the development of the tumour microenvironment (TME). Aside from containing cancerous cells, tumours communicate with nearby cells that constitute the TME, such as connective tissue and immune cells. Yet, few patient-derived organoids have been able to recapitulate the tumour microenvironment. Similarly, metastasis of cancer (formation of one or more secondary tumours) relies heavily on the growth of blood vessels (angiogenesis) toward the tumour site to allow the cancerous cells to spread through the bloodstream. However, the integration of blood vessels is another feature yet to be widely established in organoids.

Finally, most organoids have lacked inter-organ communication. Diseases often involve several organs, so the absence of these interactions in organoids limits their ability to accurately represent human systems and predict patient responses.

Moving forward

One potential solution for organoids is a microfluidics approach. Organoids can be developed on a microfluidic chip containing fluids and microchannels that recreate the extracellular environment. In 2022, Tao *et al.* developed a multi-organ microfluidic system to study type 2 diabetes mellitus. The microfluidic chip was segregated in half to create two compartments, with liver cultures on one side and kidney islets on the other, as these are the tissue types most involved in diabetes. The organoids were each contained in a single well and were connected by channels that allowed the flow of culture media, with peristaltic pumps maintaining this flow. The large number of wells per microfluidic chip makes this a high-throughput technique and the uniformity of wells promotes standardisation of the organoids generated.

Altogether, this approach holds potential for accelerating the drug discovery process and reducing the labour involved, given its high-throughput nature, as well as for inter-organ studies. 'Organoids-on-a-chip' is a novel method so more studies will be required to analyse their fidelity in recapitulating patient responses before they can become standard use.

Concluding remarks

It seems Frankenstein-esque to develop mini-organs from patient cells, yet the technology could revolutionise drug discovery. For many patients, the time and money needed for drugs to be tested on patient-derived 2D cell cultures and patient-derived xenograft animal models makes the personalisation of medicine inaccessible. A novel automated approach, such as 'organoids-on-a-chip', has significant potential for predicting patient drug responses and expediting the rollout of personalised treatments.

References and Further Reading:

- Bose, Clevers, and Shen. Promises and Challenges of Organoid-Guided Precision Medicine. (2021). doi.org/10.1016/j.medj.2021.08.005
- Kim, Koo and Knoblich. Human organoids: model systems for human biology and medicine. (2020). doi.org/10.1038/s41580-020-0259-3
- Corrò, Novellasdemunt, and Li. A brief history of organoids. (2020). doi.org/10.1152/ajpcell.00120.2020
- Fatehullah, Tan, and Barker. Organoids as an *in vitro* model of human development and disease. (2016). doi.org/10.1038/ncb3312
- Kapałczyńska et al. 2D and 3D cell cultures – a comparison of different types of cancer cell cultures. (2016). doi.org/10.5114/aoms.2016.63743
- Yin et al. Stem Cell Organoid Engineering. (2016). doi.org/10.1016/j.stem.2015.12.005
- Malik and Rao. A Review of the Methods for Human iPSC Derivation. (2013). doi.org/10.1007/978-1-62703-348-0_3
- Vlachogiannis et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. (2018). doi.org/10.1126/science.aao2774
- Tao et al. Microengineered Multi-Organoid System from hiPSCs to Recapitulate Human Liver-Islet Axis in Normal and Type 2 Diabetes. (2022) doi.org/10.1002/advs.202103495
- Wu et al. Organ-on-a-chip: recent breakthroughs and future prospects. (2020). doi.org/10.1186/s12938-020-0752-0

BUILD-A-T-CELL WITH ADOPTIVE CELL TRANSFER THERAPY

by Sarah Stubington

Cancer is responsible for nearly 1 in 6 deaths every year. This figure is evidence that the antitumour immune response is far from perfect: in the biological game of hide and seek, it appears that cancer is winning. Despite T cells constantly monitoring the body for cells presenting danger-associated antigens, cancer cells find many ways of evading detection. These include downregulating the MHC molecules that present tumour-associated antigens or expressing immune checkpoint molecules (e.g. PD-L1) that can suppress T cell activity. The tumour microenvironment also limits the immune response to solid tumours: a fibrous matrix limits immune cell infiltration, and the cells that manage to penetrate it are often suppressed by tumour-resident immunosuppressive cells such as Tregs and Myeloid-Derived Suppressor Cells (MDSCs). When cancerous cells evade the immune system, they are able to develop into a tumour. Thus, one way to solve this problem is to artificially design better T cells. Great progress has been made in the last 40 years with manipulating the antitumour immune response by transfusing carefully selected or designed tumour-responsive T cells into the patient. This is known as Adoptive Cell Transfer Therapy and it provides a highly personalised approach by using a patient's own cells and editing them to selectively target the patient's unique tumour.

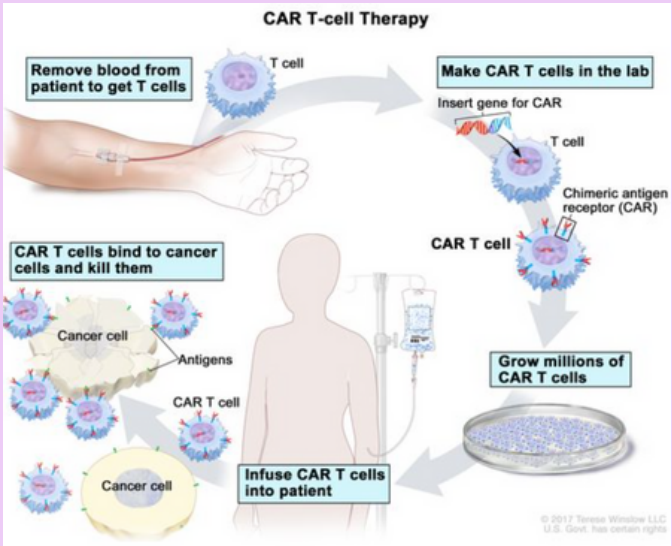
Adoptive Cell Transfer Therapy

The earliest forms of adoptive cell transfer (ACT) therapy used naturally occurring, tumour-infiltrating lymphocytes (TILs) extracted by tumour resection. Tumour-responsive T cells were then selected for before in-vitro activation and culturing. Performing this in vitro removed the cells from the immunosuppressive tumour microenvironment and allowed for the addition of various combinations of cytokines to favour survival, rapid proliferation and differentiation. The expanded activated TIL population could then be infused back into the patient to target the tumour. However, traditional ACT was limited to the T cells already present in the patient. These T cells had a propensity to express TCRs with a very low affinity for tumour antigens, and, as antigen recognition is MHC-dependent, downregulation of MHC expression would prevent an effective antitumour response. These two limitations (affinity and MHC-dependence) were overcome by gene engineering approaches to ACT.

Engineered T Cell Therapy

Gene-engineered T cells involve introducing receptors that target tumour-associated antigens which have been artificially designed to optimise the antitumour response. Engineered T Cell therapy can be divided into two types: TCR-T and CAR-T Cell therapy.

A key issue with the immune response to cancer is that cancerous cells are very similar to healthy cells. The majority of the antigens being presented by MHC complexes are also expressed by healthy tissues, thus making it difficult for T cells to distinguish between the two. Any T cells that do recognise the tumour-associated antigens will likely have TCRs with a very low affinity. This is because negative selection during T cell development ensures that T cells with a high affinity for self-proteins get destroyed; the subsequent low affinity prevents an effective anti-tumour response. **TCR-T Cell Therapy** uses a conventional T cell receptor which has been genetically edited to increase specificity and affinity for the tumour antigen, thus allowing for improved recognition and destruction of the tumour. TCRs provide MHC-restricted recognition of both intracellular and extracellular antigens.



The development of CAR-T Cells, Image from the National Cancer Institute

rTCRs and antibodies are both well-adapted for antigen recognition, but they each have limitations. TCRs effectively activate T cells through a CD3 ζ coreceptor but the binding of a CD4 coreceptor to MHC restricts their antigen recognition to MHC-presented epitopes. This poses a problem as cancer cells often downregulate their MHC expression to hide from immune detection. Conversely, the variable region of an antibody can bind to a specific, complementary antigen independent of MHC complexes, but they cannot activate T cells.

CAR-T Cell therapy overcomes these limitations by using Chimeric Antigen Receptors (CAR) which are designed from the variable region of an antibody (for antigen recognition) fused to the CD3 ζ coreceptor of the TCR (to facilitate T cell activation). This provides antigen-dependent but MHC-independent T Cell activation which bypasses the issue of MHC downregulation but restricts the antigen repertoire to surface molecules, a small fraction of the antigens normally accessible to T cells.

There have been four generations of CAR T cell development so far: the first generation contained only a CD3ζ signalling domain, while the second and third generations contained one or more costimulatory domains in addition. The fourth generation then included inducible cytokine genes allowing for the localised secretion of pro-inflammatory cytokines once the T cell has been activated by a CAR-antigen interaction. The cytokines can then recruit innate immune cells to target cancer cells that would otherwise be invisible to the CAR-T cells.

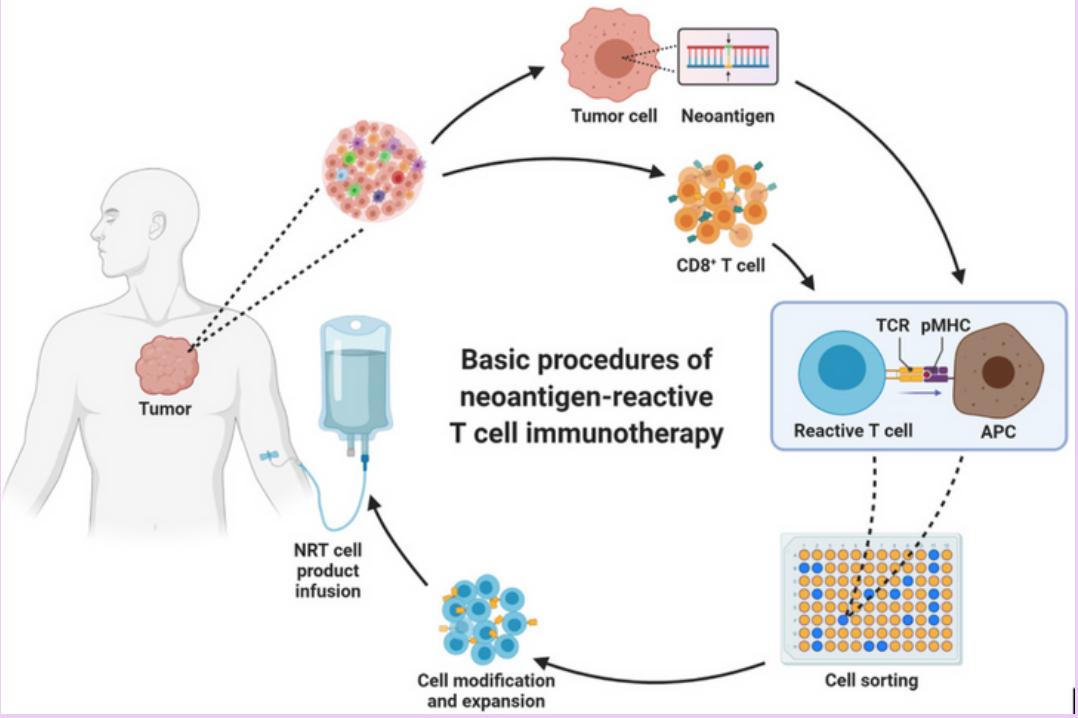
CAR-T cell therapy can be autologous using T cells extracted from the patient, or it can be allogeneic using T cells from a healthy donor. Allogeneic CAR-T cell therapy has the benefit of treating patients with defective T cells (e.g. due to chemotherapy) which is a major cause of tumour recurrence after ACT, and it has the potential of providing off-the-shelf CAR-T Cells which would allow for faster and potentially cheaper administration. However, allogeneic CAR-T Cell therapy comes with a risk of graft-versus-host disease (GVHD) if the T-cells recognise the patient as foreign and a risk of rejection if the patient's immune system recognises the allogeneic T cells as foreign. Both of these issues can be managed with careful HLA haplotype matching or genetic engineering of the T cells to remove receptors that could lead to an unwanted immune response.

Neoantigens and Personalisation

The potential of ACT has been limited by severe toxicity associated with on-target, off-tumour toxicity. This occurs because many tumour-associated antigens are proteins that are overexpressed by tumours but still expressed at a low level by normal tissues. Subsequently, the transplanted T cells can target healthy cells as well. This was seen in a severe reaction trialling CAR-T cells against HER2 which led to fatal lung T cell infiltration due to the expression of HER2 in pulmonary epithelial cells. Unexpected toxicity can also come from cross-reactivity when a TCR/CAR recognises a different but very similar epitope. For example, TCRs designed to recognise MAGE-A3 (a cancer-testes antigen not thought to be expressed by normal tissue) can show cross-reactivity with the related epitope MAGE-A12 expressed in the brain, resulting in damage to healthy grey matter. Furthermore, there is a much higher risk of toxicity with TCR-T Cell therapy as the short, MHC-presented epitopes are more likely to resemble other epitopes than a folded surface antigen recognised by a CAR-T Cell.

One way around these side effects has been to include “safety switches” in the engineered T cells. TCR-T cell therapy can use an inducible caspase 9 safety switch, making the T cells lethally sensitive to an exogenous ligand such that the T cell reaction can be terminated should side effects occur. Switchable CARs have also been designed whereby the antigen-binding domain is separated from the signal transduction domain - subsequently, they can only contact each other in the presence of an antibody. This process allows for better control of T cell activity – and, thus, the risk of toxicity - by controlling the dose of antibody. Mass-spectrometry HLA peptidomics studies could also help to identify the epitopes responsible for cross-reactivity and on-target/off-tumour toxicity, allowing patients at high risk of severe side effects to be identified through HLA-haplotyping. For example, patients with a HLA-A*0201 haplotype express MHC molecules that would present the cross-reactive MAGE-A12 epitope. These patients would therefore not be offered MAGE-A3 targeted treatments. The risk of toxicity can also be minimised for allogeneic CAR-T cell therapy by using TCRs with a CD4 coreceptor that cannot interact with the MHC molecule capable of presenting a cross-reactive epitope.

An alternative approach would be to use natural killer (NK) cells instead of T cells. NK cells lack TCRs and thus cannot cause GVHD. While NK cells would naturally have a shorter lifespan than T cells, they can be artificially encouraged to proliferate and persist in the body using IL-15 treatment or the deletion of TGFβ receptors, thus helping them to provide a longer-lasting immune response.



NEOANTIGEN-REACTIVE T CELL (NRT) PROCEDURES, IMAGE FROM HTTPS://DOI.ORG/10.1002/MCO2.41

The main focus of ACT development is currently identifying high-specificity neoantigens. The mutations that drive tumour development produce proteins with an altered amino acid sequence and, thus, unique peptide epitopes that are specific to the tumour and not expressed by any healthy cells. Developing CARs/TCRs which target these neoantigens will minimise on-target/off-tumour toxicity. Neoantigens can be identified by whole-exome sequencing of a tumour sample to identify mutated proteins before screening the candidate epitopes to determine which are presented on the surface of the tumour cell (and thus suitable for CAR targeting) and which are presented by MHC molecules (necessary for TCR-T Cell therapy). As part of this screening process, neoantigens are presented by antigen-presenting cells and T cells expressing activation markers are selected through flow cytometry. The neoantigens expressed by a tumour will be highly patient-specific, and the T cells engineered will also need to be patient-specific to ensure antigen recognition and prevent rejection. The most effective neoantigens are often those derived from the driving mutation in cancer development (often mutations to p53, KRAS, MYD88, etc) meaning that all cells descended from this initial mutated cell will express the neoantigen. This is especially important when targeting solid tumours which show considerable heterogeneity in antigen expression.

The future of Adoptive Cell Transfer Therapy

Adoptive cell transfer therapy is one of the most personalised forms of cancer treatment available, but for ACT to reach its full potential, it is important to balance maximising specificity with increasing accessibility and minimising toxicity. At the most personal level, T cells are extracted from the patient and CARs are designed to target the specific neoantigens presented by the patient's tumour. However, this approach is currently too time-consuming and expensive for wide application. Finding neoantigens shared by multiple cancer types could help to provide rational therapy to groups of patients identified by tumour genome sequencing and HLA haplotyping. Combining this approach with multi-gene edited allogeneic CAR-T Cells ("universal CAR-T Cells") could streamline the process to make it quick and cheap. For TCR T-cell therapy to play a role in this future, it is important to find ways of minimising the toxicity risk with careful HLA haplotyping and restriction. Efficacy can then be maximised with combination treatments that deliver ACT alongside immune checkpoint inhibitors to ensure the infused cells do not get suppressed by the tumour microenvironment.

References and Further Reading:

1. Rosenberg, S. A. & Restifo, N. P. Adoptive cell transfer as personalized immunotherapy for human cancer. Science 348, 62-68 (2015).
2. Zhao, L. & Cao, Y. J. Engineered T Cell Therapy for Cancer in the Clinic. Frontiers in Immunology 10, 2250 (2019).
3. King, A. The Less Personal Touch. Precision Oncology 585, 4-6 (2020).

DNA-ANALYSIS INCONCLUSIVE? RNA IS COMING TO THE RESCUE!

by Taisya Volodina

The cooperation between oncologists and genetics experts has picked up steam in recent years. Clinicians can examine tumours for actionable genetic mutations which boost drug sensitivity or test patients with colorectal cancer for microsatellite instability [1], which can improve the prognosis. A revolutionary whole genome sequencing technique was released on the market in 2014, but genetics now has much more to offer. In addition to DNA analysis, there are tools available that enable us to dive deeper and take a look at the transcriptome [2] or even the proteome [3]. One of these tools is RNA sequencing, which is discussed in this article.

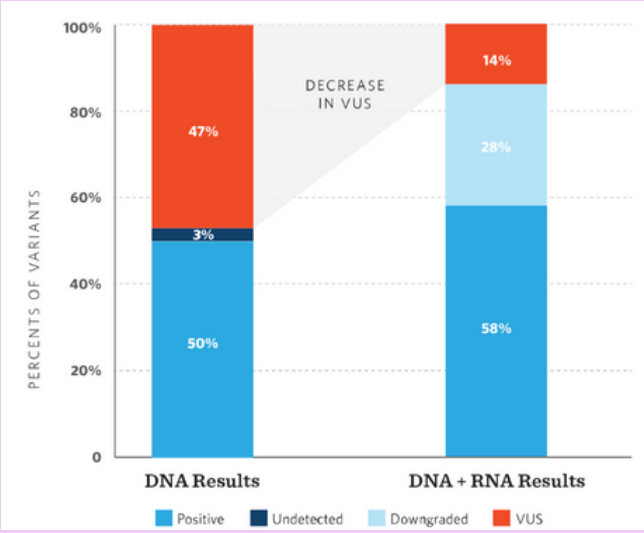
There are several reasons for an oncological patient to land in a genetic counselling session. Typically these are patients who have rare tumour forms, cancer in the family, or were diagnosed with cancer for the first time at a young age. If a tumour-causing mutation is discovered, this knowledge can support clinicians in selecting a therapy strategy. Additionally, any children of the patient may be at risk of inheriting the mutation and might have to be contacted. Clinical recommendations for them, such as more regular screening tests and preventive measures can be based on the genetic findings.

A modern up-to-date equipped lab can indeed easily find a mutation in a patient's DNA today. We can sequence entire genomes in a matter of hours thanks to Next Generation Sequencing. However, what many people don't realise is that we are still not at the point where we can interpret the consequences of every variant* that has been detected and provide patients with significant results regarding the variant's pathogenicity [4] and, accordingly, further recommendations. *variant is a correct term for mutation i.e genetic alteration

In fact, around 50% of all clinically detected variants are so-called Variants of Uncertain Significance or VUS. This indicates that, based on the data that has been published so far, we still do not have enough to make any statements regarding the pathogenicity of this specific variant. It could have been found in patients with cancer as well as healthy probands [5], so there is no evidence of the variant being associated with a disease.

It must be frustrating for a patient to leave the clinic and still be in the dark despite running all the available high-cost tests. Thankfully, one of the most exciting aspects of genetics is that it is continuously developing and novel technologies become accessible to doctors every year. For this reason, labs usually store genetic material - be it blood or hair samples - with the consent of the patients. There is always a possibility that it can be examined later on using new tools, at which point the patient will be contacted and provided with additional results.

As a doctoral intern, I have been working on the implementation of an RNA-Sequencing technique in routine diagnostics at one of the leading genetic centres in Munich. Using this tool supplementary to DNA analysis allowed us to clarify the diagnosis for cancer patients by reclassifying the VUS I addressed earlier into either pathological or benign [6] variants.



Source: https://www.ambrygen.com/providers/genetic-testing/rna

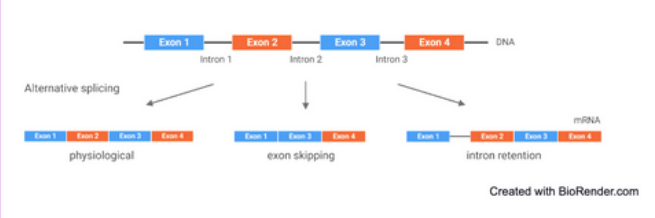
So how does RNA-Sequencing work and how can it support conventional DNA diagnostics?

Let's begin our journey with a patient sacrificing some blood or skin fibroblasts [7] at a geneticist consultation. After that, RNA is extracted in the lab and we are left with a few microliters of precious transparent liquid containing variable RNA molecules you can find in the cells (mostly rRNA).

At this point I want you to make sure you are familiar with the basics of protein synthesis, moving from DNA to RNA level and synthesising proteins based on the nucleotide sequence of mRNA. In the RNA-Seq workflow, an enzyme called Reverse Transcriptase is the key player. You have probably heard of this enzyme in the context of RNA viruses, such as COVID-19, invading human cells with its help. The role of this enzyme is to create DNA complementary to mRNA (cDNA), hence a process opposite to transcription in normal protein synthesis. Viruses exploit our cells for reproduction and synthesis of viral proteins; that's why they must convert their RNA into DNA that can eventually interact with human enzymes.

In our case, Reverse Transcriptase delivers DNA material from the RNA sample that one can amplify and easily sequence utilising Next Generation Sequencing techniques.

We are interested in mRNA sequence because, during transcription, DNA undergoes several transformations on its way to mRNA, including alternative splicing. Large enzyme complexes called spliceosomes cut out the non-coding parts of the genes so that mRNA ideally consists of a range of exons (coding sequences) in a row. Splicing can fail, just like any other highly complex process in our cells. The most common splicing pathomechanism [8] is so-called exon-skipping. When at least one of the splice sites is damaged and can not be recognized by the spliceosome, the spliceosome reaches out to the next nearest splice site in the following exon. This results in linking up of two non-successive exons and the one in between is skipped. Lately, another form of splicing has been associated with diseases, namely intron retention. In this case, introns (non-coding sequences) remain a part of a mature mRNA instead of being spliced out (see the Illustration).



RNA Sequencing, unlike DNA analysis, makes it possible to trace the impact of a DNA mutation on splicing events. Splicing patterns can indeed vary to some extent, producing different transcripts from one gene sequence. These deviations are physiological [9] and give alternative splicing its name. However, if the percentage of divergent splicing events is too high, it can be considered pathological and lead to further consequences on the protein level.

Apart from splicing, RNA transcripts also reflect the allelic [10] expression of a gene. Usually two copies of the gene –one from each parent– are actively transcribed. By the patients, the alleles may be expressed unequally or one allele may even be silenced while the other is expressed. So-called allelic imbalance or even loss is pathogenic in the majority of cases.

We can beautifully track the allelic imbalance using RNA and comparing it to DNA. There is a good possibility that one allele was "lost" during transcription if a heterozygous [11] single nucleotide mutation was found in the DNA and appeared homozygous in the RNA transcript.

As medicine is moving towards personalization of both diagnostic and treatment methods, RNA-Sequencing is one of the tools of choice when it comes to a more profound understanding of genetic variants. This functional tool enables one to comprehend the impact of a particular variant on transcription and see how it affects the splicing process as well as allelic expression. It can also be used to see which genes are active and inactive in a cell, as well as how gene expression differs depending on the sample tissue and on the point in time when the material was collected. RNA Sequencing results already support oncologists and other clinicians in therapy adjustments and diagnostic recommendations for the index patients [12] as well as for their family members. But, as we know, genetics is evolving rapidly, so there is more to come in the next few years, stay tuned!

A small glossary to refresh some terms:

1) **microsatellite instability** - high number of mutations within regions of repeated DNA as a result of impaired mismatch repair mechanism (one of the DNA repair mechanisms)
2) **transcriptome** - the set of all RNA transcripts in an individual or in a cell population
3) **proteome** - the entire set of proteins that is, or can be, expressed by a genome, cell, tissue, or organism at a certain time
4) **pathogenicity** - the potential ability to produce disease
5) **proband** - test person
6) **benign** - not harmful in effect
7) **fibroblast** - a cell in connective tissue which produces collagen and other fibres
8) **pathomechanism** - the mechanism by which a pathological condition occurs
9) **physiological** - characteristic of or appropriate to an organism's healthy or normal functioning
10) **allele** - variation of the same sequence of nucleotides at the same place on DNA molecule
11) **heterozygous** - having two different alleles of a particular gene
12) **index patient** - an individual affected with the first known case of genetically transmitted condition or mutation in a population, region, or family

WIDENING THE GAP:

THE IMPACT OF UNEQUAL ACCESS TO PERSONALIZED MEDICINE ON HEALTH INEQUALITIES

by Ashley Jackson

With every new medical advancement, there is always one goal in mind: making people's lives better. Whether it be through improving symptoms, prolonging life, or reducing side effects: researchers are constantly pushing the field of medicine forward with the goal of improving the quality of life for patients. But what if this isn't the case? Is it possible for the most cutting-edge technologies to actually do more harm than good?

Although new and innovative technologies are being developed, in the process from innovation to implementation, equitable access often gets forgotten. Unfortunately, medical advancements tend to benefit some more than others, and this is where we see the downfall of personalized medicine.

Socioeconomic Disparities

For starters, personalized medicine is not only expensive in its treatment, but also requires advanced testing to identify people who are best suited for certain therapies. Since the cost of personalized medicine, from diagnosis to treatment, is substantial, people of higher socioeconomic status will inevitably have better access to the latest technologies (1). Therefore, there is the potential to exacerbate already existing health inequalities based on wealth.

Let's look at Cystic Fibrosis (CF) as an example. Trikafta is a very effective drug used to treat CF patients with a specific mutation called F508del which occurs in approximately 90% of all cases of the disease. However, the annual cost of the medication per patient is estimated at well over £250,000 per year. In places like the UK, Ivacaftor is publicly funded for patients, but in many other countries, access to expensive drugs like these is largely dependent on private health insurance and socioeconomic status. Health inequalities are thus exacerbated within countries, and also globally as lower-income countries will face barriers to implementing these technologies (2).

There are clearly issues with access that is dependent on health insurance and wealth. But even in places like the UK, it is still very likely that wealthier people will benefit more and earlier from these innovations. It's well understood that health centres in low-income neighbourhoods are slower to receive new therapies than those in wealthier and bigger centres. In other words, geographic location and socioeconomic status can play a large role in access to personalized medicine.

It is also well known that patients of lower socioeconomic status tend to have poorer outcomes and are less likely to receive novel treatments. While access plays a large role, the social determinants of health are also a likely culprit in this finding. Social determinants of health are non-medical factors such as wealth, education and housing that influence health outcomes (3). There has been a huge push over the past few decades for better attention to the social determinants of health and how a person's environment and circumstances can impact their wellbeing. However, as medicine becomes more personalized and focused on the individual, there is fear that these important determinants will slowly be forgotten. As our focus shifts away from environmental factors and towards genes, the social intricacies of health may not receive the attention they deserve. If this is the case, there is no doubt that existing inequalities will be perpetuated.

Racial Inequalities

Speaking of CF drugs, the research landscape in personalized medicine already reflects the potential to exacerbate healthcare inequalities based on race. About 10,000 people in the UK have CF, with 1 in every 2,500 births being affected by the disease. Meanwhile, sickle cell disease (SCD), a hereditary haemoglobin abnormality that can have devastating health consequences, affects around 15,000 people in the UK. Despite the similar prevalence of these two diseases, there has been a discrepancy in the medical innovations developed to fight these diseases. There are a number of drugs that have been developed to treat various genetic mutations in CF, which has revolutionized treatment of the disease and drastically improved quality of life for these patients. Meanwhile, there have been few to no advancements to treat SCD. Of note, CF tends to affect people of European ancestry, and SCD tends to affect people of African and African-Caribbean ancestry. One can only guess that racial biases are at least partly to blame for these discrepancies in

medical advancements.

The Big Issue

What's the problem with the widening gap in health inequalities? Well, the obvious response is that healthcare is a human right and that every person should have equal access. But there are further intricacies to this argument. Healthcare is one of the most important resources to accessing other opportunities in life. Good health can open doors to better job prospects, higher education, and greater financial success. In allowing people preferential access, this not only creates a space for the exacerbation of health inequalities, but also social and economic inequalities in all aspects of life. Additionally, research has shown that the health gap between the most and least well off in a society is a big predictor of overall population health. In other words, reducing or exacerbating healthcare inequalities can have impacts on everyone.

Moving Forward

Personalized medicine has the potential to revolutionize the field of medicine. But we need to make sure that it revolutionizes the field for everyone. If we continue to allow barriers to access to persist, whether geographical, socioeconomic, or racial, then personalized medicine has the potential to exacerbate healthcare inequalities. Special attention needs to be paid to making medical advancements accessible to everyone and research needs to reflect that. Only then will we truly push the field of medicine forward.

References and Further Reading:
1. Brothers, K. B. & Rothstein, M. A. Ethical, legal and social implications of incorporating personalized medicine into healthcare. Per Med 12, 43-51 (2015). https://doi.org:10.2217/pme.14.65
2. Adeniji, A. A., Dulal, S. & Martin, M. G. Personalized Medicine in Oncology in the Developing World: Barriers and Concepts to Improve. World J Oncol 12, 50-60 (2021). https://doi.org:10.14740/wjon1345
3. Social determinants of health, <https://www.who.int/health-topics/social-determinants-of-health#tab=tab_1> (2022).
4. Geneviève, L. D., Martani, A., Shaw, D., Elger, B. S. & Wangmo, T. Structural racism in precision medicine: leaving no one behind. BMC Med Ethics 21, 17 (2020). https://doi.org:10.1186/s12910-020-0457-8

PHARMACOGENOMICS IN COMMUNITY:

PHARMACY AND ROLE OF PHARMACIST

by Hossameldin Saber

During drug development stages, drugs are generally tested on a large population of people and the average response is reported. This sort of evidence-based medicine (that is, medical decision-making based on empirical data) relies on the law of averages and statistics that do not consider individual variations. Throughout clinical practice, in many cases, we find different responses to drugs that may counter the expected results. Since patients come with different sets of characteristics, the one-size-fits-all approach of drug development fails to deliver.

Working as a pharmacist, I have always seen many patients who come with a new prescription for the same condition that the previous one showed to be ineffective. By that time, patients had already wasted money and time, and were even more impacted by their condition. Also, patients may come with serious side effects that require hospitalization, especially in elderly patients with co-morbidities and polypharmacy situations.

These types of medication errors tend to happen in primary care settings. In most cases, they can be avoided when the role of the genetics accounting for individual variation to drugs is considered.

Pharmacogenetics (PGx) describes the relationship between variations in an individual's DNA sequence and drug metabolism, transport, and response. Acknowledging these drug-gene interactions (DGI) can support individual personalized prescriptions.

This is important for both drug safety and effectiveness. The rate at which aberrant phenotypes occur in the general population is high. Over 95% of the population carry a genetic variant affecting the prescribing of at least one drug.

Now, imagine that patient had their genetics sequenced and stored to inform prescribers of the effective medications and possible side effects at the very first prescription instead of hindering the patients' health with trial and error.

This type of practice is called personalized medicine. When I came across the concept of pharmacogenomics, the first thing that came to my mind was "why this is not a standard practice already?" It would be a miracle to tailor the medication plan specifically for each individual. That is now called Medication Therapy Management MTM.

Of course, implementing pharmacogenomics into pharmacy practice has gone through many years of advancement to overcome challenges of high cost, sophisticated techniques and feasibility. Thus, that futuristic approach was limited for the patients who needed individualized pharmacotherapy the most. Drugs to deal with cancer, antipsychotics, and analgesics were the main focus of pharmacogenomics. Therefore, for the last decade, we could view the literature for pharmacogenomics applications in secondary care settings only.

As pharmacogenomics advances to solve the old challenges and makes its application more feasible, we're starting to see the spectrum widen to include more drugs and genes studied that affect drug-gene interactions that can happen and be controlled in community pharmacy settings.
In this article, we cast light on the implementation of pharmacogenomics in community pharmacies and its impact on patients' outcomes.

A case study of a 65-year-old man who underwent coronary artery stent placement post–myocardial infarction and was prescribed 75 mg of clopidogrel daily. His medical history includes hypertension and hyperlipidemia and received lisinopril, metoprolol, and pravastatin. He was presented to the pharmacy with a prescription for clopidogrel which the pharmacist screened and counseled regarding the need for a pharmacogenetic test of CYP2C19 genotyping relative to antiplatelet therapy as a part of a comprehensive medication therapy management (MTM) evaluation.

Upon consent, the patient was instructed on the proper buccal swabbing technique to provide samples, then sent to a clinical laboratory, with results returned to the pharmacist. The patient carries 1 CYP2C19 loss-of-function allele, being an intermediate metabolizer. The results were interpreted using published pharmacogenetics evidence-based antiplatelet selection guidelines, and the community pharmacist recommended that the cardiologist change the patient's antiplatelet therapy. The physician prescribed prasugrel 10 mg once daily and the patient remains on the drug.

The pharmacist intervention caused a shift in the medication plan according to patient's individual genetics to affect that particular drug. This case study shows us that a community pharmacist is a reliable medical personnel in implementing personalized medicine. Case studies of many other drugs encountered in community pharmacy level are published in the literature. It is predicted in the upcoming years to see the application of personalized medicine starting from the level of primary care settings specially community pharmacies.

References and Further Reading:

- **D. Kisor, D. Bright, M. Conaway, B. Bouts ,and G. Gerschutz. Pharmacogenetics in the Community Pharmacy: Thienopyridine Selection Post–Coronary Artery Stent Placement.** *Per Journal of Pharmacy Practice* **(2014) DOI: 10.1177/0897190014522496**
- **M. Hayashi, D. Hamdy, S. Mahmoud. Applications for pharmacogenomics in pharmacy practice: A scoping review.** *Per RSAP* **(2021). https://doi.org/10.1016/j.sapharm.2021.08.009**

DO YOUR MEDICINES *REALLY* WORK?

by Olivia Fisher

What is pharmacogenomics?

Pharmacogenomics is a term that has been used by scientists, researchers, and healthcare professionals for many years - however, what is it, and why is it important to us?

Pharmacogenomics is the study of 'pharmacology' and 'genomics'. 'Pharmacology' simply relates to the study of medicines or drugs and 'genomic' means the study of an individual organism's genome (or, more simply, the study of all of one person's DNA, made up of thousands of genes). When combining the two, we are referring to how an individual's genome can affect the way that they respond to their medications. Just as we each differ in our response to various foods, the range displayed in our response to drugs can be similar.

More than 100 drugs have been identified as having a genetic component. The way that these drugs interact with the body could cause a drug [TE3] to have an increased risk of sides effects, increased risk of toxicity, or reduced efficacy.

What are some examples of pharmacogenomics in clinical practice?

A medication that you may have heard of is the penicillin-based antibiotic, flucloxacillin. Flucloxacillin is commonly used for infections of the skin, however certain changes in some individual's genes (gene variation) have been identified which make them much more likely to experience toxicity-driven liver damage when administered the drug. Although this is rare (affecting 1-2 in every 1000 individuals), it is a potentially serious condition. In fact, flucloxacillin is not licensed in America due to this particular risk. Despite research suggesting that individuals with some variants of their genes could have an 80-fold increased chance of drug-induced liver disease in comparison to those without, there is currently no genetic test conducted before prescribing flucloxacillin in the UK.

An example of using genetic testing for a certain response to a drug in practice can be found in the use of Carbamazepine, a common anti-epileptic drug. Researchers discovered that in individuals of Han Chinese or Thai origin, they are at a much higher risk of developing potentially life-threatening, skin-related adverse drug reactions, including Stevens-Johnson Syndrome and toxic epidermal necrolysis. Therefore, it is now recommended by the Medicines and Healthcare products Regulatory Agency (MHRA) that screening in these populations should occur to rule out this gene variation before considering treatment. More recently, research has indicated that this risk may extend to other Asian populations and even to those of European descent. However, there is currently insufficient data to support pre-treatment screening in these populations. This example illustrates the importance of ensuring that clinical trial data is representative of different populations worldwide and of increasing the diversity of participants. [TE1]

Pharmacogenomics also plays an important role in medications used for pain management, a medical area that is notoriously debilitating and difficult to treat. In particular, understanding pharmacogenomics could help to combat the opioid crisis by helping clinicians predict those that could be more at risk of addiction. CYP2D6, a well-known human metabolizing gene, is involved in the metabolism of around 25% of the drugs used in clinical practice. Hundreds of variations of this gene have been identified. Some commonly used opioids, including codeine, tramadol, and oxycodone are metabolised by CYP2D6. Codeine is one of the most prescribed pain medications in both hospitals and in the community. Individual responses to codeine by the body can vary greatly as it is broken down into morphine. CYP2D6 plays a role in this metabolism – thus, gene variation can lead to a reduced or increased amount of morphine in the body. Poor metabolizers, for example, are unlikely to achieve adequate pain control in comparison to ultrarapid metabolizers (greater than >20% of the population in some Asian and African communities). Hence, these individuals are more likely to experience increased side effects or, in more serious cases (and particularly in younger patients), respiratory depression.

Where are we now?

Unfortunately, due to many barriers such as cost, education, research, and ethics, we are yet to accomplish routine genetic testing for many of the

medications that we know to have a genetic component. In many countries across the globe, pharmacogenomic testing services have been delivered in community pharmacies, though this is yet to happen in the UK. Education of healthcare professionals, notably pharmacists, is crucial to allow them to be at the forefront of implementing pharmacogenomic testing.

Genomics England is currently rolling out a new study called the 'Newborn Genomes Programme' which aims to sequence over 100,000 new-born genomes for information on rare diseases, susceptibility to disease, and pharmacogenomics. The data from this project could help us to build a future where medicines can be best optimised for every patient.

References & Further Reading

- *https://www.nature.com/articles/nature15817*
- *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7504675/*
- *https://www.gov.uk/drug-safety-update/carbamazepine-oxcarbazepine-and-eslicarbazepine-potential-risk-of-serious-skin-reactions*
- *https://bnf.nice.org.uk/drugs/carbamazepine/*
- *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6613192/*
- *https://www.tandfonline.com/doi/full/10.1080/15622975.2021.2012397?cookieSet=1*
- *https://bmchealthservres.biomedcentral.com/articles/10.1186/s12913-022-07730-y*
- *https://www.genomicsengland.co.uk/initiatives/newborns*